Non-Symmetrically-Masked Tri*PPP*ro Prodrugs

and

γ-Modified Nucleoside Triphosphate Compounds

as Potential Antivirals against HIV

Dissertation

zur Erlangung des naturwissenschaftlichen Doktorgrades

von

Chenglong Zhao

Vorgelegt dem Fachbereich Chemie

der Universität Hamburg

Hamburg 2019

Die vorliegende Arbeit wurde in der Zeit von Oktober 2014 bis Dezember 2018 am Institut für Organische Chemie der Universität Hamburg im Arbeitskreis von Prof. Dr. Chris Meier angefertigt.

1. Gutachter: Prof. Dr. Chris Meier

2. Gutachter: Prof. Dr. Dr. h. c. mult. Wittko Francke

Datum der Disputation: 08.02.2019

Acknowledgement

First of all, I would like to thank Prof. Dr. Chris Meier to be my supervisor. His patient and kind supervise provides me a free research atmosphere. I am grateful for his recognition of my work for the last four years. Further I would also like to thank Prof. Dr. Dr. h. c. mult. Wittko Francke as the second reviewer of my dissertation.

I would like to thank Prof. Dr. Jan Balzarini and Prof. Dr. Dominique Schols for the antiviral test and preparing the cell extracts for research. I would also like to thank the NMR-team and MS-team for the efficient technical support.

I would like to thank all the members in the Meier group. We are like a family and supporting each other all the time. Hereby, I would like to thank Tristan Gollnest, Tobias Nack and Thiago Dinis de Oliveira for introducing me into the project and primer extension experiment. Thank Dr. Johanna Huchting, Ivo Sarac, Simon Weising, Nils Jeschik and Alexander Laubach for helping me on communicating with university and making things work. I would like to thank Nora Constanze Fohrmann for the contribution with work on computational chemistry. I also want to thank Yara Angeloni for the synthesis contribution of my project when studying in the Meier group. Here is a special thanks to Matthias Winkler, not only I learnt everything in the lab when we are sharing the lab in room OC524, but also for helping me every aspects of the life in Hamburg. I also thank you for the proof reading of my thesis. Our friendship will last forever.

Last but not the least, I would like to thank my parents, my wife and all my family for supporting my decision to study abroad and encouraging me when there are difficulties. I am a lucky person that I meet my wife in Hamburg. She is the loveliest girl I have ever met which is also full of energy and enthusiasm. She always encourages me to keep on fighting when difficulty comes. Loving you!

Time flies. My four years Ph.D. life in Hamburg is coming to an end. It is also my 30th birthday four days later. There is a Chinese old saying that a man should be independent at the age of 30. At this time point, new life will go on and beautiful hope is ahead. Like a greeting conversation in native German in the lab: ``*Was geht ab, Digga? Alles Gute!´´* I believe my following life will be better and better. Thank everyone who has helped me. They are the best people in the world!

21.10.2018 in Hamburg

List of abbreviations and symbols:

(HIV) RT	(HIV) reverse transcriptase
3TC	lamivudine (NRTI)
9AA	9-aminoacridine
AB	acyloxybenzyl
ABC	abacavir (NRTI)
Ac	acetyl
AIDS	acquired immunodeficiency syndrome
ANS	1-amino-naphthalene-5-sulfonate
araC	cytarabine
ART	antiretroviral therapy
ATP	Adenosine triphosphate
AZT	3'-Azido-3'-deoxythymidine, Zidovudine (NRTI)
BAB	bis-acyloxybenzyl
BIC	bictegravir (INSTI)
br	broad signal (NMR)
c	cobicistat (Cytochrome P450 inhibitor)
CA	capsid protein
cART	combination antiretroviral therapy
CatA	cathepsin A
CC_{50}	50% cytostatic concentration
CCR5	C-C chemokine receptor type 5
CD4	cluster of differentiation 4
$CDCl_3$	chloroform
CEM/0	Human $CD4^+$ T-lymphocytes cell line (wildtype)
CES1	carboxylesterase
Chol-ATP	Cholesteryloxycarbonyl-ATP
CYP3A4	cytochrome P450 enzyme
d	doublet (NMR)
d4T	2′,3′-didehydro-2′,3′-dideoxythymidine, stavudine (NRTI)
d4U	2',3'-Didehydro-2',3'-dideoxyuridine (NRTI)
DAG	diacylglycerol
DCM	dichloromethane
dd	doublet of doublets (NMR)
ddC	zalcitabine (NRTI)
ddI	didanosine (NRTI)
ddT	2',3'-Dideoxythymidine (NRTI)
DHB	2,5-dihydroxybenzoic acid
DMF	dimethylformamide

DMSO	dimethyl sulfoxide
DP	diphosphate
DPP	diphenyl-*h*-phosphonate
dq	doublet of quartets (NMR)
DSG	distearoylglycerol
dt	doublet of triplets (NMR)
dT or T	thymidine
DTE	dithioethanol
DTG	dolutegravir (INSTI)
EA	ethyl acetate
EC_{50}	50% effective concentration
EFV	efavirenz (NNRTI**)**
EI	electron ionization
Eq. or equiv.	equivalent
ESI	electrospray ionization
EVG	elvitegravir (INSTI)
FDA	The US Food and Drug Administration
FI	fusion inhibitor
FTC	Emtricitabine (NRTI)
GP	general procedure
HAART	Highly Active Antiretroviral Therapy
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HIV	human immunodeficiency virus
HIV-1	HIV type 1
HIV-2	HIV type 2
HPLC	High-performance liquid chromatography
HRMS	high resolution mass spectrometry
IN	Integrase
INSTI	integrase strand transfer inhibitor
*i*Pr	*iso*-propanol
IR	Infrared spectroscopy
J	coupling constant
m	multiplet (NMR)
MA	matrix protein
MAB	mono-acyloxybenzyl
MALDI	matrix-assisted laser desorption/ionization
Me	methyl
MeCN	acetonitrile
MeOH	methanol

MP	monophosphate
MS	mass spectrometry
NC	nucleocapsid protein
NCS	N-Chlorosuccinimide
NDP	nucleoside diphosphate
Nef	negative regulatory factor
NMP	nucleoside monophosphate
NMR	nuclear magnetic resonance
NNRTI	Non-nucleoside reverse transcriptase inhibitor
NRTI	nucleoside reverse transcriptase inhibitor
NTP	nucleoside triphosphate
NuCl or Nu.	nucleoside
PAI	post-attachment inhibitor
PBMC	peripheral blood resting cells
PBS	phosphate buffer solution
PE	petroleum ether 50-70
PE	pharmacokinetic enhancer
Ph	phenyl
PHA	phytohemagglutinin
PI	protease inhibitors
PI	protease inhibitor
PLE	Pig liver esterase
POC	*iso*-propyoxycarbonyloxymethyl
Pol	DNA polymerase (Human)
POM	pivaloyoxymethyl
PR	protease
q	quartet (NMR)
r. t.	Room temperature
Rev	regular of virion protein
RNA	Ribonucleic acid
RP	reverse phase
RPMI	Roswell Park Memorial Institute medium
RPV	rilpivirine hydrochloride (NNRTI)
s	singlet (NMR)
SATE	S-acylthioethyl ester
SIV	simian immunodeficiency virus
SIVcpz*Ptt*	chimpanzee SIV *P. troglodytes* troglodytes
t	triplet (NMR)
$t_{1/2}$	half life
TAF	tenofovir alafenamide (NRTI)

Tat	trans-activator of transcription protein
TBAA	tetrabutylammonium acetate
TBAH	tetrabutylammonium hydroxide
*t*Bu	*tert*-butyl
TDF	tenofovir disoproxil fumarate (NRTI)
TEA	triethylamine
TFAA	trifluoroacetic anhydride
THF	Tetrahydrofuran
TK	thymidine kinase
TK^-	thymidine kinase deficient
TLC	thin layer chromatography
TMS	trimethylsilyl
TP	triphosphate
TTP	thymidine 5'-triphosphate
TXL	tenofovir exalidex
UV	Ultra Violet
Vif	viral infectivity factor
Vpr	viral protein r
Vpu	viral protein u
VZV	Varicella zoster virus
α	alfa position
β	beta position
γ	gamma position
λ	wavelength

CONTENTS

1 INTRODUCTION 1

2 LITERATURE REVIEW 4

2.1 Background 4

2.1.1 Basic Knowledge of Nucleobase, Nucleoside and Nucleotide Analogues 4

2.1.2 Human Immunodeficiency Virus (HIV) and AIDS 4

2.1.3 The Epidemic of AIDS and Antiretroviral Therapy (cART) of HIV 9

2.1.4 Nucleoside Reverse Transcriptase Inhibitors (NRTIs) 13

2.1.5 HIV-RT versus Human DNA Polymerases 14

2.2 The Development of Nucleotide Prodrugs or Pronucleotides 22

2.2.1 Nucleoside Monophosphate Prodrugs 22

2.2.2 Nucleoside Diphosphate Prodrugs 27

2.2.3 Nucleoside Triphosphate Prodrugs 30

3 MOTIVATIONS AND OBJECTIVES 35

4 DISCUSSION AND CONCLUSION 37

4.1 General Synthesis Route 37

4.2 Synthesis of Starting Materials 39

4.2.1 Synthesis of 4-(Hydroxymethyl)-phenyl esters 44 39

4.2.2 Synthesis of Nucleoside Monophosphate 41

4.3 Non-symmetric Tri*PPP*ro-compounds γ-(AB,ab)-d4TTPs 56 43

4.3.1 Synthesis of non-symmetric (AB,ab) *H*-phosphonates 55 43

4.3.2 Synthesis of non-symmetric Tri*PPP*ro γ-(AB,ab)-dNTPs 56 44

4.3.3 Chemical and Biological Hydrolysis 50

4.3.4 Anti-HIV activities in CEM/0 and CEM/TK$^-$ cells 57

4.4 Non-symmetrically-modified γ-(AB,alkyl)-d4TTPs 58 59

4.4.1 Synthesis of Non-Symmetrically-Modified (AB,alkyl)-*H*-phosphonate 57 60

4.4.2 Synthesis of γ-(AB,alkyl)-d4TTP 58 61

4.4.3 Chemical and Biological Hydrolysis 65

4.4.4 Anti-HIV Activities in CEM/0 and CEM/Tk$^-$ Cells 72

4.5 γ-(alkyl)-dNTPs 60,62 74

4.5.1 Synthesis of γ-(alkyl)-dNTPs 74

4.5.2 Chemical and Biological Hydrolysis 76

4.5.3 Anti-HIV Activities 81

4.6 Other γ-Modified NTPs 83

4.6.1 γ-(AB-C4,alkyl-C18)-d4UTP 64er 83

4.6.2 γ-(alkyl-C18)-ddTTP 67r 86

4.6.3 γ-(AB-C17,alkyl-EEE)-d4TTP 70r 88

4.7 Primer Extension Assays 91
4.7.1 Primer Extension Assay with HIV-RT 92
4.7.2 Primer Extension Assay with Human DNA Pol β 100
4.7.3 Primer Extension Assay with Human DNA Pol γ 101
4.8 A trial of explanation on the Selectivity through Computational Chemistry 102
5 CONCLUSION 104
6 EXPERIMENT SECTION....106
6.1 Chemicals and Instruments....106
6.2 General Synthetic Procedures 107
6.3 Hydrolysis Study 109
6.3.1 HPLC Method 109
6.3.2 Chemical Hydrolysis in PBS....109
6.3.3 Enzyme-catalyzed Hydrolysis in CEM cell extracts 109
6.3.4 Enzyme-catalyzed Hydrolysis in Pig Liver Esterase (PLE) 110
6.3.5 Preparation of Cell Extracts:....110
6.3.6 Data Analysis....110
6.4 Antiviral Assay against HIV 110
6.5 Chemicals and solutions for primer extension experiment....111
6.5.1 Enzyme....111
6.5.2 Sequence of Primer and Template....112
6.5.3 Chemicals and Solutions....112
6.6 Primer Extension Essay Condition 115
6.7 Experiment Data of Synthesized Compounds 116
7 REFERENCE 180
8 ATTACHMENT....191
8.1 Chemicals an Hazards....191
8.1.1 Hazardous Substances Directory with HP-statements 191
8.1.2 GHS Hazards Pictograms 197
8.1.3 Hazard Statements 197
8.1.4 Precautionary Statements....199
8.2 Overview of the Compound Structures....202
8.3 Curriculum Vitae 204
8.4 Publication List....205
8.5 Eidesstattliche Versicherung....206

Zusammenfassung

Die Entwicklung von Nukleosidtriphosphatprodrugs ist eines der herausragenden Ziele bei der Anwendung von antiviral wirksamen Nukleosid-Reverse-Transkriptase-Inhibitoren. In dieser Arbeit wurden Nukleosidanaloga hergestellt, welche zwei verschiedene Acyloxybenzyl-masken (AB-Masken) am γ-Phosphat aufweisen. Das Ziel besteht dabei in einer Erhöhung der Stabilität des Triphosphats in einem zellularen Medium, bei einer gleichzeitig schnelleren Spaltung der Acylesterbindung. Die dabei darstellten Verbindungen wurden in Zelltests gegen HIV-1 und HIV-2 getestet und zeigten Aktivitäten in natürlichen humanen $CD4^+$T-Lympocyten (CEM/0) wie auch in Thymidinkinase defizienten CD4* T-Zellen (CEM/TK). In den durchgeführten Hydrolysestudien in Phosphatpuffer und CEM/0-Zellextrakt konnte kein selektives Spaltverhalten zwischen den verschiedenen verwendeten AB-Masken beobachtet werden. Wenn jedoch eine AB-Maske mit einer Methoxytriglycol-Gruppe versehen wurde, konnte eine bevorzugte Spaltung der verbleibenden AB-Maske, welche eine Esterfunktion aufweist, beobachtet werden. Es zeigte sich jedoch, dass diese Verbindungstypen im Vergleich zu Verbindungen mit zwei klassischen AB-Masken eine deutlich geringere Aktivität aufweisen.

Darauf aufbauend wurde das γ-Phosphat von d4T-Triphosphat mit einem lypophilen Alkylrest versehen und *primer-extension assays* mit der viralen HI-Transkriptase und verschiedenen DNA-Polymerasen durchgeführt. Es konnte festgestellt werden, dass die Alkyl-modifizierten NTPs Substrate für die virale HI-Transkriptase, nicht jedoch für die DNA-Polymerase β, sind. Durch diese Unterscheidung werden bei einer potentiellen Verabreichung der Alkyl-modifizierten NTPs weniger Nebenwirkungen auftreten. Zusätzliche wurden die Alkyl-modifizierten NTPs mit einer weiteren Acyloxybenzyl-Gruppe am γ-Phosphat versehen. Im Gegensatz zum bisherigen Tri*PPP*ro-Konzept wird dabei jedoch nur das Alkyl-modifizierten NTP freigesetzt und nicht das Nucleosid-Triphosphat. Die Freisetzung des stabilen Alkyl-modifizierten NTP konnte in CEM/0 Zellextrakten nachgewiesen werden und im Gegensatz zu d4TTP zeigte das modifizierte NTP eine deutlich höhere Stabilität gegenüber einer Dephosphorylierung. In weiteren antiviralen Untersuchungen zeigte sich auch eine potente Inhibierung von HIV-1 und HIV-2 in Zelkulturen von infizierten CEM/0 Zellen und viel wichtiger auch in CEM/TK^- Zellen.

Abstract

The development of nucleoside triphosphate prodrugs is one of the outstanding goals in the application of antivirally active nucleoside reverse transcriptase inhibitors. Here, we firstly report on a nucleoside triphosphate analogue in which contain two different acyloxybenzyl-masks (AB-masks). The aim is to increase the degradation rate for the fast formation of NTP and the stability for cell delivery. Thus, γ-non-symmetric-dimasked Tri*PPP*ro-compounds was synthesized and they are active against HIV-1 and HIV-2 in cultures of infected wild-type human $CD4^+$ T-lymphocyte (CEM/0) cells and more importantly in thymidine kinase-deficient $CD4^+$ T-cells (CEM/TK^-). However, from the hydrolysis studies both in PBS and CEM cell extracts, there is no obvious selective cleavage behaviour between different AB-masks. Interestingly, when one AB-mask was modified with methoxytriglycol group with a diester linker, the other AB-mask with alkanoate was cleaved predominately. Unfortunately, these compounds are less potent than the Tri*PPP*ro-compounds with two alkanoate AB-masks.

Furthermore, the γ-phosphate was covalently modified by a lipophilic alkyl residue and d4TTP as the nucleotide analogue. Primer extension assays using HIV's reverse transcriptase (RT) and different cellular DNA-polymerases show a high selectivity of this type of γ-modified nucleoside triphosphates (NTPs) to act as substrates for RT while the compounds proved to be non-substrates for cellular DNA-polymerases β. Thus, the delivery of these γ-alkyl-triphosphate derivatives might potentially lead to a higher selectivity of these NTPs to act in infected vs. non-infected cells which might result in lower side effects. Additionally, a series of acyloxybenzyl (AB)-prodrugs of these γ-modified nucleoside triphosphates was prepared. In contrast to our previously disclosed Tri*PPP*ro-approach, here the intracellular delivery of a stable γ-alkyl-nucleoside triphosphate is envisaged. Successful and selective delivery of γ-alkyl-d4TTP was demonstrated in CEM cell extracts. In contrast to d4TTP, γ-alkyl-d4TTPs showed a very high stability in cell extracts towards dephosphorylation. In antiviral assays, the compounds were potent inhibitors of HIV-1 and HIV-2 in cultures of infected wild-type CEM/0 cells and more importantly in CEM/TK^- cells.

1 Introduction

Over the last decades a variety of nucleoside analogues were applied in antitumor and antiviral therapy and still play an important role to combat HIV, herpes virus, hepatitis B and hepatitis C virus infections.[1,2] The targets of these nucleoside analogue drugs are the virus-encoded DNA- or RNA-polymerases, such as the HIV reverse transcriptase (RT)[3,4] or the HCV-encoded RNA-dependent RNA-polymerase NS5B.[5] Till now, several nucleoside analogues have been approved as HIV reverse transcriptase inhibitors (NRTIs)[6] and they are used as the backbone of the combined antiretroviral therapy (cART). However, the antiviral efficacy of nucleoside analogues such as 3´-deoxy-2´,3´-didehydrothymidine **4** (d4T), is dependent on the in-vivo phosphorylation by host cell kinases into their nucleoside triphosphates (NTP), e.g. d4TTP **4t** via the nucleoside mono- (**4m**, NMP) and the diphosphate (**4d**, NDP).[7,8] The stepwise transformation into the corresponding triphosphates from **4** to **4t** often occurs insufficiently due to the substrate specificity of the involved kinases. Furthermore, limitations such as poor biological half-lives due to catabolic elimination, variable bioavailability after oral administration or selection of drug resistance, which reduce their clinical efficacy, have been observed for nucleoside analogues.[9] To overcome some of these hurdles prodrugs of the first phosphorylated metabolite, the nucleoside monophosphate (NMP), have been explored in the past and resulted in orally administered forms of some antiviral NMPs and are currently under continuing development.[10-13] Examples of efficient NMP-prodrugs are the phosphoramidates and *cyclo*Sal-phosphate triesters.[14-19]

A further issue with nucleoside analogues as antivirals is the need of a marked selectivity of the corresponding nucleoside triphosphate to act as substrates for the viral polymerase but not for cellular polymerases, e.g. DNA polymerase α, β and the mitochondrial DNA-polymerase γ. Particularly, the inhibition of DNA polymerase γ is crucial and often associated with marked toxicity effects. However, all these approaches delivered the monophosphorylated forms of the nucleosides which still need further phosphorylation into the triphosphate forms by cellular kinases to inhibit the corresponding polymerases. Recently, a successful NDP delivery approach by modifying at β-phosphate with lipophilic masks was developed but these NDP prodrugs (Di*PP*ro-approach) are still partially charged.[20-22]

Introduction

Although for a long time it was thought that it would be impossible to develop nucleoside triphosphate prodrugs[23], we recently disclosed the first delivery system of NTPs through a prodrug technology (Tri*PPP*ro-approach).[24-27] It was proven for d4T **4** and other nucleoside analogues that the corresponding Tri*PPP*ro-compounds even retained pronounced anti-HIV activity in CEM/TK$^-$ cell cultures whereas the parent d4T **4** was virtually inactive in these cells due to the lack of phosphorylation. The membrane permeability was achieved by covalently attaching two 4-acceptor-substituted benzyl esters at the γ-phosphate group. The enzyme-driven cleavage of the two masks in Tri*PPP*ro-compounds **41** by an initial cleavage of the acyloxy moiety and a subsequent spontaneous cleavage of the remaining part of the mask led to the formation of d4TTP **4t**. The cellular uptake of these compounds was proven by using fluorescent nucleoside analogues.[26] Then, we synthesized a series of non-symmetric-masked Tri*PPP*ro-prodrugs **56** bearing one long and one short AB-mask. We propose that the short mask should be fast cleaved in cells and the intermediate with one AB-mask would be formed, delivered into the cells and finally showed activity.

Further studies showed that the γ-dimasked Tri*PPP*ro-compounds were not substrates for polymerases such as HIV-RT or DNA-pol β. Nevertheless, the development of NTP prodrugs is still highly desirable because this would lead to the bypass of *all* steps of phosphorylation and would in principle maximize the intracellular concentration of the ultimately bioactive NTP.[28] Thus, the important unsolved issues are: i) the need of a sufficient selectivity of the NTPs to act as a substrate for viral polymerases and not for cellular polymerases and ii) decreased sensitivity of NTPs for enzymatic dephosphorylation. With regard to the lack of enzyme selectivity, it was observed in a previous study that pyrimidine nucleoside triphosphates including d4TTP **4t** inhibited Pol β slightly and was even more inhibitory to Pol γ, while not being a substrate for Pol α.[29]

To address these two issues, γ-(AB,alkyl)-NTPs prodrugs **58** and γ-alkyl-NTPs compounds **58**,**60**. Krayevsky and his coworker reported that, although the describe compounds were not fully characterized, the replacement of the γ-phosphate group by a methyl- or a phenyl-phosponate moiety had almost no effect on the substrate properties towards retroviral RT, but abolished their ability to function towards DNA polymerases α and β.[30,31] Moreover, the authors reported that such compounds led to an increase in stability of the triphosphate unit in human serum (about a 10-fold).[32]

However, their compounds proved to be antivirally inactive and were not studied in combination with a prodrug approach. A further motivation that such an approach is worth to explore was obtained from our own previous work. In contrast to Tri*PPP*ro-compounds **41**, the intermediate that comprised only one masking group proved to be a substrate for HIV-RT and d4TMP was incorporated into the primer strand. Moreover, although at a lesser extent than the Tri*PPP*ro-derivatives themselves, the mono-masked intermediate bearing a lipophilic octadecyloxybenzyl-moiety proved to be antivirally active in the cell assay using TK-deficient CEM cells (EC_{50} = 1.5 µM). Recently, we replaced the bioreversible acyloxybenzyl moiety in the intermediate by a non-cleavable ketobenzyl group and again these compounds proved to act on retroviral reverse transcriptase but not on DNA-polymerases β or γ. These observations guided us to the design of new γ-modified NTP prodrugs **58** which comprise simple γ-alkyl chains of different length in combination with a biodegradable acyloxybenzyl group. Enzymatic cleavage of the latter group will lead to γ-alkyl-NTPs **60**.

The synthesis of these new prodrug compounds **58** bearing different stable γ-alkyl groups in combination with a bio-reversible acyloxybenzyl group at the γ-phosphate moiety as well as their hydrolysis products **60**. Their hydrolysis properties in different media and their anti-HIV activity will be described. In addition, primer extension assays were conducted with compounds **60**,**62**. As a nucleoside analogue d4T **4** was used to allow a comparison with Tri*PPP*ro-compounds **41**,**56** bearing *two* enzyme-cleavable γ-acyloxybenzyl groups which led to the formation of the unmasked NTP.

2 Literature Review

2.1 Background

2.1.1 Basic Knowledge of Nucleobase, Nucleoside and Nucleotide Analogues

Nucleobases, also known as nitrogenous bases, are nitrogen-containing heterocyclic compounds that can be grouped into purines (adenine (A), guanine (G)) and pyrimidines (adenine (A), thymine (T) and uracil (U)). Figure 2-1 shows the general structure of nucleobase, nucleoside and nucleotide. A nucleoside consists of a nucleobase attached to a five-carbon sugar (ribose or deoxyribose) and nucleotide has an extra phosphate group (monophosphate, diphosphate and triphosphate) which is linked to the sugar moiety.

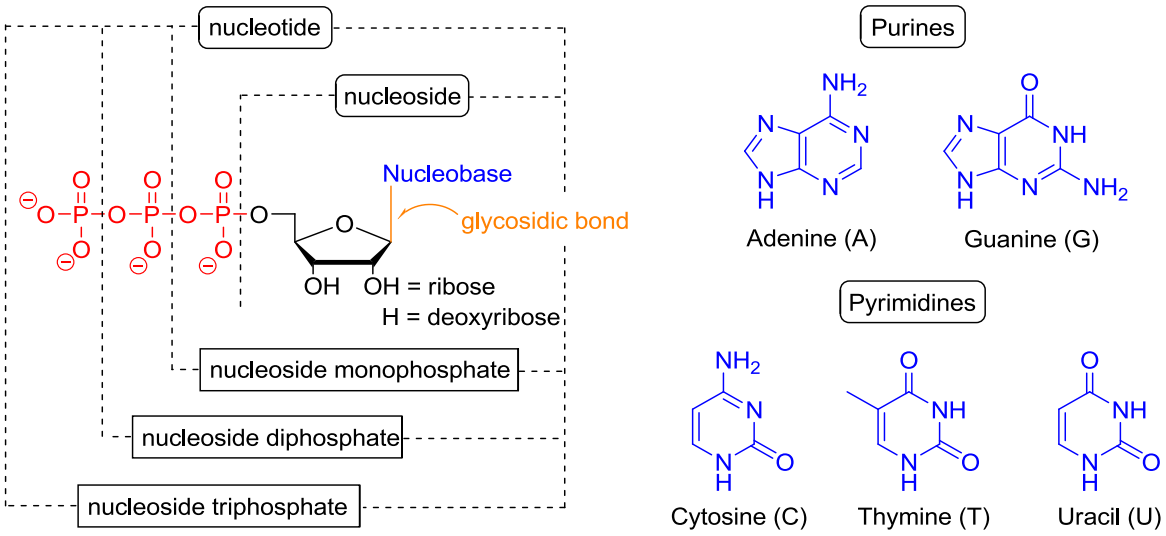


Figure 2-1: General structure of nucleobase, nucleoside and nucleotide

2.1.2 Human Immunodeficiency Virus (HIV) and AIDS

Human Immunodeficiency Virus (HIV) is a lentivirus in the retrovirus family that attacks human immune system, especially $CD4^+$ T cells and causes HIV infection. As time goes on, HIV will gradually destroy the immune system and advance to the final stage of HIV infection, which is called as Acquired Immunodeficiency Syndrome (AIDS).[33-36] The depletion of $CD4^+$ cells[37,38] will make the organism vulnerable to infections and certain cancers. Once a person becomes infected with HIV within 2 weeks to 4 weeks, flu-like symptoms like fever will appear. Then HIV continues multiply in human body. Without the treatment with anti-HIV medicines, HIV infection moves in to clinical latency stage (also called chronic HIV infection) without severe

symptoms and develops to AIDS within 10 years. At the final stage of HIV infection, the symptoms include: rapid weight loss; recurring fever or profuse night sweats; extreme and unexplained tiredness; prolonged swelling of the lymph nodes in the armpits, groin, or neck; diarrhea that lasts more than a week; sores of the mouth, anus, or genitals; pneumonia; red, brown, pink, or purplish blotches on or under the skin or inside the mouth, nose, or eyelids; memory loss, depression, and other neurologic disorders. HIV is spread through direct contact with HIV infected person's body fluids which include blood, semen, pre-semen fluid, vaginal fluid, rectal fluids and breast milk.[39-41]

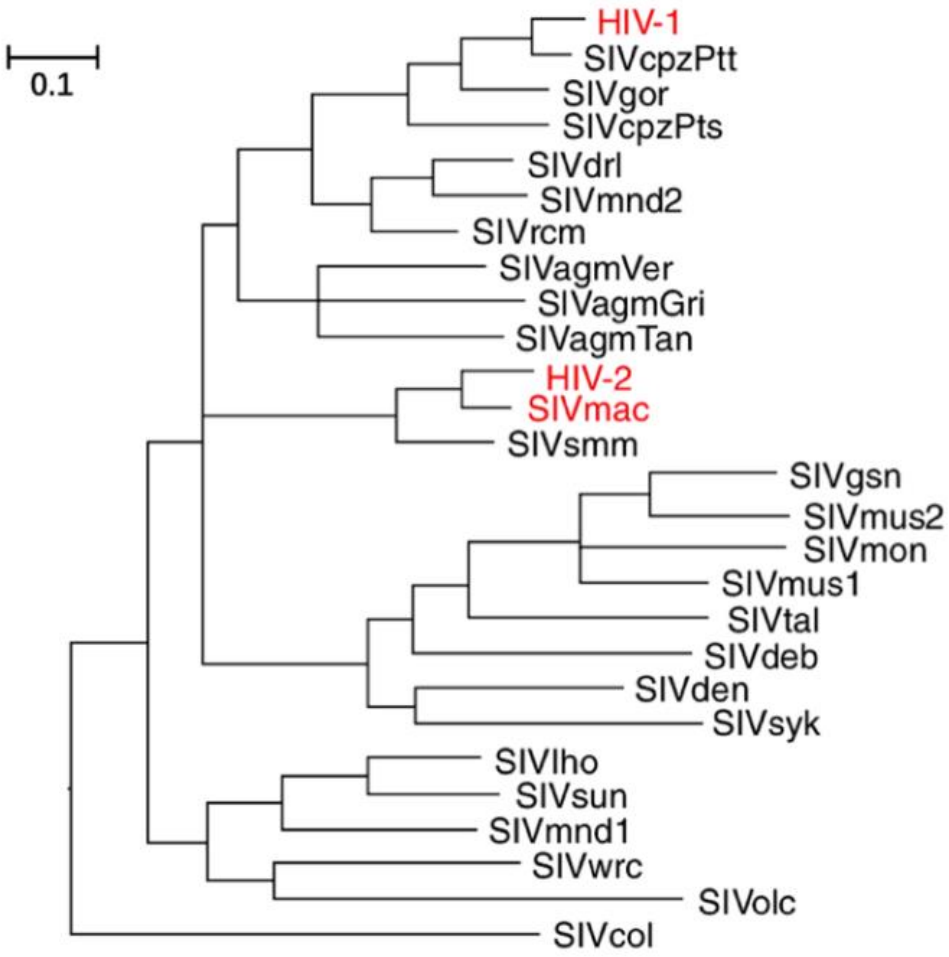


Figure 2-2: Phylogeny of lentiviruses in Primates. HIV-1, HIV-2 and SIVmac are highlighted in red. SIVmac is not a natural pathogen of macaques but has been generated inadvertently in US primate centers. The phylogenetic tree was estimated by Guindon and Gascuel in 2003. The scale bar represents 0.1 amino acid replacements per site.[42]

There are two major types of HIV: HIV type 1 (HIV-1) and HIV type 2 (HIV-2). Both are lentiviruses and they are a result of cross-species transmissions from African primates which naturally infecting simian immunodeficiency viruses (SIVs). HIV-1 include four distinct lineages groups M (the main cause of the AIDS pandemic), N, O and P. HIV-1 groups N and M are closely related to *P. t. troglodytes* (SIVcpz*Ptt)* strains from central chimpanzees in southern Cameroon. HIV group P is related to SIV*gor* strains from western gorilla, but the region is unclear due to there is no sufficient characterization where the transmission occurs. The immediate source of HIV group O is also unknown. However, the closer relationship between HIV group O, P virus and SIVcpz*Ptt* suggests that both groups originated in west central Africa. Interestingly, compared with HIV-1, HIV-2 is more related to SIVsmm strains from sooty mangabey. The origin of HIV-2 is sooty mangabey which was firstly proposed by Hirsch *et al*.[43] in 1989 and then confirmed by Gao[44] and Chen[45]. HIV-2 infections are predominantly found in West Africa. Unlike HIV-1 infection, HIV-2 has lower infectivity; most HIV-2 infections have lower viral loads and take longer time to progress to AIDS. All above shows that HIV-2 has a different natural history from HIV-1. Up to now, at least eight subtypes of HIV-2 have been identified (group A-H) and only HIV-2 group A and B are considered epidemic.[42]

The structure biology of HIV is shown in Figure 2-3.[46] It includes two strands of RNA, 15 types of viral proteins (and a few proteins from last host cell it infected) and a lipid bilayer membrane. The viral protein can be divided into three species: structural proteins, accessory proteins and viral enzymes. For structural proteins, there are glycoproteins gp120 and gp41 which help HIV binding itself to CD4 receptors, capsid protein (CA) which delivers viral RNA into the cell during fusion, matrix protein (MA) and nucleocapsid protein (NC). Accessory proteins consist of viral protein u (Vpu), viral infectivity factor (Vif), viral protein r (Vpr), P6, negative regulatory factor (Nef), regulator of virion protein (Rev) and trans-activator of transcription protein (Tat). Viral enzymes include reverse transcriptase (RT) which converts viral RNA into DNA, Integrase (IN) which inserts the copied DNA strand into cellular genome and HIV protease (PR). These three enzymes (RT, IN, PR) are the main biological targets of the corresponding anti-HIV medicines (NRTI, NNRTI, INSTI and PI).

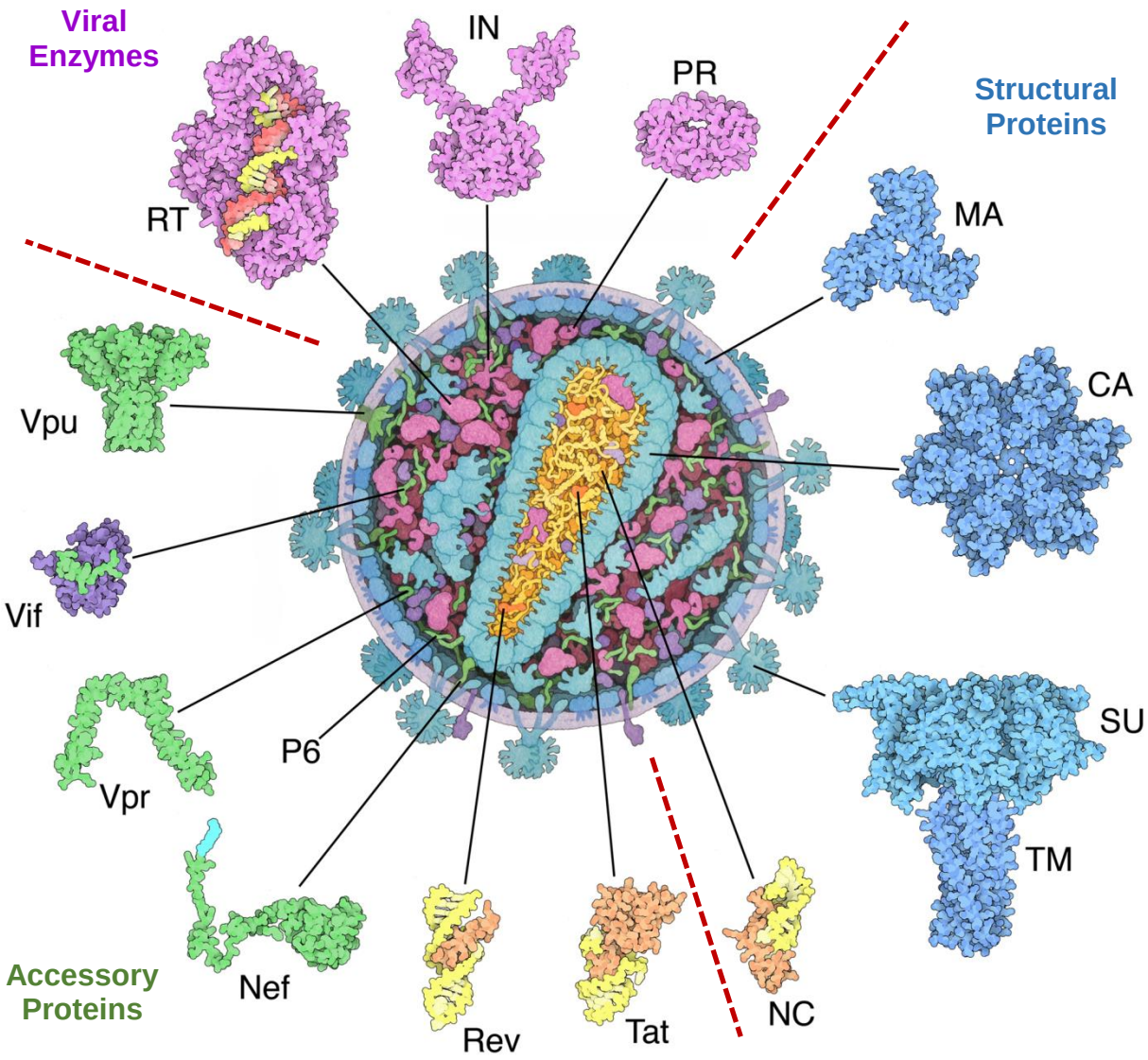


Figure 2-3: The structure biology of HIV.[46]

The HIV life cycle contains seven stages as follows (Figure 2-4):[47]

1) **Binding**: CD4 (Cluster of Differentiation 4) is a glycoprotein that can be found on the surface of most T lymphocytes. As an important step for virus entry, gp120 start to bind with CD4 receptor. CCR5 antagonist (maraviroc) and post-attachment inhibitor (Ibalizumab) are the HIV medicines that prevent HIV binding to CD4 cell.

2) **Fusion**: Next, the connection between gp120 and CD4 receptor lead a more effective interaction between gp120 and its co-receptor. Then gp41 plays an important role in the fusion of viral and the host cell membrane. Membrane fusion allows HIV enters the CD4 cell. In this step, Enfuvirtide (T-20) is a fusion inhibitor (FI) which was developed and became an FDA approved HIV medicines in 2003.

3) **Reverse Transcription**: Once HIV get into the host cell, HIV RNA is converted into HIV DNA by using HIV reverse transcriptase (HIV RT). The non-integrating HIV DNA accumulates in the cell and can be transported into the nucleus after cellular activation. Nucleoside reverse transcriptase inhibitors (NRTIs) and non-nucleoside reverse transcriptase inhibitors (NNRTIs) are widely used in clinical treatment by preventing HIV RNA reverse transcription.

4) **Integration**: After activation of $CD4^+$ cells, viral DNA is integrated into the host cell genome and produces new virions. Thus, integrase strand transfer inhibitors (INSTI), such as raltegravir (approved by the FDA in 2007) and dolutegravir (approved by the FDA in 2013), were developed to prevent the integration of HIV.

5) **Replication**: After integrated into host DNA, all resources in $CD4^+$ cell can be used to produce HIV proteins.

6) **Assembly**: Produced HIV proteins and HIV RNA move to the inner surface of cell and assemble into immature HIV. This immature HIV binds to the cell surface and it is still noninfectious.

7) **Budding**: New formed immature HIV leave the host cell. The long protein chains are cut into small pieces by protease. Those smaller HIV proteins become the building blocks of HIV. Thus, mature and infectious HIV are formed. Saquinavir, ritonavir, atazanavir, fosamprenavir, tipranavir and darunavir are protease inhibitors (PIs) that were approved by the FDA as HIV medicines used in this stage.

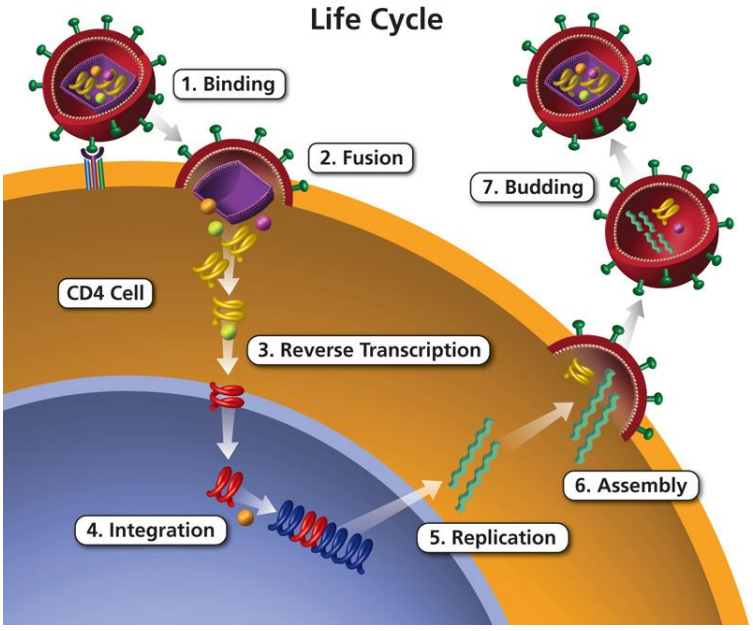


Figure 2-4: The life cycle of HIV.[47]

2.1.3 The Epidemic of AIDS and Antiretroviral Therapy (cART) of HIV

The initial report of AIDS was recognized as *Pneumocystis pneumonia* (PCP) in the United States in June 1981[48,49] and soon known as AIDS. In 1983, researchers succeeded in the isolation of HIV-1. Two years later, an antibody test for the presence of HIV in body was developed. Those two breakthroughs made it possible to take a deep look of the HIV infection mechanism and provided a possibility to establish antiretroviral therapy. Until 1987, FDA approved NRTI zidovudine (AZT) as the first medicine used in the antiretroviral therapy against HIV. The first protease inhibitor (PI), which was approved by the FDA in 1995, was Saquinavir mesylate (trade name Invirase®). One year later the FDA approved nevirapine (trade name Viramune®) as the first non-nucleoside reverse transcriptase inhibitor (NNRTI). In March 2003, another class of drugs was introduced as fusion inhibitor (FI). Enfuvirtide (T-20, trade name Fuzeon®) is the only fusion inhibitor which was approved by the FDA until May 2018. In 2007, maraviroc (trade name Celsentri®, CCR5 antagonist) and raltegravir (brand name Isentress®, integrase inhibitor) were approved by the FDA as two new drug classes. Until 2018, ibalizumab (TMB-355, trade name Trogarzo®), a post-attachment inhibitors (PAI) which is another new class of HIV medicine, was approved by the FDA after 15 years of research and clinic trials by TaiMed Biologics. Thus, from 1987 to 2018, eight classes (fix dose combination is not included) of HIV medicine have been developed. They are CA, FI, INSTI, NNRTI, NRTI, PE (pharmacokinetic enhancer), PI and PAI. The terms of Highly Active AntiRetroviral Therapy (HAART) were introduced in the initial years. Gradually, the name of the therapy was replaced by a more appropriate name, antiretroviral therapy (ART) or combination antiretroviral therapy (cART).[50]

The statistics of Joint United Nations Programme on HIV/AIDS (UNAIDS) are shown in Table 2-1 and Figure 2-5. There were 36.9 million people living with HIV all around the world in 2017. Among them, around 70% people were in Africa. Asia and pacific area took around 14% of all population infected with HIV. The annual number of the people who were infected with HIV and deaths due to AIDS decreased during the period of 2008 to 2017. It shows that efforts on fighting against HIV and AIDS become efficient.[51]

(data source: UNAIDS/WHO estimates)		2015	2016	2017
Number of people living with HIV	Total	36.7	36.7	36.9
	Adults	31.8	34.5	35.1
	Women	16.0	17.8	18.2
	Children (<15 years)	3.2	2.1	1.8
People newly infected with HIV	Total	2.1	1.8	1.8
	Adults	1.9	1.7	1.6
	Children (<15 years)	0.24	0.16	0.18
Deaths caused by AIDS	Total	1.1	1.0	0.94
	Adults	1.0	0.89	0.83
	Children (<15 years)	0.19	0.12	0.11

Table 2-1: Summary of global HIV epidemic in 2015, 2016 and 2017.[51]

According to the Antiretroviral Guidelines for Adults and Adolescents, which issued by U.S. Department of Health and Human Services (HHS) Panel in October 2017, recommended initial cART regimen for most people with HIV is as follows: two NRTIs (e.g. ABC/3TC, 3TC/TDF, TAF/FTC or TDF/FTC) combined with a third antiretroviral HIV drug from either the NNRTI, protease inhibitor (PI), or integrase strand transfer inhibitors (INSTI). NRTIs block Reverse Transcriptase's enzymatic function and prevent completion of the double-stranded viral DNA synthesis, thus preventing HIV from replication. NRTIs are still playing an important role as the backbone in cART therapy (Figure 2-6).

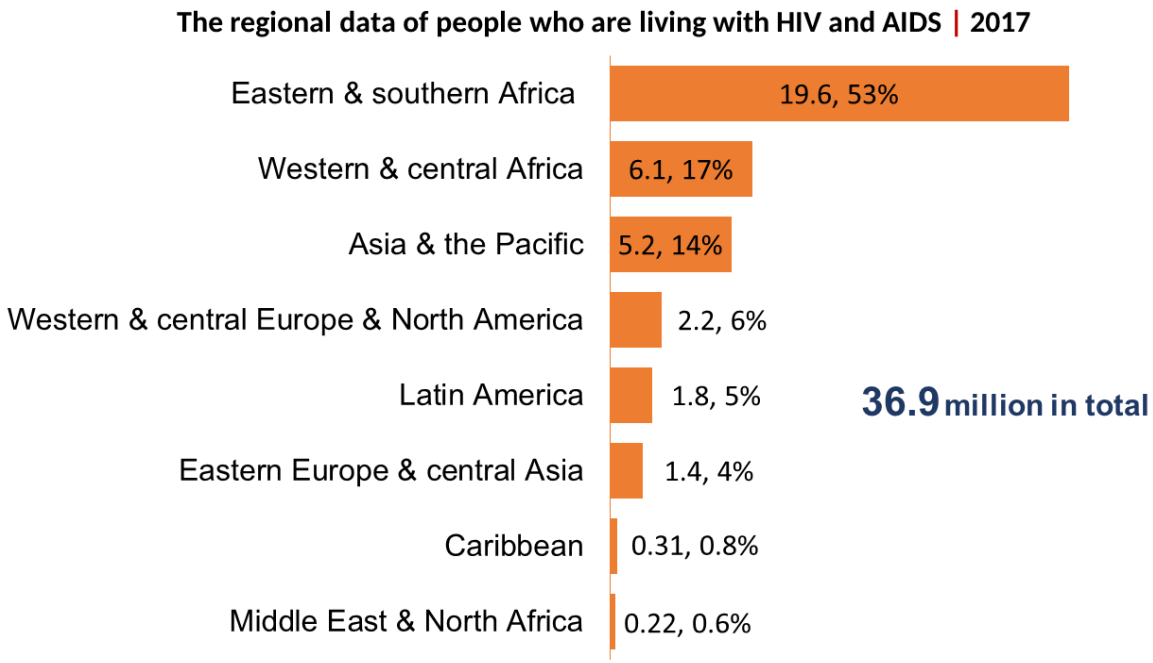


Figure 2-5: Regional HIV and AIDS statistics and features in 2017.[51]

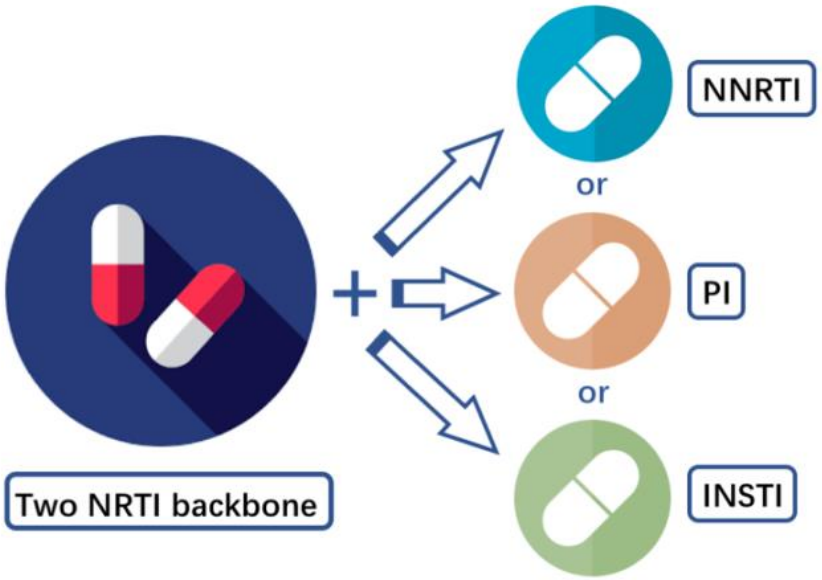


Figure 2-6: NRTI is still the backbone of cART treatment.

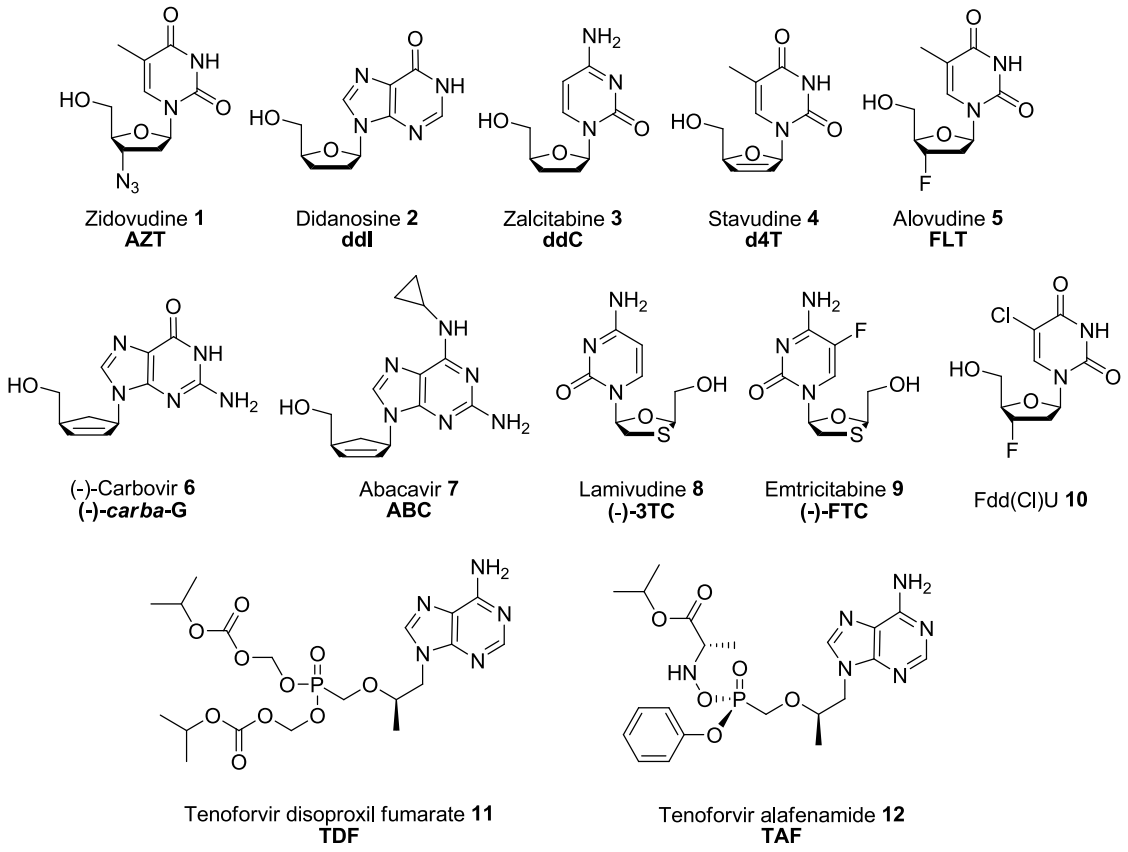
In Table 2-2, FDA approved NRTIs including their fix-dose combination since 1987 were listed. After 2004, anti-HIV medicines with only one NRTI were replaced by fixed-dose combination. FTC, TDF, TAF and 3TC are most frequently used in the clinical formulations.

Approval Date	Active Ingredient	Proprietary Name	Application Number	Applicant Holder
19.03.1987	AZT	RETROVIR	N019655	ViiV Healthcare
09.10.1991	ddI	VIDEX	N020155	Bristol-Myers Squibb
19.06.1992	ddC*	HIVID	N020199	Hoffmann-La Roche
24.06.1994	d4T*	ZERIT	N020412	Bristol-Myers Squibb
17.11.1995	3TC	EPIVIR	N020596	ViiV Healthcare
26.09.1997	AZT/3TC	COMBIVIR	N020857	ViiV Healthcare
17.12.1998	ABC	ZIAGEN	N020977	ViiV Healthcare
31.10.2000	ddI	VIDEX EC	N021183	Bristol-Myers Squibb
14.11.2000	ABC/3TC/AZT	TRIZIVIR	N021205	ViiV Healthcare
26.10.2001	TDF	VIREAD	N021256	Gilead Sciences
02.07.2003	FTC	EMTRIVA	N021500	Gilead Sciences
02.08.2004	ABC/FTC	EPZICOM	N021652	ViiV Healthcare
02.08.2004	FTC/TDF	TRUVADA	N021752	Gilead Sciences
12.07.2006	EFV/FTC/TDF	ATRIPLA	N021937	Gilead Sciences
10.08.2011	RPV/FTC/TDF	COMPLERA	N202123	Gilead Sciences
27.08.2012	c/EVG/FTC/TDF	STRIBILD	N203100	Gilead Sciences
22.08.2014	DTG/ABC/3TC	TRIMEQ	N205551	ViiV Healthcare
05.11.2015	c/EVG/FTC/TAF	GENVOYA	N207561	Gilead Sciences
04.04.2016	FTC/TAF	DESCOVY	N208215	Gilead Sciences
07.02.2018	BIC/FTC/TAF	BIKTARVY	N210251	Gilead Sciences
28.02.2018	3TC/TDF	CIMDUO	N022141	Mylan Laboratories
05.02.2018	EFV/3TC/TDF	SYMFI LO	N208255	Mylan Pharmaceuticals
22.03.2018	EFV/3TC/TDF	SYMFI	N022142	Mylan Laboratories

Table 2-2: FDA approved NRTIs medicines from March 1987 to March 2018. **Abbreviations: AZT**: zidovudine; **ddI**: didanosine; **ddC**: zalcitabine; **d4T**: stavudine; **3TC**: lamivudine; **ABC**: abacavir; **TDF**: tenofovir disoproxil fumarate; **FTC**: emtricitabine; **EFV**: efavirenz (NNRTI**)**; **RPV**: rilpivirine hydrochloride (NNRTI); **c**: cobicistat (Cytochrome P450 inhibitor); **EVG**: elvitegravir (INSTI); DTG: dolutegravir (INSTI); **TAF**: tenofovir alafenamide; **BIC**: bictegravir (INSTI); **NRTI**: nucleoside reverse transcriptase inhibitor; **NNRTI**: non-nucleoside reverse transcriptase inhibitor; **INSTI**: integrase strand transfer inhibitor; **FDA**: U.S. Food and Drug Administration.

2.1.4 Nucleoside Reverse Transcriptase Inhibitors (NRTIs)

As the structure and the life cycle of HIV are revealed after decades, several concepts and methods were built to prevent HIV infection and AIDS. Until now, nucleoside analogues for interfering reverse transcription are commonly used both in scientific research and clinical treatment. The modifications are usually based on nucleobase and sugar. The methods of chemical modifications include azidation, halogenation, N-conjugation, sugar ring opening, sugar ring oxygen replacement, saturation, dehydroxylation, etc..[52] These chemical diversities provide a possibility to treat HIV infection and AIDS.[2-4,6,53,54]



Scheme 2-1: Representative nucleoside and nucleotide analogues.

NRTIs share the natural metabolism pathway and produce corresponding triphosphate degradation products as active antiretroviral form. Because of the lack of 3'-OH in the NRTIs, the viral DNA elongation is terminated after NRTIs incorporated into the growing DNA chain. As is shown in Table 2-5, most nucleoside analogs have beta-D-configurations, which are similar to natural nucleosides, except for 3TC **8** (2´,3´-dideoxy-3´-thiacytidine) and FTC **9** ((-)-2´,3´-dideoxy-5-fluoro-3´-thiacytidine) with beta-L-configurations.

As the first FDA proved HIV medicine, AZT **1** (3´-azido-3´-deoxythymidine) was also the first NRTI discovered as antiretroviral compound. D4T **4** (2´,3´-didehydro-2´,3´-dideoxythymidine) is another thymidine analog which was approved by the FDA in 1994. Both AZT and d4T enter the cell by non-facilitated diffusion.[55] AZT **1**, d4T **4** and FLT **5** (3´-fluoro-3´-deoxythymidine) are thymidine analogs which are phosphorylated to mono-, di- and triphosphates by thymidine kinase (TK), thymidylate kinase (TMP-K) and nucleoside diphosphate kinase (NDP-K) respectively.[7] From AZT to AZTTP, the diphosphorylation of AZTMP to AZTDP is the rate-limiting step. [56] The low phosphorylation rate of AZTMP is possibly due to the bulky azido group ($-N_3$) which induced P-loop movement.[57,58] Previous studies showed that after incubated with AZT in the cell, the intracellular level of TTP was reduced, which means that phosphorylation of thymidine and AZT share the enzymes. The thymidine phosphorylation was also inhibited by AZT.[56] Unlike AZT, the rate-limiting step of d4T to d4TTP is the TK catalyzed d4TMP formation from d4T. According to the phosphorylation profiles, AZT and d4T were more efficiently phosphorylated to their triphosphate form in activated cells (phytohemagglutinin-stimulated peripheral blood mononuclear cells, PHA-stimulated PBMC) and they were little phosphorylated in resting cells (resting nondividing peripheral blood mononuclear cells, R-PBMC).[59] However, ddC **3** (2´,3´-dideoxycytidine), 3TC **8** and ddG (2´,3´-dideoxyguanosine) produced higher ratios of NRTI triphosphate/dNTP in resting cells. The half-life of AZTTP in PHA-stimulated PBMC is 2.8 ± 0.6 h.[60] In CEM cells, the half-life of AZTTP and d4TTP is almost the same, which is about 3.3 h.[61,62]

2.1.5 HIV-RT versus Human DNA Polymerases

Human immunodeficiency virus reverse transcriptase (HIV-RT) is responsible for the reverse transcription of HIV RNA to DNA. To develop a selective anti-HIV compounds that only targets HIV-RT but not human polymerases is necessary.[63]

HIV-RT consists of two subunits, p66 (560 amino acids in length) and p51 (440 amino acids in length). p66 is composed of two domains: polymerase and RNase H, and the polymerase domain has four subdomains: fingers, palm, thumb and connection.[64,65] The polymerase active site consists of three catalytic carboxylates binding with two Mg^{2+} (*in vivo*) or Mn^{2+} (*in vitro*) in the p66 palm subdomain (Figure 2-7 B).[66] Experiments showed that dNTP binding is slightly more efficient on DNA/DNA template/primer (K_d 4 µM) than RNA/DNA template/primer (K_d 14 µM).[67]

RNase H is responsible for the degradation of the RNA portion and removal of priming tRNA.[68,69] *In vivo,* the nuclease active site of RNase H shows function with two Mg^{2+}. RNase H inactive viruses do not show infectious activity. Mutations in the connections or RNase H subdomain enhance resistance to NRTI.[70,71] For example, AZT-resistant RTs have the ability to remove the incorporated AZTMP from the DNA template strand.[72-74] Previous study showed that AZTTP was 100-fold more efficient as substrate for HIV-RT than human lymphocyte (H9 cell) DNA polymerase α by using activated calf thymus DNA as template.[56]

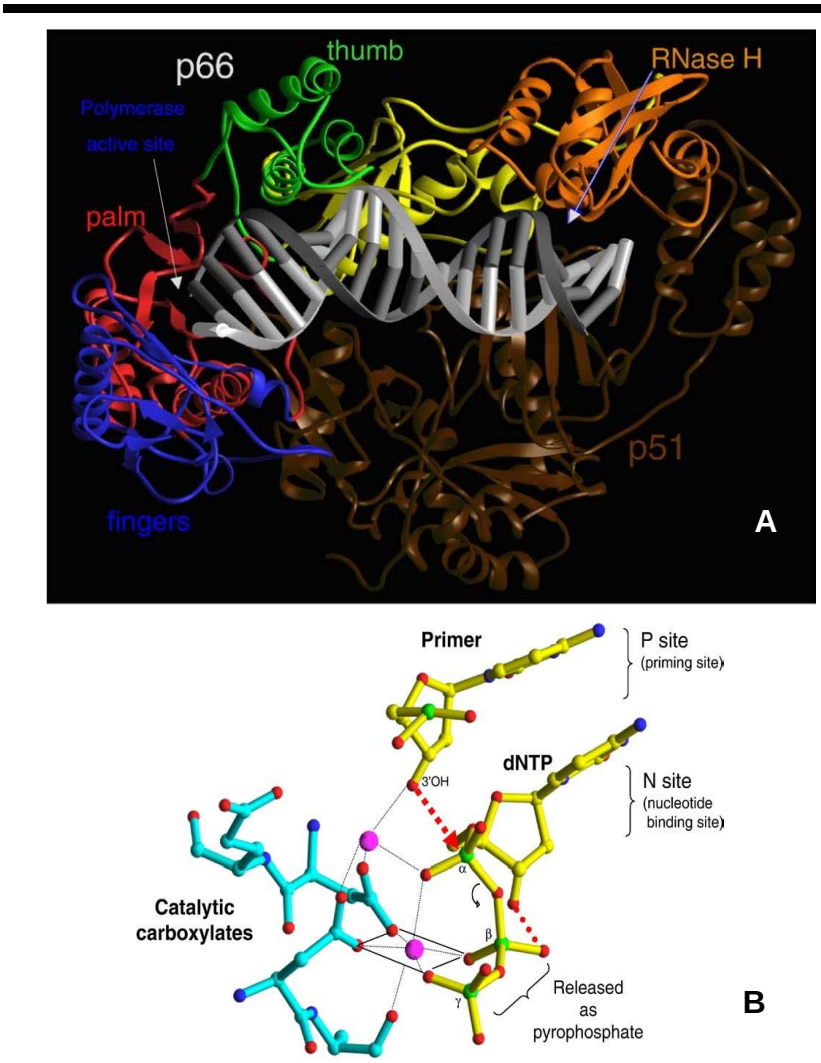
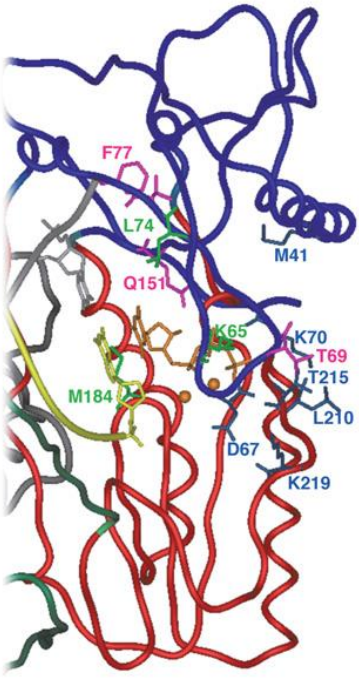


Figure 2-7: Interactions of HIV-RT with DNA complex and dNTP. (A) Ribbon structure of HIV-1 RT with nucleic acids. Fingers, palm, thumb, connection and RNase H of subunit p66 are shown in blue, red, green, yellow and orange, respectively. Subunit p51 is showed in brown. The template is in light gray and primer is in dark gray. (B) The complex of carboxylates/Mg^{2+}(or Mn^{2+})/DNA/dNTP in the polymerase active site. Carboxylates, Mg^{2+}(or Mn^{2+}) and DNA/dNTP are shown in light blue, purple and yellow, respectively.[66]

The evolution or mutation of HIV-RT causes the drug resistance towards NRTIs. Most of the mutations of HIV-RT occurred at the 'palm' and 'finger' subdomains. AZT got high-level resistance after one of the mutations of M41L, D67N, K70R, L210W, T215Y/F and K219Q. For resistance of d4T, it is predominately about the V75T mutation. K65R mutation is responsible for TDF, ABC, ddI, ddC resistance. Furthermore, the mutations of M184V produced more than 100-fold resistance to 3TC and FTC. The crystal structure of DNA/DNA-M184I RT complex revealed that the replacement of the Met side chain by Val or Ile caused steric hindrance with the sulphur atom in the ribose ring of 3TC and FTC.[75]

NRTI	Position of mutated residues in HIV-1 RT											
AZT	M41L	*	D67N	T69D	K70R	*	V75T	Q151M	*	L210W	T215F	K219Q
d4T	M41L	K65R	D67N	T69D	K70R		V75T	Q151M	*	L210W	T215F	K219Q
TDF	M41L	K65R	D67N	T69D			V75T	Q151M	*	L210W	T215F	K219Q
ABC	M41L	K65R	D67N	T69D		L74V	V75T	Q151M	M184V	L210W	T215F	K219Q
ddI	M41L	K65R	D67N	T69D		L74V	V75T	Q151M	M184V	L210W	T215F	K219Q
ddC	M41L	K65R	D67N	T69D	K70R	L74V	V75T	Q151M	M184V	L210W	T215F	K219Q
3TC	M41L	K65R		T69D	K70R			Q151M	M184V	L210W	T215F	
FTC	M41L	K65R		T69D			V75T	Q151M	M184V	L210W	T215F	

High-level resistance
Intermediate resistance
Low-level resistance
Contributes to resistance
No resistance
* Hypersusceptibility
X000X Main mutations associated to the NRTI resistance
X000X Less frequent mutations



Note: In the figure, the palm and finger subdomains of HIV-1 RT are in red and blue, respectively. The primer/template backbone is in yellow/grey. Only base pair near the active site are shown in balls and sticks. The coming dTTP and the Mg^{2+} ions are in orange. M41, K70, T215, L210, D67 and K219 are in blue and responsible for the resistance to AZT. L74, K65 and M184 are in green and responsible to ddI, ddC and 3TC. F77, Q151 and T69 are in pink and responsible for the cross-resistance to AZT and ddI or ddC.

Figure 2-8: Mutations of HIV-RT and resistance toward NRTIs.[75]

Though the main target of NRTIs is HIV-RT, the triphosphate forms of NRTIs also have effects on human DNA polymerases (Pol α, β, γ and ε) during DNA synthesis and produce *in vivo* activity and toxicity.[76] Generally, the effects of NRTI on

polymerase are: HIV-RT >> Pol γ > Pol β > Pol α = Pol ε. The release of the pyrophosphate from triphosphate is based on a two-metal ion-catalytic mechanism.[77]

Pol α is mainly responsible for chromosomal DNA synthesis or replication and is located in the nucleus.[78,79] One evidence is that Pol α raised to the highest level when lymphocytes stimulated by a mitogen, and drops to a low level in resting cells.[80,81] However, the level of Pol β did not increased when the cells grow rapidly. During catalysis process, Pol α firstly interacting with template (single-strand DNA), then primer and finally with dNTPs.[82]

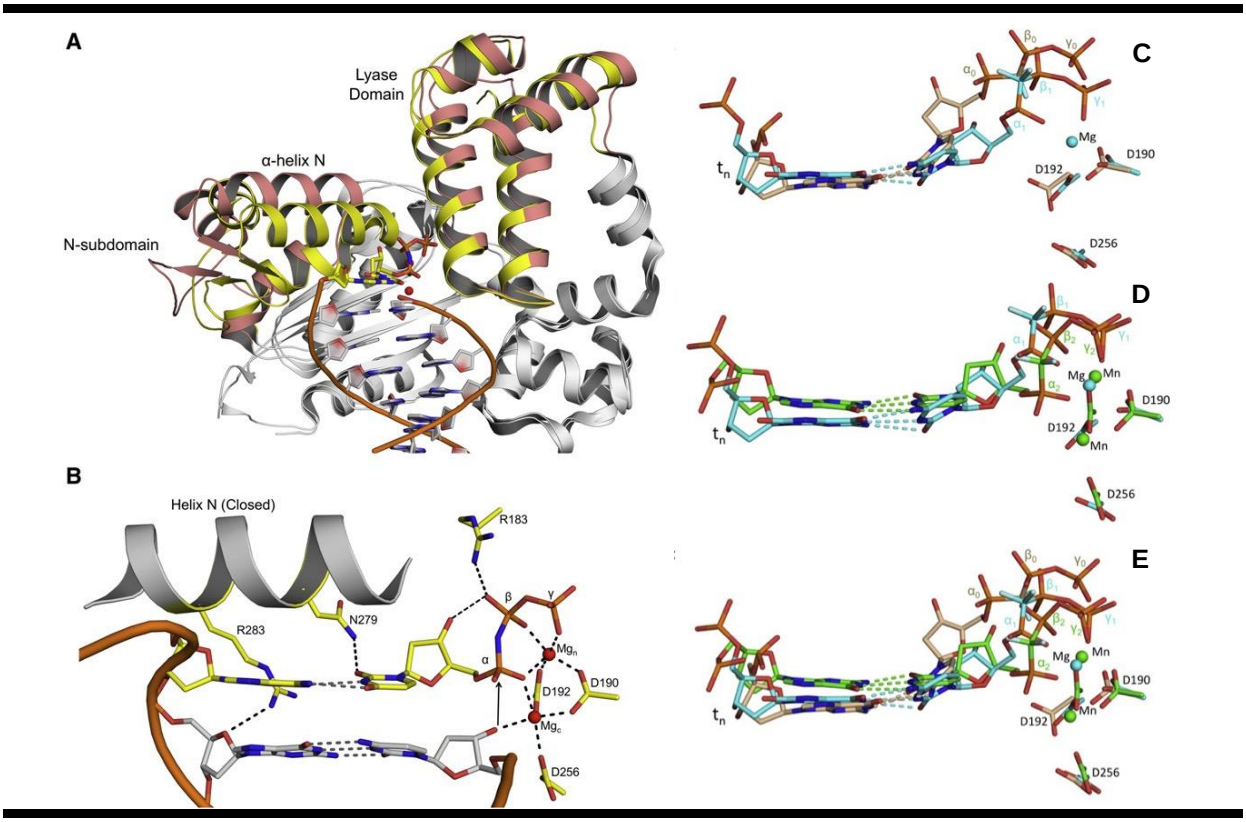


Figure 2-9: Interactions of wild-type Pol β with DNA complex and dNTP. (A) Structural overlay of the open binary Pol β/DNA complex (salmon) and the closed ternary Pol β/DNA/dNTP complex (yellow). The incoming nucleotide and templating base are shown in yellow with the Mg^{2+} ions in red. The DNA backbone of the upstream duplex is represented as an orange ribbon. (B) Closed ternary pol β active site. Key amino acids, templating base, and incoming nucleotide are shown in yellow, and important interactions are indicated (dashed lines). Mg_C and Mg_N represent the catalytic and nucleotide-binding magnesium ions, respectively. (C) Overlay of the metal-free and one-metal Pol β structures with dCMP(CF_2)PP. (D) Overlay of the one-metal and two-metal Pol β structures with dCMP(CF_2)PP. (E) Overlay of the metal-free, one-metal, and two-metal Pol β structures with dCMP(CF_2)PP.

DNA Pol β is the simplest DNA polymerase in both size and catalysis. Pol β plays an important role in base excision repair.[83] It can also fill gaps and nicks.[82,84] Figure 2-9

(A, B) shows that Pol β alone is in an "open" conformation (salmon) and transferred to a "closed" configuration (yellow) after binding to nucleotide. From Figure 2-9 (C, D, E), it is understood that the process of nucleotide attaching to the active site of Pol β is undergoing a metal-free, one-metal and two-metal interaction process.

DNA Pol γ is the only DNA polymerase which was detected in mammalian mitochondria among 16 discovered eukaryotic DNA polymerase. Pol γ is responsible for mitochondria DNA replication and repair with high degree of nucleoside insertion fidelity.[82,85] During *in vitro* chain elongation, ddNTP and d4TTP are as efficient as natural NTPs utilized by Pol γ, while AZTTP, 3TCTP and Tenofovir are only moderate inhibitors. After incorporating with ddNMP, d4TMP or AZTMP, Pol γ is inefficient to remove these terminal NRTI from DNA.[86,87] Research showed that high level of d4T was detected during AZT treatment, revealing the fact that AZT was converted to d4T and thus the toxicity increased. Data showed when 0.05% of AZT converted to d4T; it will bring the same toxicity of d4T therapy alone.[77,88-91]

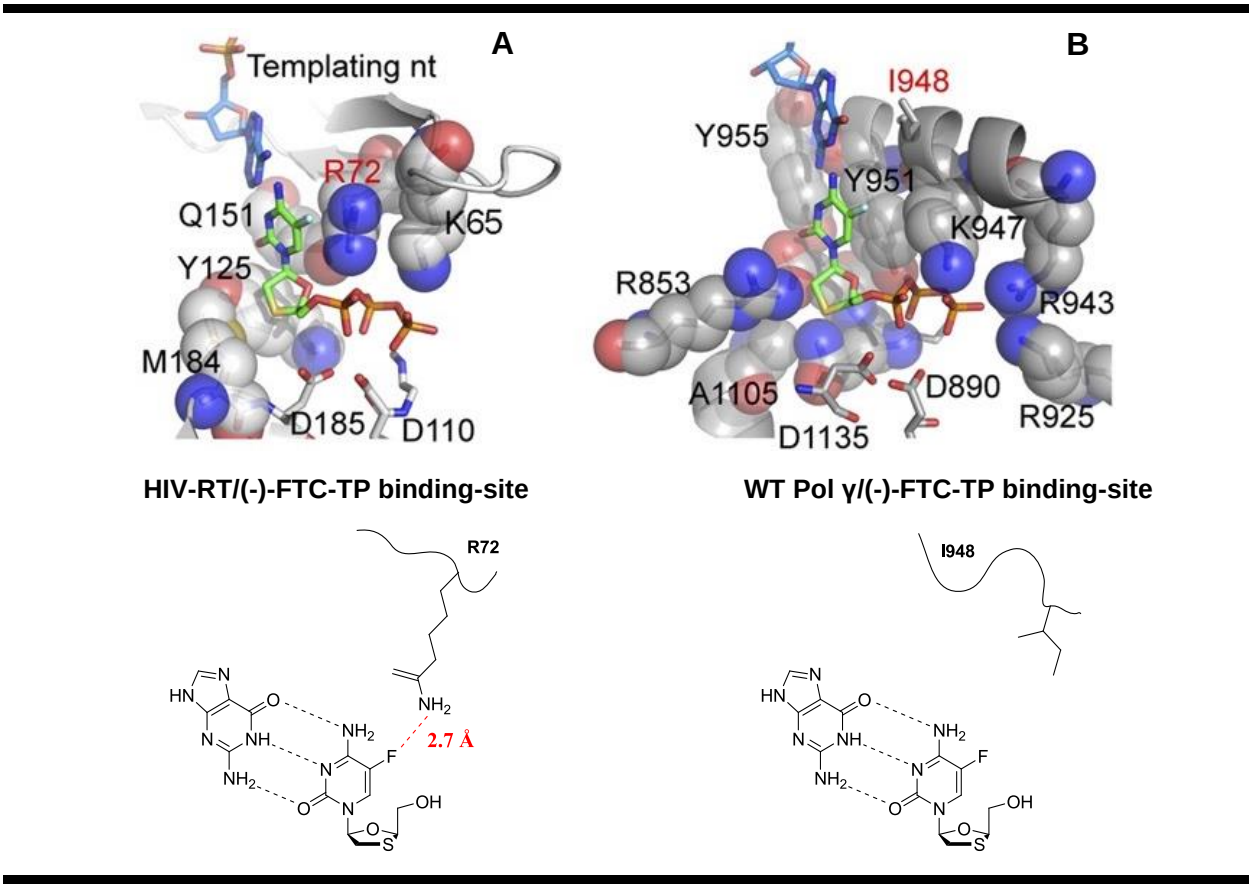


Figure 2-10: (A) The binding site of RT and incoming (-)-FTC-TP. (B) The binding site of human wild-type Pol γ and incoming (-)-FTC-TP. Interactions between (-)-FTC-TP with HIV-RT R72 and Pol γ I948 are showed below.[92]

In 2015, C. D. Sohl, *et al*. reported the crystals of complex of D24/D48 primer/template, (+)-FTC-TP or (-)-FTC-TP with human mitochondrial DNA Pol γ, and compared the complex crystal structure with C-terminal His-tagged R721 HIV-RT. As shown in Figure 2-10, the binding site of HIV-RT/(-)-FTC-TP is more flexible than that of WT Pol γ/(-)-FTC-TP, thus indicates that HIV-RT is less selective than human Pol γ among nucleoside triphosphates. As (-)-FTC-TP is the 5-fluoro modification of (-)-3TC-TP, they have comparable activity against HIV, but (-)-FTC-TP is 100-fold less toxic than (-)-3TC-TP. This may be explained by no hydrogen bond interactions between 5-F from (-)-FTC-TP with wild-type human Pol γ I948.[92]

The crystal structures of the complex of Pol β and γ with DNA and NRTI triphosphates are showed in Figure 2-11. The shape and size of the pockets for incoming nucleoside triphosphates to incorporate into DNA template are clearly observed.

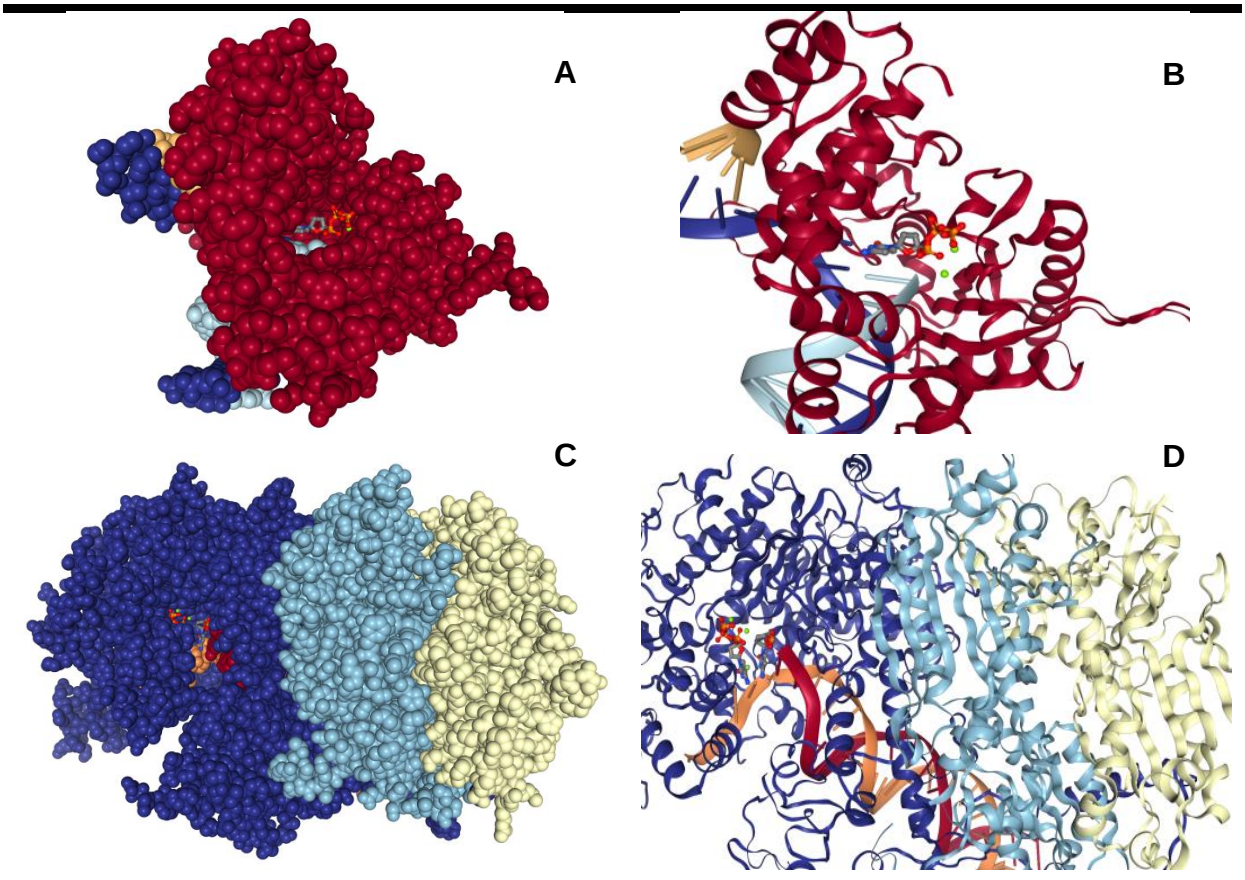


Figure 2-11: (A, B) Crystal structure of Human R283K DNA Pol β complex with gapped DNA and ddC-TP. A and B are from the same angel of view.[93] (C, D) Crystal structure of Human mitochondrial Pol γ in complex with (-)-FTC-TP. C and D are from the same angel of view,[92] The 3D view program is NGL which powered by MMTF.[94,95] The shape and size of the pockets for incoming nucleoside triphosphates to incorporate with DNA template are clearly observed.

DNA Pol ε is a mammalian DNA polymerase that tightly associated 3'-5' exonuclease activity. It is a repair enzyme that removes the mistake from DNA leading strands.[96-98]

J. L. Martin *et al.* determined inhibition constants for 16 NTPs against human DNA polymerases α, β, γ and ε. Seven nucleoside analogs were tested with mitochondrial DNA synthesis in human Molt-4 cell culture. By comparing of K_i/K_m among FLTTP **5t**, Fdd(Cl)UTP **10t** and all four ddNTPs, or between 3TCTP **8t** and FTCTP **9t**, the replacement of 5-methyl group from thymine to a chloro group, 5-hydrogen from cytidine base to a fluoro atom or 3'-hydroxy group from the sugar ring to a fluoro atom did not affect the potency of inhibition, which indicated that improving the steric effects of this modification is needed. Generally, Pol α and Pol ε are much less sensitive than Pol β and Pol γ on structure changes of NRTIs. Moreover, the IC_{50} values of 7 NRTIs for inhibition of mtDNA synthesis were tested. Except the example of Fdd(Cl)U **10**, when NRTI strongly inhibited both Pol β and γ, it will lead to the result of inhibition of mtDNA synthesis.[99]

Another study showed that AZTTP is not a substrate for Pol α, β and ε while moderately inhibiting Pol γ. Results also showed that d4TTP inhibited Pol β and was a particularly strong inhibitor of Pol γ, but not a substrate for Pol α.[29] D4TTP also do not incorporate with pol ε, which means d4TMP was not able to be removed from the 3´-end DNA by DNA pol ε.[100] Cheng *et al*. also reported the interaction of AZTTP and TTP on HIV-RT and Human Pol α, β, γ by using activated calf thymus DNA as template. Results showed that the K_i/K_m ratios of AZTTP/TTP with HIV-RT, Pol α, β and γ were 0.05, 34.6, 0.16 and 0.58, respectively (for K_i/K_m of ddCTP/dCTP were 0.02, 122, 0.60 and 0.05), which means that AZTTP inhibited HIV-RT efficiently but not incorporated well with Pol α.[101] In conclusion, the inhibition of Pol β and γ by NRTI triphosphates is the most important aspect that produces toxicity and side effects during cART. As different templates and polymerases from different cells are used, the kinetic constant values are not comparable among different studies and the potencies of inhibition may vary.

dNTP	NRTI	Pol α			Pol β			Pol γ			Pol ε		
		K_m	K_i	K_i/K_m	K_m	K_i	K_i/K_m	K_m	K_i	K_i/K_m	K_m	K_i	K_i/K_m
TTP		2.4			1.6			0.2			3.0		
	AZTTP **1t**		140	58		290	180		8.7	51		400	130
	d4TTP **4t**		120	50		1.2	0.8		0.05	0.3		59	20
	FLTTP **5t**		9	4		1.7	1.1		0.04	0.2		ND	ND
	Fdd(Cl)UTP **10t**		24	10		2.5	1.6		0.07	0.4		44	15
	ddTTP		90	38		1.3	0.8		0.03	0.2		70	23
dGTP		0.9			1.4			0.1			2.5		
	ABCTP **7t**		7	8		340	240		14	100		410	160
	ddGTP		27	30		1.7	1.2		0.02	0.1		67	27
dCTP		1.4			1.4			0.2			2.4		
	3TCTP **8t**		110	79		13	9		4	24		120	50
	FTCTP **9t**		130	86		17	12		6	35		150	63
	ddCTP		90	64		1.2	0.9		0.02	0.1		70	29
dATP		1.3			1.2			0.2			3.0		
	ddATP		64	49		1.1	0.9		0.02	0.1		67	22

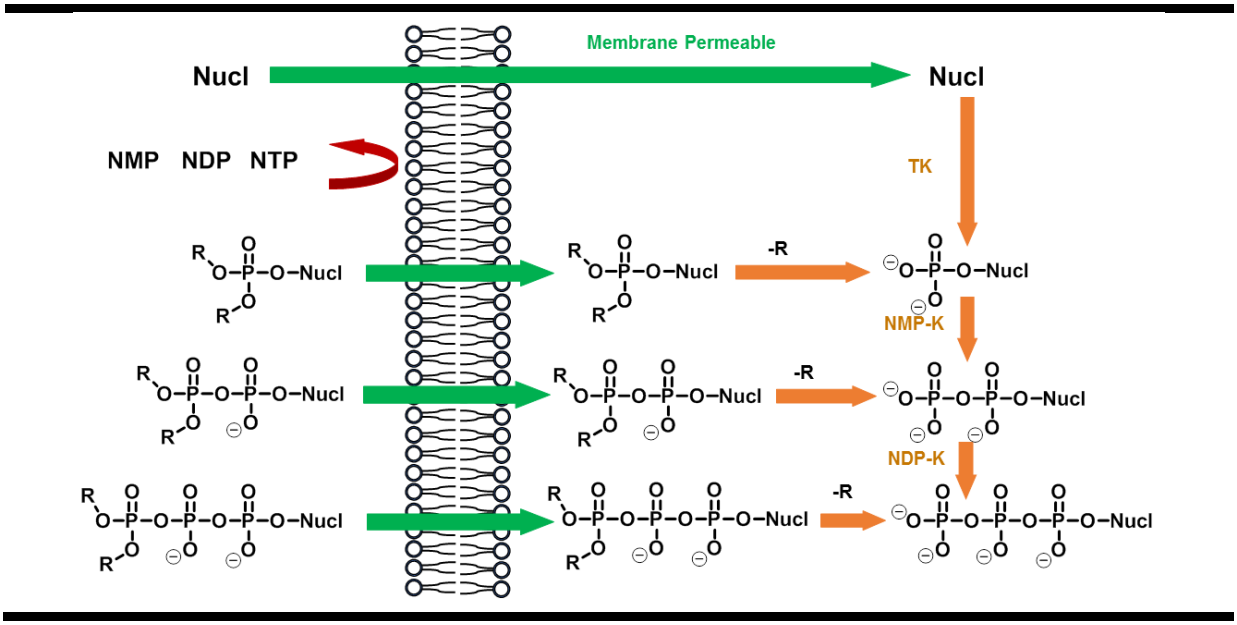
Table 2-3: Human DNA polymerase K_m, K_i values and their ratios for nucleotide analogs.[99]

NRTI	Inhibition of Pol α[a]	Inhibition of Pol β[a]	Inhibition of Pol γ[a]	Inhibition of Pol ε[a]	IC_{50} (μM) for inhibition of mtDNA synthesis[b]
AZT **1**	- -	- -	+	- -	>100[c]
d4T **4**	- -	+	+++	-	10
FLT **5**	+	+	+++	ND[e]	0.02[d]
Fdd(Cl)U **10**	-	+	+++	-	>100
3TC **8**	- -	-	+	- -	>200
FTC **9**	- -	-	+	- -	>100
ddC	-	+	+++	-	0.002

Table 2-4: Human DNA polymerase K_m, K_i values and their ratios for nucleotide analogs.[99] [a] *Ki* value of < 0.1μM (+++), 0.1 to 1.0 μM (++), 1.0 to 10 μM (+), 10 to 100 μM (-), >100 μM (- -). [b] IC50s were determined from day 7 exposure values, except ddC (day 5 harvest values) and for d4T (day 6 harvest values). [c] Highest concentration tested. [d] Cell death was observed at 5 μM without selective depletion of mitochondrial DNA. [e] ND: not determined.

2.2 The Development of Nucleotide Prodrugs or Pronucleotides

With the development of HIV chemotherapeutics during last decades, prodrugs became more and more important as HIV drugs. Chemical modified nucleotide prodrugs have better membrane penetration efficacy and are also able to improve the anti-HIV activity. Unlike most nucleosides, nucleotides (NMPs, NDPs and NTPs) have limited or no membrane penetration efficacy because of the negative charge of the phosphate moiety. Thus, the nucleotide prodrug or pronucleotide approach was developed. The aim of this approach is to deliver NMPs, NDPs and NTPs into the cell, to bypass some phosphorylation steps and finally produce activity.[11,12,102]

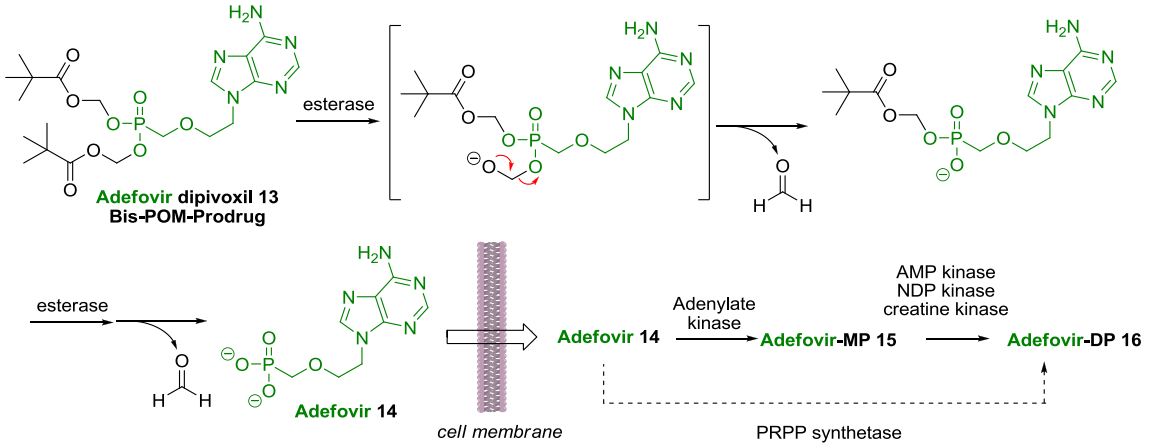


Scheme 2-2: Metabolism of NRTIs and prodrugs. Nucl: nucleoside.

2.2.1 Nucleoside Monophosphate Prodrugs

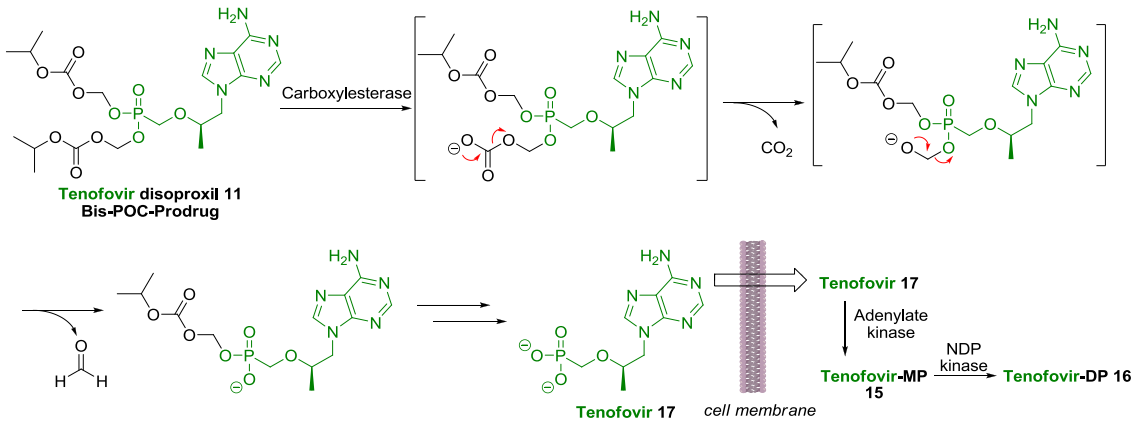
In 2001, McGuigan *et al*. reported the synthesis and evaluation of amidate prodrugs of Adefovir **14** and Tenofovir **17**.[103] One year later in 2002, Adefovir dipivoxil **13** (Hepsera®, ADV, bis-POM-prodrug) became a FDA approved medicine and used to treat chronic hepatitis B infection.[104] The bis-(pivaloyloxymethyl)-ester (bis-POM) moiety greatly improved oral bioavailability in human (Adefovir, < 12%, Adefovir dipivoxil, 59%).[105] After involved in circulation, POM-ester moiety was cleaved fast to form Adefovir **14** by extracellular esterases and then enter the cell.[106,107] Once inside the cell, Adefovir **14** is phosphorylated to ADV-MP and ADV-DP.[108-111] Another example is bis-POM-5-FdUTP (bis-POM 2'-deoxy-5-fluorouridine 5'- monophosphate). Its half-life is more than 40 hours under acidic and neutral pH condition and it was

hydrolyzed to 5-FdU-MP in mouse plasma by liver carboxylate esterases, plasma phosphodiesterases.[112]

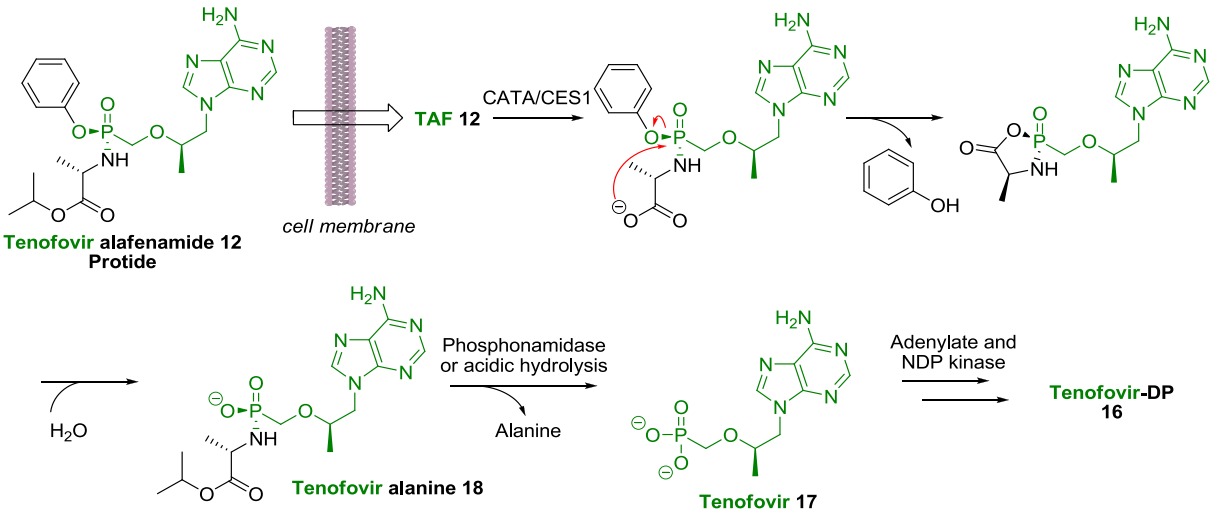


Scheme 2-3: Metabolism of Adefovir dipivoxil **13** (ADV).

Tenofovir disoproxil fumarate **11** (TDF, bis-POC-prodrug)[113] was developed against HIV-1 and HBV and was approved by the FDA in 2001. The existence of fumaric acid in the formulation reduced the rate of mask cleavage and the dimerization of the adenine moiety.[114,115] Until April 2018, seven HIV medicines in fix-dose combination class which contain TDF have been approved by the FDA (Table 2-2). Tenofovir disoproxil **11** is the oral prodrug of Tenofovir **17** like ADV **13**. Similarly, once after TDF **11** crossed the intestinal barrier, the bis-(isopropyloxycarbonyloxymethyl)-ester (bis-POC) masks are cleaved rapidly and form Tenofovir **17**.[116,117] After cellular uptake, Tenofovir **17** is metabolized to Tenofovir monophosphate and finally to the active Tenofovir-DP.[113] The bis-POC approach allows TDF **13** have higher oral bioavailability and lower cytotoxicity than its bis-POM counterpart.[116,118] During the deprotection of the Bis-POM-prodrug[119] and the bis-POC-prodrug, formaldehyde was generated as byproduct. Thus, as these drugs were normally used for long term treatment, side effects occurred and cannot be ignored.



Scheme 2-4: metabolism of Tenofovir disoproxil **11** (TDF).



Scheme 2-5: metabolism of Tenofovir alafenamide **9** (TAF)

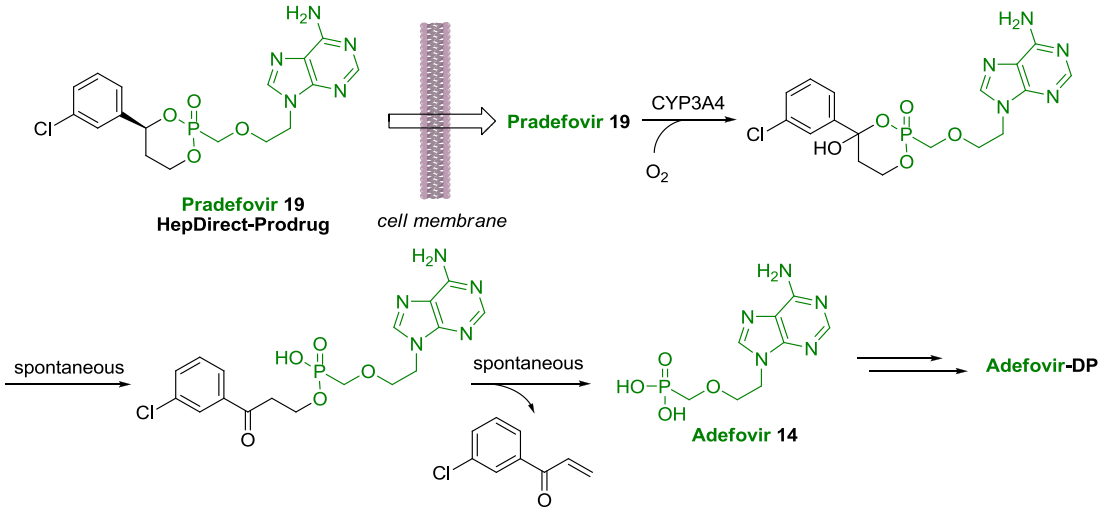
The discovery of Tenofovir alafenamide **12** (TAF, proTide approach) further increased the stability of the prodrug and decreased the concentration of Tenofovir **17** in human circulating plasma. The lipophilic phosphoramidate moiety increased cell permeability by passive diffusion and nucleoside transporters.[120] Once TAF **12** delivered into the cell, the hydrolysis is catalyzed by cathepsin A (CatA) or carboxylesterase (CES1) and followed a non-enzymatic chemical reaction forming Tenofovir alanine **18** as an intermediate. The alanine group was then cleaved. Lastly, Tenofovir **17** is converted to the active Tenofovir diphosphate **16** after phosphorylation steps. Other proTide derivatives from other nucleosides such as d4U and ddU were also developed.[121,122]

	CEM/TK$^-$ (HIV-1[a] or HIV-2[b])	
	EC_{50} [µM][c]	CC_{50} [µM][d]
bis-Me-SATE-d4TMP	0.012 ± 0.006[a]	60 ± 22
d4T	10 ± 10[a]	>100
bis-*t*Bu-SATE-AZTMP	0.45[a]	>10
AZT	>100[a]	>100
3-Me-cycloSal-d4TMP **22**	0.05[b]	32
5-di-AM-cycloSal-d4TMP **24**	10.5 ± 3[b]	99
d4T	47.5 ± 26.3[b]	234
3-MePr-cycloSal-d4TMP **23**	0.26 ± 0.14[b]	42.8 ± 13.5
d4T	15.0 ± 7.71	58.8 ± 24.2

Table 2-5: EC_{50} and CC_{50} values of bis-SATE prodrugs of d4TMP and AZTMP in CEM/TK$^-$ cells.[124,125] [a] EC_{50} values against HIV-1. [b] EC_{50} values against HIV-2. [c] Antiviral activity in CD4$^+$ T-lymphocytes: 50% effective concentration. [d] Cytotoxicity: 50% cytostatic concentration or compound concentration required to inhibit CD4$^+$ T-cell (CEM) proliferation by 50%.

The bis-S-acylthioethyl ester prodrug (bis-SATE-prodrug) and bis-(dithioethyl)-ester prodrugs (bis-DTE-prodrug) were developed with the monophosphates of ddU, ddA, AZT, d4T, ddC, 3TC and Acyclovir. The first bis-SATE-prodrug was modified from Adefovir **14** reported in 1996. As compared to bis-POM-Adefovir **13**, bis-*t*-Bu-SATE prodrug have similar cellular antiviral activity and greater stability in human gastric juice and human serum.[123] As is shown in Table 2-5, bis-SATE prodrugs of d4TMP and AZTMP greatly increased their EC_{50} values in thymidine-kinase-deficient cells (CEM/TK^-).[124,125]

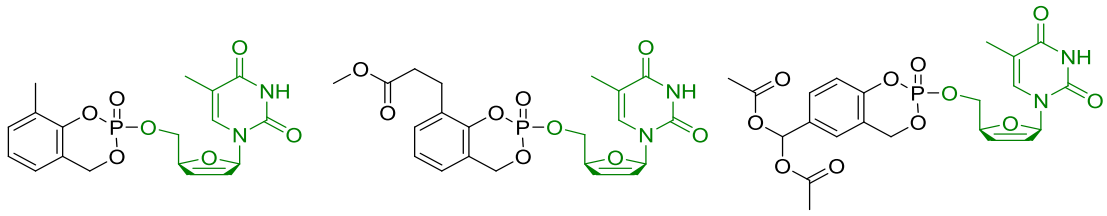
All prodrug systems mentioned above are cleaved in tissues and bloodstream. Cyclic 1-aryl-1,3-propanyl ester prodrugs (HepDirect) were designed to afford four characteristics: high oral absorption, rapid activation by one enzyme in the liver, high stability in blood and tissue and no by-product related toxicity. The HepDirect prodrug was activated by cytochrome P450 enzyme (CYP3A4) in the liver and deliver the monophosphate by spontaneous ring opening and β-elimination.[126,127] Pradefovir is one example of HepDirect prodrugs which exhibited a good bioavailability and good clinical anti-HIV activity in phase 2 trials.



Scheme 2-6: metabolism of pradefovir 1**9**

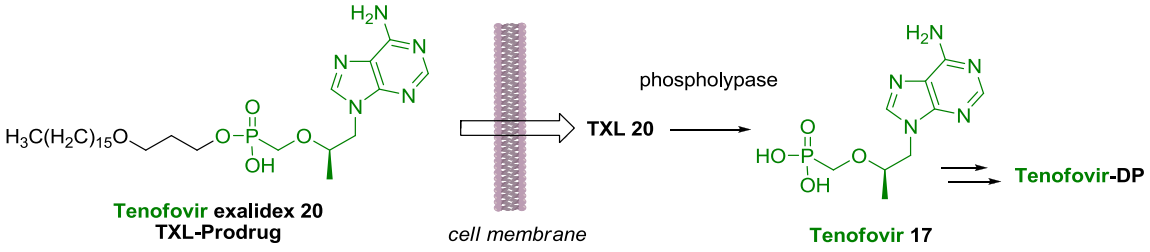
In 1996, C. Meier *et al*. synthesized and tested a series of prodrugs against HIV by introducing the *cyclo*Sal-approach.[128] The so-called *cyclo*Sal-pronucleotide approach that is one of the leading pronucleotide systems world-wide. This prodrug is sensitive to sodium phosphate buffer (pH 7.3) and sodium borate buffer (pH 8.9) and it was the first example of a chemically activated prodrug system.[129] Later, a second[130] and third-generation[131] of *cyclo*Sal-"Lock-in"-modified prodrugs were proposed and

developed. The *cyclo*Sal-"Lock-in"-prodrugs produces polar intermediate with negative charge that resulted in trapping the compound inside cells.



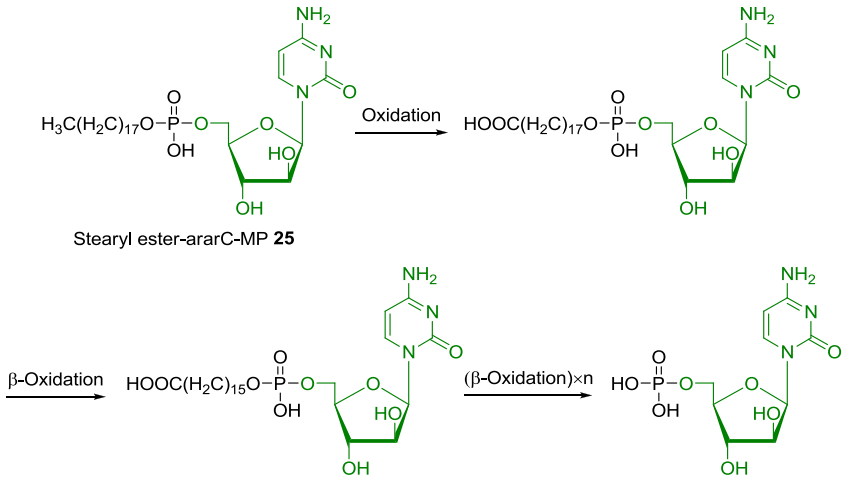
Scheme 2-7: Examples of 1st, 2nd and 3rd generation of *cyclo*Sal-prodrugs

Tenofovir exalidex (TXL) is a mono-hexadecyloxypropyl (mono-HDP) prodrug in phase 2 clinical studies against HIV-1 and HBV.[132] As a monoester prodrug to mimic lysophosphatidylcholine, TXL is efficiently absorbed in gastrointestinal tract and it is stable in peripheral circulation. The HDP moiety is then cleaved intracellular by phospholipase *C* to form Tenofovir **17**.[102]



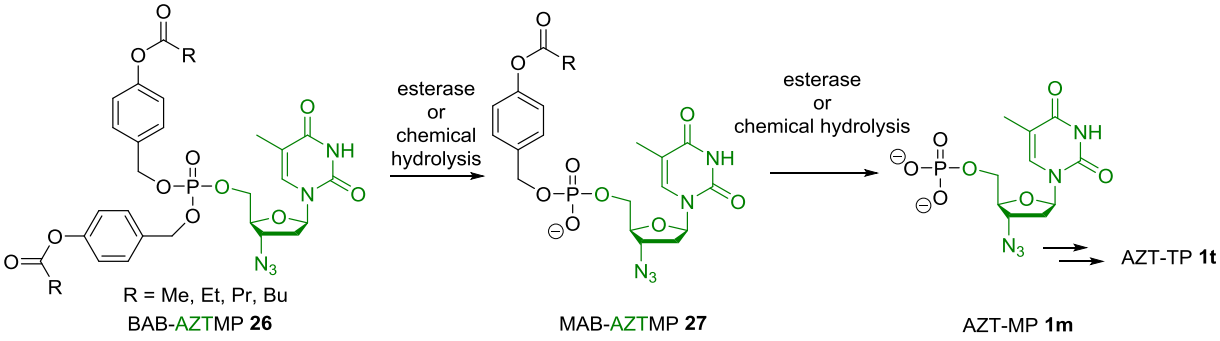
Scheme 2-8: Metabolism of Tenofovir exalidex **20** (TXL)

The concept of using stearyl ester in prodrugs was proposed to improve metabolic stability of Cytarabine (araC) against leukemia and was first approved by the FDA in 1992.[133] Similarly, a glycerolipid ester prodrug of AZT (fozivudine tidoxil) was developed as a once-a-day HIV treatment.[134]



Scheme 2-9: Metabolism of stearyl ester prodrugs

The bis-acyloxybenzyl (BAB) nucleoside monophosphate prodrugs were reported in 1993. This BAB-prodrug was designed for targeting to the central nervous system. After the successful synthesis, anti-HIV-1 activity of BAB-AZTMP-prodrugs was tested in C8166 cells and no increasing activity was observed comparing with AZT and AZTMP. Interestingly, the mono-acyloxybenzyl masked (MAB) prodrug showed higher activity than BAB-prodrugs.[135]

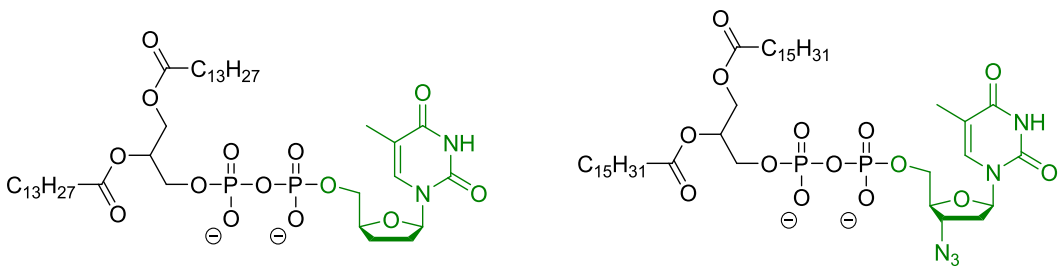


Scheme 2-10: Metabolism of BAB-AZTMP prodrugs

2.2.2 Nucleoside Diphosphate Prodrugs

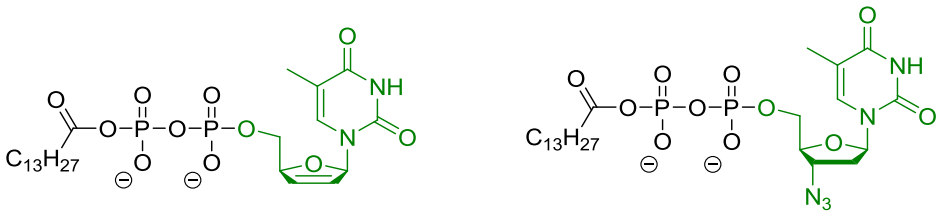
To bypass the phosphorylation steps to NDP, nucleoside diphosphate prodrugs were proposed and deliver NDP directly.[22]

In 1990, K. Y. Hostetler *et al.* reported diacylglycerol NDP prodrugs (DAG-prodrug) and tested their antiretroviral activity in HIV-infected U937 and CEM cells. The *in vivo* efficacy of ddT-DP prodrug was more effective than ddT. However, AZT-DP and ddC-DP prodrugs showed lower activity than the corresponding nucleosides.[136,137] Five years later, D. Bonnaffe *et al*. synthesized acyl pyrophosphate prodrugs of d4T and AZT.[138,139] Biological data for the antiviral activity against HIV-1 did not reveal any difference between the acyl-NDP (and NMP) prodrugs and corresponding parent nucleoside. This was maybe because of the short half-life (less than 2 h) of the acyl-AZTDP prodrugs in RPMI culture media.[140]



DAG-ddTDP **28** DAG-AZTDP **29**

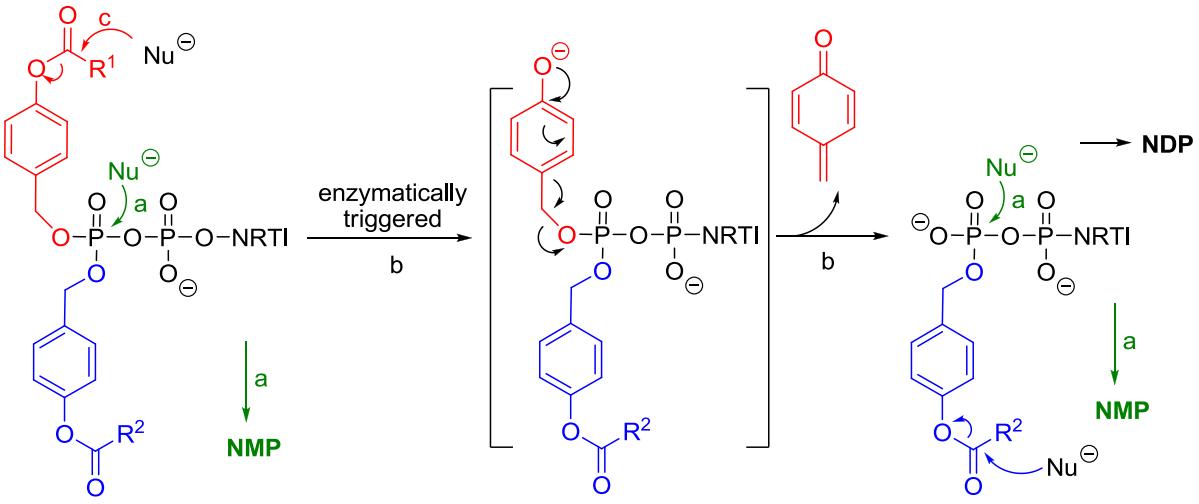
Scheme 2-11: Structures of NDP diglyceride prodrugs.



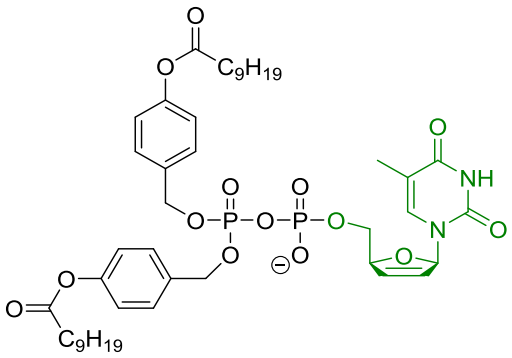
Acyl-d4TDP **30** Acyl-AZTDP **31**

Scheme 2-12: Structures of Acyl-NDP prodrugs.

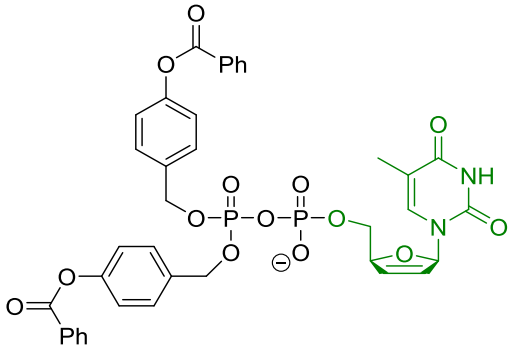
A new class of nucleoside diphosphate prodrugs are bis-acyloxybenzyl (BAB) prodrug (Di*PP*ro-approach) developed from C. Meier *et al*.. They firstly synthesized a series of symmetric-BAB-d4TDP prodrugs (Di*PP*ro) and evaluated the activity against HIV-1 and 2 in wild-type CEM/0 cells and CEM/TK$^-$ cells. Later, BBB-Di*PP*ro (bis-(benzoyloxybenzyl)-Di*PP*ro) and non-symmetric-BAB-Di*PP*ro were also studied. In contrast to the *cyclo*Sal-technology also developed by them, the delivery mechanism of BAB-prodrugs relies on an enzymatically triggered process. Chemical methods did not lead to a selective release of the corresponding nucleoside diphosphate. The lead structure of these prodrugs is shown below as well as the proposed mechanism of hydrolysis. Compounds **32**, **33** and **34** are representative compounds of these three series of Di*PP*ro, respectively. Their activities against HIV-2 in CEM/TK$^-$, cytotoxic activities, half-life in PBS (pH = 7.3) and CEM cell extracts are listed in Table 2-6. Results showed that longer acyl residues increased Di*PP*ro-activities compared to shorter ones. This result can be explained as the necessity of having long acyl residues for the membrane permeability.[20,21,141,142]



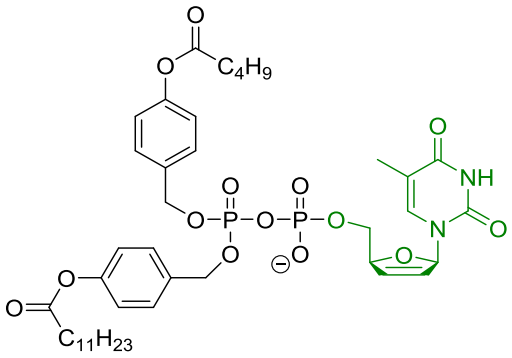
Scheme 2-13: Proposed hydrolysis mechanism of Di*PP*ro-compounds.



BAB-bis-C9-d4TDP **32**



BBB-bis-Ph-d4TDP **33**



BAB-(AB-C4, ab-C11)-d4TDP **34**

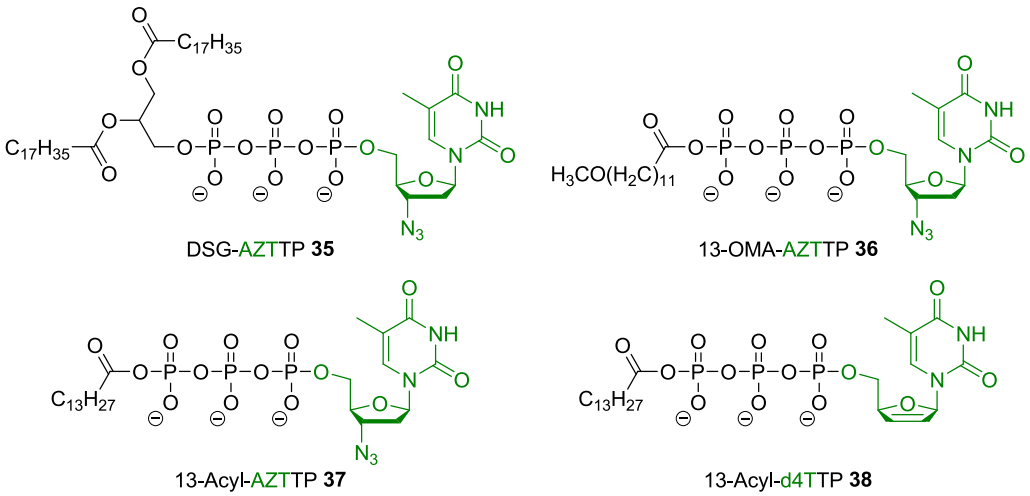
	EC_{50} (µM)[a]	CC_{50} (µM)[b]	Half-life (h)	
	HIV-2, CEM/TK$^-$	CEM/0	PBS (pH 7.3)	CEM cell extracts
BAB-bis-C9-d4T-Di*PP*ro **32**	0.11 ± 0.042	72 ± 9.9	63	7.6[c]
d4T	173 ± 70	> 250		
BBB-bis-Ph-d4T-Di*PP*ro **33**	0.85	36 ± 5	82	7
d4T	70	> 100		
BAB-(AB-C4,ab-C11)-d4T-Di*PP*ro **34**	0.13 ± 0.07	30 ± 17	52	1.9
d4T	150 ± 9	79 ± 3		

Table 2-6: Characteristics of bioreversible protection of nucleoside diphosphates. [a] Antiviral activity in $CD4^+$ T-lymphocytes: 50% effective concentration; values are the mean ±SD of n=2-3 independent experiments. [b] Cytotoxicity: 50% cytostatic concentration or compound concentration required to inhibit $CD4^+$ T-cell (CEM) proliferation by 50%; values are the mean ±SD of n=2-3 independent experiments. [c] (3:7 CEM cell extracts/PBS).

2.2.3 Nucleoside Triphosphate Prodrugs

Nucleoside triphosphate prodrugs bypass all phosphorylation steps from free nucleoside to NTP and directly deliver the triphosphate form of nucleoside analogs as active antiviral compounds.

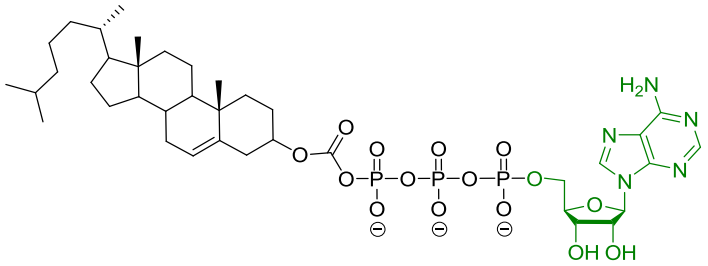
After K. Y. Hostetler *et al*. studied 1,2-diacylglycerol AZTMP and AZTDP prodrugs (DAG-AZTMP and DAG-AZTDP **29**), distearoylglycerol AZTTP prodrug (DSG-AZTTP **35**) was studied. The *in vitro* IC_{50} values of DSG-AZTTP prodrugs were 0.79 µM in HT4 cells and 0.33 µM in CEM cells, which was less active than DSG-AZTDP. Additionally, the DSG-AZTTP prodrug was less toxic to CEM cells than DSG-AZTMP and DSG-AZTDP.[143] D. Bonnaffe *et al*. also continued on the synthesis of two series of acyl NTP prodrugs.[138,139] The antiviral activity data on HIV-1 infected cells did not reveal any difference between acyl AZTTP prodrugs and AZT. Because of the short half-life (less than 2 h) of acyl AZTTP prodrugs in RPMI culture media where aminolysis reaction happens.[140]



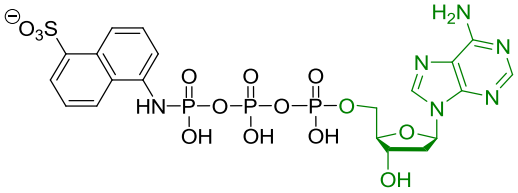
Scheme 2-14: DSG-AZTTP and Acyl NTP prodrugs as examples

In 1998, A. Kreimeyer synthesized cholesteryloxycarbonyl-ATP (Chol-ATP) **39** and they focused on the study on NTP transference through membrane. Chol-ATP was incubated in a phosphate buffer containing liposomes and the mixture was monitored by ^{31}P NMR. By adjusting pH value and without destroying the phospholipids, resonances of ATP molecules located inside and/or outside the liposomes were distinguished. Results showed that after incubating Chol-ATP with liposomes, ATP was observed inside the liposomes without destroying the phospholipids via passive diffusion. However, there is no NRTI applied in this modification method with antiviral data.[144,145]

In 2005, B. A. Mulder reported the synthesis of a γ-modified nucleoside triphosphate. The γ-phosphate of dNTPs was attached with 1-amino-naphthalene-5-sulfonate (ANS). ANS is also a strongly fluorescent compound with an emission at 460 nm and its excited state lifetime is 20 nsec. The cleavage of α,β-phosphoryl bond produces a 15 nm red shift. This property is useful for studying enzymatic reaction.[146] Primer extension experiment showed that all four ANS-dNTPs can be incorporated with HIV-RT, while the incorporation rates of ANS-dNTPS are slower than their natural dNTP counterparts. Furthermore, they also constructed a molecular model of the HIV-RT binding with an ANS-dNTP attached to the primer/template. Results showed that the modification of γ-phosphate can increase the fidelity of HIV-RT.



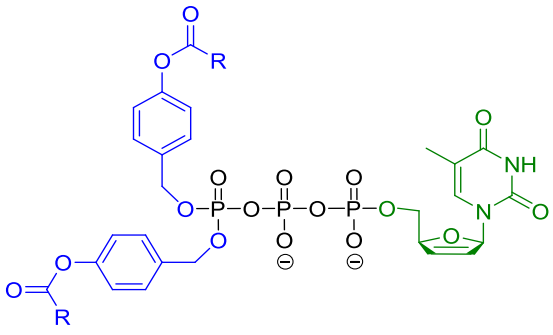
Chol-ATP **39**



ANS-dATP **40**

Scheme 2-15: Chol-ATP and ANS-dATP as examples.

In 2015, T. Gollnest in our group developed a NTP prodrug concept which was called the Tri*PPP*ro-concept (or Tri*PPP*ro-approach). He synthesized a series of Tri*PPP*ro-compounds containing two 4-(hydroxymethyl)-phenylalkyl groups (lipophilic AB-masks) attached to the γ-phosphate. The structures are listed as examples in Scheme 2-16.



Scheme 2-16: Bis(AB)-Tri*PPP*ro-compounds **41** as examples.

The half-lives of the Tri*PPP*ro-compounds were determined in phosphate buffer solution (PBS, pH = 7.3) and in CEM cell extracts as biological condition (Table 2-7). In both PBS and CEM cell extracts, the stability of Tri*PPP*ro-compounds γ-(AB,ab)-d4TTPs **41** increased with the increasing alkyl chain length from the AB-mask moiety. However, the $t_{1/2}$ of Tri*PPP*ro-compounds **41** in CEM cell extracts were much lower than those in PBS, which means enzymatic cleavage process is the main hydrolysis route in CEM cell extracts.

Compound	Nucl.	R	PBS pH=7.3 [h]	CEM/0 Cell extracts [h]
			$t_{1/2}(1)$[a]	$t_{1/2}(1)$[a]
41b	d4T	CH_3	18	0.05
41c	d4T	C_2H_5	17	0.12
41e	d4T	*n*-C_4H_9	22	0.43
41g	d4T	*n*-C_6H_{13}	26	0.98
41i	d4T	*n*-C_8H_{17}	52	2.5
41j	d4T	*n*-C_9H_{19}	44	2.8
41l	d4T	*n*-$C_{11}H_{23}$	68	2.2
41m	d4T	*n*-$C_{13}H_{27}$	90	4.6
41p	d4T	*n*-$C_{15}H_{31}$	73	5.3
41r	d4T	*n*-$C_{17}H_{35}$	50	13

Table 2-7: Hydrolysis half-lives of Tri*PPP*ro-compounds (AB,ab)-d4TTPs **41**.

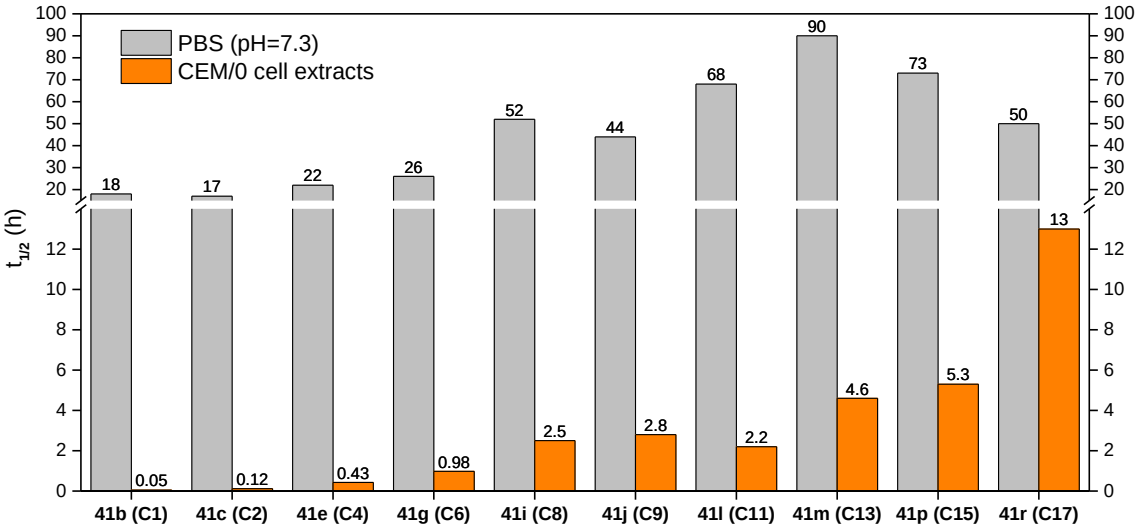
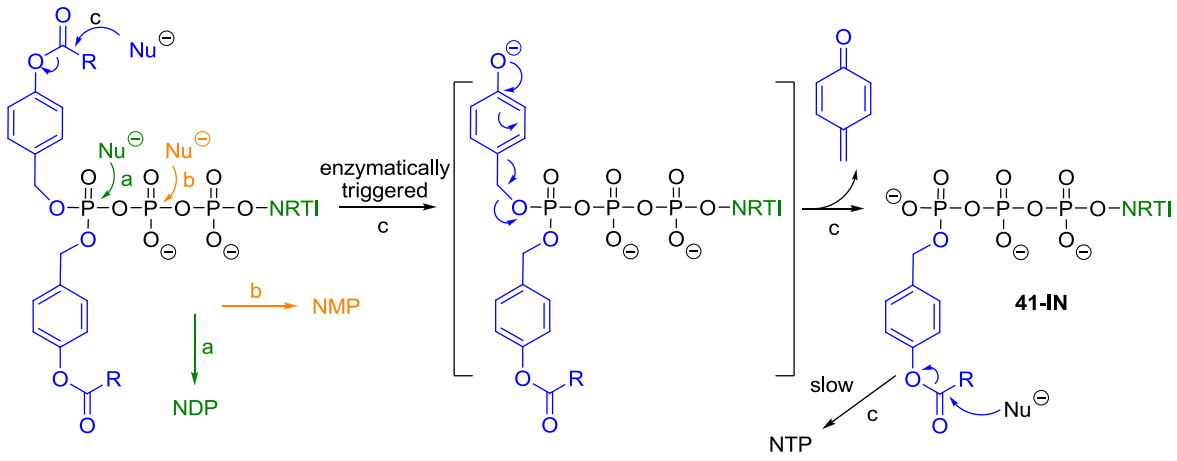


Figure 2-12: Hydrolysis half-lives of Tri*PPP*ro-compounds (AB,ab)-d4TTPs **41** in bar charts.

The proposed hydrolysis mechanism is shown in Scheme 2-17. The calculation of half-life of Tri*PPP*ro-compounds are based on the cleavage of first AB-mask moiety.

D4TTP **4t** and d4TDP **4d** were detected as major hydrolysis product in PBS and CEM cell extracts. D4TMP **4m** was formed only in very small amounts in PBS. In cell extracts, the peak of d4TMP **4m** was overlapped with the peaks of cell extracts in RP-18-HPLC chromatography and it is not possible to get the exact peak area of d4TMP **4m**. Thus, for hydrolysis in CEM cell extracts, the exact amount of d4TMP **4m** was unknown because of technical difficulties. As the $t_{1/2}$ of intermediate-(AB-C8)-d4TTPs **41-IN-i** is 1.8 h in cell extracts and d4TTP **4t** is labile under the same conditions ($t_{1/2}$ = 0.63 h), the formation of d4TDP **4d** in cell extract was mainly because of the rapid degradation of d4TTP, not following the **Path a** in Scheme 2-17.



Scheme 2-17: Proposed hydrolysis mechanism of Tri*PPP*ro-compounds **41**.

Antiviral assay revealed that Tri*PPP*ro-compounds **41** are potent inhibitors of HIV-1 and HIV-2 in CEM/0 cell lines (Table 2-8 and Figure 2-13). Similar to the Di*PP*ro-compounds, longer alkyl chain residues of the Tri*PPP*ro-compounds increased its membrane permeability and resulted in higher antiviral activity in CEM/TK$^-$ cells. When the alkyl chain of the AB-mask is shorter than C8, Tri*PPP*ro-compounds **41** lost activity against HIV-2 in CEM/TK$^-$ cells.

Later, this Tri*PPP*ro-approach was applied to different nucleoside analogs against HIV. The formed NTPs from Tri*PPP*ro-compounds were proved to be an enzyme-triggered degradation by pig liver esterase or in human T-lymphocyte (CEM/0) cell extracts. Some HIV-inactive nucleoside analogues showed marked anti-HIV activity after they were transferred to their Tri*PPP*ro-form.[25,26] Then by using a fluorescent Tri*PPP*ro-compound, cellular uptake studies proved that Tri*PPP*ro-compound delivered the triphosphorylated metabolite to intact CEM cells. Thus, it can be

concluded that, with this Tri*PPP*ro-approach, prodrugs directly deliver NTPs into cells, and thus bypass all the phosphorylation steps and finally produce antiviral activity.[24]

Compound	EC_{50} [µM][a]			CC_{50} [µM][b]
	CEM/0		CEM/TK$^-$	CEM/0
	HIV-1	HIV-2	HIV-2	
41b (C1)	0.43 ± 0.25	0.72 ± 0.16	>10	63 ± 2
41c (C2)	0.46 ± 0.21	1.16 ± 0.15	>10	57 ± 6
41e (C4)	0.40 ± 0.00	1.05 ± 0.30	>10	58 ± 3
41g (C6)	0.36 ± 0.06	0.94 ± 0.16	10 ± 0.00	74 ± 2
41i (C8)	0.31 ± 0.01	0.62 ± 0.30	2.26 ± 1.03	52 ± 1
41j (C9)	0.25 ± 0.07	0.33 ± 0.03	0.50 ± 0.14	34 ± 5
41l (C11)	0.21 ± 0.01	0.27 ± 0.06	0.72 ± 0.16	26 ± 0
41m (C13)	0.50 ± 0.14	1.10 ± 0.23	1.63 ± 0.52	28 ± 7
41p (C15)	0.62 ± 0.30	0.66 ± 0.08	0.72 ± 0.16	61 ± 3
41r (C17)	0.17 ± 0.00	0.31 ± 0.00	0.28 ± 0.04	29 ± 9
d4T **4**	0.33 ± 0.11	0.89 ± 0.00	150 ± 9	79 ± 3

Table 2-8: Antiviral data of Tri*PPP*ro-compounds γ-(AB,ab)-d4TTPs **41** in comparison of d4T **4**. [a] Antiviral activity in $CD4^+$ T-lymphocytes: 50% effective concentration; values are the mean ±SD of n=2-3 independent experiments. [b] Cytotoxicity: 50% cytostatic concentration or compound concentration required to inhibit $CD4^+$ T-cell (CEM) proliferation by 50%; values are the mean ±SD of n=2-3 independent experiments.

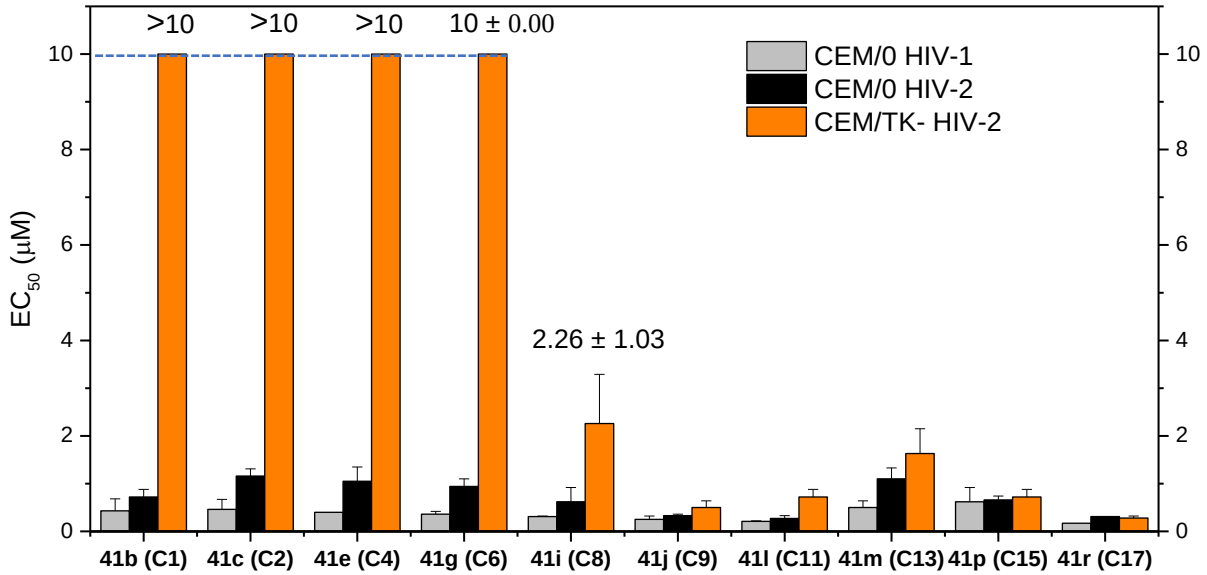


Figure 2-13: Antiviral data of Tri*PPP*ro-compounds γ-(AB,ab)-d4TTPs **41** in bar charts.

3 Motivations and Objectives

As is well known, the aim of nucleotide prodrug or pronucleotide approach is to deliver NMPs, NDPs and NTPs into the cell, to bypass some phosphorylation steps and finally produce activity. The antiviral data of NMP, NDP and NTP prodrugs as examples were listed in Table 3-1. Result showed that Di*PP*ro- and Tri*PPP*ro-compounds greatly increased the antiviral activity as compared to the parent nucleoside d4T **4**.

		CEM/TK$^-$		CEM/0	Half-life (h)	
		EC_{50} (µM) (HIV-1[a] or HIV-2[b])	EC_{50} ratio: NRTI/prodrug	CC_{50} (µM)	PBS (pH 7.3)	CEM/0 Cell Extracts
NMP prodrug	bis-Me-SATE-d4TMP	0.012 ± 0.006[a]	833	60 ± 22		
	d4T	10 ± 10[a]		> 100		
	bis-*t*Bu-SATE-AZTMP	0.45[a]	222	>10		
	AZT	> 100[a]		>100		
	3-Me-cycloSal-d4TMP **22**	0.05[b]	950	32		
	d4T	47.5 ± 26.3[b]		234		
	5-di-AM-cycloSal-d4TMP **24**	10.5 ± 3[b]	5	99		
	d4T	47.5 ± 26.3[b]		234		
	3-MePr-cycloSal-d4TMP **23**	0.26 ± 0.14[b]	58	42.8 ± 13.5		
	d4T	15.0 ± 7.71[b]		58.8 ± 24.2		
NDP prodrug	BAB-bis-C9-d4T-Di*PP*ro **32**	0.11 ± 0.04[b]	1572	72 ± 9.9	63	7.6 (3:7 CEM/PBS)
	d4T	173 ± 70		> 250		
	BBB-bis-Ph-d4T-Di*PP*ro **33**	0.85[b]	82	36 ± 5	82	7
	d4T	70		> 100		
	BAB-C4/C11-d4T-Di*PP*ro **34**	0.13 ± 0.07[b]	1153	30 ± 17	52	1.9
	d4T	150 ± 9		79 ± 3		
NTP prodrug	BAB-bis-C9-d4TTP **41i**	0.50 ± 0.14	300	34 ± 5	44	2.8
	BAB-bis-C17-d4TTP **41q**	0.28 ± 0.04	535	29 ± 9	50	13
	BAB-bis-OC11-d4TTP **41z**	1.26 ± 0.00	119	41 ± 12	99	3
	d4T	150 ± 9		79 ± 3		

Table 3-1: Antiviral data of NMP, NDP and NTP prodrugs. [a] EC_{50} values against HIV-1. [b] EC_{50} values against HIV-2.

Previews studies also showed that symmetrically modified Tri*PPP*ro-compounds **41** with a longer acyloxybenzylalkanoate mask exhibited higher antiviral activity, while also exhibited longer half-life in the hydrolysis in PBS and CEM cell extracts.

Thus, the first part of this work is to study a series of non-symmetrically modified Tri*PPP*ro-compounds **56** to search a compound with higher antiviral activity. In this idea, Tri*PPP*ro-compounds with short chain alkanoyl esters led to a fast hydrolysis by

chemical or enzymatic process. The ester group in the second prodrug mask comprised long lipophilic alkyl chain provided lipophilicity and enabled the prodrug to penetrate the cell membrane. The introduction of two different groups allowed a controlled stepwise removal of the prodrug moieties to achieve a highly selective delivery of the NDP in CEM cell extracts. The compounds were highly active against HIV even in thymidine kinase-deficient CEM cells. Thus, the compounds, although charged at the α- and β-phosphate group, were taken up by the cells and released NDPs.

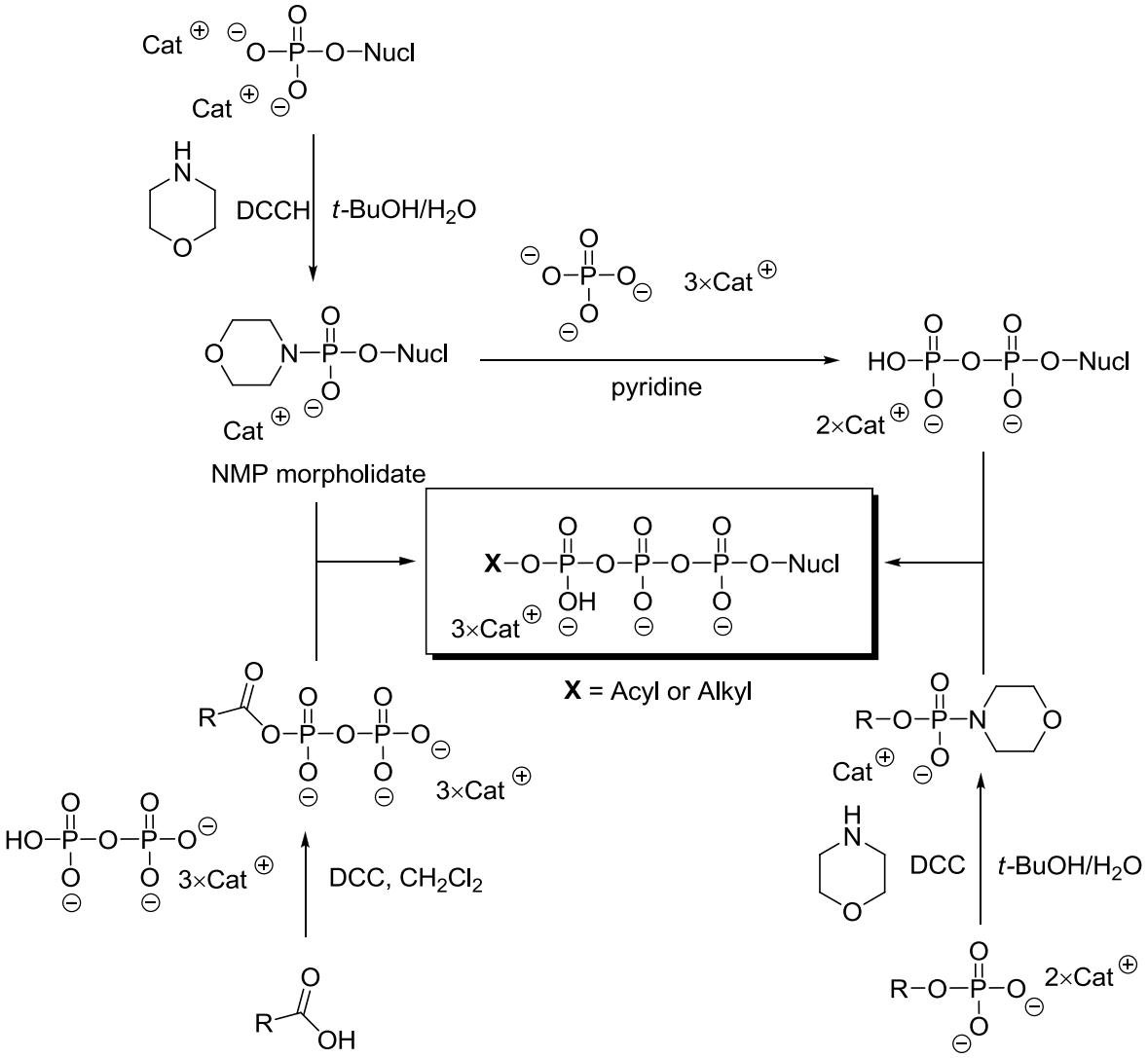
A preview study also showed that d4TTP inhibited Pol β and was a particularly strong inhibitor of Pol γ, but not a substrate for Pol α. Thus, there are two important issues related with the delivery of NTPs from Tri*PPP*ro-compounds: i) the need of a sufficient selectivity of the NTP to act as a substrate for the viral polymerases but not for the cellular polymerases and ii) their high sensitivity for enzymatic dephosphorylation. To address these two issues, we decided to explore γ-alkyl-NTPs and its corresponding lipophilic prodrugs. The idea is that such compounds led to an increased stability of the triphosphate in biological condition. For Tri*PPP*ro-compounds **41**, the intermediate proved to be a substrate for HIV-RT and d4TMP was incorporated into the primer strand.

These observations and new ideas guided us to the design of new γ-modified NTP prodrugs **58** and γ-modified NTPs **60** which comprise simple γ-alkyl chains of different length in combination with a biodegradable acyloxybenzyl group. The final aim of this work is to find γ-modified NTP prodrugs that could produce active γ-modified NTP against HIV. At the same time, they are not substrate for human polymerases and thus will not interact with normal DNA synthesis.

4 Discussion

4.1 General Synthesis Route

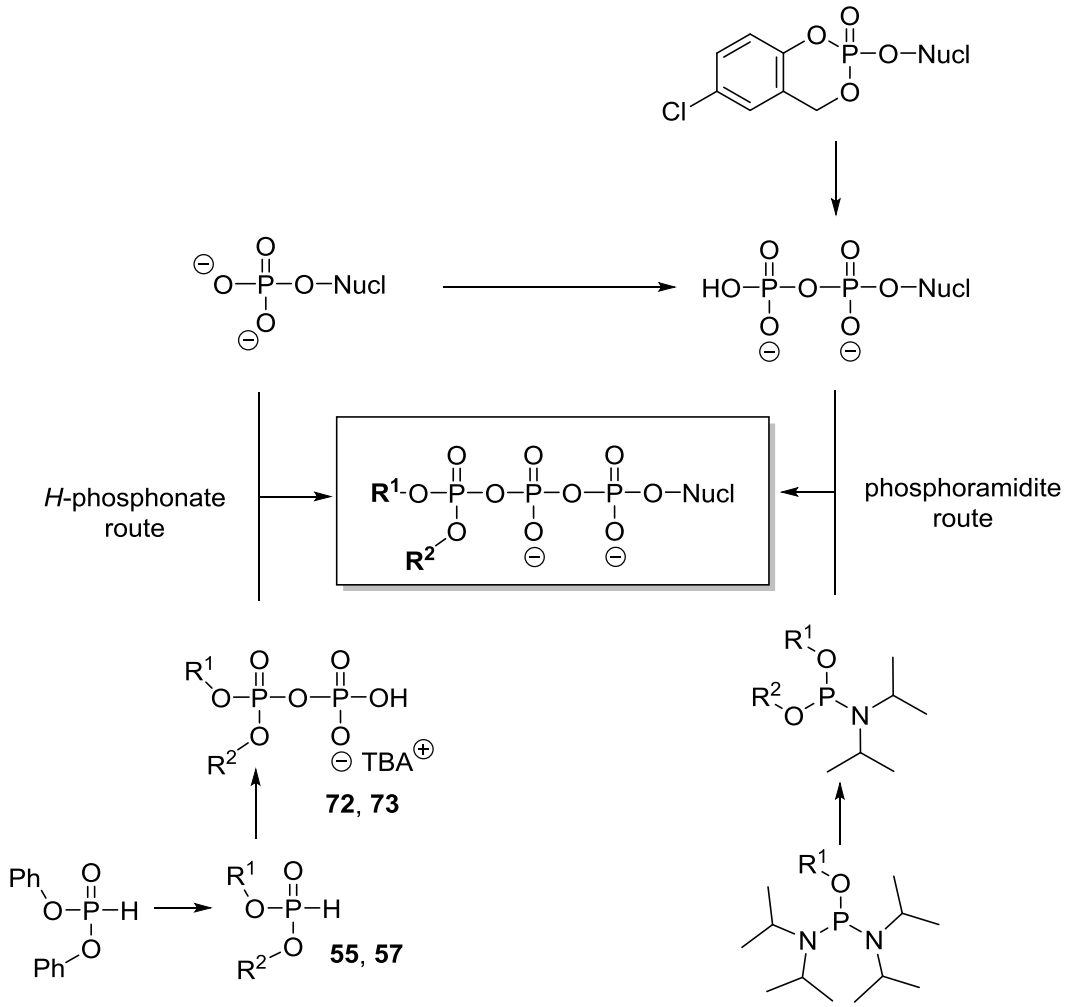
To synthesize esterified triphosphate prodrugs, the morpholidate route, phosphoramidite route and *H*-phosphonate route can be used. Using the morpholidate route, the monophosphate morpholidate was used as a building block in the coupling reaction to form the NTP prodrug. The coupling process was reported by Hostetler *et al.* and D. Bonnaffe *et al*..[136,138,140] The corresponding morpholidate was reacted with an acyl pyrophosphate. The NDP was also used to react with an ester monophosphate morpholidate to form NTP prodrugs.



Scheme 4-1: Morpholidate route to synthesize NTP prodrugs

To synthesize BAB-triphosphate prodrugs (Tri*PPP*ro-compounds) and γ-modified-triphosphate prodrugs, a phosphoramidite based route was developed first. A

dicyanoimidazol-mediated coupling was conducted using a phosphoramidite and a NDP. The NDP was prepared via the *cyclo*Sal approach.



Scheme 4-2: Phosphoramidite and *H*-phosphonate route to synthesize NTP prodrugs

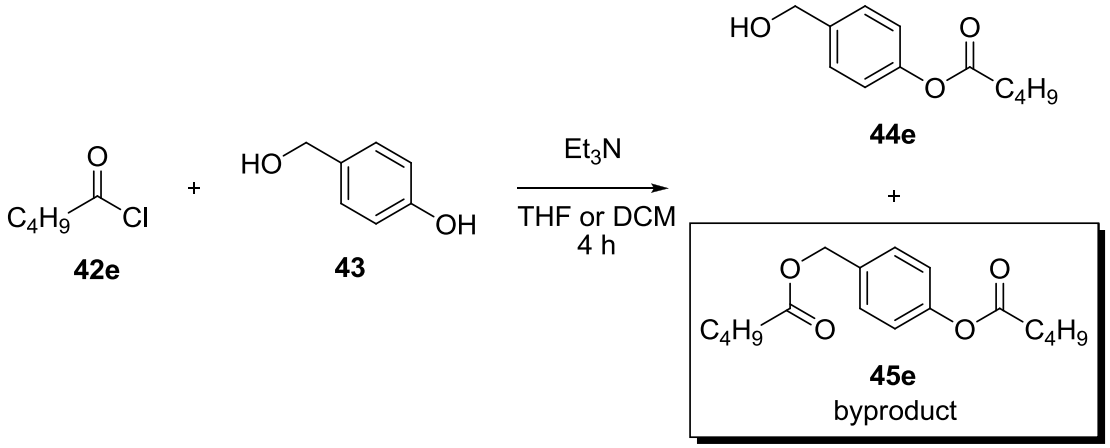
As on the phosphoramidite route, the NDP are used as substrate. The instability of the nucleoside diphosphate increases the difficulty of purification and is the disadvantage of this route. Then T. Gollnest used the *H*-phosphonate route by using a NMP and a modified pyrophosphate for the final coupling. The diester modified *H*-phosphonates **55**,**57** were firstly converted into the chloridate by an oxidative chlorination using NCS. Then the modified pyrophosphates **72**,**73** were prepared by the phosphorylation of **55**,**57** with 2×TBA monophosphate. The conversion in this phosphorylation reaction is almost quantitative and after extraction in DCM/water, the product can be used for the next coupling step without further purification. As compared to the phosphoramidite approach, the *H*-phosphonate route offers two main advantages: i) the non-symmetric *H*-phosphonates **55** were found to be stable at -20 °C for more than two years and ii) in our hands d4TMP **4m** was easier to

synthesize and is more stable compared to the d4TDP. The non-symmetric Tri*PPP*ro-compounds γ-(AB,ab)-NTPs and γ-modified NTPs were synthesized through the *H*-phosphonate route in this work.

4.2 Synthesis of the Starting Materials

4.2.1 Synthesis of 4-(Hydroxymethyl)-phenyl esters 44

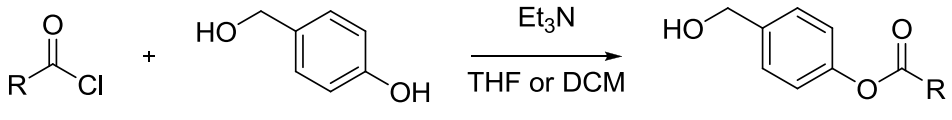
From literature, the synthesis of 4-(hydroxymethyl)phenyl esters **44** is based on the Schlenk technique and involved dry THF as solvent (see entry a in Table 4-1). A simpler esterification condition to synthesize compounds **44** is needed. Then the reaction was optimized by changing the solvents and temperature.



Entry	43	Solvents[a]	Base (dry)	Temp.	Yield
a	1.1 eq.	dry THF[b]	1 eq. Et_3N	0 °C	50%
b	1.1 eq.	dry DCM	1 eq. Et_3N	0 °C - r.t.	80%
c	1.2 eq.	dry DCM[b]	1.2 eq. Et_3N	r.t.	60%
d	1.1 eq.	dry DCM	1 eq. Et_3N	0 °C	69%
e	1.1 eq.	DCM[c]	1.1 eq. Et_3N	0 °C - r.t.	65%
f	1.2 eq.	DCM[c]	1.2 eq. Et_3N	r.t.	78%

Table 4-1: Optimization of the synthesis of 4-(hydroxymethyl)phenylalkanoate. [a] 0.17 mol/L of **42e** was used without further noticed. [b] 0.84 mol/L of **42e** was used in the reaction. [c] HPLC grade DCM.

1.1 Equivalents of 4-hydroxybenzyl alcohol **43** were used in this reaction to prevent the formation of di-esterified byproduct **45e**. When using HPLC grade DCM and non-dry conditions (reaction e in Table 4-1) instead of dry THF and dry DCM under N_2 (reaction **d** and **a** in Table 4-1), the yield of **44e** was increased to 65%. With HPLC grade DCM as solvent, it was possible to increase the reaction temperature to room temperature and the yield increased from 65% to 78% (reaction e and f in Table 4-1). This yield is only 2% lower than that found in reaction b. This means that the ice bath is not necessary at the beginning of reaction. Thus, new synthesis conditions were found, which is easy to handle at room temperature without using dry solvent and Schlenk technique.



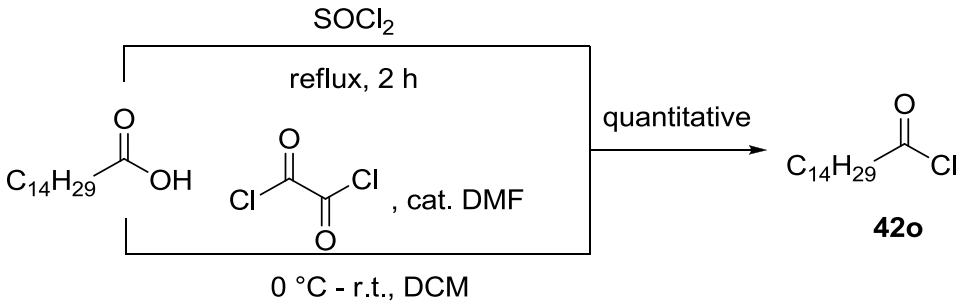
42 **43** **44**

	R	Yield
44b	CH_3	56%
44c	C_2H_5	60%
44e	*n*-C_4H_9	78%
44e*i*	*iso*-C_4H_9	64%
44g	*n*-C_6H_{13}	58%
44o	*n*-$C_{14}H_{29}$	63%
44u	*n*-$C_{15}H_{31}$	80%
44w	*n*-$C_{17}H_{35}$	78%

Table 4-2: Synthesis of 4-(hydroxymethyl)phenylalkanoate **44**.

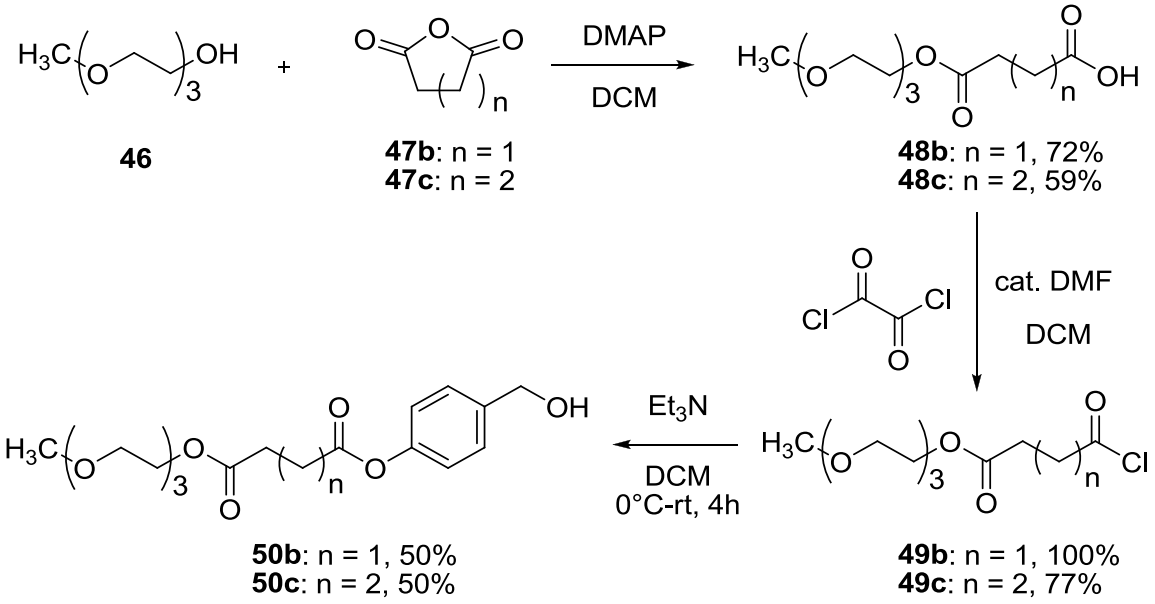
During the optimization, a series of 4-(hydroxymethyl)phenylalkanoates **44** were synthesized. The yield ranged from 56% to 80%. The reaction time was prolonged to overnight when R group is longer than C6. The yield of compound **44o** was 63%, which is identical with the result already published.[147] Pentadecanoyl chloride **42o** was synthesized from pentadecanoyl acid in a quantitative yield and was directly used in the next synthesis without further purification. Compared to the method of

thionyl chloride, the reaction condition with oxalyl chloride[147] was mild and produced nontoxic CO_2 as byproduct instead of toxic SO_2 or SO_3.



Scheme 4-3: Synthesis of acyl chloride.

As compounds **44o**-**s** are compounds with a lipophilic moiety, masks with hydrophilic moieties **50** were also an important aspect for the studies the prodrug activity. The hydrophilic moieties of the mask were synthesized starting from 2-(2-(2-methoxyethoxy)ethoxy)ethan-1-ol **46** (**MEEE**). Succinic anhydride **47b** (**S**) and glutaric anhydride **47c** (**G**) were used to form a di-ester linker between the PEG moiety and the 4-hydroxybenzyl alcohol **43**.[148]

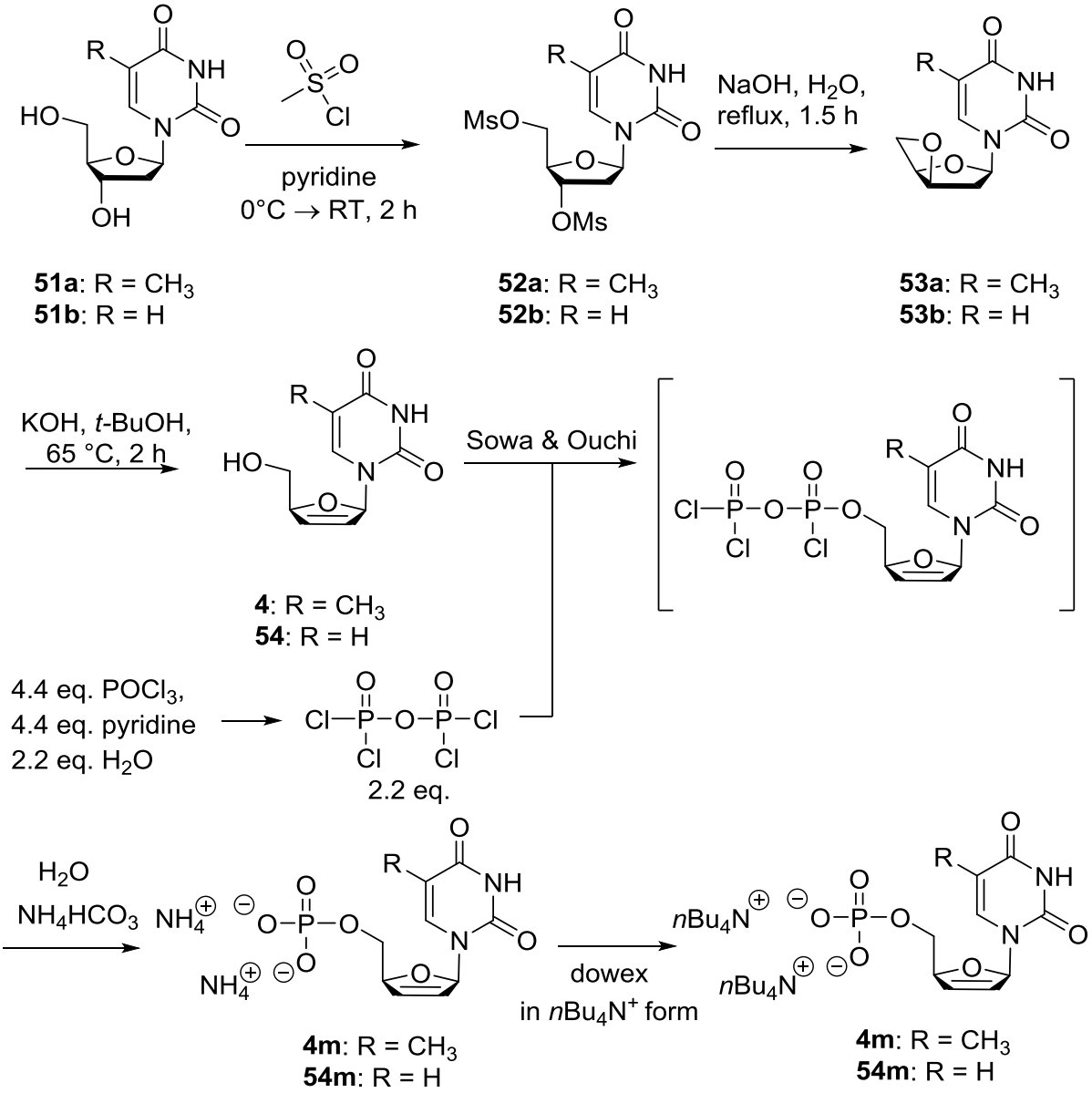


Scheme 4-4: Synthesis of masks with PEG moiety.

4.2.2 Synthesis of Nucleoside Monophosphate

In this work, d4T **4** and d4U **54** was prepared by using the method of McGuigan *et al.*[149] and Horwitz *et al*..[121,122] As an example, the compound of thymidine **51** was firstly 3´,5´-dimesylated and then reacted with aqueous sodium hydroxide to form compounds **53**. Afterwards, compounds **53** were treated with sodium hydride in

tert-butanol to give the target compound (d4T and d4U) in a moderate yield. Additionally, ddT can be synthesized by hydrogenation of d4T in the presence of palladium on activated carbon.[25,26] D4TMP **4m** and d4UMP **54m** were prepared from d4T **4** and d4U **54** applying the method described by Sowa and Ouchi.[150] The d4TMP 2×NH_4^+ salt was isolated and converted into its acid form by a Dowex 50WX8 (H^+ form) ion exchange and then titrated with tetra-*n*-butylammonium hydroxide solution to pH = 7 followed by freeze-drying. The di($n$$Bu_4N$)$^+$ salt of d4TMP **4m** was highly hygroscopic and needed to be rigorously dried before use.



Scheme 4-5: Synthesis of d4TMP **4m** and d4UMP **54m**.

4.3 Non-symmetric Tri*PPP*ro-compounds γ-(AB,ab)-d4TTPs 56

In alteration to the synthesis of the phosphoramidite route, non-symmetric Tri*PPP*ro-compounds γ-(AB,ab)-d4TTPs **56** were synthesized preferably using the *H*-phosphonate route. The non-symmetric *H*-phosphonates were first converted to pyrophosphates **72** and then coupled with nucleoside monophosphate to form Tri*PPP*ro-compounds **56**.

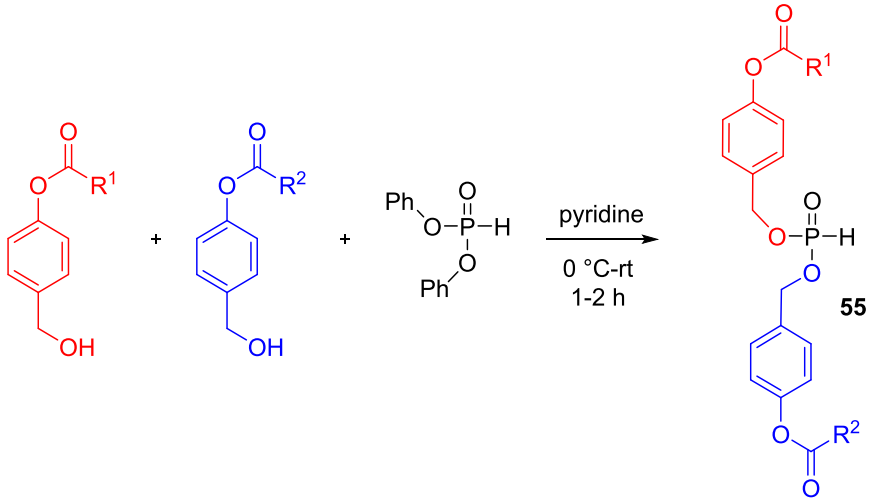
4.3.1 Synthesis of non-symmetric (AB,ab) *H*-phosphonates 55

In the first step, diphenylhydrogenphosphonate (DPP) was cooled to 0 °C and then reacted with alcohol **44** or **50**. This step was fast and was finished within 10 min at 0 °C. Thus, an alcohol with a long alkyl chain moiety was preferred to use in this step. The long alkyl chain moiety decreased the ester exchange rate and diester exchange byproduct can be reduced. In some cases, if 4-(hydroxymethyl)phenylalkanoate with short alkyl chain moiety has to be used in the first ester exchange step, the reaction temperature can be reduced to -10 °C or even -20 °C. Mask with long alkyl chain moiety such as **44u** and **44w** have low solubility in pyridine at low temperature, the first ester exchange step was conducted at 0 °C using an ice bath and the temperature was slowly increased to room temperature within 2 h.

Next, the non-symmetric *H*-phosphonates **55** were formed by adding a second alcohol at room temperature and the reaction mixture was heated to 40 °C for 2 h or overnight. The reaction can be monitored by TLC and the product was purified by silica column chromatography. The R_f values of **55** and its two symmetric byproducts are similar but distinguishable on the TLC plate. The relationship of R_f values by using **55eo** as example is as follows: symmetric *H*-phosphonate byproduct with long alkyl moiety **55oo** > **55eo** > symmetric *H*-phosphonate byproduct with short alkyl moiety **55ee**. If we used less equivalents of the alcohol in the first ester exchange step and excess equivalents of alcohol in second ester exchange step, the amount of symmetric byproduct was reduced to a minimum. Thus, the byproduct was mainly **55oo** and the separation was much easier when there are only two spots (**55eo** and **55oo**) near to each other on TLC plate.

As can be seen in Table 4-3, the yields of non-symmetric *H*-phosphonates **55** were only moderate (44% to 52%). The reaction to synthesize symmetric *H*-phosphonate **55uu** was easier than that of non-symmetric *H*-phosphonate. 1.0 equivalent of DPP and 2.2 equivalents of alcohol were stirred in pyridine at 40 °C for 2-4 h and purified

by recrystallization in hexane/methanol instead of silica column chromatography. The yield of **55uu** was only 34%. Recrystallization for the purification step is probably the reason to decrease the yield of **55uu**.

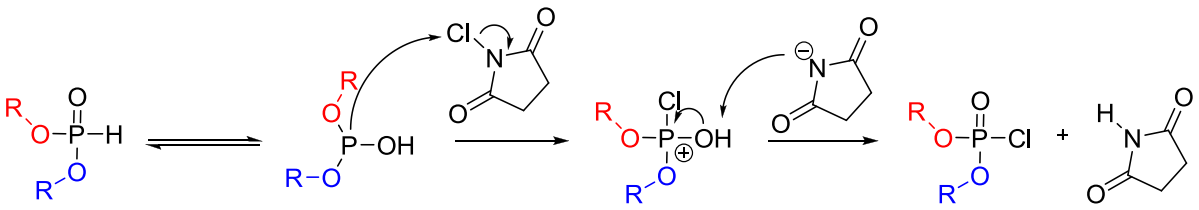


Compound	R_1	R_2	Yield
55co	C_2H_5 **44c**	*n*-$C_{14}H_{29}$ **44o**	50%
55eo	*n*-C_4H_9 **44e**	*n*-$C_{14}H_{29}$ **44o**	48%
55go	*n*-C_6H_{13} **44g**	*n*-$C_{14}H_{29}$ **44o**	52%
55e*i*o	*iso*-C_4H_9 **44e*i***	*n*-$C_{14}H_{31}$ **44o**	46%
55eu	*n*-C_4H_9 **44e**	*n*-$C_{15}H_{31}$ **44u**	44%
55ew	*n*-C_4H_9 **44e**	*n*-$C_{17}H_{35}$ **44w**	43%
55e-MEEES	*n*-C_4H_9 **44e**	PEG-S2 **50b**	52%
55o-MEEES	*n*-$C_{14}H_{29}$ **44o**	PEG-S2 **50b**	52%
55o-MEEEG	*n*-$C_{14}H_{29}$ **44o**	PEG-G3 **50c**	46%
55uu	*n*-$C_{15}H_{31}$ **44u**	*n*-$C_{15}H_{31}$ **44u**	34%

Table 4-3: Synthesis and yield of (AB,ab)-*H*-phosphonate.

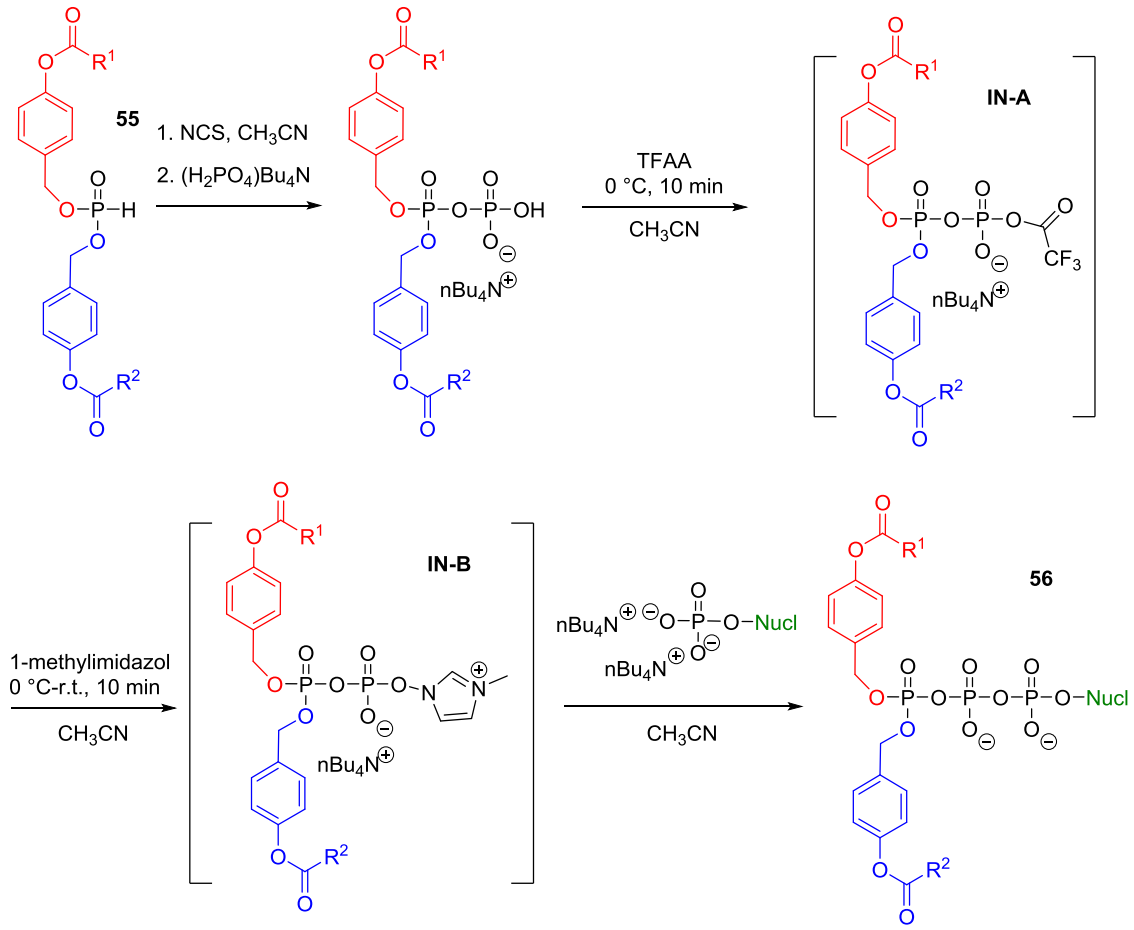
4.3.2 Synthesis of non-symmetric Tri*PPP*ro compounds γ-(AB,ab)-dNTPs 56

After the non-symmetric *H*-phosphonates **55** were obtained, they were reacted with *N*-chlorosuccinimide (NCS) to form the corresponding phosphorochloridates. The reaction time may vary from 2 h to 12 h at room temperature. The mechanism of the oxidative chlorination is shown in Scheme 4-6. The Chlorophosphate were formed and then reacted with tetra-*n*-butylammonium phosphate to yield the corresponding pyrophosphates in almost quantitative yields. Due to its chemical instability, the pyrophosphates were quickly purified by extraction and directly used in the next step.



Scheme 4-6: The chlorination mechanism of *H*-phosphonate.

The final reaction was started from a stepwise activation of the pyrophosphates with trifluoroacetic acid anhydride (TFAA) to form intermediate **IN-A** and then transferred to **IN-B** with *N*-methylimidazol. **IN-B** was coupled with a NMP to give the non-symmetric Tri*PPP*ro γ-(AB,ab)-dNTPs **56**. After a reverse-phase column chromatography, then an ion-exchange was conducted using Dowex 50WX8 (NH_4^+ form) and followed by a second reverse-phase column chromatography and freeze-drying, non-symmetric Tri*PPP*ro γ-(AB,ab)-dNTPs **56** (NH_4^+-form) were isolated as colorless cotton.[25,26]

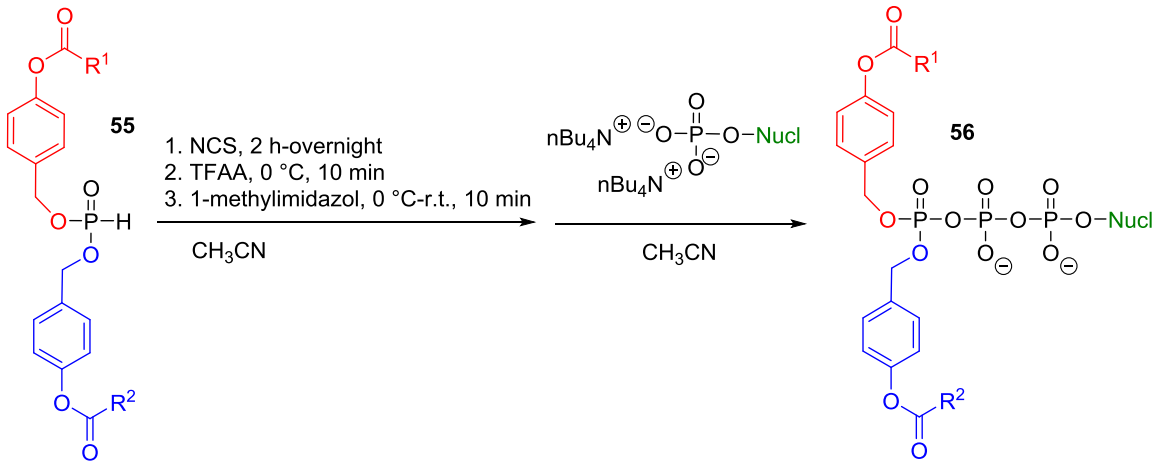


Scheme 4-7: Synthesis route of non-symmetric Tri*PPP*ro-compounds.

The total yields obtained in the conversions of compounds NMP to **56** varied between 28%-64% (Table 4-4). When an excess of the pyrophosphate (1.0 equiv. of

pyrophosphate to 0.75 equiv. of d4TMP **4m**) was used, the conversion of d4TMP **4m** was more efficient and the yields of target compounds **56** were improved as compared to those obtained when a 1:1 ratio was used.

Compound **56e-MEEES**, **56o-MEEES** and **56o-MEEEG** are Tri*PPP*ro-compounds that contained masks with one hydrophilic mask moiety. By introducing this moiety, it will help us to know whether a hydrophilic mask have influence on the character of the prodrugs. These compounds are hygroscopic and must be carefully handled when exposed to air. Tri*PPP*ro γ-(AB-C15,ab-C15)-d4UTP **56uu** is based on d4U and the synthesis method and purification protocol are as same as those with d4T **4**.



Compound	Nucl.	R_1	R_2	Yield
56co	d4T	C_2H_5 **44c**	*n*-$C_{14}H_{29}$ **44o**	64%
56eo	d4T	*n*-C_4H_9 **44e**	*n*-$C_{14}H_{29}$ **44o**	28%
56go	d4T	*n*-C_6H_{13} **44g**	*n*-$C_{14}H_{29}$ **44o**	52%
56e*io*	d4T	*iso*-C_4H_9 **44e*i***	*n*-$C_{14}H_{31}$ **44o**	39%
56eu	d4T	*n*-C_4H_9 **44e**	*n*-$C_{15}H_{31}$ **44u**	47%
56ew	d4T	*n*-C_4H_9 **44e**	*n*-$C_{17}H_{35}$ **44w**	40%
56e-MEEES	d4T	*n*-C_4H_9 **44e**	MEEES **50b**	45%
56o-MEEES	d4T	*n*-$C_{14}H_{29}$ **44o**	MEEES **50b**	39%
56o-MEEEG	d4T	*n*-$C_{14}H_{29}$ **44o**	MEEEG **50c**	35%
56uu	d4U	*n*-$C_{15}H_{31}$ **44u**	*n*-$C_{15}H_{31}$ **44u**	50%

Table 4-4: Synthesis and yield of Tri*PPP*ro γ-(AB,ab)-d4TTPs

Discussion

The ^{1}H-NMR, ^{13}C-NMR and ^{31}P-NMR spectra of Tri*PPP*ro γ-(AB-C4,ab-C15)-d4TTP **56eu** are depicted below as an example. In the ^{1}H-NMR spectra (Figure 4-1), the protons, which belong to the two AB-masks and d4T **4**, are colored in red, blue and green respectively. It is worth to note that the peaks of H-t and H-u are overlapped. Similar, the peaks of H-b and H-q are also overlaping. In ^{13}C-NMR (Figure 4-2), C-1´´, C-2´´, C-3´´, C-4´ and C-5´ are doublet peaks due to $J_{C,P}$ coupling.

The ^{31}P-NMR spectra of **56eu** are shown in Figure 4-3. The peaks of P-α and P-β are broad singlet in this case. Except the only example of **56eu**, ^{31}P-NMR of Tri*PPP*ro **56** are more similar to the spectra of **56ew**, in which the signal for P-α appears as a doublet and P-β appears as a triplet due to $J_{p,p}$ coupling (Figure 4-4).

The ^{1}H-NMR of Tri*PPP*ro-compound γ-(AB-C14,ab-MEEES)-d4TTP **56o-MEEES** is shown in Figure 4-5 as an example. In this spectrum, we can clearly observe that the peaks of H-b, H-q, H-c and H-r are separate because of the extra ester group.

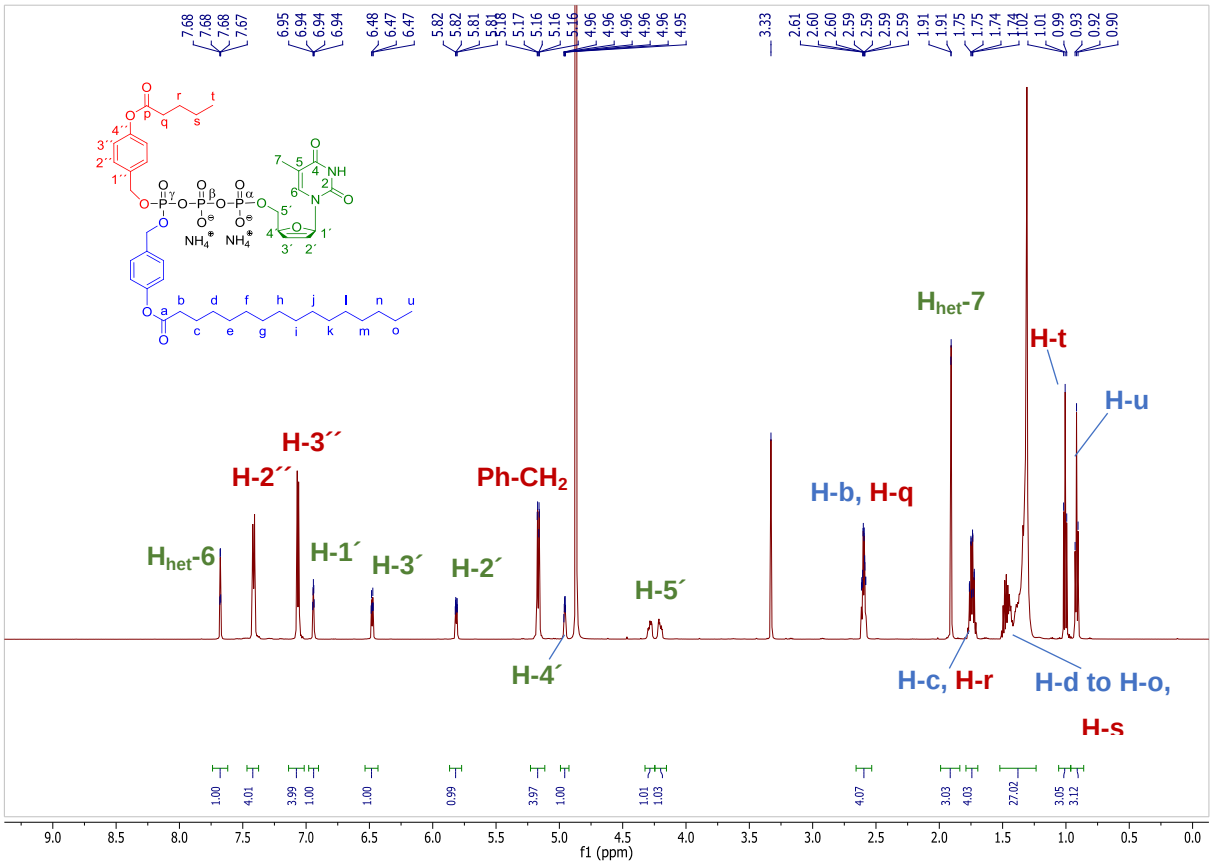


Figure **4-1:** ^{1}H-NMR spectra of Tri*PPP*ro-compounds γ-(AB-C4,ab-C15)-d4TTP **56eu** in CD_3OD.

Discussion

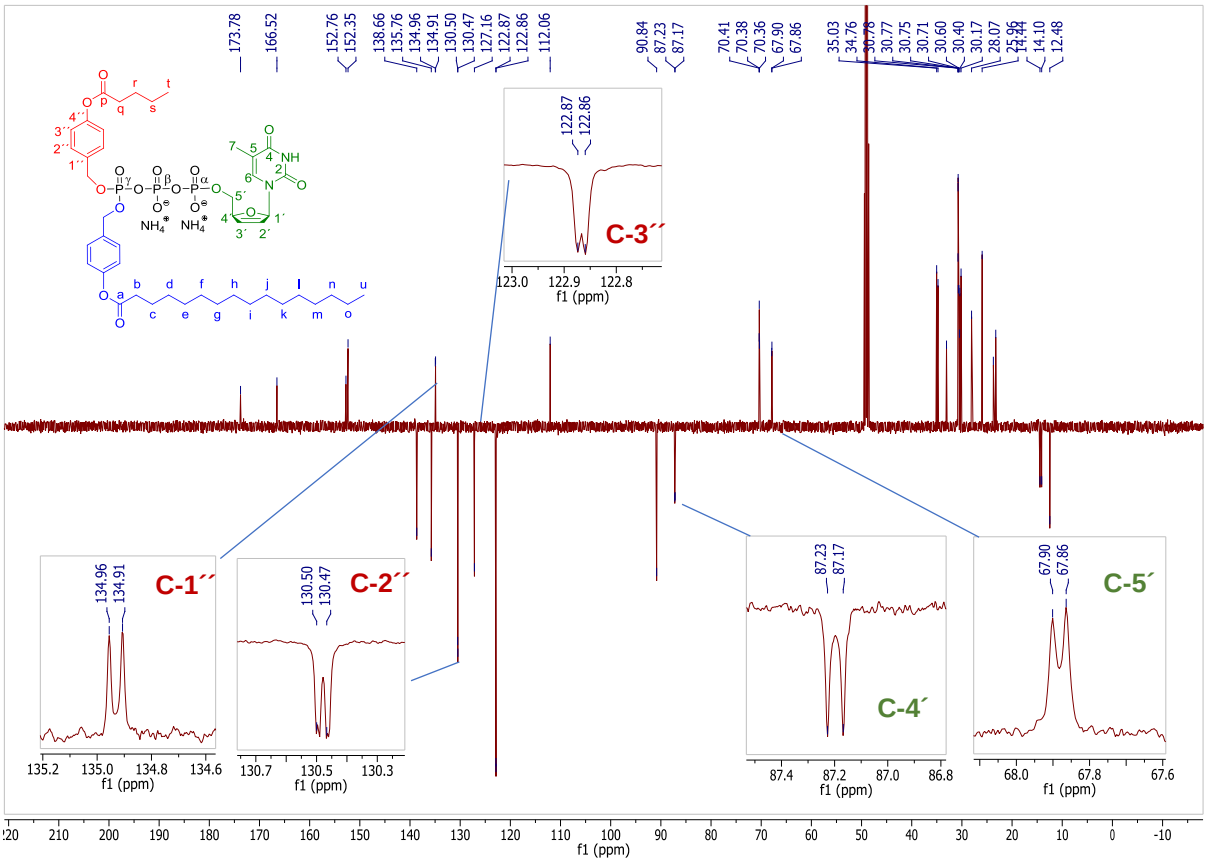


Figure 4-2: ^{13}C-NMR spectra of Tri*PPP*ro-compounds γ-(AB-C4,ab-C15)-d4TTP **56eu** in CD_3OD.

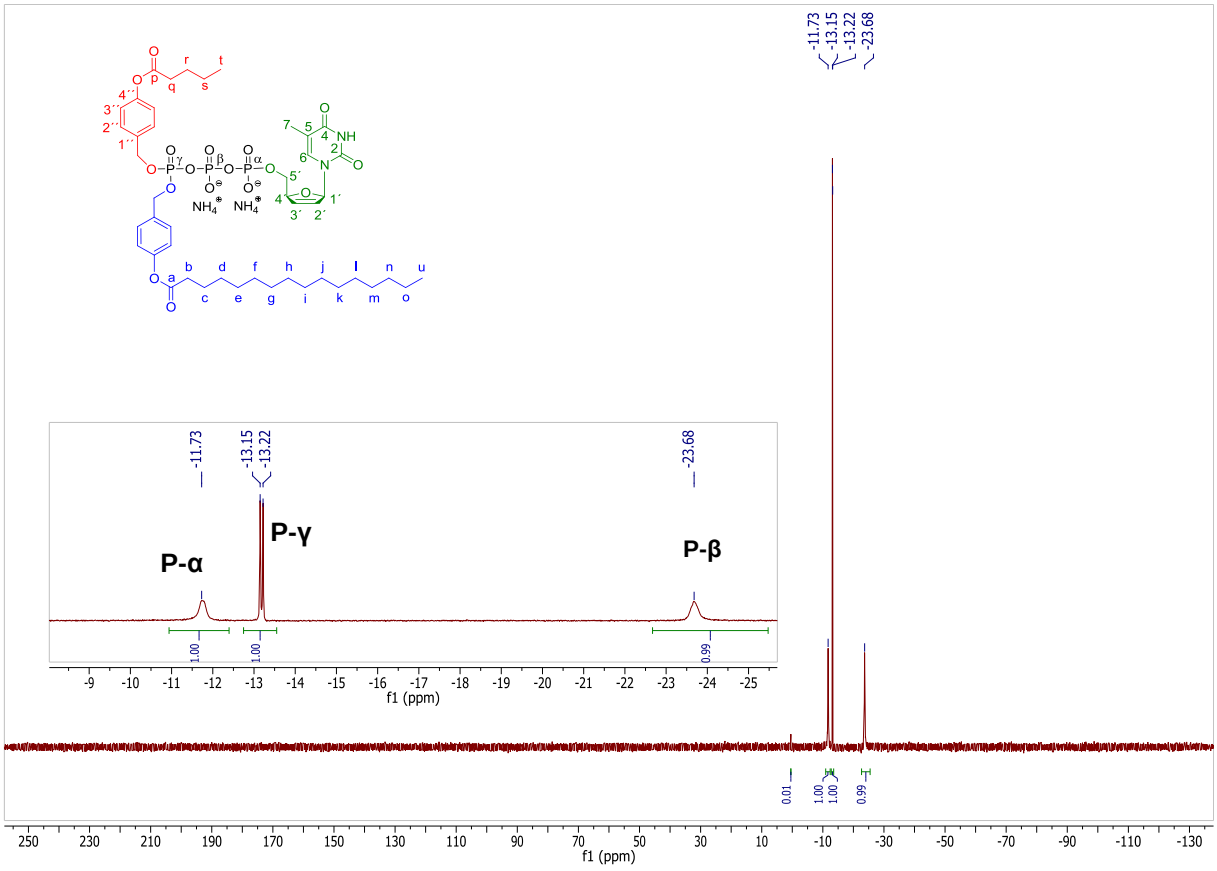


Figure 4-3: ^{31}P-NMR spectra of Tri*PPP*ro γ-(AB-C4,ab-C15)-d4TTP **56eu** in CD_3OD.

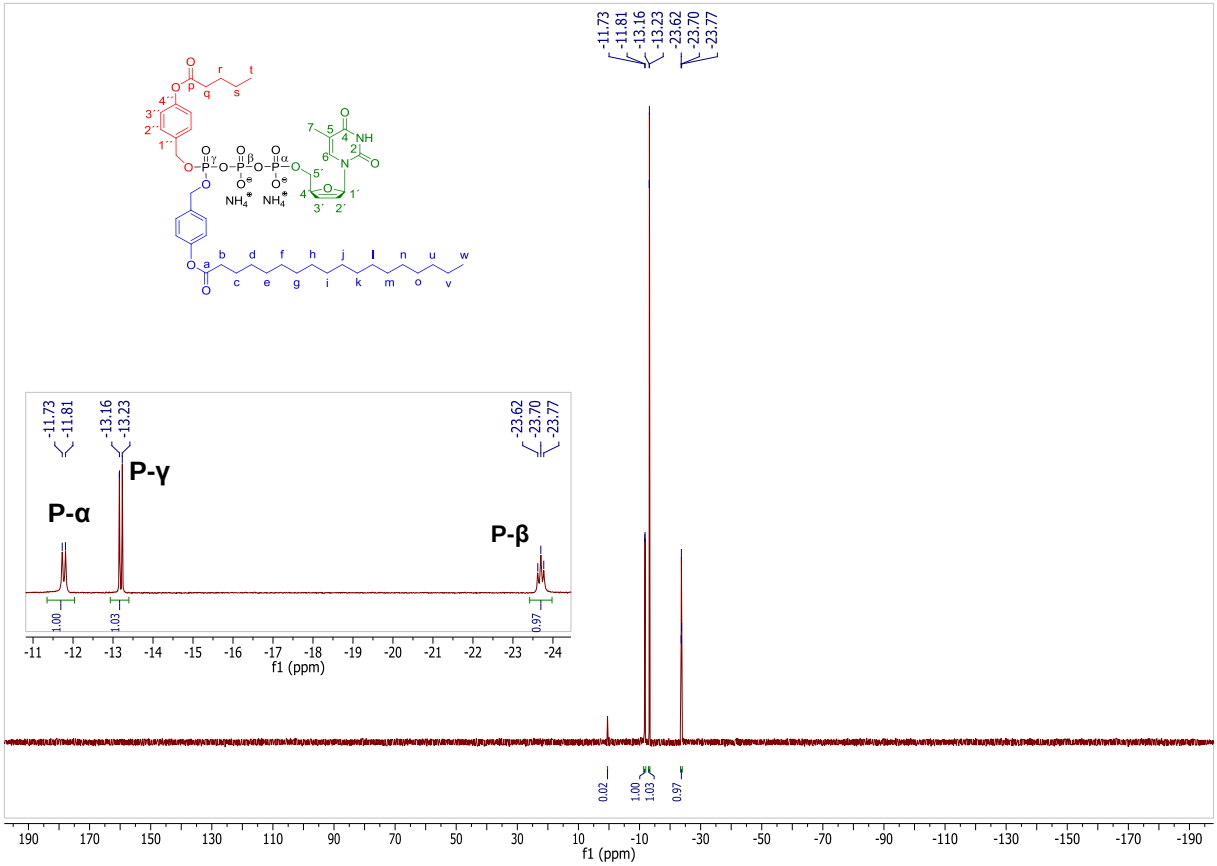


Figure 4-4: ^{31}P-NMR spectra of Tri*PPP*ro γ-(AB-C4,ab-C17)-d4TTP **56ew** in CD_3OD.

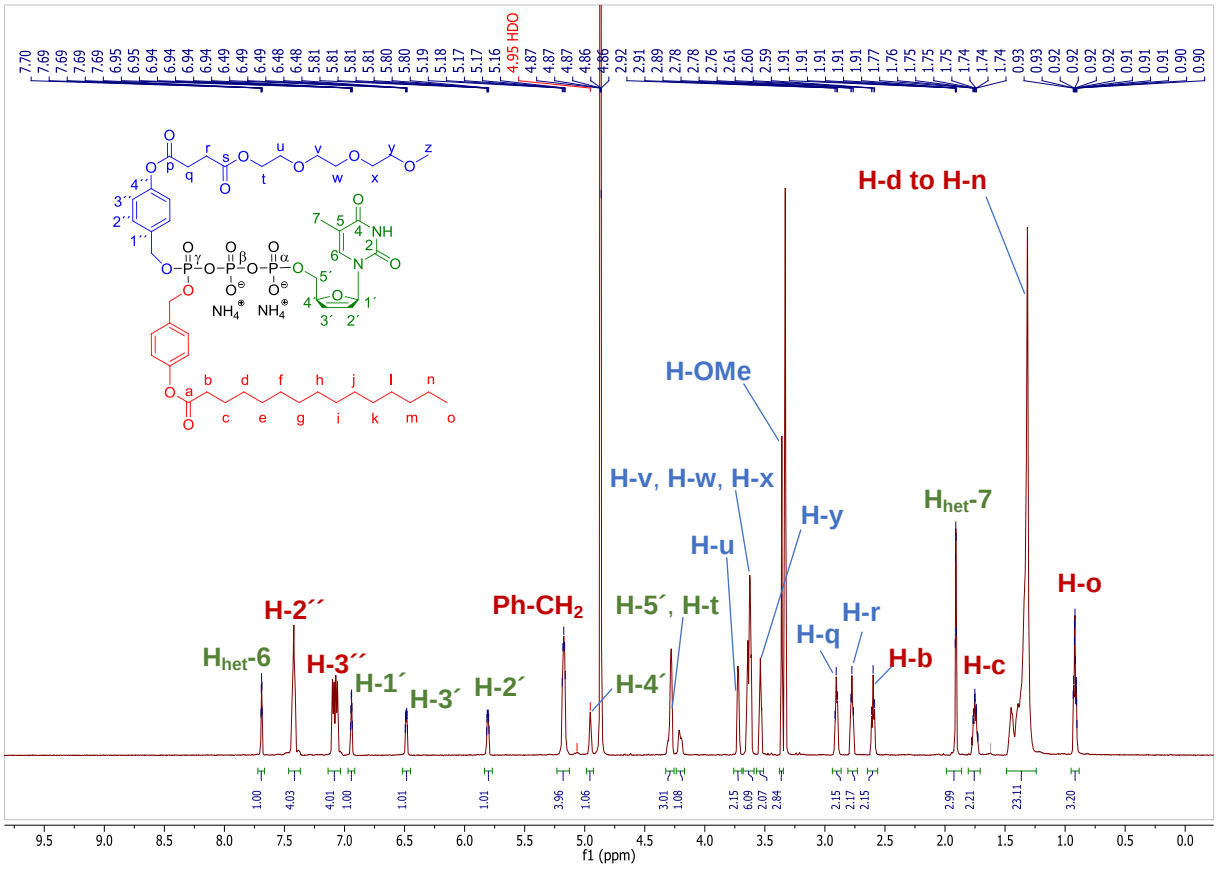
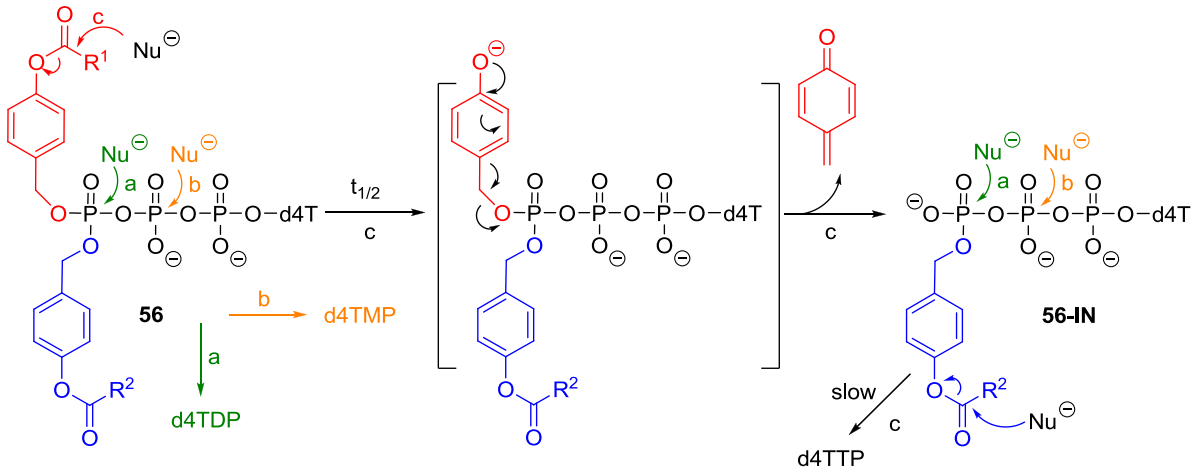


Figure 4-5: ^{1}H-NMR spectra of Tri*PPP*ro γ-(AB-C14,ab-MEEES)-d4TTP **56o-MEEES** in CD_3OD.

4.3.3 Chemical and Biological Hydrolysis

To study the hydrolytic stability of prodrugs, Tri*PPP*ro-compounds **56** were incubated in phosphate buffer (PBS, 25 mM, pH 7.3) and human CD_4^+ T-lymphocyte cell extracts. After certain incubation periods, the hydrolysis mixtures were analyzed by analytical RP18-HPLC. The compound peak area was calculated from the integrated peaks in the chromatogram. Then the half-lives ($t_{1/2}$) of the prodrugs compound and the corresponding intermediates were calculated from the fitted curve. The calculated half-lives of Tri*PPP*ro-compounds **56** (Table 4-5, $t_{1/2}$) reflect the removal of the bioreversible AB-group to yield the monomasked intermediate **56-IN**.

It was observed that the $t_{1/2}$ of the intermediate **56-IN** was much longer than that of prodrug **56** both in PBS and CEM/0, which means **56-IN** is much more stable than **56**. The $t_{1/2}$ of Tri*PPP*ro-compound γ-(AB-C15,ab-C15)-d4UTP **56uu** was not tested as the solubility of this compound was too low in the solvent of water or DMSO.



Scheme 4-8: Hydrolysis mechanism of Tri*PPP*ro.

The half-lives ($t_{1/2}$) of prodrugs **56** in phosphate buffer (PBS, 25 mM, pH 7.3) and human CD_4^+ T-lymphocyte cell extracts are summarized in Table 4-5 and Figure 4-6.

Compound	Nucl.	R_1	R_2	PBS pH=7.3 [h]	CEM cell extracts [h]
				$t_{1/2}(1)$[a]	$t_{1/2}(1)$[a]
56co	d4T	C_2H_5	*n*-$C_{14}H_{29}$	59	0.8
56eo	d4T	*n*-C_4H_9	*n*-$C_{14}H_{29}$	45	2.5
56go	d4T	*n*-C_6H_{13}	*n*-$C_{14}H_{29}$	61	2.3
56e*io*	d4T	*iso*-C_4H_9	*n*-$C_{14}H_{31}$	64	3.8
56eu	d4T	*n*-C_4H_9	*n*-$C_{15}H_{31}$	49	3.1
56ew	d4T	*n*-C_4H_9	*n*-$C_{17}H_{35}$	50	3.3
56e-MEEES	d4T	*n*-C_4H_9	MEEES	22	0.8
56o-MEEES	d4T	*n*-$C_{14}H_{29}$	MEEES	65	1.1
56o-MEEEG	d4T	*n*-$C_{14}H_{29}$	MEEEG	86	2.4
56uu	d4U	*n*-$C_{15}H_{31}$	*n*-$C_{15}H_{31}$	n.d.	n.d.

Table 4-5: The hydrolysis half-lives of Tri*PPP*ro γ-(AB,ab)-d4TTPs.

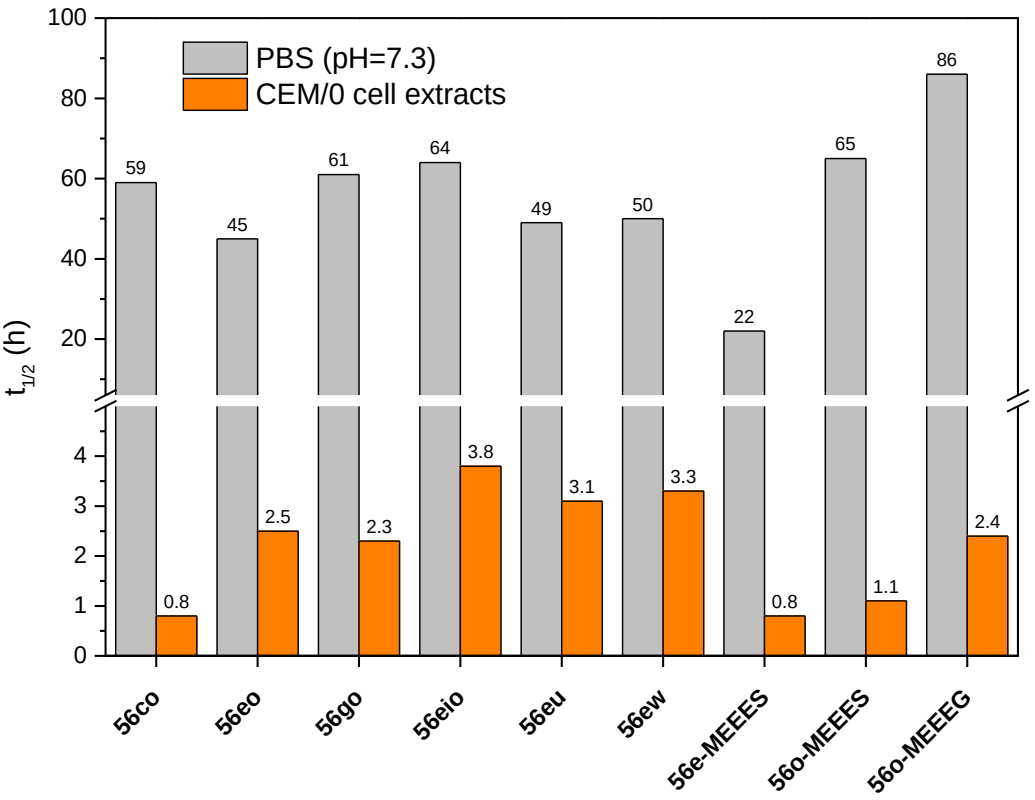


Figure 4-6: The hydrolysis half-lives of Tri*PPP*ro γ-(AB,ab)-d4TTPs in bar chart.

4.3.3.1 Chemical stability in phosphate buffer, pH 7.3

In PBS, the stability of prodrugs of the Tri*PPP*ro-compounds **56** increased with elongation alkyl chain lengths of the masks (Table 4-5) and therefore with the lipophilicity of the compounds. Compared to the half-lives of Tri*PPP*ro-derivatives **56eo** (45 h) and **56e*i*o** (64 h), it suggested that a mask with a branched alkyl chain increased the prodrug stability as well.

Figure 4-7-a showed the hydrolysis behavior of Tri*PPP*ro-compound γ-(AB-C4,ab-C15)-d4TTP **56eu** as an example. The initial cleavage step of the hydrolysis mechanism proceeded similarly to the previously published cleavage pathway for symmetrically-masked Tri*PPP*ro-compounds **41** using d4T.[25,26] D4TDP **4d** and d4TTP **4t** was detected as main hydrolysis product. D4TMP **4m** was also observed but only in very low concentrations. Thus, d4TTP **4t**, which is the active compound against HIV, was formed in high concentrations from Tri*PPP*ro-compounds **56** during PBS hydrolysis.

Intermediate-γ-(ab-C15)-d4TTP **56-IN-u** and intermediate-γ-(AB-C4)-d4TTP **56-IN-e** were formed in the first step of the hydrolysis. The amount of these two intermediates increased at the beginning and decreased after 200 h simultaneously. Furthermore, the concentration of intermediate-γ-(ab-C15)-d4TTP **56-IN-u** is always higher than that of intermediate-γ-(AB-C4)-d4TTP **56-IN-e**. This result demonstrated that, under PBS hydrolysis condition, there is a slightly selective effect between the formation of **56-IN-u** and **56-IN-e**. After long incubation times with compounds bearing d4T, a further side reaction occurred that led to the formation of thymine by the cleavage of the glycosidic bond as reported before.[25,26] This side reaction is a zero-order reaction. After 150 h incubation while both intermediates got the highest concentration, the ratio of **56-IN-u** and **56-IN-e** was 1:1.6.

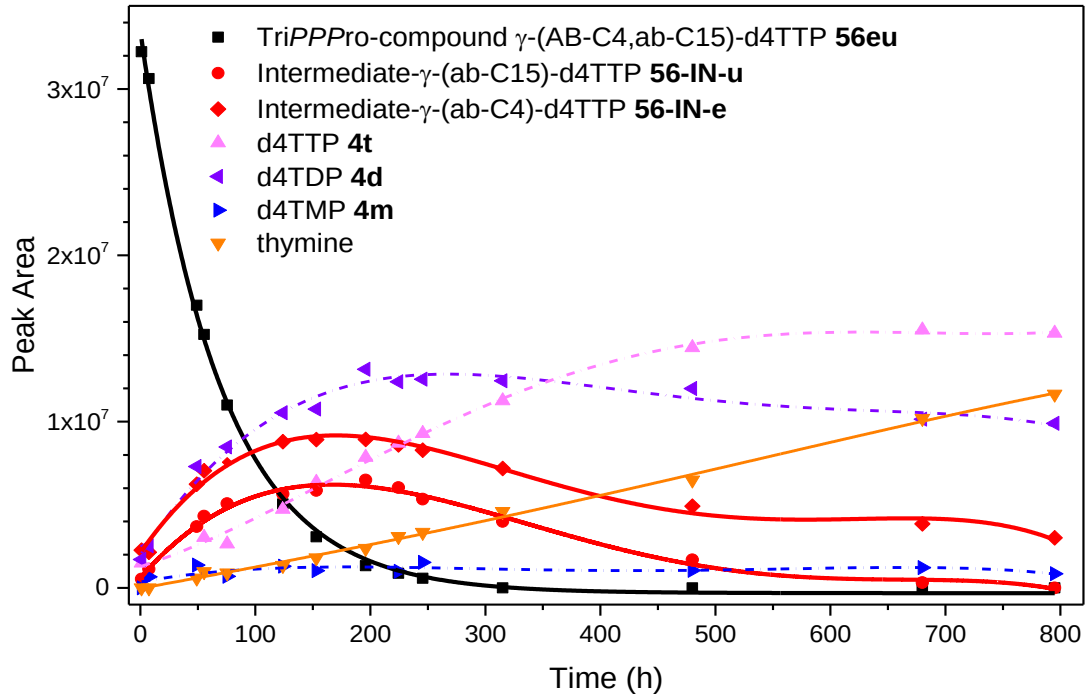


Figure 4-7-a: Chemical hydrolysis of Tri*PPP*ro-compound γ-(AB-C4,ab-C15)-d4TTP **56eu** (black square) in **PBS**. Intermediate-γ-(Ab-C15)-d4TTP **56-IN-u** is in red dots. Intermediate-γ-(AB-C4)-d4TTP **56-IN-e** is in red diamond. D4TTP, d4TDP, d4TMP and thymine are in pink, purple, blue and orange triangle, respectively.

4.3.3.2 Hydrolysis in CEM cell extracts

The stability of Tri*PPP*ro-prodrugs **56** was further investigated in human CD_4^+ T-lymphocyte CEM cell extracts. In cell extracts, an enzymatic cleavage reaction took place. Half-lives ranging from 0.8 h (**56co**, **56e-MEEES**) to 3.8 h (**56e*io***) were determined. Compounds **56co** and **56e-MEEES**, comprising a propanoylester or a combination of propanoylester/**MEEES** moiety respectively, were found to be the less stable compounds. This is in accordance to our previous results of the Di*PP*ro- (**32**, **34**) or the Tri*PPP*ro-compounds **41**. Particularly short alkylester group in the mask proved extremely labile in cell extracts ($t_{1/2}$ of 0.4 and 1.6 h, respectively). Again, the half-lives of the prodrugs **56** correlated well with the mask chain length and were significantly lower than the half-lives in PBS buffer (Table 4-5). In all cases we observed the predominate formation of d4TDP **4d**. Intermediate **56-IN** and d4TTP **4t** was only observed in very low concentration. This result was identical with the previous study from T. Gollnest. It showed that, in CEM cell extracts, the half-life of d4TDP **4d** is 60 h and that of d4TTP **4t** is only 38 min.[151] Thus, the low level of

d4TTP **4t** in the hydrolysis was the consequence of instability of d4TTP **4t** in CEM cell extracts.

As the incubation time is 9-10 h for all prodrugs, no thymine was detected during the hydrolysis. The peak of d4TMP **4m** was overlapped with the peaks from cell extracts. Thus, it is not possible to calculate the peak area of d4TMP **4m** under this HPLC elution condition.

As an example (Figure 4-7-b), **56eu** showed a $t_{1/2}$ of 3.1 h, which is 16-fold lower than its $t_{1/2}$ in PBS (49 h). D4TDP **4d** was detected as largely predominate product. It was almost impossible to detect d4TTP due to its fast dephosphorylation to form first d4TDP **4d** and finally d4TMP **4m** in cell extracts.[151] No thymine was detected after 9 h incubation. Both of the intermediates **56-IN-u** and **56-IN-e** were observed in very low concentration. Unfortunately, no selective cleavage of the different masks was observed in the hydrolysis in CEM cell extracts.

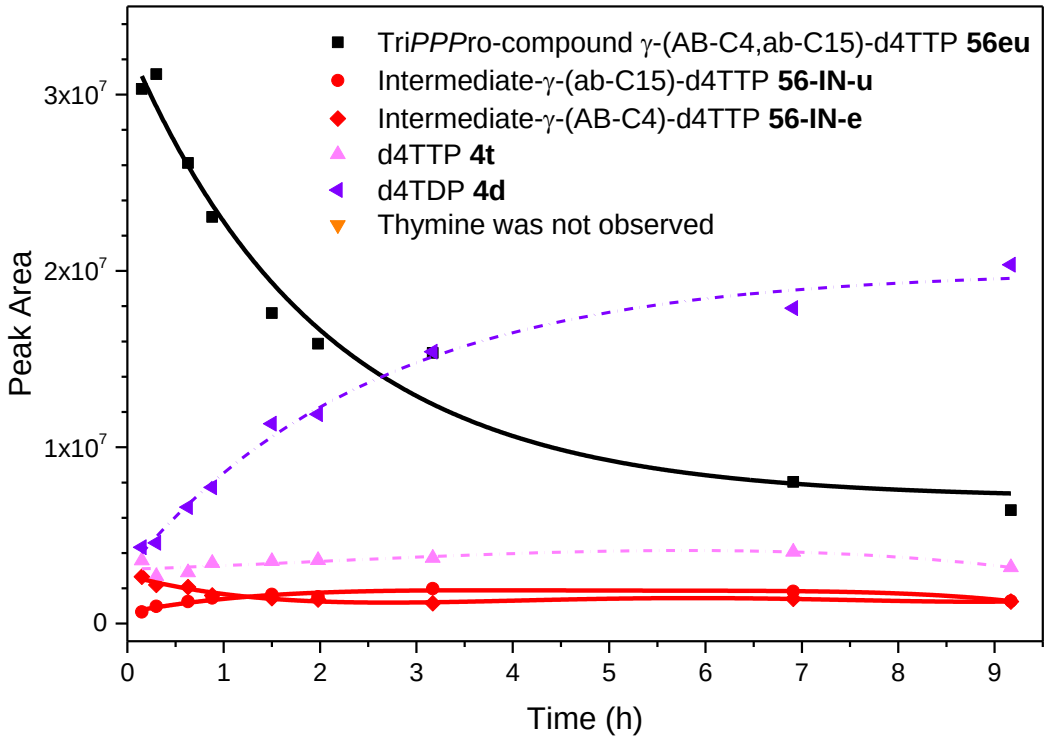


Figure 4-7-b: Hydrolysis of Tri*PPP*ro-compounds γ-(AB-C4,ab-C15)-d4TTP **56eu** (black square) in **CEM cell extracts**. Intermediate-γ-(Ab-C15)-d4TTP **56-IN-u** is in red dots. Intermediate-γ-(AB-C4)-d4TTP **56-IN-e** is in red diamond. D4TTP, d4TDP and thymine are in pink, purple and orange triangle, respectively.

4.3.3.3 Hydrolysis in Pig Liver Esterase (PLE)

Tri*PPP*ro-compound γ-(AB-C4,ab-C15)-d4TTP **56eu** was further treated with pig liver esterase (PLE) to demonstrate the release of d4TTP **4t** by esterase (Figure 4-8).

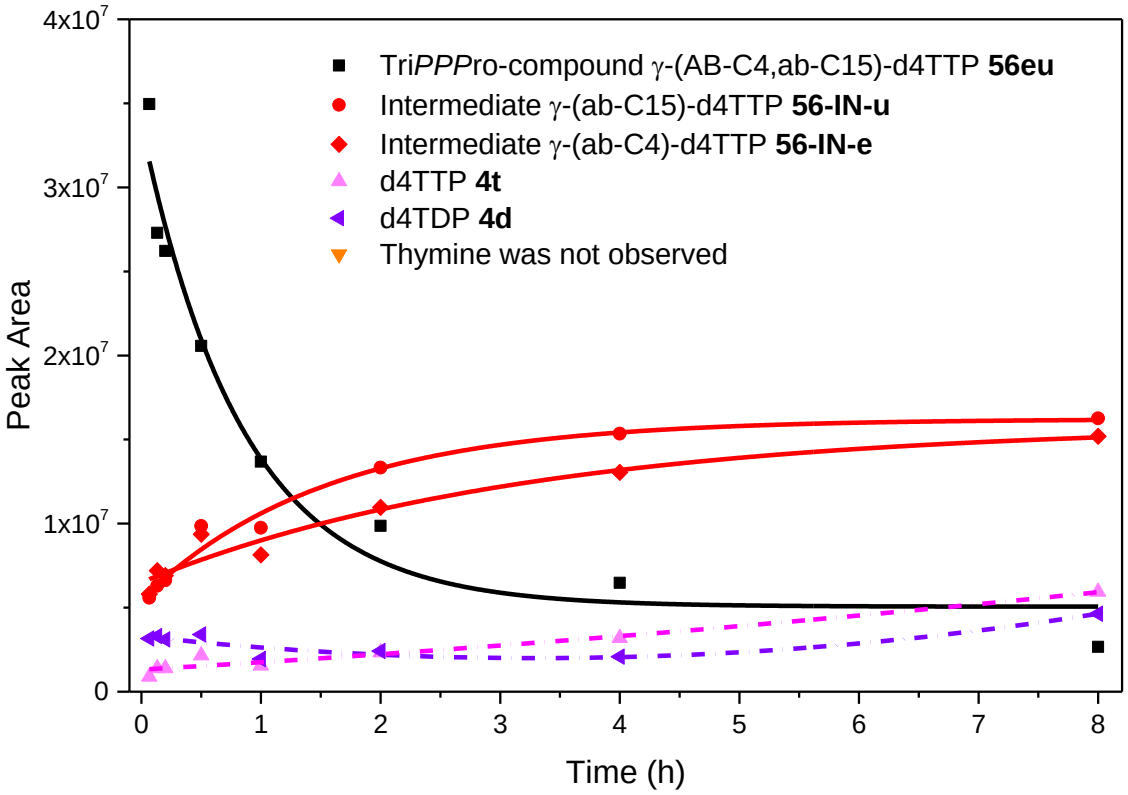


Figure 4-8: Hydrolysis of Tri*PPP*ro-compounds γ-(AB-C4,ab-C15)-d4TTP **56eu** (black square) in **Pig liver Esterase (PLE)**. Intermediate-γ-(Ab-C15)-d4TTP **56-IN-u** is in red dots. Intermediate-γ-(AB-C4)-d4TTP **56-IN-e** is in red diamond. D4TTP and d4TDP are in pink and purple triangle, respectively.

Result showed the half-life of prodrug **56eu** in PLE is 0.75 h. The cleavage of the masking unit in **56eu** occurred much faster than that in PBS ($t_{1/2}$ = 49 h) and in CEM cell extracts ($t_{1/2}$ = 3.1 h). This result suggested that the release of nucleotides undergo an enzymatic process. The two intermediates **56-IN-u** and **56-IN-e** were formed in identical concentrations. D4TTP **4t** and d4TDP **4d** were both observed in low level after 8 h hydrolysis. Thymine was not detected in the PLE hydrolysis with a period of 8 hours.

4.3.3.4 The hydrolysis characters of Tri*PPP*ro-compounds γ-(AB,ab)-d4TTPs with PEG moiety (MEEES and MEEEG)

As Tri*PPP*ro-compounds γ-(AB,ab)-d4TTPs with two acyl chain residues showed no selectivity in the cleavage of the two different masks, developing a new Tri*PPP*ro-mask combination became the primary task. Thus, a new class of AB-masks with

hydrophilic PEG residues was developed. From the hydrolysis data, it can be concluded that the $t_{1/2}$ of Tri*PPP*ro-compounds with a hydrophilic PEG moiety still correlated well with the mask chain length. For **56o-MEEES** and **56o-MEEEG**, when the linker structure changed from succinic diester (**56o-MEEES**) to glutaric diester (**56o-MEEEG**), the $t_{1/2}$ of the prodrug increased to 1.3-fold (from 65 h to 85 h) in PBS and 2.2-fold (from 1.1 h to 2.4 h) in CEM cell extracts.

The hydrolysis character of Tri*PPP*ro-compound γ-(AB-C14,ab-MEEES)-d4TTP **56o-MEEES** is shown in Figure 4-9 and Figure 4-10. In PBS solution, the product distribution of the hydrolysis product was similar to the result of Tri*PPP*ro-compound γ-(AB-C4,ab-C15)-d4TTP **56eu**. In CEM cell extracts, a very low concentration level of d4TTP **4t** and intermediate-γ-(ab-C14)-d4TTP **56-IN-o** was detected. Interestingly, the predominant hydrolysis product was intermediate-γ-(ab-MEEES)-d4TTP **56-IN-MEEES**. This result means that in cell extracts, the presence of a MEEES mask **50b** increased cleavage efficiency of the masking group bearing the acyl residue (**44o**). Thus, we successfully developed a new hydrophilic mask approach which involved selective mask cleavage activity which is promoted by enzymes.[25]

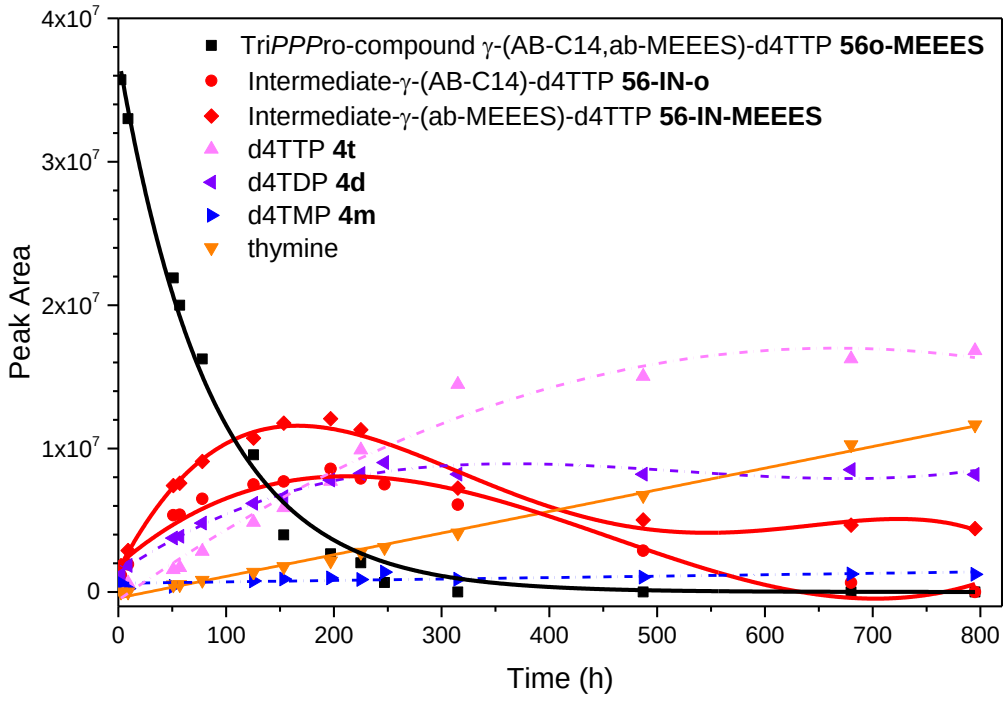


Figure 4-9: Hydrolysis of Tri*PPP*ro-compound γ-(AB-C14,ab-MEEES)-d4TTP **56o-MEEES** (black square) **in PBS**. Intermediate-γ-(AB-C14)-d4TTP **56-IN-o** is in red dots. Intermediate-γ-(Ab-MEEES)-d4TTP **56-IN-MEEES** is in red diamond. D4TTP, d4TDP, d4TMP and thymine are in pink, purple, blue and orange triangle, respectively.

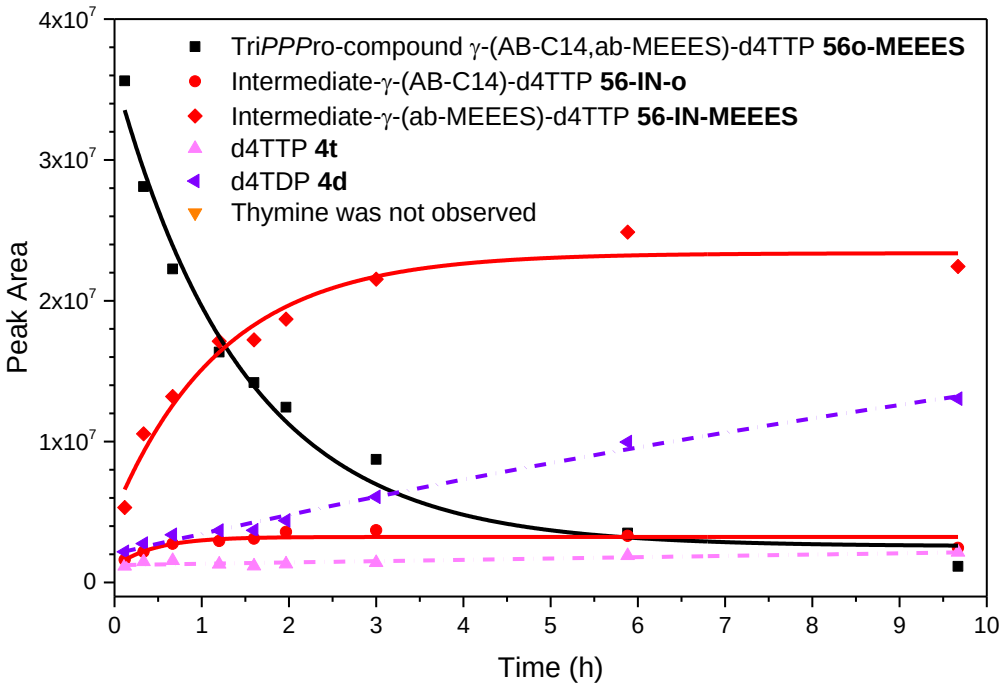


Figure 4-10: Biological hydrolysis of Tri*PPP*ro-compound γ-(AB-C14,ab-MEEES)-d4TTP **56o-MEEES** (black square) **in CEM cell extracts**. Intermediate-γ-(Ab-C14)-d4TTP **56-IN-o** is in red dots. Intermediate-γ-(AB-MEEES)-d4TTP **56-IN-MEEES** is in red diamond. D4TTP, d4TDP and thymine are in pink, purple and orange triangle, respectively.

4.3.4 Anti-HIV activities in CEM/0 and CEM/TK$^-$ cells

Tri*PPP*ro-compounds **56** and were evaluated for their ability to inhibit the replication of HIV. For this purpose, HIV-1- or HIV-2-infected wild-type CEM/0 as well as mutant thymidine kinase-deficient CEM cell cultures (CEM/TK$^-$) were treated with the compounds **56**.

As can be seen in Table 4-6, in CEM/0 cell line, all Tri*PPP*ro-compounds with d4T as parent nucleoside showed similar or slightly lower activities against HIV-2 and better activities against HIV-1 as the parent nucleoside d4T **4** and d4TTP **4t**. More importantly, almost all d4T prodrugs **56** (except **56uu**) were highly potent in CEM/TK$^-$ cells whereas d4T **4** and d4TTP **4t** lacked any relevant anti-HIV activity in this thymidine kinase-deficient cell model (EC_{50}: >50 µM for d4T **4** and >100 µM for d4TTP **4t**). Only **56e-MEEES** (C4/PEG) lost most of the activity in CEM/TK$^-$ cells as compared to other Tri*PPP*ro-prodrugs. After increasing the lipophilicity of the mask (from **44e** to **44o**), Tri*PPP*ro-compound **56o-MEEES** is 10-fold more active than **56e-MEEES** against HIV-2 in CEM/TK$^-$ cells. This phenomenon may be explained as when masks are not lipophilic and stable enough (like C4/PEG combination), the

biodegradation of Tri*PPP*ro-compounds **56** is fast, and prodrugs decompose to nucleoside tri-, di- and monophosphates extracellularly. Finally, the nucleotides probably lost membrane penetration ability and resulted in lower antiviral activity. To prevent extremely fast hydrolysis of non-symmetrically-masked Tri*PPP*ro-compounds **56**, at least one mask with long alkyl chain moiety is necessary to ensure that the prodrug is active against HIV.

Compound	EC_{50} [µM][a]			CC_{50} [µM][b]
	CEM/0		CEM/TK$^-$	CEM/0
	HIV-1	HIV-2	HIV-2	
56co (C2/C14)	0.24 ± 0.17	0.39 ± 0.22	1.1 ± 0.82	20 ± 0
56eo (C4/C14)	0.14 ± 0.11	0.32 ± 0.26	0.69 ± 0.52	18 ± 0
56go (C6/C14)	0.15 ± 0.087	0.29 ± 0.24	0.74 ± 0.46	22 ± 3
56e*i*o (iso-C4/C14)	0.17 ± 0.085	0.23 ± 0.18	0.39 ± 0.21	24 ± 3
56eu (C4/C15)	0.15 ± 0.12	0.27 ± 0.25	1.1 ± 0.81	24 ± 15
56ew (C4/C17)	0.12 ± 0.05	0.10 ± 0.03	0.54 ± 0.41	33 ± 7
56e-MEEES (C4/PEG)	0.34 ± 0.23	0.76 ± 0.58	16 ± 4.0	23 ± 9
56o-MEEES (C14/PEG)	0.26 ± 0.14	0.43 ± 0.30	1.3 ± 0.64	28 ± 3
56o-MEEEG (C14/PEG)	0.31 ± 0.16	0.65 ± 0.59	2.0 ± 1.1	34 ± 14
56uu (C15/C15)	> 10	> 10	> 10	32 ± 4
d4T **4**	0.33 ± 0.13	0.97 ± 0.50	>50	>50
d4TTP **4t**	1.05 ± 0.35	0.40 ± 0.26	>100	>100

Table 4-6: Antiviral activity and cytotoxicity of Tri*PPP*ro-compounds **56** in comparison to the parent nucleoside d4T **4** and d4TTP **4t** in CEM/0 and CEM/TK$^-$ cells. [a] Antiviral activity in CD4$^+$ T-lymphocytes: 50% effective concentration; values are the mean ±SD of n=2-3 independent experiments. [b] Cytotoxicity: 50% cytostatic concentration or compound concentration required to inhibit CD4$^+$ T-cell (CEM) proliferation by 50%; values are the mean ±SD of n=2-3 independent experiments.

Without thymidine kinase (TK), d4T **4** was not converted to d4TMP **4m**. Thus, under this condition, without the formation d4TMP **4m**, d4T **4** could not be phosphorylated to d4TDP **4d** and d4TTP **4t**. This led finally to poor activities in the CEM/TK$^-$ cells. As a result, compound **56e*i*o** is the most potent compound of all the listed derivatives (128-fold more active than d4T). According to the hydrolysis data in Table 4-5, compound **56e*i*o** is 1.5-fold more stable than compound **56eo** in both PBS and CEM cell extracts. This increase in stability is probably the reason of higher antiviral activity.

Compounds **56eo** and **56ew** also showed high activities in CEM/TK$^-$ cells, which means the mask combinations C4/C14, C4/C17 provided enough stability and lipophilic properties for the prodrugs. All Tri*PPP*ro-compounds **56** showed a higher cytotoxicity than the parent d4T **4** and d4TTP **4t**. In the end, Tri*PPP*ro-Compound γ-(AB-C15,ab-C15)-d4UTP **56uu** was surprisingly not active in either CEM/0 cells or CEM-TK$^-$ cells (EC_{50}: >10 µM). The result is maybe the consequence of low solubility of Tri*PPP*ro-Compound **56uu** in methanol, water and DMSO.

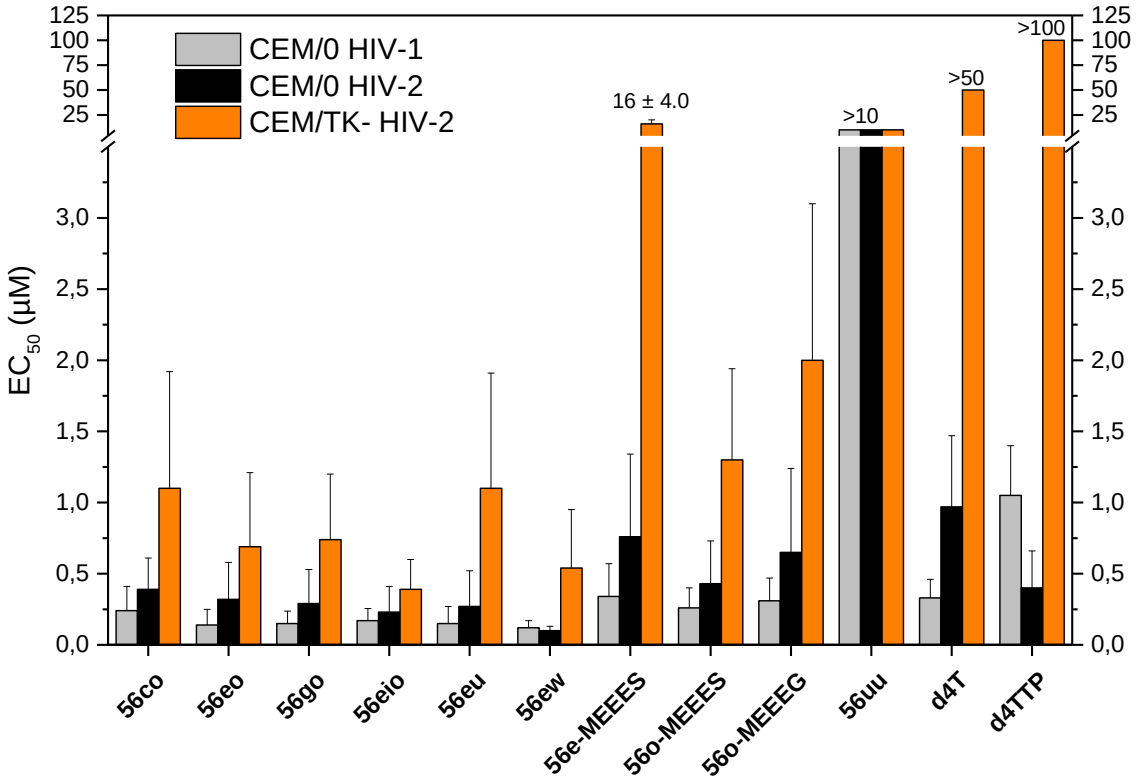


Figure 4-11: Antiviral activity of Tri*PPP*ro-compounds **56** in comparison to the parent nucleoside d4T **4** and d4TTP **4t** in CEM/0 and CEM/TK$^-$ cells.

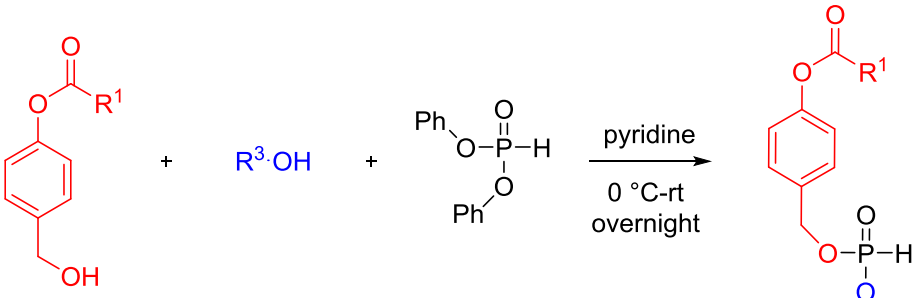
4.4 Non-symmetrically-modified γ-(AB,alkyl)-d4TTPs 58

It is already known that selective cleavage behavior between different masks of non-symmetrically-masked Tri*PPP*ro-compounds **56** was not observed in CEM cell extracts. Even though prodrug **56o-MEEES** showed selective cleavage, its activity against HIV-2 in CEM/TK$^-$ cells is lower than **56eo**. A new class of prodrugs is needed. The new prodrug must have at least one lipophilic group (long alkyl chain) for efficient membrane penetration ability. To produce selective cleavage behavior of prodrugs, one of the AB-masks need to be replaced by a totally different group. Thus, γ-non-symmetrically-modified (AB,alkyl)-d4TTPs **58** were designed and will be

described as γ-(AB,alkyl)-d4TTPs **58** for short. Prodrugs **58** have only one AB-mask and one alkoxy group is linked to γ-phosphate as well and we expected that γ-(alkyl)-d4TTP **60** has a higher stability than γ-(AB)-d4TTP **56-IN**. By analyzing the antiviral activity of prodrugs γ-(AB,alkyl)-d4TTP **58** that contain only one cleavable mask and compound γ-(alkyl)-d4TTP **60** without masking group, the assumption of application new prodrugs can be verified if they show antiviral activities. For the synthesis of γ-(AB,alkyl)-d4TTPs **58**, the *H*-phosphonate route which has already described was used preferably.

4.4.1 Synthesis of Non-Symmetrically-Modified (AB,alkyl)-*H*-phosphonate 57

In the first step, diphenylhydrogenphosphonate (DPP) was successively reacted with aliphatic alcohols (1-butanol, 1-pentadecanol, 1-octadecanol) and compound 4-acyloxybenzylalkanoates **44** to form the non-symmetric *H*-phosphonates **57**. The yields are moderate and are listed in Table 4-7.



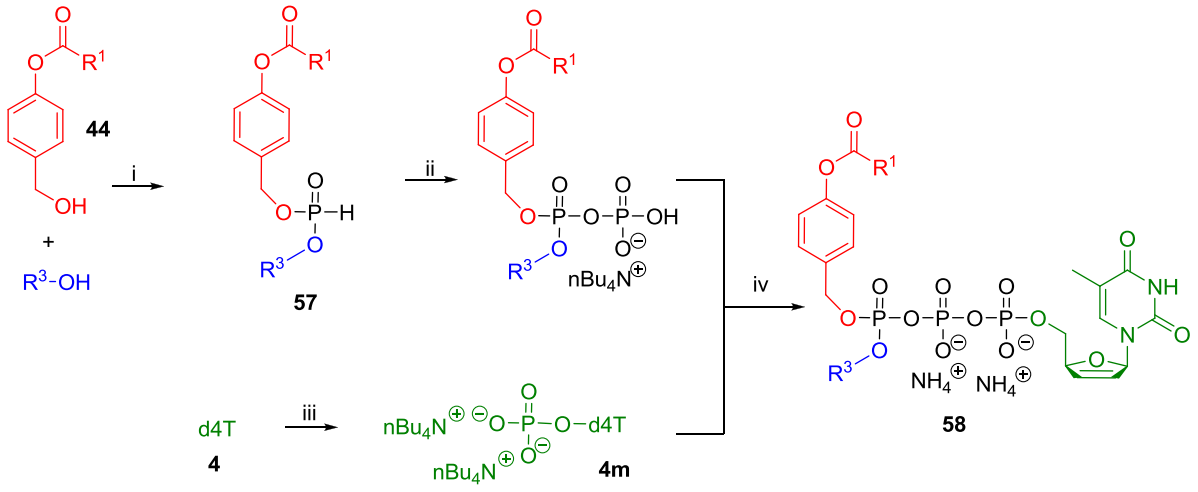
Compound	R^1	R^3	Yield
57ed	*n*-C_4H_9 **44e**	*n*-C_4H_9	31%
57ud	*n*-$C_{15}H_{31}$ **44u**	*n*-C_4H_9	39%
57bo	CH_3 **44b**	*n*-$C_{15}H_{31}$	65%
57eo	*n*-C_4H_9 **44e**	*n*-$C_{15}H_{31}$	43%
57go	*n*-C_6H_{18} **44g**	*n*-$C_{15}H_{31}$	31%
57uo	*n*-$C_{15}H_{31}$ **44u**	*n*-$C_{15}H_{31}$	46%
57br	CH_3 **44b**	*n*-$C_{18}H_{37}$	48%
57cr	C_2H_9 **44c**	*n*-$C_{18}H_{37}$	52%
57er	*n*-C_4H_9 **44e**	*n*-$C_{18}H_{37}$	57%
57gr	*n*-C_6H_{18} **44g**	*n*-$C_{18}H_{37}$	63%

Table 4-7: Synthesis and yields of non-symmetrically-modified (AB,alkyl)-*H*-phosphonates **57**.

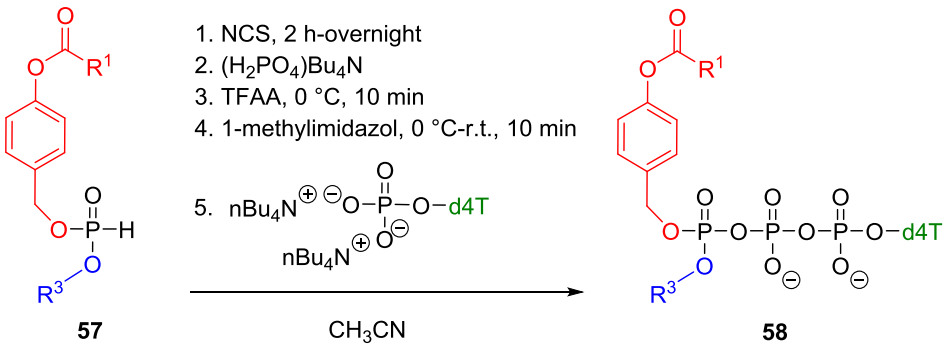
When using 1-butanol as substrate, the yields were 31% (**57ed**) and 39% (**57ud**), which were lower than those with 1-pentadecanol and 1-octadecanol. The yield of **57go** is 31% and it is much lower than the yields of **57bo** and **57eo**. It is because the separation via SiO_2 column chromatography only could afford a crude product with 70% purity which still included the symmetrically di-esterified byproducts (**57gg** with two **44g** mask moiety and **57oo** with two **44o** mask moiety) and another recrystallization in DCM/hexane at -26 °C was necessary for further purification.

4.4.2 Synthesis of γ-(AB,alkyl)-d4TTP 58

Next, non-symmetrically-modified (AB,alkyl)-*H*-phosphonates **57** were reacted with *N*-chlorosuccinimide (NCS) to form the corresponding phosphorochloridates which were then reacted with tetra-*n*-butylammonium phosphate to yield the corresponding pyrophosphates in almost quantitative yields. Due to its chemical instability, the pyrophosphates were quickly purified by extraction and directly used in the next step. The final reaction was started from a stepwise activation of pyrophosphates with trifluoroacetic acid anhydride (TFAA) and *N*-methylimidazole and coupling with d4TMP **4m** to give γ-modified-NTP. After a reverse-phase column chromatography and a Dowex 50WX8 (NH_4^+ form) ion exchange, followed by a second reverse-phase column chromatography and freeze-drying, the (AB,alkyl)-NTP prodrugs (NH_4^+-form) **58** were isolated. γ-(AB-C4,alkyl-C18)-d4UTP **64er** was also synthesized and will be discussed later separately.



Scheme 4-9. Reagents and conditions: i) diphenylphosphoric acid (DPP), pyridine, 0 °C-38 °C, 3.3 h; ii) a. NCS, CH_3CN, rt, 2 h, b) $(H_2PO_4)Bu_4N$, CH_3CN, rt, 1 h; iii) d4T **4**, $POCl_3$, pyridine, H_2O, CH_3CN, 0 °C-rt, 5h; iv) a. TFAA, Et_3N, CH_3CN, 0 °C, 10 min, b. 1-methylimidazol, Et_3N, CH_3CN, 0 °C-RT, 10 min, c. d4TMP **4m**, rt, 3-5 h, rp-chromatography, Dowex 50WX8 (NH_4^+ form) ion exchange, rp-chromatography.



Compound	R^1	R^3	Yield
58ed	*n*-C_4H_9 **44e**	*n*-C_4H_9	59%
58ud	*n*-$C_{15}H_{31}$ **44u**	*n*-C_4H_9	33%
58eo	*n*-C_4H_9 **44e**	*n*-$C_{15}H_{31}$	33%
58go	*n*-C_6H_{18} **44g**	*n*-$C_{15}H_{31}$	59%
58uo	*n*-$C_{15}H_{31}$ **44u**	*n*-$C_{15}H_{31}$	44%
58br	CH_3 **44b**	*n*-$C_{18}H_{37}$	47%
58cr	C_2H_9 **44c**	*n*-$C_{18}H_{37}$	63%
58er	*n*-C_4H_9 **44e**	*n*-$C_{18}H_{37}$	30%
58gr	*n*-C_6H_{18} **44g**	*n*-$C_{18}H_{37}$	36%

Table 4-8: Synthesis and yields of γ-(AB,alkyl)-d4TTPs **58**.

The overall yields obtained in the conversions of compounds **57** to **58** varied between 33%-63%. When an excess of the pyrophosphate (1.33 equiv. of pyrophosphate to 1 equiv. of **4m**) was used, the conversion of d4TMP **4m** was more efficient and the yields of target compounds **58** were improved as compared to those obtained when just a 1:1 ratio was used.

The ^{1}H-NMR, ^{13}C-NMR and ^{31}P-NMR spectra of γ-(AB-C4,alkyl-C18)-d4TTP **58er** are showed in Figures 4-12 below. All peaks were successfully assigned by analyzing 1D- and 2D-NMR (^{1}H-^{1}H COSY, ^{1}H-^{13}C HSQC and ^{1}H-^{13}C HMBC). In the ^{1}H-NMR spectra (Figure 4-12), the protons, which belong to the AB-mask, alkoxy group and d4T **4**, are color-labeled in red, blue and green, respectively. The relationship of ^{1}H-NMR peaks is marked with different colors in ^{1}H-^{1}H COSY spectra (Figure 4-16). In ^{13}C-NMR (Figure 4-13), C-1´´ is the doublet of doublets, and C-2´´, C-4´, C-5´, Ph-CH_2, C-a and C-b are doublet peaks due to $J_{C,P}$ coupling.

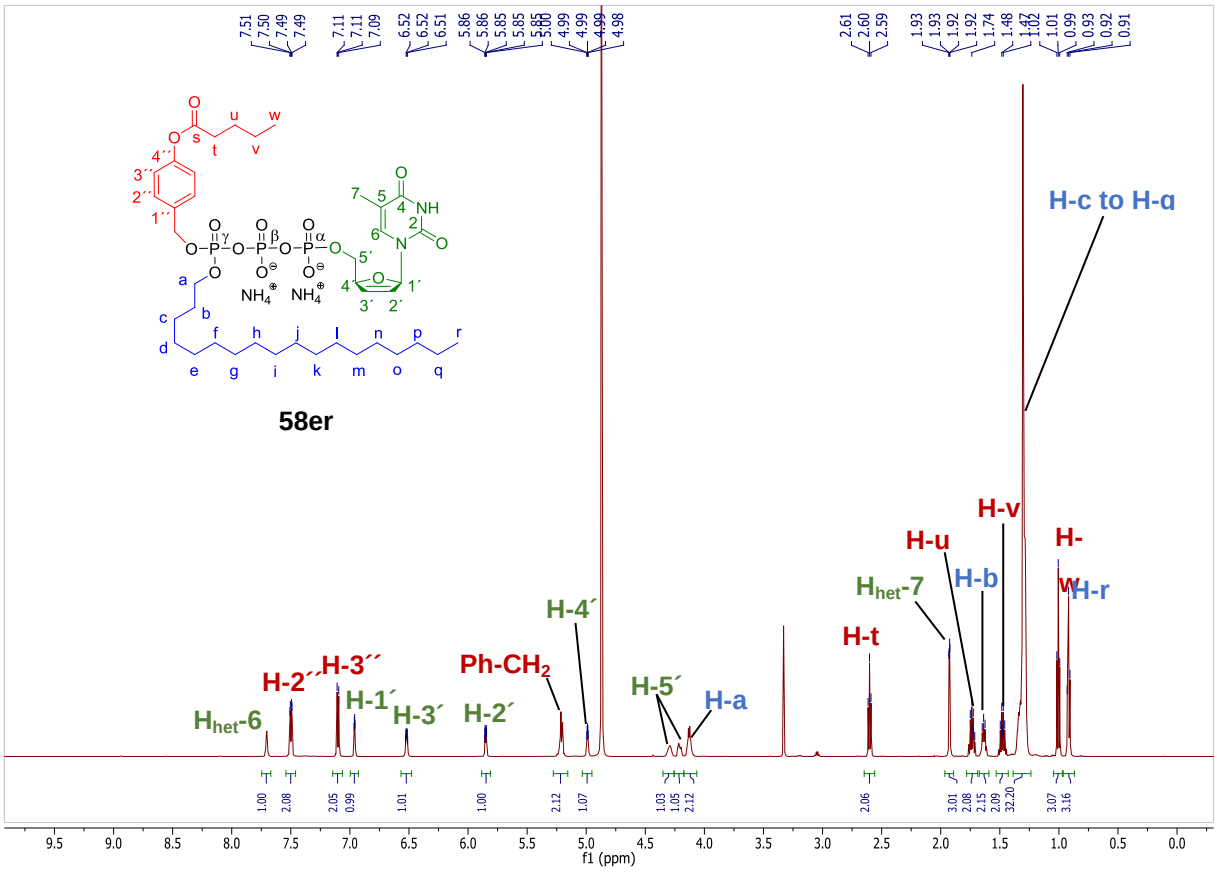


Figure 4-12: ^{1}H-NMR spectra of γ-(AB-C4,alkyl-C18)-d4TTP **56er** in CD_3OD.

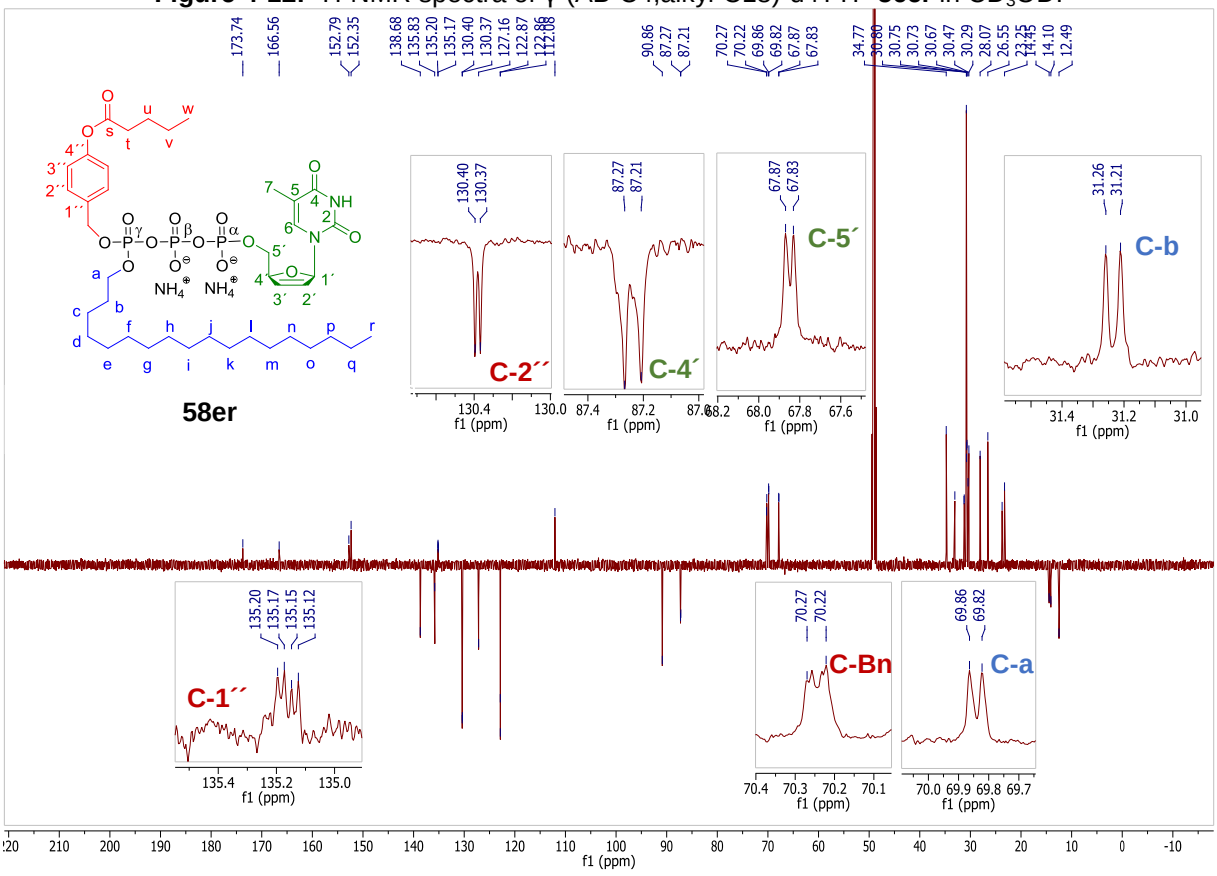


Figure 4-13: ^{13}C-NMR spectra of γ-(AB-C4,alkyl-C18)-d4TTP **58er** in CD_3OD.

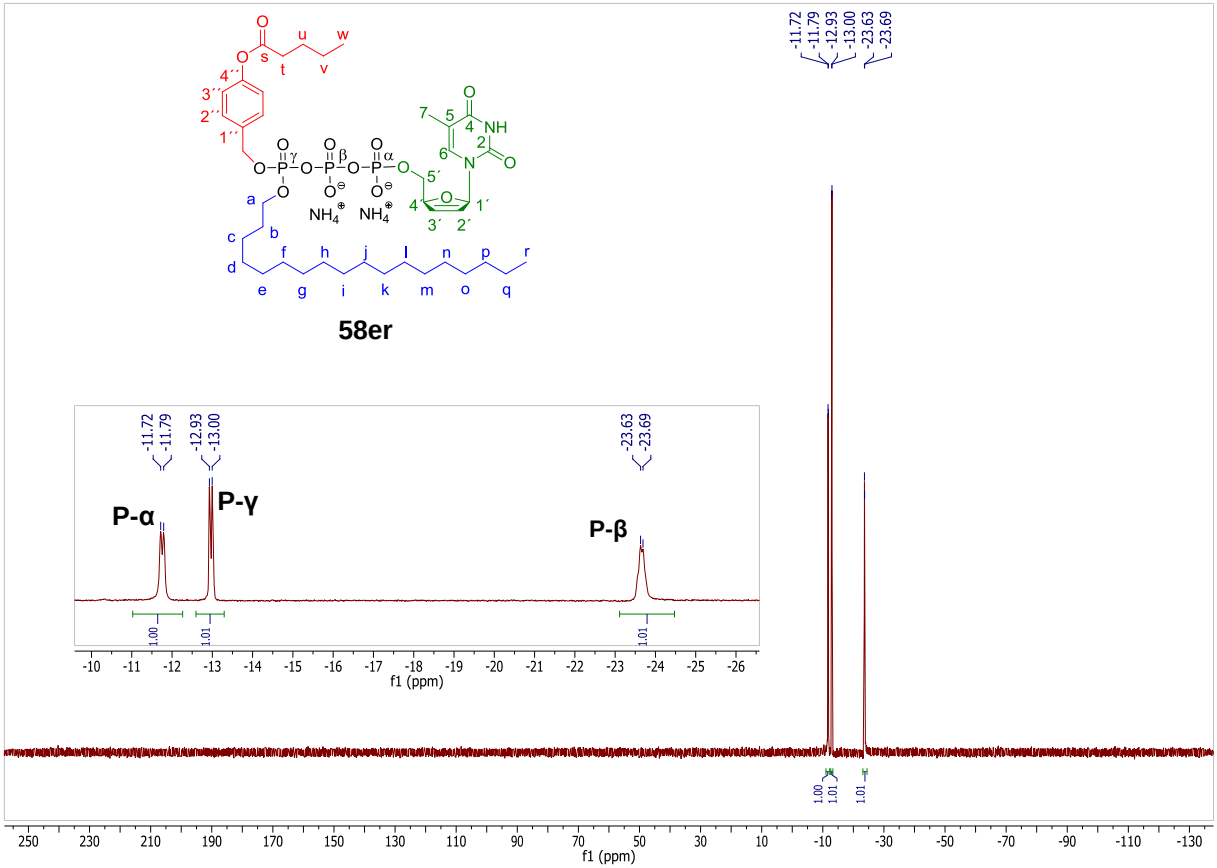


Figure 4-14: ^{31}P-NMR spectra of γ-(AB-C4,alkyl-C18)-d4TTP **58er** in CD_3OD.

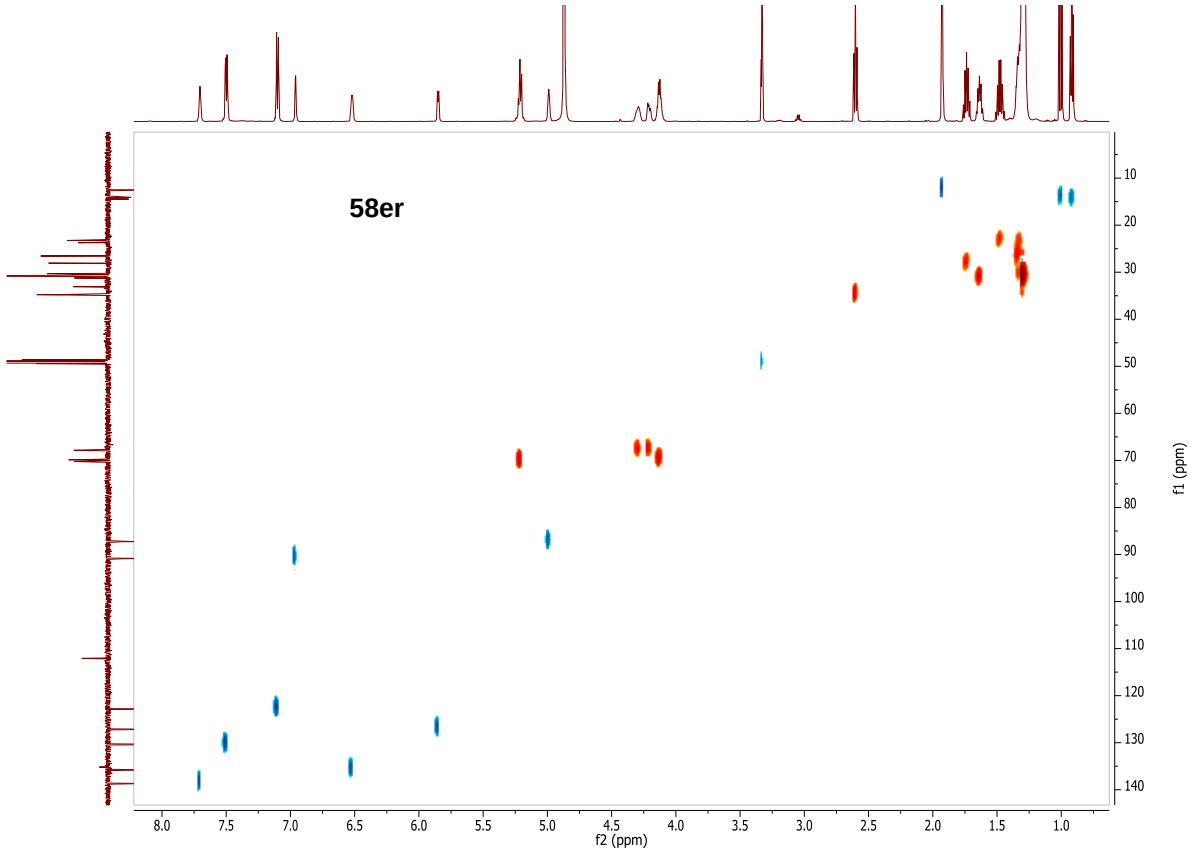


Figure 4-15: ^{1}H-^{13}C HSQC spectra of γ-(AB-C4,alkyl-C18)-d4TTP **58er** in CD_3OD.

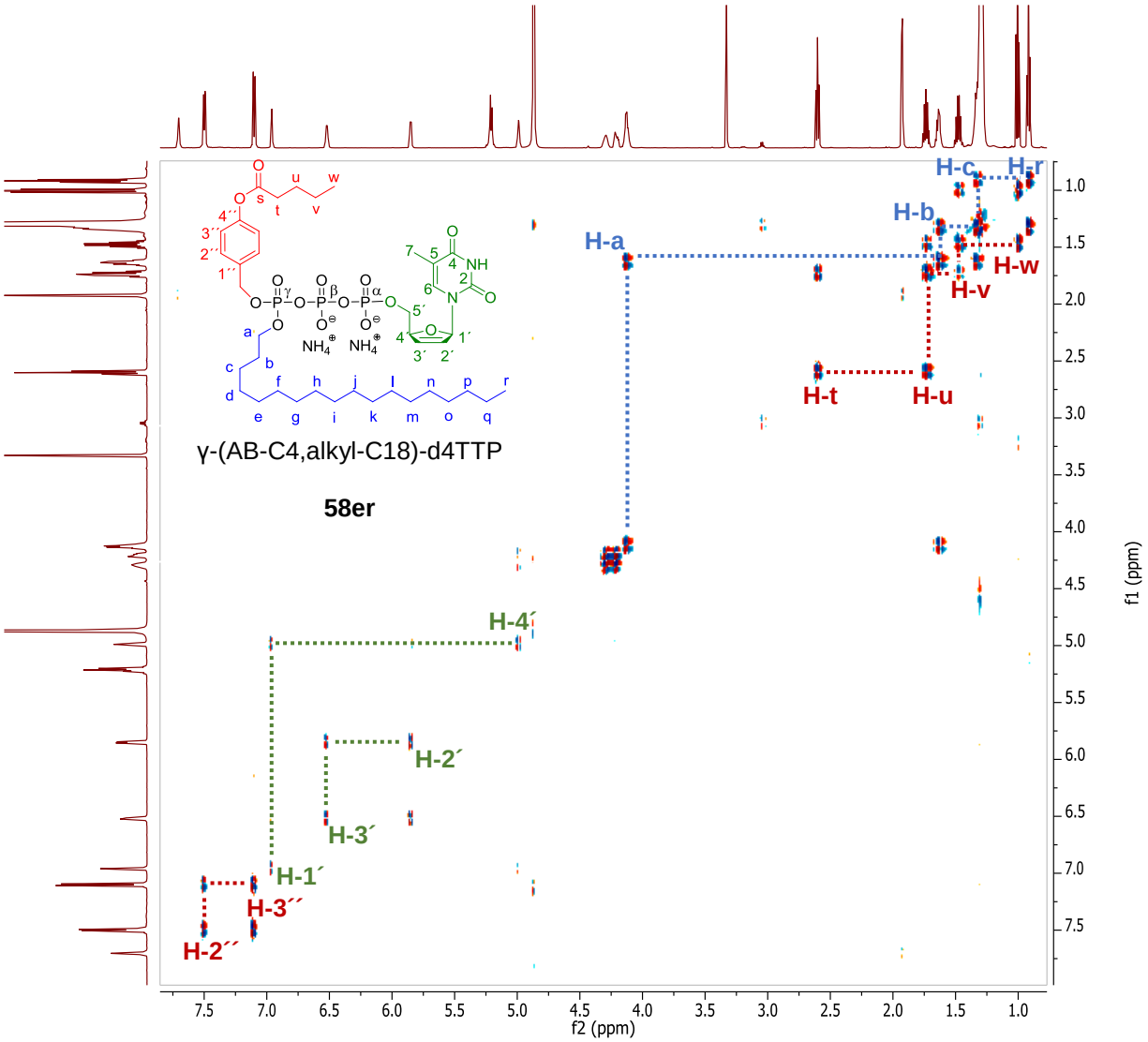
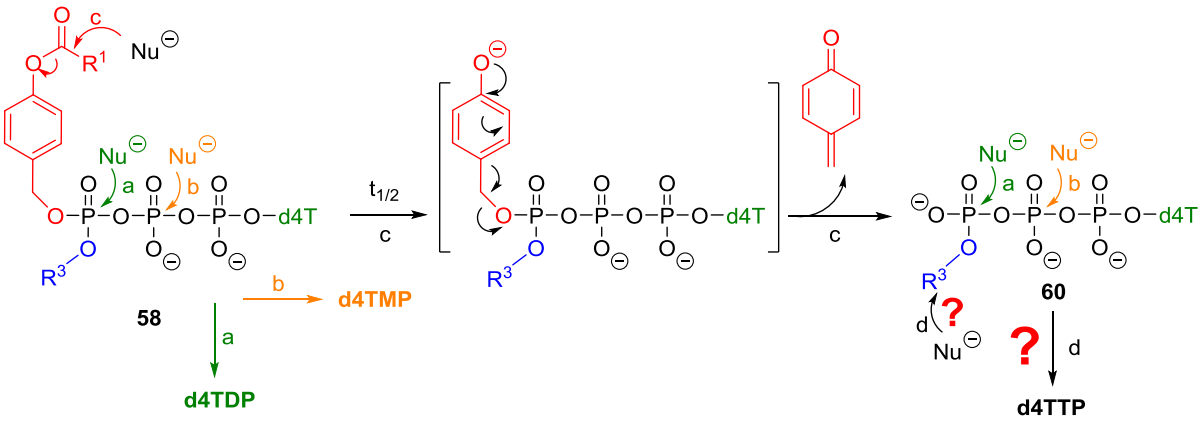


Figure 4-16: ^{1}H-^{1}H COSY spectra of γ-(AB-C4,alkyl-C18)-d4TTP **58er** in CD_3OD.

4.4.3 Chemical and Biological Hydrolysis

The prodrugs of the γ-(AB,alkyl)-NTP prodrugs **58** were incubated in phosphate buffer (PBS, 25 mM, pH 7.3) and human CD_4^+ T-lymphocyte cell extracts to study their stability and the product distribution. The hydrolysis mixtures were analyzed by means of analytical RP18-HPLC. In all cases the calculated half-lives of γ-(AB,alkyl)-NTP prodrugs **58** (Table 4-9, $t_{1/2}$) reflect the removal of the bio-reversible AB-group to yield the γ-(alkyl)-NTPs **60**. It is important to know if γ-(alkyl)-NTPs **60** can be further hydrolyzed to yield d4TTPs (Scheme 4-10, path d). Moreover, the stability and the antiviral activity of γ-(alkyl)-NTPs **60** need to be evaluated.



Scheme 4-10: Hydrolysis mechanism of γ-(AB,alkyl)-d4TTP prodrugs **58**.

Comp.	R^1	R^3	PBS pH=7.3 [h]	CEM cell extracts [h]
			$t_{1/2}(1)$[a]	$t_{1/2}(1)$[a]
58ed	*n*-C_4H_9 **44e**	*n*-C_4H_9	94	0.7
58ud	*n*-$C_{15}H_{31}$ **44u**	*n*-C_4H_9	198	2.2
58eo	*n*-C_4H_9 **44e**	*n*-$C_{15}H_{31}$	197	3.4
58go	*n*-C_6H_{18} **44g**	*n*-$C_{15}H_{31}$	212	3.6
58uo	*n*-$C_{15}H_{31}$ **44u**	*n*-$C_{15}H_{31}$	249	2.5
58br	CH_3 **44b**	*n*-$C_{18}H_{37}$	196	0.4
58cr	C_2H_9 **44c**	*n*-$C_{18}H_{37}$	206	1.6
58er	*n*-C_4H_9 **44e**	*n*-$C_{18}H_{37}$	237	4.8
58gr	*n*-C_6H_{18} **44g**	*n*-$C_{18}H_{37}$	269	5.2

Table 4-9: Half-lives of γ-(AB,alkyl)-d4TTP prodrugs **58** in PBS and CEM cell extracts.

In Figure 4-17, the half-lives of the hydrolysis in PBS and CEM cell extracts are shown using a bar chart. The half-life/structure relationship will be discussed next. Additionally, γ-(AB-C4,alkyl-C18)-d4UTP **61er** is also listed and could be compared with d4TTP prodrugs **58**. The hydrolysis properties of **64er** will be discussed separately in Chapter 4.6 in details.

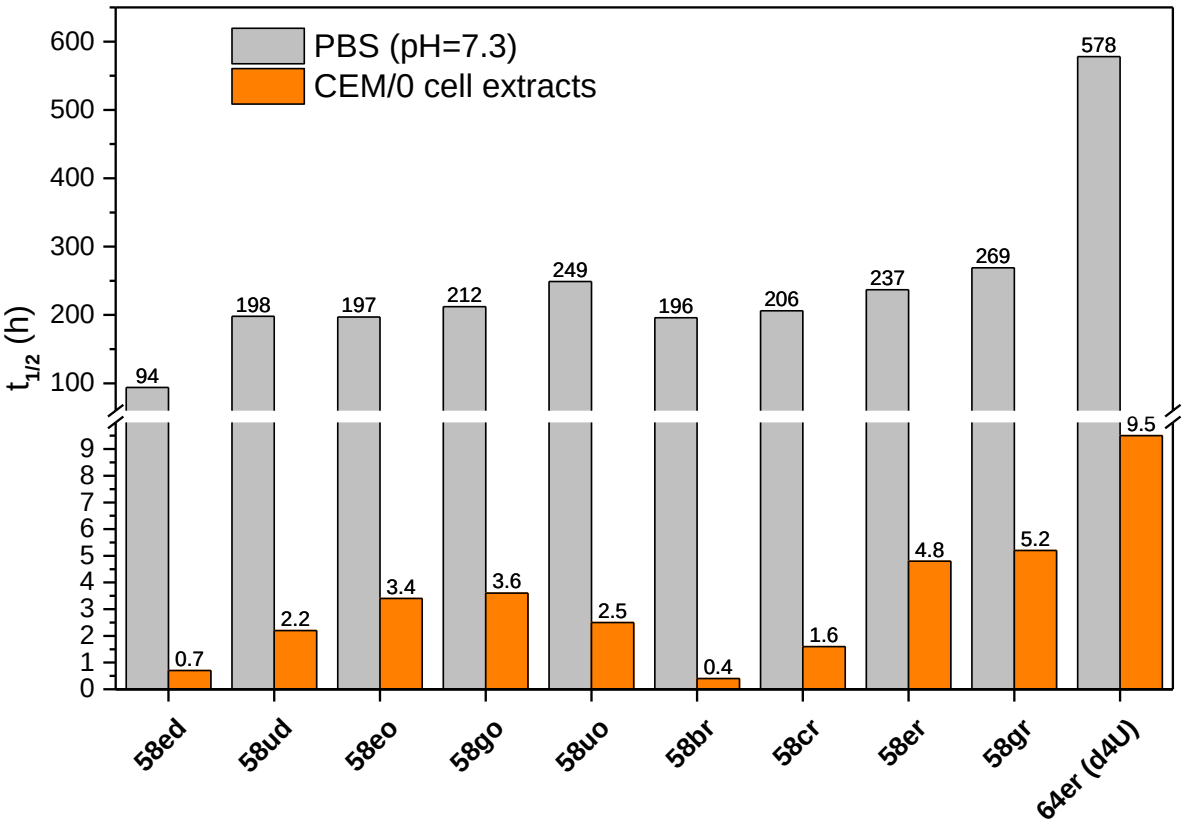


Figure 4-17: Half-lives of γ-(AB,alkyl)-d4TTP prodrugs **58 in PBS and CEM/0** in bar chart.

4.4.3.1 Chemical stability in phosphate buffer, pH 7.3

In PBS, the initial cleavage step of the hydrolysis mechanism proceeded similarly to the previously studied cleavage pathway for the Tri*PPP*ro-NTPs **56**. The stability of the γ-(AB,alkyl)-d4TTP prodrugs **58** increased with the increase of alkyl chain lengths within the AB-masks (**58br**, **58cr**, **58er** and **58gr** in Table 4-9 and Figure 4-17). When the length of the AB-masks is fixed, prodrug **58** with the longer alkyl chain length of alkoxy moiety exhibited higher stability. For example, prodrugs **58ed**, **58eo** and **58er** with a C4-AB-mask, the stability increased with the increase of the length of alkyl group (the half-lives are 94 h, 197 h, 237 h). The hydrolysis product starting from the γ-(AB,alkyl)-d4TTP prodrugs **58** resulted in a predominant formation of the γ-(alkyl)-d4TTP **60** and a very small amount of d4TDP **4d**. After consumption of the starting material, no further increase of d4TDP **4d** concentrations was observed. This supports again the hypothesis that only the γ-(AB,alkyl)-d4TTP prodrugs **58** were prone to a breakage between the γ- and β-phosphate. In contrast to the studies with Tri*PPP*ro-NTPs **56** containing a second cleavable mask (first generation NTP prodrugs), no d4TTP was detected in these studies using **58**.

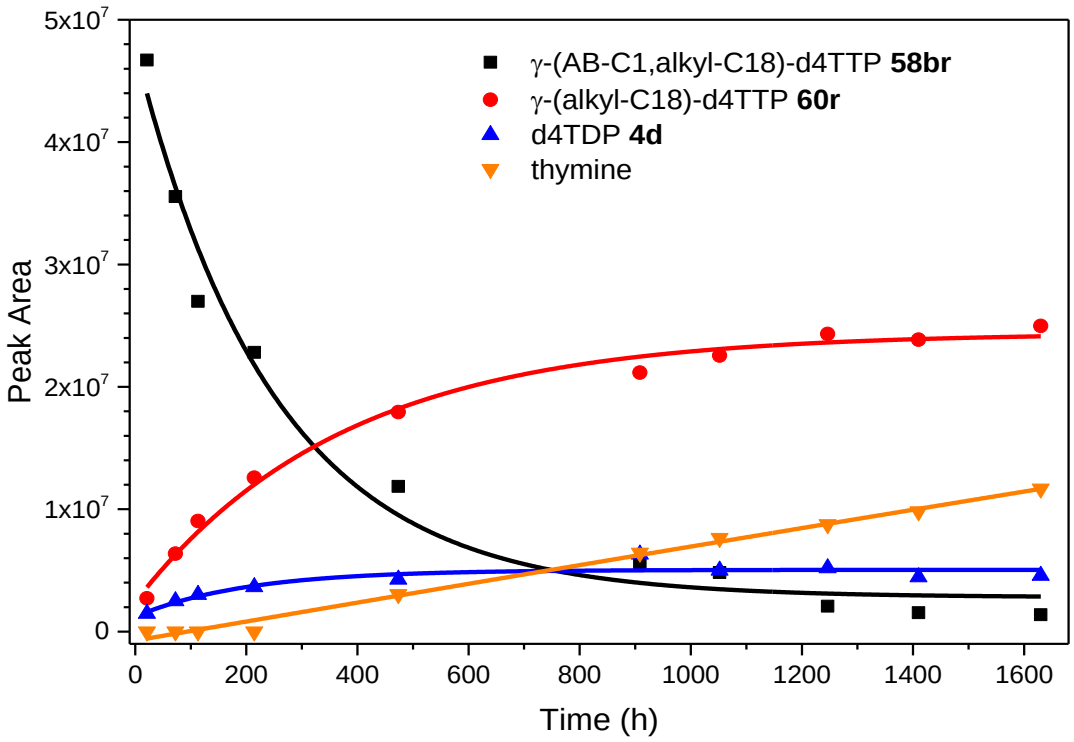


Figure 4-18: Hydrolysis of γ-(AB-C1,alkyl-C18)-d4TTP prodrug **58br** (black square) in **PBS**. γ-(Alkyl-C18)-d4TTP **60r** is in red dots. The d4TDP **4d**, and thymine are in blue and orange triangle, respectively.

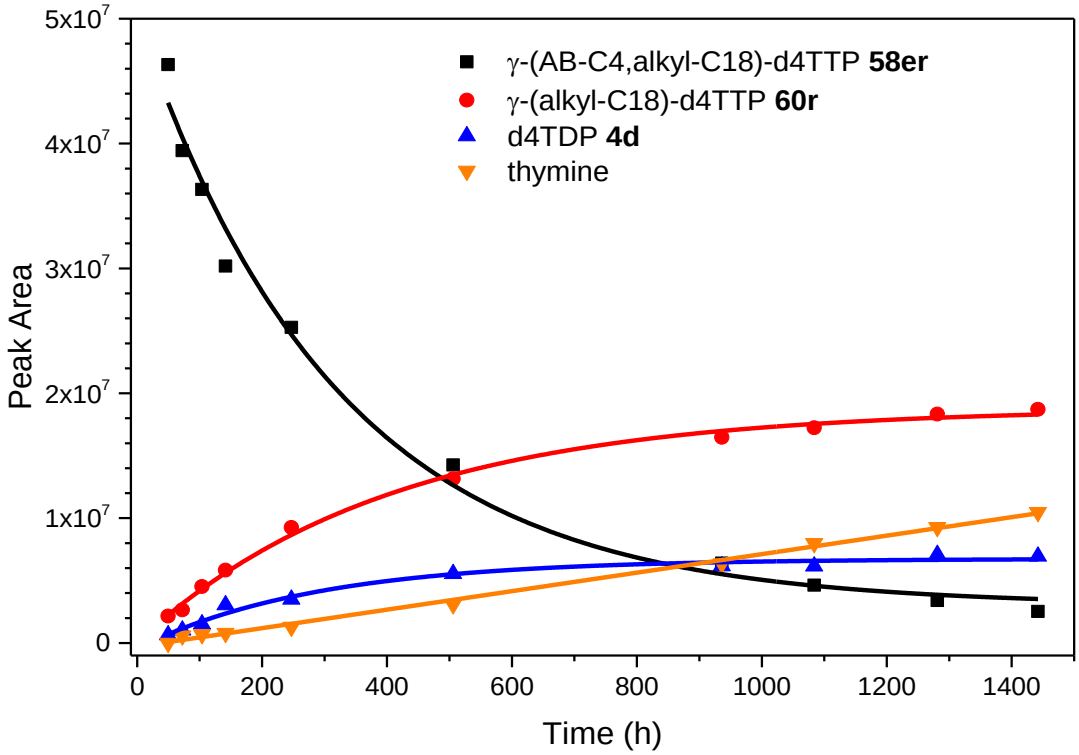


Figure 4-19: Hydrolysis of γ-(AB-C4,alkyl-C18)-d4TTP prodrug **58er** (black square) in **PBS**. γ-(Alkyl-C18)-d4TTP **60r** is in red dots. The d4TDP **4d**, and thymine are in blue and orange triangle, respectively.

The hydrolysis of compounds **58br** and **58er** is summarized as examples in Figure 4-18 and Figure 4-19. The $t_{1/2}$ of **58br** and **58er** are 196 h and 237 h, which is 4-5-fold more stable than Tri*PPP*ro-NTPs **56** ($t_{1/2}$ = 45-64 h). The hydrolysis was followed over a time period of 1400-1600 h. Clearly, the starting material disappeared and the expected γ-(alkyl)-d4TTP **60** was formed. Compounds **60** had shorter retention time than the parent prodrugs. A very small amount of d4TDP **4d** was formed and no d4TTP was detected during hydrolysis.

In the case of compounds using d4T as parent nucleoside, a further side reaction occurred that led to the formation of thymine by the cleavage of the glycosidic bond as discussed before (Chapter 4.3.3.1). The formation of thymine in long term PBS hydrolysis was identical with those with Tri*PPP*ro-NTPs **56**.

4.4.3.2 Hydrolysis of γ-(AB,alkyl)-d4TTP prodrugs 58 in CEM cell extracts, comparison with the Tri*PPP*ro-NTPs 56

The stability of the γ-modified-d4TTP prodrugs **58** was then determined in human CD_4^+ T-lymphocyte CEM cell extracts. Again, the half-lives of prodrugs **58** still correlated well with the chain length (both R^1 and R^3) and were significantly lower than their half-lives in PBS buffer (Table 4-9). As an enzymatic cleavage reaction took place, half-lives as low as 0.4 h (**58br**) to 5.2 h (**58gr**) were determined. In all cases we observed the predominate formation of γ-(alkyl)-d4TTP **60**. D4TDP **4d** was observed in very low concentration. No d4TTP **4t** was detected. This was in sharp difference to the studies performed with the Tri*PPP*ro-compounds **56**. There, it was almost impossible to detect significant concentrations of d4TTP due to its fast dephosphorylation to form first d4TDP **4d** and finally d4TMP **4m**.

Here, prodrugs γ-(AB-C4,alkyl-C4)-d4TTP **58ed** and γ-(AB-C1,alkyl-C18)-d4TTP **58br** were found to be the least stable compounds. This is also in accordance to our previous results. Particularly short acylester modifications in the mask moiety (AB group) proved extremely labile in cell extracts ($t_{1/2}$ of **58br** is 0.4 and 1.6 h). Moreover, the $t_{1/2}$ of prodrug **58er**, **58eo** and **58ed** are 4.8 h, 3.4 h and 0.7 h, respectively. This result suggested that the length of alkyl groups at the γ-position has a certain impact on the compound stability. When long alkoxy group (C18) was replaced by a short chain such as C4, the $t_{1/2}$ of prodrugs decreased dramatically. As example of γ-(AB-C15,alkyl-C15)-d4TTP **58uo** which is a very lipophilic compound, the $t_{1/2}$ of prodrug **58uo** also decreased comparing to **58go**.

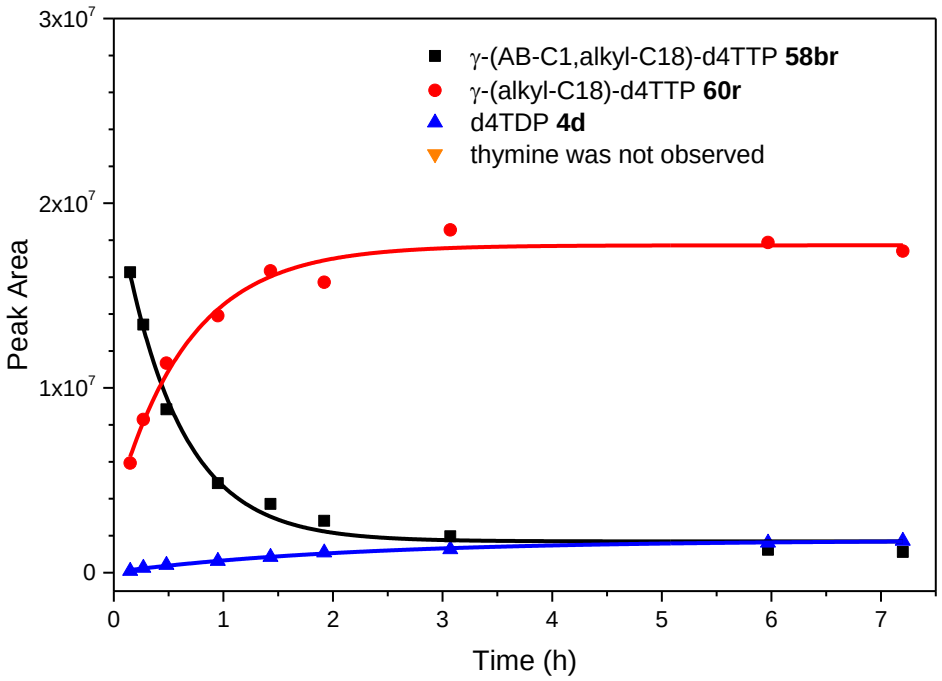


Figure 4-20-a: Hydrolysis of γ-(AB-C1,alkyl-C18)-d4TTP prodrug **58br** (black square) in **CEM cell extracts**. γ-(Alkyl-C18)-d4TTP **60r** is in red dots. D4TDP **4d**, and thymine are in blue and orange triangle, respectively.

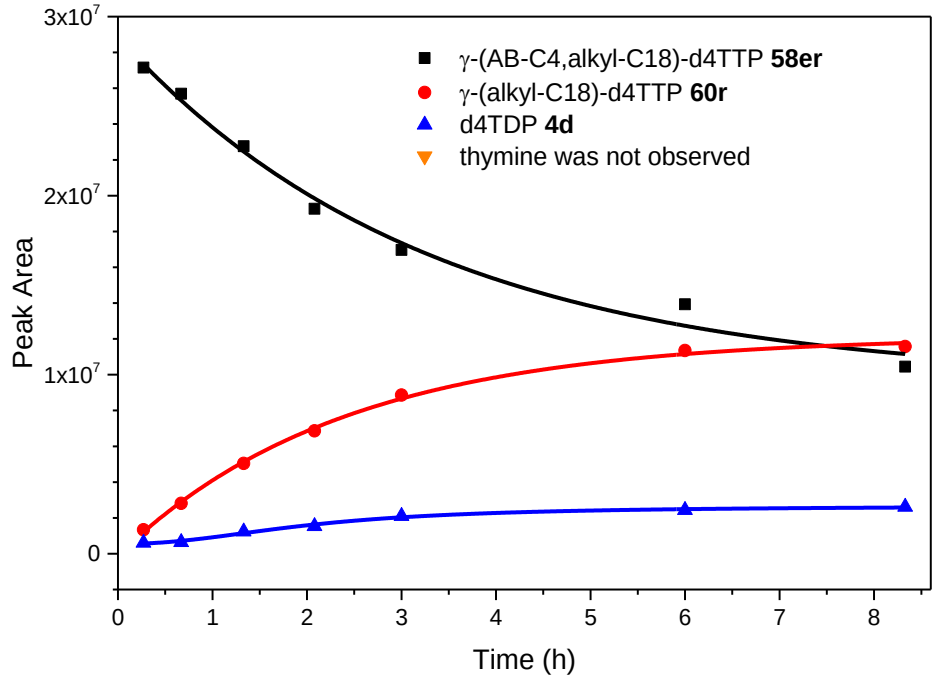


Figure 4-20-b: Hydrolysis of γ-(AB-C4,alkyl-C18)-d4TTP prodrug **58er** (black square) in **CEM cell extracts**. γ-(Alkyl-C18)-d4TTP **60r** is in red dots. D4TDP **4d**, and thymine are in blue and orange triangle, respectively.

4.4.3.3 Hydrolysis of Prodrug 58er and 58ud in PLE

Discussion

Next, we examined the enzymatic stability of prodrugs γ-(AB-C4,alkyl-C18)-d4TTP **58er** and γ-(AB-C15,alkyl-C4)-d4TTP **58ud** by incubation with pig liver esterase (PLE) in phosphate buffer, pH 7.3 at 37 ºC (Figure 4-21-a, 4-21-b). The cleavage of the masking unit in **58er** occurred fast and the $t_{1/2}$ of prodrug **58er** is 8.1 min. For prodrug **58ud** containing a long alkyl chain mask, its half-life is 25.5 min. This result was in full agreement to the studies of the Tri*PPP*ro-compounds **56eu** described before and proved a significant contribution of the enzymatic cleavage. Moreover, in these enzymatic hydrolysis studies, no cleavage of the alkyl residue in compounds **60** was observed. This proves our initial concept of introducing an enzyme-stable group to the γ-phosphate unit.

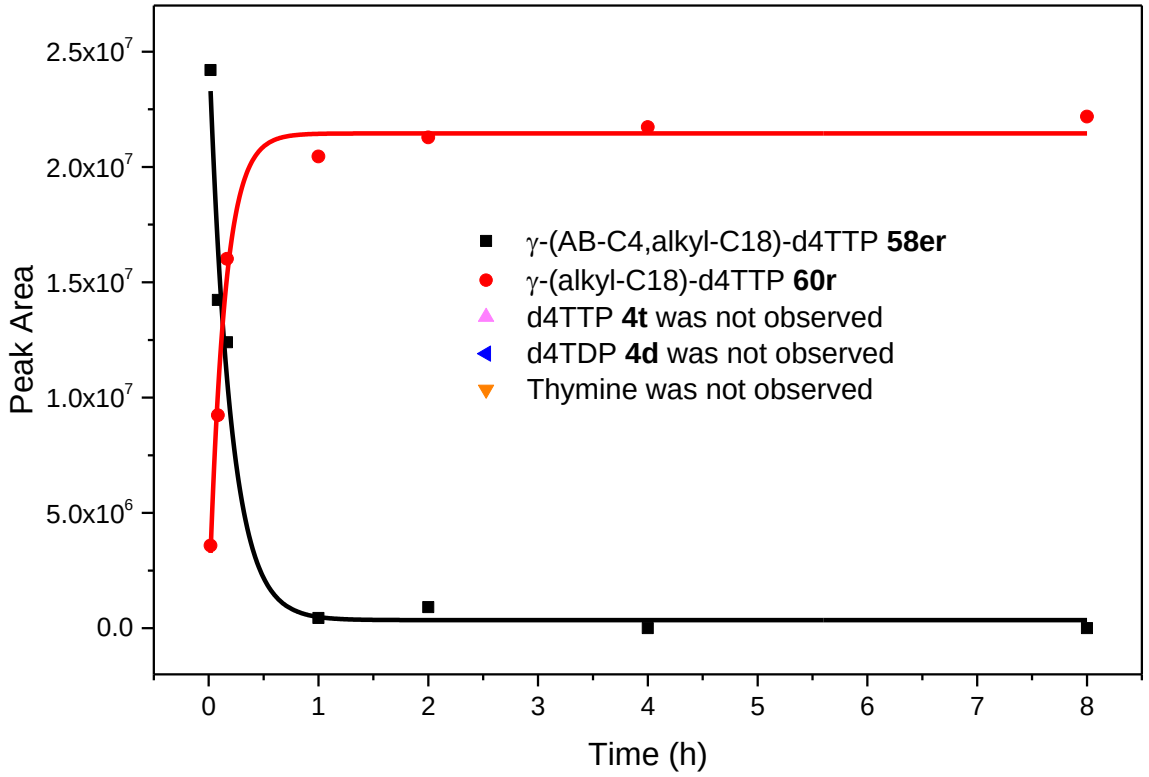


Figure 4-21-a: Hydrolysis of γ-(AB-C4,alkyl-C18)-d4TTP prodrug **58er** (black square) in **pig liver extracts (PLE)**. γ-(Alkyl-C18)-d4TTP **60r** is in red dots. D4TTP **4t**, d4TDP **4d**, and thymine are in pink, blue and orange triangle, respectively.

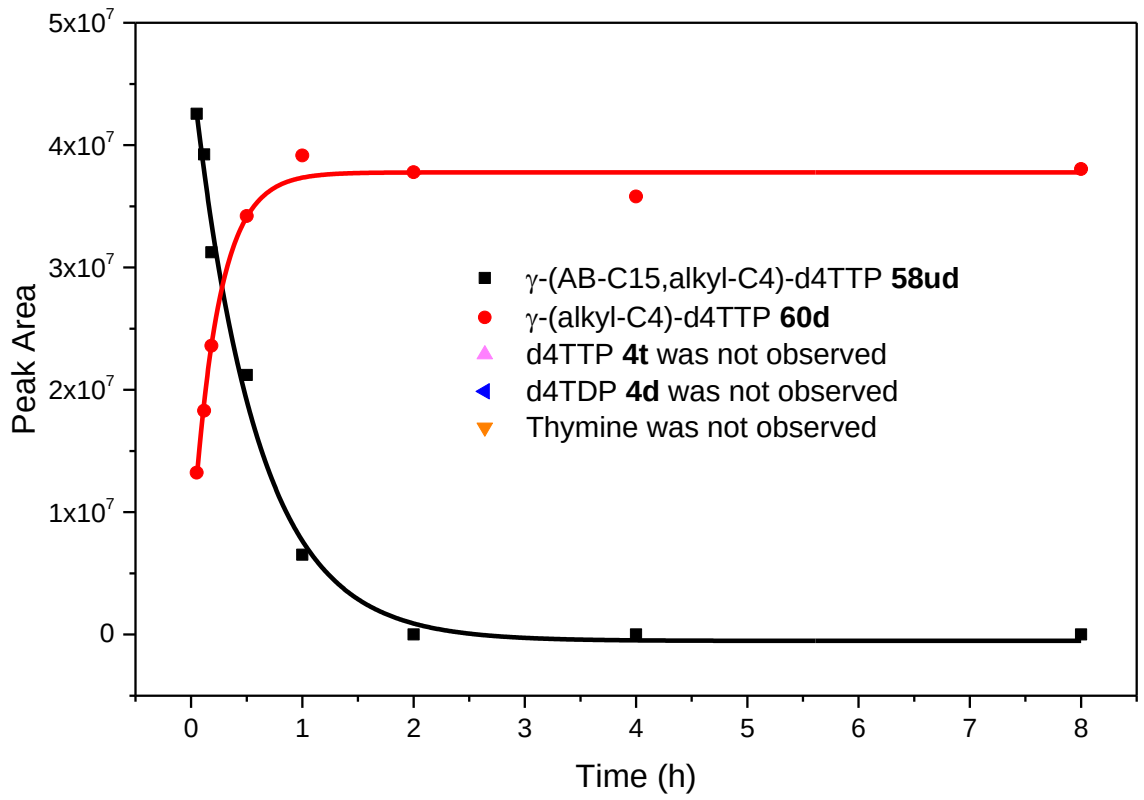


Figure 4-21-b: Hydrolysis of γ-(AB-C15,alkyl-C4)-d4TTP prodrug **58ud** (black square) in **pig liver extracts (PLE)**. γ-(Alkyl-C4)-d4TTP **60d** is in red dots. D4TTP **4t**, d4TDP **4d**, and thymine are in pink, blue and orange triangle, respectively.

4.4.4 Anti-HIV Activities in CEM/0 and CEM/Tk$^-$ Cells

The γ-(AB,alkyl)-d4TTPs **58** were evaluated for their ability to inhibit the replication of HIV in CEM cell line. HIV-1- or HIV-2-infected wild-type CEM/0 as well as mutant thymidine kinase-deficient CEM cell cultures (CEM/TK$^-$) were treated with compounds **58**. For comparison, two Tri*PPP*ro-derivatives **41h** and **56ew** were also included in this series. The results are summarized in Table 4-10. As can be seen, all compounds showed virtually similar or even slightly better activities against HIV-1 and HIV-2 as the parent nucleoside d4T **4** and d4TTP **4t**. More importantly almost all prodrugs **58** were also highly potent in CEM/TK$^-$ cells whereas d4T **4** and d4TTP **4t** lacked any relevant anti-HIV activity in this thymidine kinase-deficient cell model (EC_{50}: >50 µM and > 100 µM, respectively). γ-(AB-C4,alkyl-C18)-d4TTP **58er** is the most potent prodrugs against HIV in CEM/0 and especially in CEM/TK$^-$ cells. With these data in Table 4-10 and Figure 4-22, with the increasing lipophilicity of the prodrugs in a certain range, the activity also increased.

Discussion

Prodrugs **58ed** (C4/C4) and **58uo** (C15/C15) lost some of the activity in wild-type CEM/0 cells as compared to the other prodrugs. More importantly, prodrugs **58ed** (C4/C4), **58ud** (C15/C4) and **58uo** (C15/C15) were found to be inactive in CEM/TK$^-$ cells. For prodrugs **58ed** (C4/C4) and **58ud** (C15/C4), it is easy to understand that very short alkyl moieties could not provide enough membrane permeation ability.

Interestingly, **58uo** (C15/C15) lost activity both in CEM/0 and CEM/TK$^-$ cells and showed a different behavior even it should be lipophilic enough to provide membrane penetration ability. In the hydrolysis studies, γ-(AB-C15,alkyl-C15)-d4TTP **58uo** (C15/C15) was more stable than γ-(AB-C6,alkyl-C15)-d4TTP **58go** (C6/C15) in PBS but with an unexpected shorter half-life than **58go** in cell extracts. It reveals AB-mask with alkyl-C15 moiety has a hydrolysis mechanism or path way which is different in PBS and cell extracts. It finally led to a loss of antiviral activity in CEM/TK$^-$ cells.

Compound (AB,alkyl)	EC_{50} [μM][a]			CC_{50} [μM][b]
	CEM/0		CEM/TK$^-$	CEM/0
	HIV-1	HIV-2	HIV-2	
58ed (C4 / C4)	0.50 ± 0.21	0.67 ± 0.88	9.74 ± 3.44	64 ± 11
58ud (C15 / C4)	0.33 ± 0.11	0.24 ± 0.01	>10	21 ± 2
58eo (C4 / C15)	0.25 ± 0.00	0.23 ± 0.18	0.39 ± 0.30	21 ± 7
58go (C6 / C15)	0.18 ± 0.01	0.16 ± 0.11	0.92 ± 0.12	18 ± 4
58uo (C15 / C15)	0.62 ± 0.30	0.86 ± 0.04	3.90 ± 3.40	48 ± 31
58br (C1 / C18)	0.22 ± 0.05	0.50 ± 0.14	0.39 ± 0.30	17 ± 5
58cr (C2 / C18)	0.19 ± 0.09	0.25 ± 0.00	0.35 ± 0.25	11 ± 4
58er (C4 / C18)	0.18 ± 0.09	0.16 ± 0.10	0.17 ± 0.00	13 ± 1
58gr (C6 / C18)	0.17 ± 0.00	0.21 ± 0.06	0.76 ± 0.11	27 ± 4
41h (AB-C8 / ab-C8)	0.31 ± 0.01	0.62 ± 0.30	2.26 ± 1.03	52 ± 1
56ew (AB-C4 / ab-C17)	0.12 ± 0.05	0.10 ± 0.03	0.54 ± 0.41	33 ± 7
d4T **4**	0.43 ± 0.25	0.22 ± 0.05	>50	>50
d4TTP **4t**	1.05 ± 0.35	0.40 ± 0.26	>100	>100

Table 4-10: Antiviral activity and cytotoxicity of γ-(AB,alkyl)-d4TTP prodrugs **58** in comparison to the parent nucleoside d4T **4**, d4TTP **4t** and bis(AB)-d4TTP **41h** and **56ew**. [a] Antiviral activity in CD4$^+$ T-lymphocytes: 50% effective concentration; values are the mean ±SD of n=2-3 independent experiments. [b] Cytotoxicity: 50% cytostatic concentration or compound concentration required to inhibit CD4$^+$ T-cell (CEM) proliferation by 50%; values are the mean ±SD of n=2-3 independent experiments.

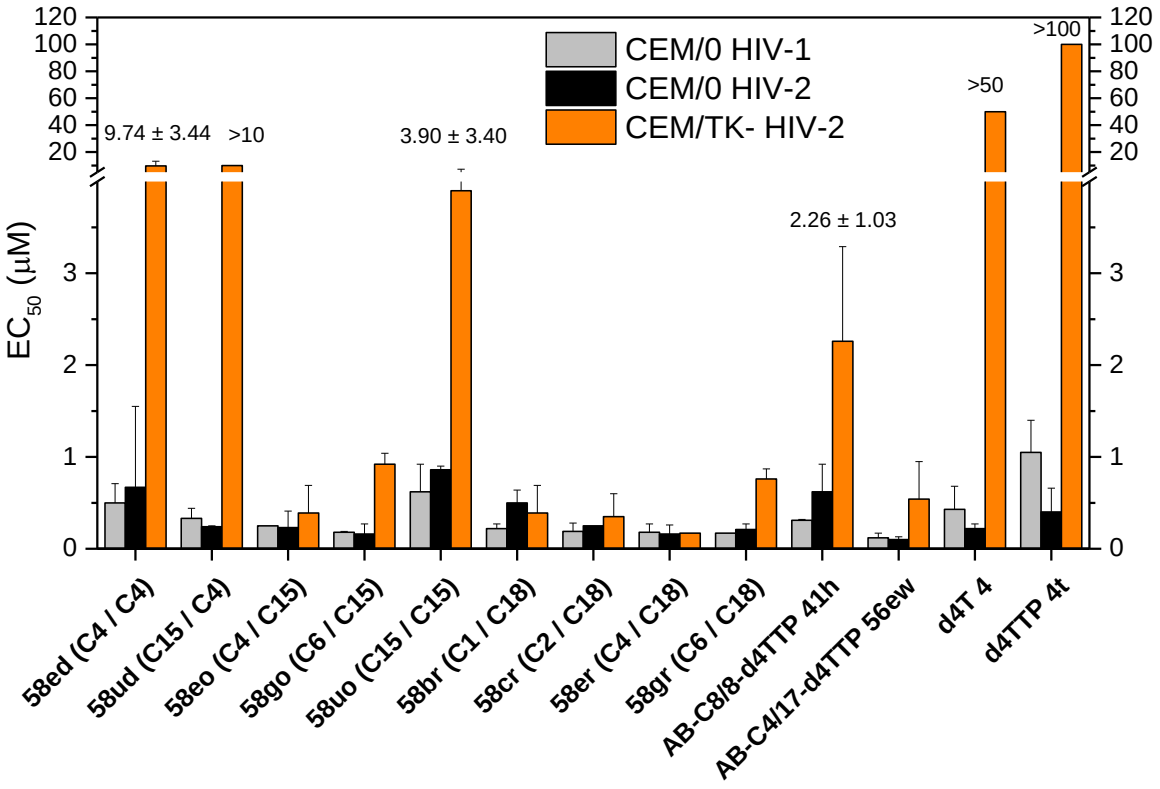


Figure 4-22: Antiviral activity of γ-(AB,alkyl)-d4TTP prodrugs **58** in comparison to the parent nucleoside d4T **4**, d4TTP **4t** and bis(AB)-d4TTP **41h** and **56ew** in bar chart.

It should also be mentioned that all the prodrugs **58** were slightly more toxic than the parent d4T **4** and d4TTP **4t**. The two Tri*PPP*ro-compounds comprising either two C8 chains (**41h**) or a mixture of a short C4 and a long C17 alkyl group (**56ew**) were active in the TK-deficient CEM cells but less active than γ-(AB-C4,alkyl-C18)-d4TTP prodrug **58er**. Again, the compound **56ew** bearing the C17 chain proved to be more active as compared to the other one (C8 chain) **41h** that is showed in the Table 4-10.

4.5 γ-(alkyl)-dNTPs 60,62

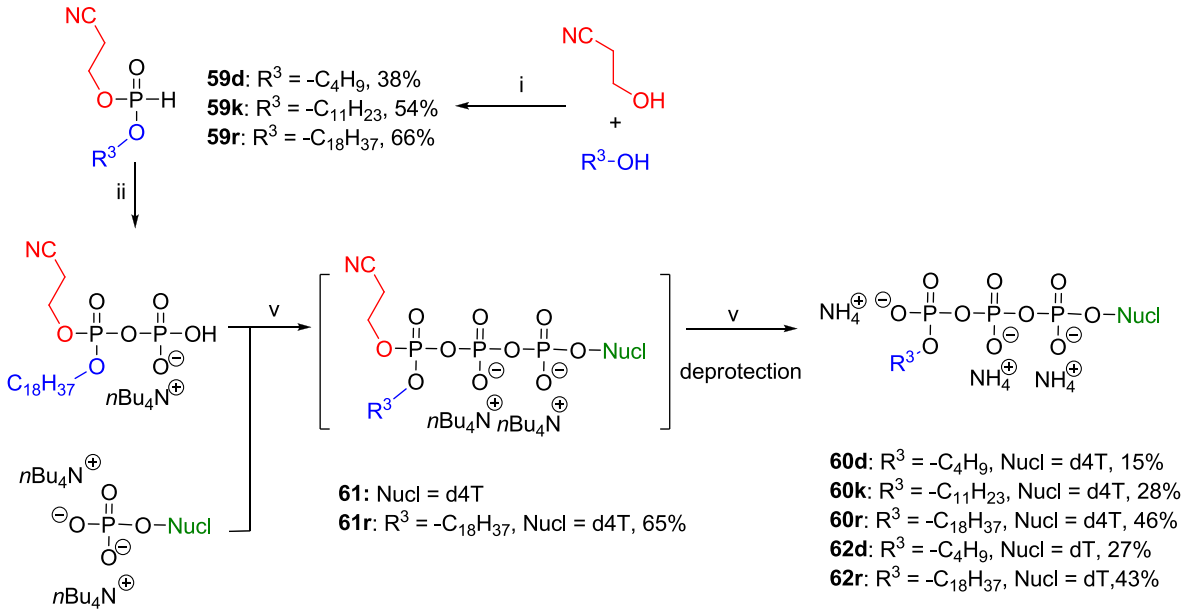
To look deep into the properties of γ-(AB,alkyl)-d4TTP **58**, compound γ-(alkyl)-d4TTP **60** is an important derivative that need to be synthesized and tested regarding their stability and antiviral activity. Unlike γ-(AB,alkyl)-d4TTP **58**, γ-(alkyl)-d4TTP **60** and γ-(alkyl)-TTP **62** are potential substrates for the polymerases used in primer extension experiment.

4.5.1 Synthesis of γ-(alkyl)-dNTPs

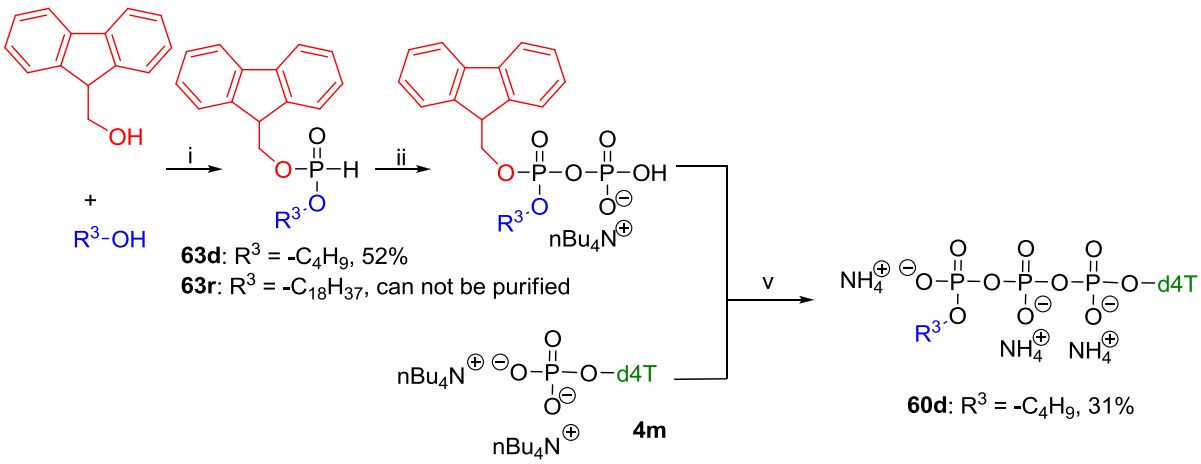
To obtain γ-(alkyl)-d4TTP **60**, 3-hydroxypropionitrile (β-cyanoethanol) was used as a base labile protection group in the synthesis. In the first step, non-symmetric *H*-

phosphonates **59** were synthesized. Next, *H*-phosphonates **59** were converted to its pyrophosphate form. After the coupling reaction between the pyrophosphates and d4TMP **4m**, γ-(β-cyanoethyl,alkyl)-d4TTP **61** (with nBu$_4$N$^+$ as counter ion) were synthesized and was purified and transformed to γ-(β-cyanoethyl,alkyl)-d4TTP **61** (NH_4^+ as counter ion). The yields from NMP to **60** and **62** increased if γ-(alkyl)-d4TTP contained longer alkyl group (**60r** and **62r**).

For the synthesis of compounds **60d**, **60k**, **62d** and **62r**, the crude product after the coupling to give compounds **61** was dried in vacuum and was directly stirred in a mixture of 40% nBu$_4$N$^+$OH$^-$ water solution and acetonitrile at room temperature to yield target compounds **60**, **62**. As judged by HPL-chromatography, the reaction was stopped after 8-20 hours. After purification, γ-(alkyl)-d4TTPs **60** were isolated in 27-46% yield. For the deprotection of **61r**, a water solution of ammonium hydroxide was also used and stirred at room temperature for 2 hours. However, this led to a mixture of 19% γ-(alkyl-C18)-d4TTP **60r,** 24% γ-(β-cyanoethyl,alkyl-C18)-d4TTP **61r**, and 57% d4TMP **4m**. Additionally, triethylamine was used as an alternative reagent, but no conversion was observed after 2 hours.



Scheme 4-11: Reagents and conditions: i) alcohol, DPP, 3-hydroxypropionitrile, pyridine, 0 °C-38 °C, 3.3 h; ii) a. NCS, CH_3CN, RT, 2 h, b) $(H_2PO_4)Bu_4N$, CH_3CN, RT, 1 h; v) a. pyrophosphate, TFAA, Et_3N, CH_3CN, 0 °C, 10 min, b. 1-methylimidazole, Et_3N, CH_3CN, 0 °C-RT, 10 min, c. NMP, RT, 3-5 h, n-$Bu_4N^+OH^-$, RP Chromatography, Dowex 50WX8 (NH_4^+ form) column, RP Chromatography.



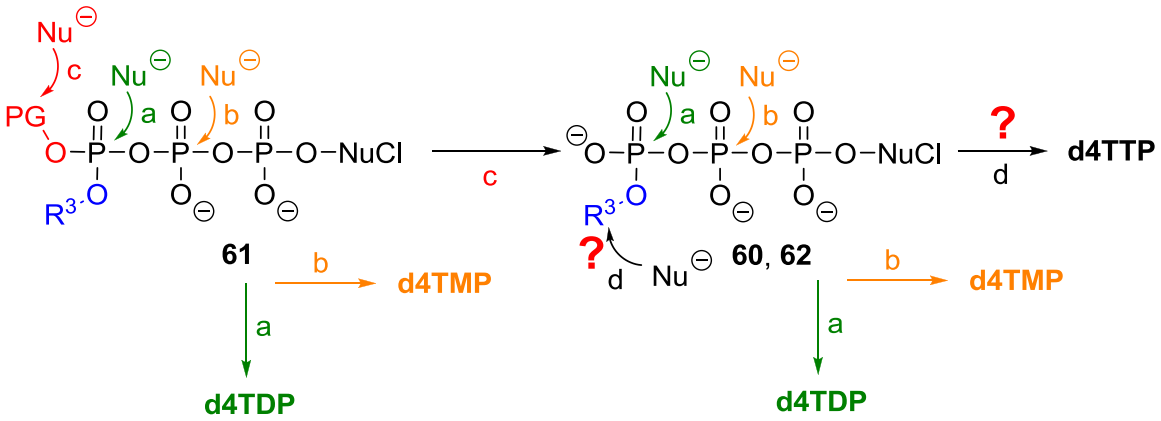
Scheme 4-12: Reagents and conditions: i) 1-Butanol, DPP, 9-fluorenylmethanol, pyridine, 0 °C-38 °C, 3.3 h; ii) a. NCS, CH_3CN, RT, 2 h, b) $(H_2PO_4)Bu_4N$, CH_3CN, RT, 1 h; iii) **7**, $POCl_3$, pyridine, H_2O, CH_3CN, 0 °C-RT, 5h; v) a. pyrophosphate, TFAA, Et_3N, CH_3CN, 0 °C, 10 min, b. 1-methylimidazole, Et_3N, CH_3CN, 0 °C-RT, 10 min, c. NMP, RT, 3-5 h, *n*-$Bu_4N^+OH^-$, RP Chromatography, Dowex 50WX8 (NH_4^+ form) column, RP Chromatography.

As can be seen in Scheme 4-11, the yield of compound **60d** is only 15% when 3-hydroxypropionitrile (β-cyanoethanol) was used as protection group. In order to improve the yield, 9-fluorenylmethanol was used as alternative for protection and the yield of *H*-phosphonate **63d** and γ-(alkyl-C4)-d4TTP **60d** were increased to 52% and 31% respectively (Scheme 4-12). It probably can be explained as follows. When *H*-phosphonate **59** contains two short alkoxy groups (e.g. compound **59d** with C4 and β-CN-ethyl groups), the extraction efficacy of pyrophosphate in DCM/water decreased. The 9-fluorenylmethyl group is more lipophilic than the β-cyanoethyl group. Furthermore, the purification by column chromatography of **63d** was much easier than that of **59d**. The method of using 9-fluorenylmethanol as protection group was also conducted in synthesizing *H*-phosphonate **63r**. Unfortunately, it was impossible to purify the product **63r** as its R_f value on TLC is almost identical with its symmetrically-esterified byproduct.

4.5.2 Chemical and Biological Hydrolysis

The hydrolysis study of γ-(AB,alkyl)-NTP prodrugs **58** revealed that no d4TTP **4t** formed during the hydrolysis either in PBS or in CEM cell extracts. But this result is maybe due to the extreme slow cleavage rate of the alkoxy group and enzyme in cell extracts may lose activity after the cleavage of AB-mask moiety. The best way to study the decomposed product of γ-(AB,alkyl)-NTP prodrugs **58** is to use γ-(alkyl)-NTP **61**, **62** as substrate in hydrolysis study. Thus, the hydrolysis activity can be

observed. γ-(β-Cyanoethoxy,alkyl-C18)-d4TTP **61r** was isolated as it is only one example to proof its formation. γ-(Alkyl)-TTP **62** with a thymidine moiety was sybtrhesized mainly for the primer extension experiment. The possible hydrolysis mechanism is shown in Scheme 4-13. Whether the alkoxy group can be cleaved is the most important aspect which is related to the antiviral activity.



PG: protection grouop

Scheme 4-13: Hydrolysis mechanism of γ-(PG,alkyl)-NTP **61** and γ-(alkyl)-NTP **61**, **62**.

Comp.	Nucl.	PG	R^3	PBS pH=7.3 [h]	CEM Cell extracts [h]
				$t_{1/2}$	$t_{1/2}$
60d	d4T	/	*n*-C_4H_9	no hydrolysis	>30
60k	d4T	/	*n*-$C_{11}H_{23}$	no hydrolysis	>30
60r	d4T	/	*n*-$C_{18}H_{37}$	no hydrolysis	>30
61r	d4T	β-CN-ethyl	*n*-$C_{18}H_{37}$	170	8.9
62d	dT	/	*n*-C_4H_9	no hydrolysis	>30
62r	dT	/	*n*-$C_{18}H_{37}$	no hydrolysis	>30

Table 4-11: Half-lives of γ-(alkyl)-NTP **60**,**62** and γ-(β-CN-ethyl,alkyl)-d4TTP **60** in PBS and CEM cell extracts.

From the hydrolysis data in Table 4-11, it can be seen that in PBS and CEM cell extracts, γ-(alkyl)-NTP **60**,**62** are much more stable than Tri*PPP*ro-compounds γ-(AB,ab)-NTPs **56** and γ-(AB,alkyl)-NTPs **58**. There was no obvious difference in the stability of γ-(alkyl)-NTP **60**,**62** with long alkoxy group (C18) or short alkoxy group (C4) in PBS. This means that **60d** and **60r** have same stability under chemical hydrolysis condition. When the nucleoside d4T of **60r** was changed to thymidine (T, **62r**), the stability remained the same. In CEM cell extracts, all compounds of γ-(alkyl)-NTP **60**, **62** are stable after 24 h incubation. In conclusion, γ-(alkyl)-NTP **60** and **62** are stable in PBS and CEM cell extracts.

4.5.2.1 Hydrolysis of γ-(β-cyanoethanoxy,alkyl-C18)-d4TTP 61r

In PBS, the hydrolysis process of γ-(β-cyanoethanoxy,alkyl-C18)-d4TTP **61r** was monitored for a period of 700 h. D4TTP **4t** was not detected even after 700 h. A very small amount of d4TMP **4m** was formed in the beginning and kept at the same concentration throughout the hydrolysis. γ-(Alkyl-C18)-d4TTP **60r** and d4TDP **4d** were formed from the beginning of the hydrolysis and their concentration was increasing at the same level. Thymine was formed and was observed clearly during long time incubation and this side reaction is a zero-order reaction. After 300 h of hydrolysis, the concentration of γ-(alkyl-C18)-d4TTP **60r** and d4TDP **4d** did not further increased. It is suggested that after 300 h, the decomposition of γ-(β-cyanoethanoxy,alkyl-C18)-d4TTP **61r** was mainly because of the cleavage of the glycosidic bond.

In CEM cell extracts, the level of γ-(β-cyanoethoxy,alkyl-C18)-d4TTP **61r** decreased gradually. γ-(Alkyl-C18)-d4TTP **60r** and d4TDP **4d** was formed at the beginning of hydrolysis and the concentration did not increased from 0.5 h to 8 h. Thymine was not observed as the incubation time is too short in CEM cell extracts compared to PBS.

As a result, γ-(β-cyanoethoxy,alkyl-C18)-d4TTP **61r** is less stable than γ-(alkyl)-NTP **60**, **62**. The $t_{1/2}$ of **61r** is 170 h in PBS, which is less stable than γ-(AB-C4,alkyl-C18)-d4TTP **58er** ($t_{1/2}$ = 237 h). But in CEM cell extracts, the $t_{1/2}$ of **61r** is 8.9 h, which is almost 2-fold higher than the $t_{1/2}$ of **58er** (4.8 h).

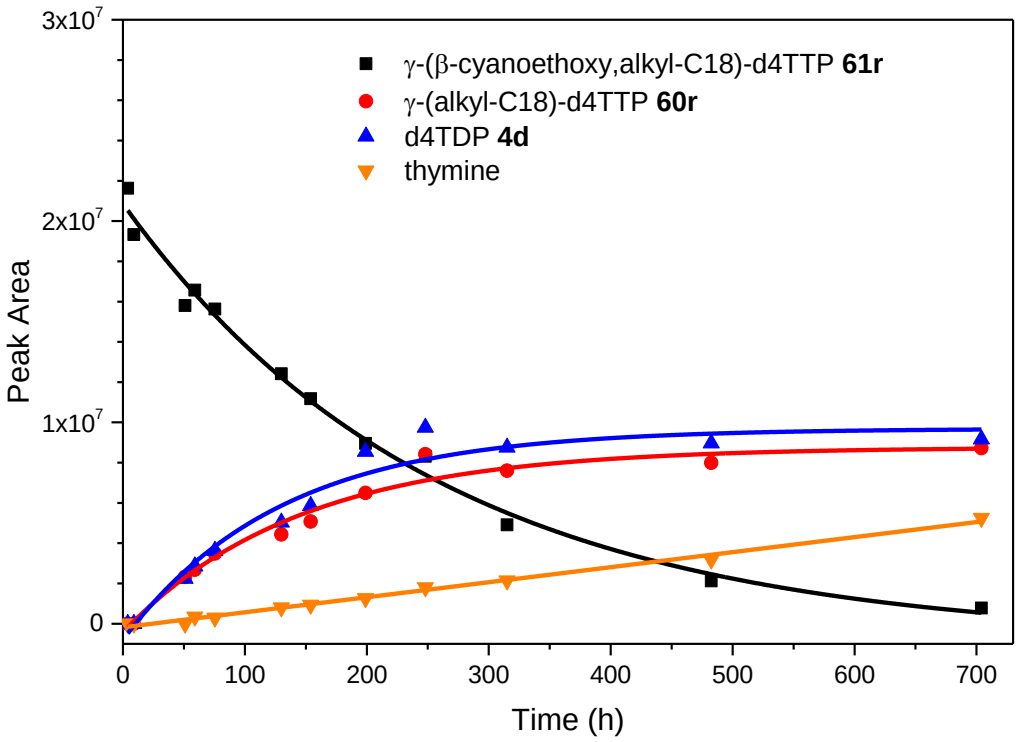


Figure 4-23: Hydrolysis of γ-(β-cyanoethoxy,alkyl-C18)-d4TTP **61er** (black square) in **PBS**. γ-(alkyl-C18)-d4TTP **60r** is in red dots. D4TDP **4d**, and thymine are in blue and orange triangle, respectively.

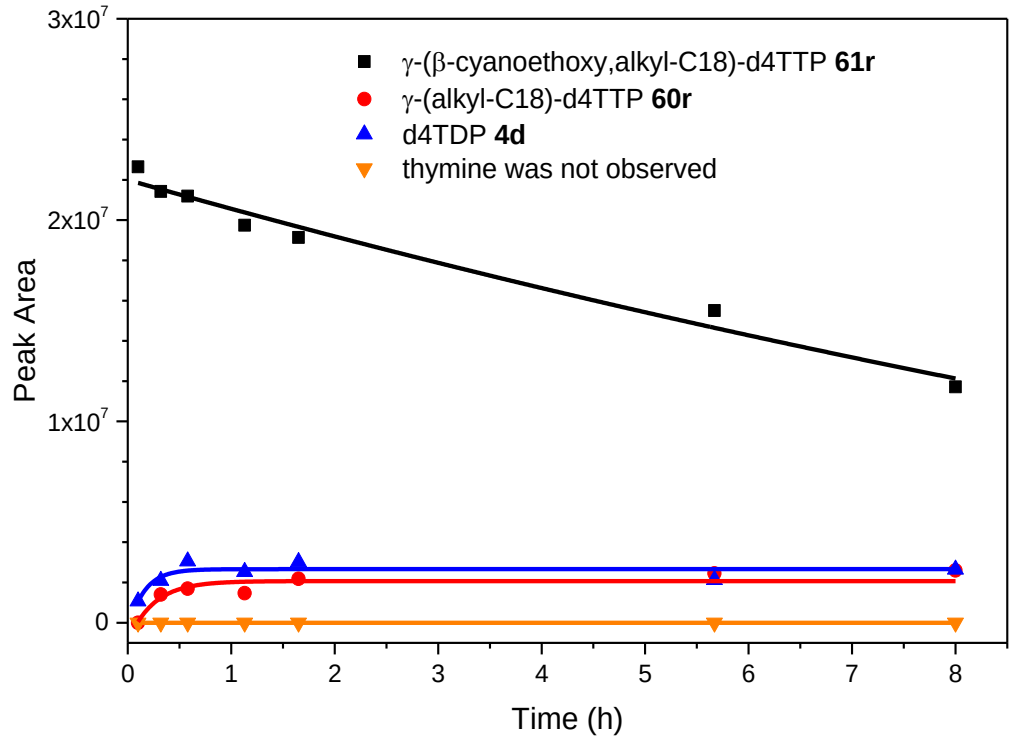


Figure 4-24: Hydrolysis of γ-(β-cyanoethoxy,alkyl-C18)-d4TTP **61er** (black square) in **CEM cell extracts**. γ-(alkyl-C18)-d4TTP **60r** is in red dots. D4TDP **4d**, and thymine are in blue and orange triangle, respectively.

4.5.2.2 Hydrolysis of γ-(alkyl-C18)-d4TTP 60r

The hydrolysis of γ-(alkyl-C18)-d4TTP **60r** was then performed. From Table 4-11, it is can be seen that compound **60r** is stable both in PBS and CEM cell extracts. More details of the chemical and biological hydrolysis were showed in Figure 4-25 and Figure 4-26.

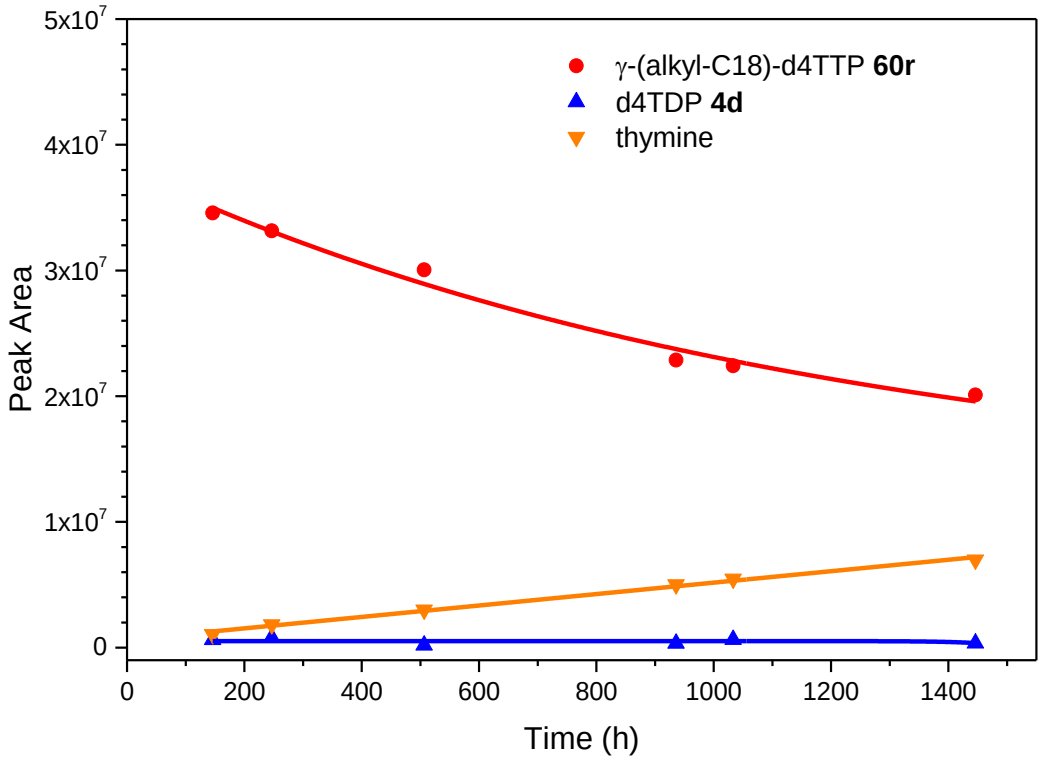


Figure 4-25: Chemical hydrolysis of γ-(alkyl-C18)-d4TTP **60r** (red dots) **in PBS**. D4TDP **4d**, and thymine are in blue and orange triangle, respectively.

In PBS, the incubation time took more than 1400 h. D4TDP **4d** was detected in an extremely low concentration at the beginning and kept constant until the end of the study. Thymine was formed during hydrolysis and its concentration increased gradually following a zero-order reaction property. The half-life here for γ-(alkyl-C18)-d4TTP **60r** in PBS was calculated as 1639 h. However, becuase the degradation of **60r** is based on the cleavage of the glycosidic bond, the meaning of half-life here is totally different from that of prodrugs. No d4TTP **4t** was observed during the hydrolysis.

In CEM cell extracts hydrolysis, γ-(alkyl-C18)-d4TTP **60r** was stable after 9 h incubation. When the incubation time was extended to 32 h still no decomposition of **60r** was observed. D4TDP **4d** was formed in a very low concentration and kept at the

same level until the end of hydrolysis. Again, thymine was not observed in CEM cell extracts hydrolysis. Identical results were also afforded in PLE hydrolysis.

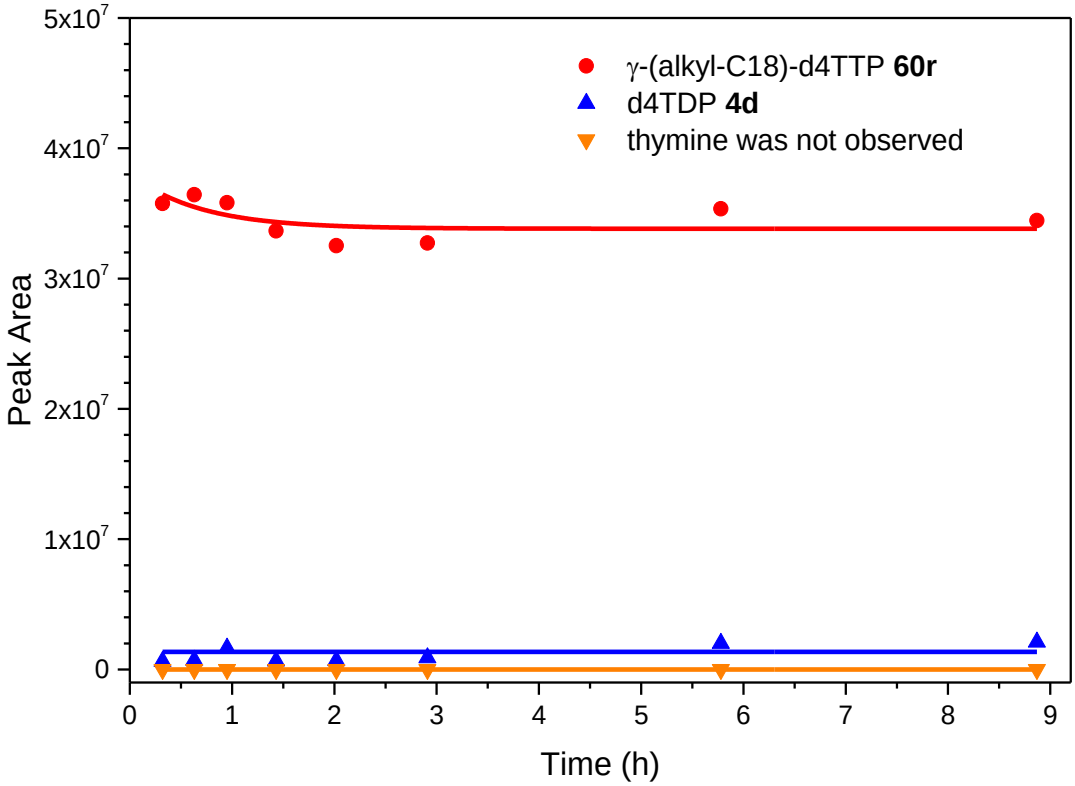


Figure 4-26: Hydrolysis of γ-(alkyl-C18)-d4TTP **60r** (red dots) **in CEM cell extracts**. D4TDP **4d**, and thymine are in blue and orange triangle, respectively.

4.5.3 Anti-HIV Activities

The γ-(β-cyanoethoxy,alkyl-C18)-d4TTP **61r** and γ-(alkyl)-NTPs **60**, **62** were evaluated for their ability to inhibit the replication of HIV in CEM cell line. HIV-1- or HIV-2-infected wild-type CEM/0 as well as mutant thymidine kinase-deficient CEM cell cultures (CEM/TK$^-$) were treated with the compounds **60**-**62**. For comparison, d4T **4**, d4TTP **4t**, two Tri*PPP*ro-derivatives **41h** and **56ew** and one γ-(AB-C4,alkyl-C18)-d4TTP **58er** were also included in the anti-HIV testing in Table 4-12.

As can be seen in Table 4-12, with the increase of chain length, the antiviral activity in CEM/TK$^-$ cells increased as well (e.g. **60d**, **60k** and **60r**). For the activity of γ-(alkyl-C4)-d4TTP **60d** and γ-(alkyl-C11)-d4TTP **60k** in CEM/TK$^-$ cells, lower activity were observed compared with those in CEM/0 cells. When the alkyl chain length increased to C18, compound **60r** became more active in CEM/TK$^-$ cells compared with those in CEM/0 cells. This result revealed that 1-octadecyl group (C18) provided enough

lipophilicity that γ-(alkyl-C18)-d4TTP **60r** are permeable through membrane. It is worth to note that the cytotoxicity of **60r** is 6.6-fold less toxic than γ-(AB-C4,alkyl-C18)-d4TTP **58er** and also less toxic than d4T **4** and d4TTP **4t**. Till now, γ-(alkyl-C18)-d4TTP **60r** is the most active compound against HIV in this study.

Compound	EC_{50} [µM][a]			CC_{50} [µM][b]
	CEM/0		CEM/TK$^-$	CEM/0
	HIV-1	HIV-2	HIV-2	
60d (C4-d4TTP)	0.95 ± 0.51	0.29 ± 0.15	35.81 ± 29.88	>100
60k (C11-d4TTP)	0.89 ± 0.28	0.26 ± 0.083	16.19 ± 4.71	78 ± 10
60r (C18-d4TTP)	0.47 ± 0.014	0.30 ± 0.085	0.11 ± 0.09	86 ± 3
61 (CN/C18-d4TTP)	0.054 ± 0.023	0.070 ± 0.019	0.20 ± 0.26	28 ± 2
62d (C4-TTP)	>21	5.51 ± 0.82	>21	21 ± 0
62r (C18-TTP)	>54	8.05 ± 4.04	>54	54 ± 4
58er (C4 / C18)	0.18 ± 0.09	0.16 ± 0.10	0.17 ± 0.00	13 ± 1
41h (AB-C8,ab-C8)	0.31 ± 0.01	0.62 ± 0.30	2.26 ± 1.03	52 ± 1
56ew (AB-C4,ab-C17)	0.12 ± 0.05	0.10 ± 0.03	0.54 ± 0.41	33 ± 7
d4T **4**	0.47 ± 0.29	0.25 ± 0.13	21.60 ± 10.95	> 50
d4TTP **4t**	1.05 ± 0.35	0.40 ± 0.26	>100	>100

Table 4-12: Antiviral activity and cytotoxicity of γ-(alkyl)-d4TTP **60**, γ-(β-Cyanoethoxy,alkyl)-d4TTP **61** and γ-(alkyl)-TTP **62** in comparison to the parent nucleoside d4T **4**, d4TTP **4t**, γ-(AB-C4,alkyl-C18)-d4TTP **58er** and γ-(AB,ab)-d4TTPs **41h**,**56ew**. [a] Antiviral activity in $CD4^+$ T-lymphocytes: 50% effective concentration; values are the mean ±SD of n=2-3 independent experiments. [b] Cytotoxicity: 50% cytostatic concentration or compound concentration required to inhibit $CD4^+$ T-cell (CEM) proliferation by 50%; values are the mean ±SD of n=2-3 independent experiments.

To our surprise, γ-(β-cyanoethoxy,alkyl-C18)-d4TTP **61r** is also active and have the similar activity against HIV-2 in CEM/TK$^-$ cells. However, **61r** is more toxic than γ-(alkyl-C18)-d4TTP **60r** and d4T **4**. It is probably a consequence of the existence of β-cyanoethyl group. As expected, with the existence of the 3´-OH group in thymidine (T), the polymerase could use thymidine triphosphate derivatives to elongate DNA strand and finally γ-modified-TTP compounds (**62d** and **62r**) are not active either in CEM/0 cells or CEM/TK$^-$ cells.

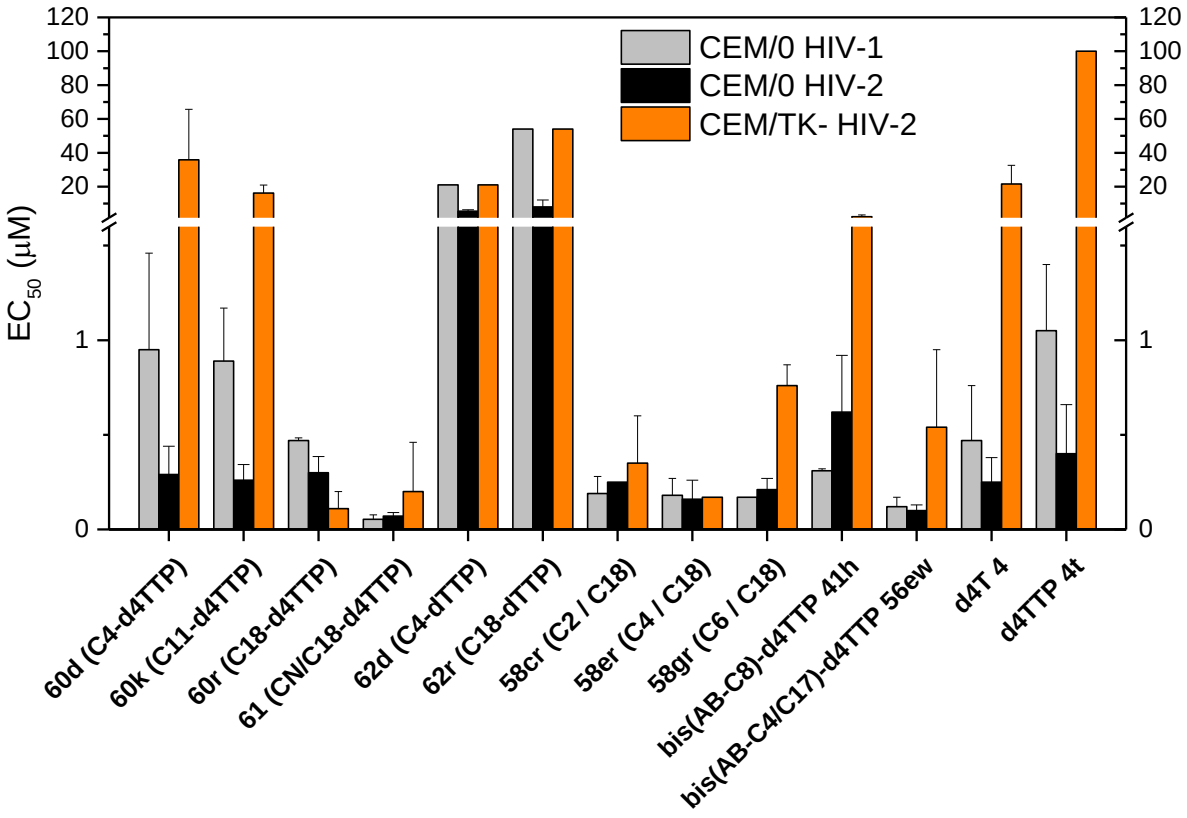
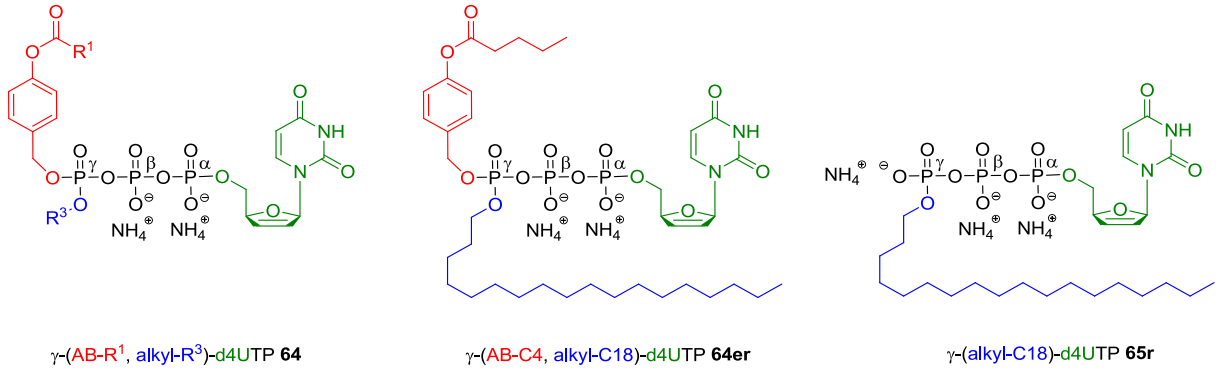


Figure 4-27: Antiviral activity of γ-(β-Cyanoethoxy,alkyl-C18)-d4TTP **61r** and γ-(alkyl)-NTP **60**, **62** in comparison to the parent nucleoside d4T **4**, d4TTP **4t**, γ-(AB-C4,alkyl-C18)-d4TTP **58er** and γ-(AB,ab)-d4TTPs **41h**, **56ew** in bar chart.

4.6 Other γ-Modified NTPs

4.6.1 γ-(AB-C4,alkyl-C18)-d4UTP 64er

γ-(AB-C4,alkyl-C18)-d4UTP **64er** was synthesized following the *H*-phosphonate route which has discussed before in Chapter 4.4.1 and 4.4.2. The yield from d4UMP 5**4m** to **64er** was 33%. The corresponding chemical structures are shown in Figure 4-28.



γ-(AB-R[1], alkyl-R[3])-d4UTP **64** γ-(AB-C4, alkyl-C18)-d4UTP **64er** γ-(alkyl-C18)-d4UTP **65r**

Figure 4-28: The structure of γ-(AB-C4,alkyl-C18)-d4UTP **64er**. D4U is in green. AB-mask is in red. 1-octadecyloxy group is in blue.

The hydrolysis data (Table 4-13) showed that, γ-(AB-C4,alkyl-C18)-d4UTP **64er** is more stable than γ-(AB-C4,alkyl-C18)-d4TTP **58er**. The half-lives of **64er** are more than 2-fold higher than those of **58er** both in PBS and CEM cell extracts.

Comp.	Nucl.	R^1	R^3	PBS pH=7.3 [h]	CEM cell extracts [h]
				$t_{1/2}$	$t_{1/2}$
58er	d4T	*n*-C_4H_9 **44e**	*n*-$C_{18}H_{37}$	237	4.8
64er	d4U	*n*-C_4H_9 **44e**	*n*-$C_{18}H_{37}$	578	9.5

Table 4-13: Half-lives of γ-(AB-C4,alkyl-C18)-d4UTP prodrugs **64er** in comparison with γ-(AB-C4,alkyl-C18)-d4TTP prodrugs **58er** in PBS and CEM cell extracts.

In PBS, the incubation time took 1600 h and only small amounts of γ-(alkyl-C18)-d4UTP **65r** and trace amount of d4UDP **4d** were detected during hydrolysis. Uracil was observed, and its concentration increased gradually. All these results suggested that the hydrolysis of prodrug γ-(AB-C4,alkyl-C18)-d4UTP **64er** is a result of the cleavage of the glycosidic bond.

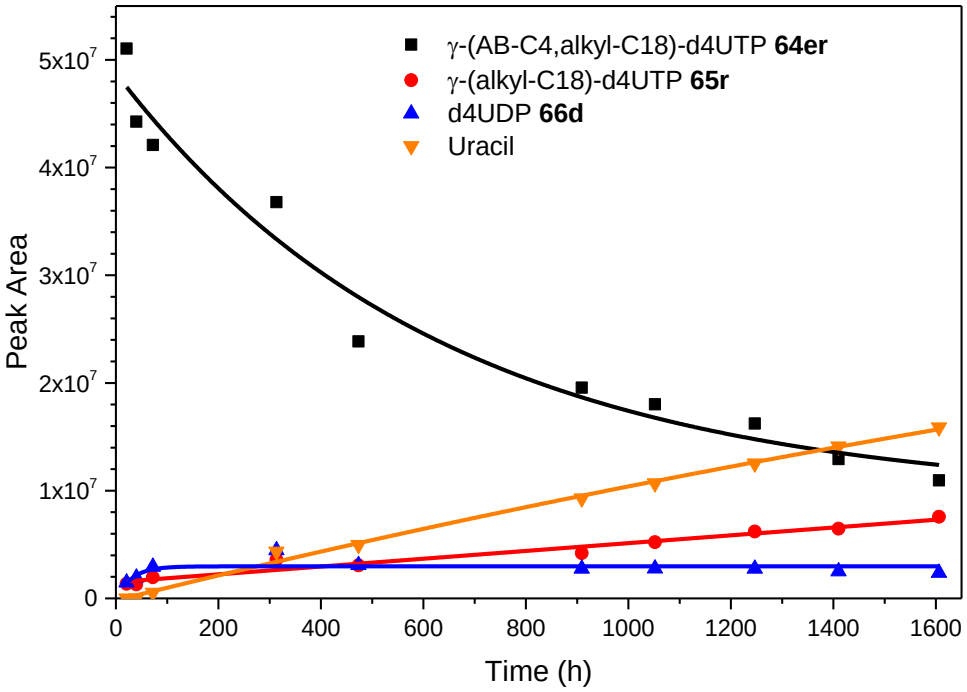


Figure 4-29: Hydrolysis of γ-(AB-C4,alkyl-C18)-d4UTP **64er** (black square) in **PBS**. γ-(alkyl-C18)-d4UTP **65r** is in red dots. D4UDP **54d**, and uracil are in blue and orange triangle, respectively.

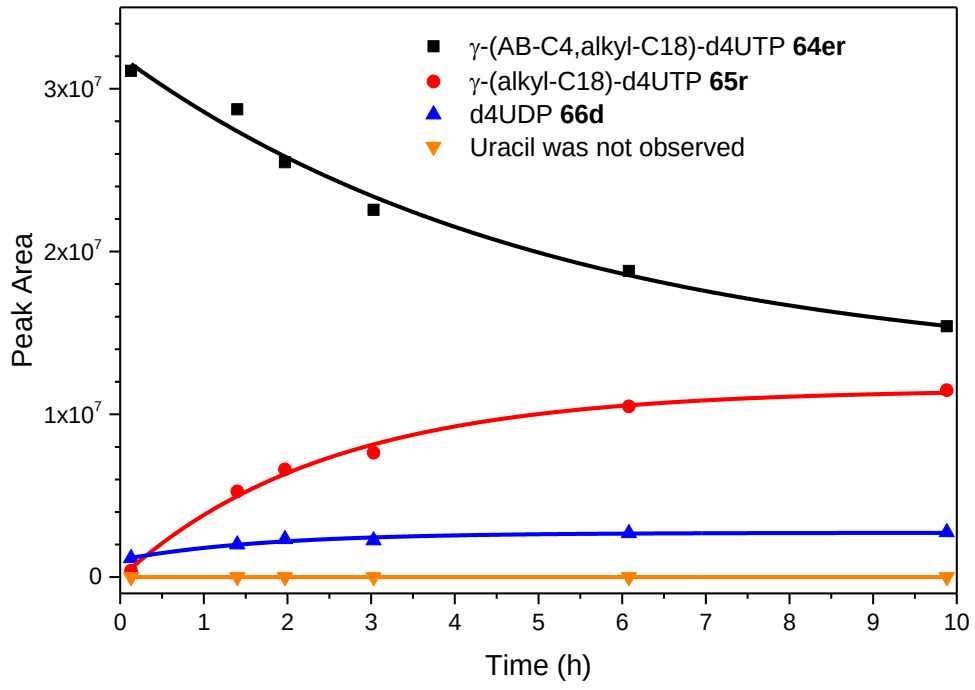


Figure 4-30: Hydrolysis of γ-(AB-C4,alkyl-C18)-d4UTP **64er** (black square) in **CEM cell extracts**. γ-(alkyl-C18)-d4UTP **65r** is in red dots. D4UDP **54d**, and uracil are in blue and orange triangle, respectively.

In CEM cell extracts, γ-(AB-C4,alkyl-C18)-d4UTP **64er** was also found to be as twice stable as γ-(AB-C4,alkyl-C18)-d4TTP **58er**. Except the difference of half-life, the

hydrolysis patterns of these two compounds were almost identical in CEM cell extracts.

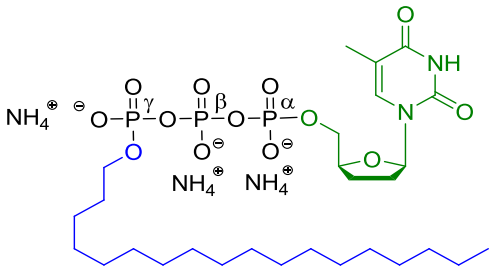
Even though γ-(AB-C4,alkyl-C18)-d4UTP **64er** was more stable than **58er** and these two compounds have similar structure and lipophilicity, γ-(AB-C4,alkyl-C18)-d4UTP **64er** is not an active prodrug against HIV in either CEM/0 cells or CEM/TK$^-$ cells (Table 4-14).

Compound	EC_{50} [µM][a]			CC_{50} [µM][b]
	CEM/0		CEM/TK$^-$	CEM/0
	HIV-1	HIV-2	HIV-2	
58er (C4 / C18, d4T)	0.18 ± 0.09	0.16 ± 0.10	0.17 ± 0.00	13 ± 1
64er (C4 / C18, d4U)	13 ± 4.3	9.6 ± 0.064	11 ± 2.6	40 ± 14
d4T **4**	0.43 ± 0.25	0.22 ± 0.05	>50	>50
d4U **54**	>250[c]	>250[c]	>250[c]	>250[c]

Table 4-14: Antiviral activity and cytotoxicity of γ-(AB-C4,alkyl-C18)-d4UTP prodrugs **64er** in comparison to the parent nucleoside d4T **4**, d4U **54** and γ-(AB-C4,alkyl-C18)-d4TTP prodrugs **58er**. [a] Antiviral activity in CD4$^+$ T-lymphocytes: 50% effective concentration; values are the mean ±SD of n=2-3 independent experiments. [b] Cytotoxicity: 50% cytostatic concentration or compound concentration required to inhibit CD4$^+$ T-cell (CEM) proliferation by 50%; values are the mean ±SD of n=2-3 independent experiments. [c] Data was afforded from reference.[152]

4.6.2 γ-(Alkyl-C18)-ddTTP 67r

γ-(Alkyl-C18)-ddTTP **67r** is another γ-(alkyl)-NTP compound. It was synthesized from the *H*-phosphonate **59r** and ddTMP **68m**. The chemical structure of **67r** is shown in Figure 4-31.



γ-(alkyl-C18)-ddTTP **67r**

Figure 4-31: The chemical structure of γ-(alkyl-C18)-ddTTP **67r**. Nucleoside ddT is in green. The 1-octadecyloxy group is in blue.

Discussion

The hydrolysis data showed that γ-(alkyl-C18)-ddTTP **67r** as well as γ-(alkyl-C18)-d4TTP **60r** were stable in PBS and CEM cell extracts. The stabilities of these two compounds were similar.

Comp.	Nucl.	R^1	R^3	PBS pH=7.3 [h]	CEM cell extracts [h]
				$t_{1/2}$	$t_{1/2}$
67r	ddT	/	*n*-$C_{18}H_{37}$	No hydrolysis	>30
60r	d4T	/	*n*-$C_{18}H_{37}$	No hydrolysis	>30

Table 4-15: Half-lives of γ-(alkyl-C18)-ddTTP **67r** in comparison with γ-(alkyl-C18)-d4TTP **60r** in PBS and CEM cell extracts.

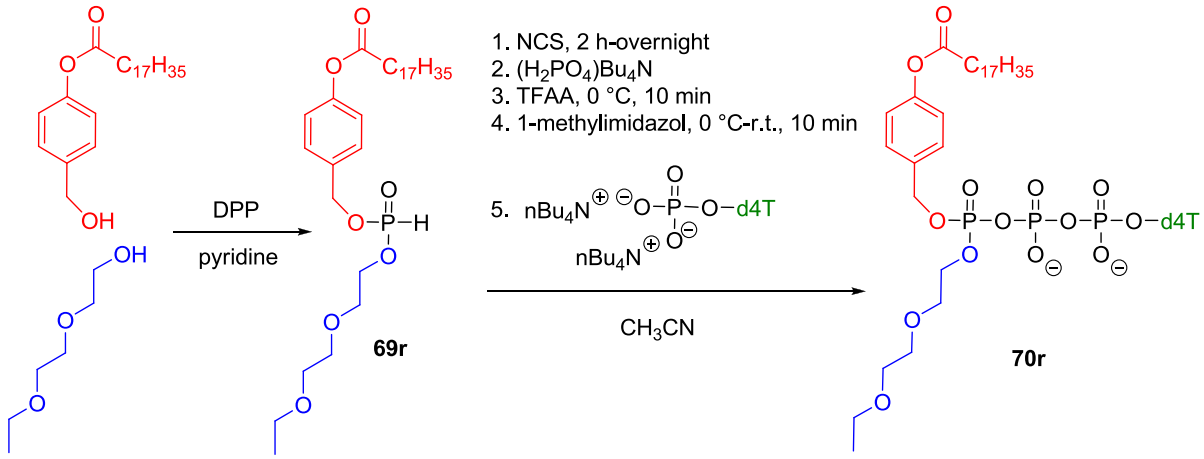
In the antiviral test, it was observed that γ-(alkyl-C18)-ddTTP **67r** expressed weak activity in CEM/0 against HIV-2. However, it is not an active compound against HIV-1 in CEM/0 and more importantly not active against HIV-2 in CEM/TK$^-$ cell (Table 4-16). Following to this result, ddT **68** is not an ideal NRTI substrate in this prodrug concept.

Compound	EC_{50} [µM][a]			CC_{50} [µM][b]
	CEM/0		CEM/TK$^-$	CEM/0
	HIV-1	HIV-2	HIV-2	
60r (C18, d4T)	0.47 ± 0.014	0.30 ± 0.085	0.11 ± 0.09	86 ± 3
67r (C18, ddT)	>100	7.39±4.02	>100	>100
d4T **4**	0.43 ± 0.25	0.22 ± 0.05	>50	>50
ddT **68**	8.32 ± 2.42	3.42 ± 0.51	>100	>100

Table 4-16: Antiviral activity and cytotoxicity of γ-(alkyl-C18)-ddTTP **67r** in comparison to the parent nucleoside d4T **4**, ddT **68** and γ-(alkyl-C18)-d4TTP **60r**. [a] Antiviral activity in $CD4^+$ T-lymphocytes: 50% effective concentration; values are the mean ±SD of n=2-3 independent experiments. [b] Cytotoxicity: 50% cytostatic concentration or compound concentration required to inhibit $CD4^+$ T-cell (CEM) proliferation by 50%; values are the mean ±SD of n=2-3 independent experiments.

4.6.3 γ-(AB-C17,alkyl-EEE)-d4TTP 70r

As discussed before, γ-(AB-C15,alkyl-C4)-d4TTP **58ud** were surprisingly found to be inactive against HIV-2 in CEM/TK$^-$ cells and this might be due to an insufficient lipophilicity of the compound combined with a relatively fast cleavage of the bioreversible moiety which led to the formation of short-chain γ-(alkyl-C4)-d4TTP **60d**. Compound **60d** was almost inactive in this assay. In addition, the AB-mask moiety with a C15 alkyl chain also showed unexpected properties as compared to C14 and C17 in the hydrolysis (**56eo**, **56eu** and **56ew**). The prodrug γ-(AB-C15,alkyl-C15)-d4TTP **58uo** also exhibited no activity against HIV-2 in CEM/TK$^-$ cells. AB-mask moiety with C15 alkyl chain is not suitable for studying new γ-modified NTPs. Thus, a further prodrug was developed. It is a d4TTP derivative which is modified with an AB-C17-mask and one 2-(2-ethoxyethoxy)ethan-1-oxy group at γ-phosphate. The AB-mask moiety was designed to provide lipophilicity of the triphosphate prodrug for membrane permeability. The 2-(2-ethoxyethoxy)ethan-1-oxy group replaced the alkyl moiety used before and it is supposed to be stable at chemical and biological condition as a non-cleavable group. As 2-(2-ethoxyethoxy)ethan-1-oxy group does not provide lipophilicity as alkyl moieties, the membrane permeability of this prodrug can mostly be attributed the AB-mask. Thus, by studying this compound, it will help us to understand whether one AB-C17-mask is enough for membrane penetration. The synthesis of γ-(AB-C17,alkyl-EEE)-d4TTP **70r** is shown below in Scheme 4-14.



Scheme 4-14: Synthesis of γ-(AB-C17,alkyl-EEE)-d4TTP **70r**. 1). NCS, CH_3CN, rt, 2 h; 2) $(H_2PO_4)Bu_4N$, CH_3CN, rt, 1 h; 3). TFAA, Et_3N, CH_3CN, 0 °C, 10 min; 4) 1-methylimidazol, Et_3N, CH_3CN, 0 °C-RT, 10 min; 5) d4TMP **4m**, rt, 3-5 h, rp-chromatography, Dowex 50WX8 (NH_4^+ form) ion exchange, rp-chromatography.

Discussion

The half-life of prodrug γ-(AB-C17,alkyl-EEE)-d4TTP **70r** is 86 h in PBS and 1.4 h in CEM cell extracts. Compared to prodrug γ-(AB-C15,alkyl-C4)-d4TTP **58ud** ($t_{1/2}$ = 198 h), the modification with 2-(2-ethoxyethoxy)ethan-1-oxy group greatly increased the hydrolysis rate of prodrug **70r**. However, the half-lives of these two compounds in CEM cell extracts were found to be in the same range.

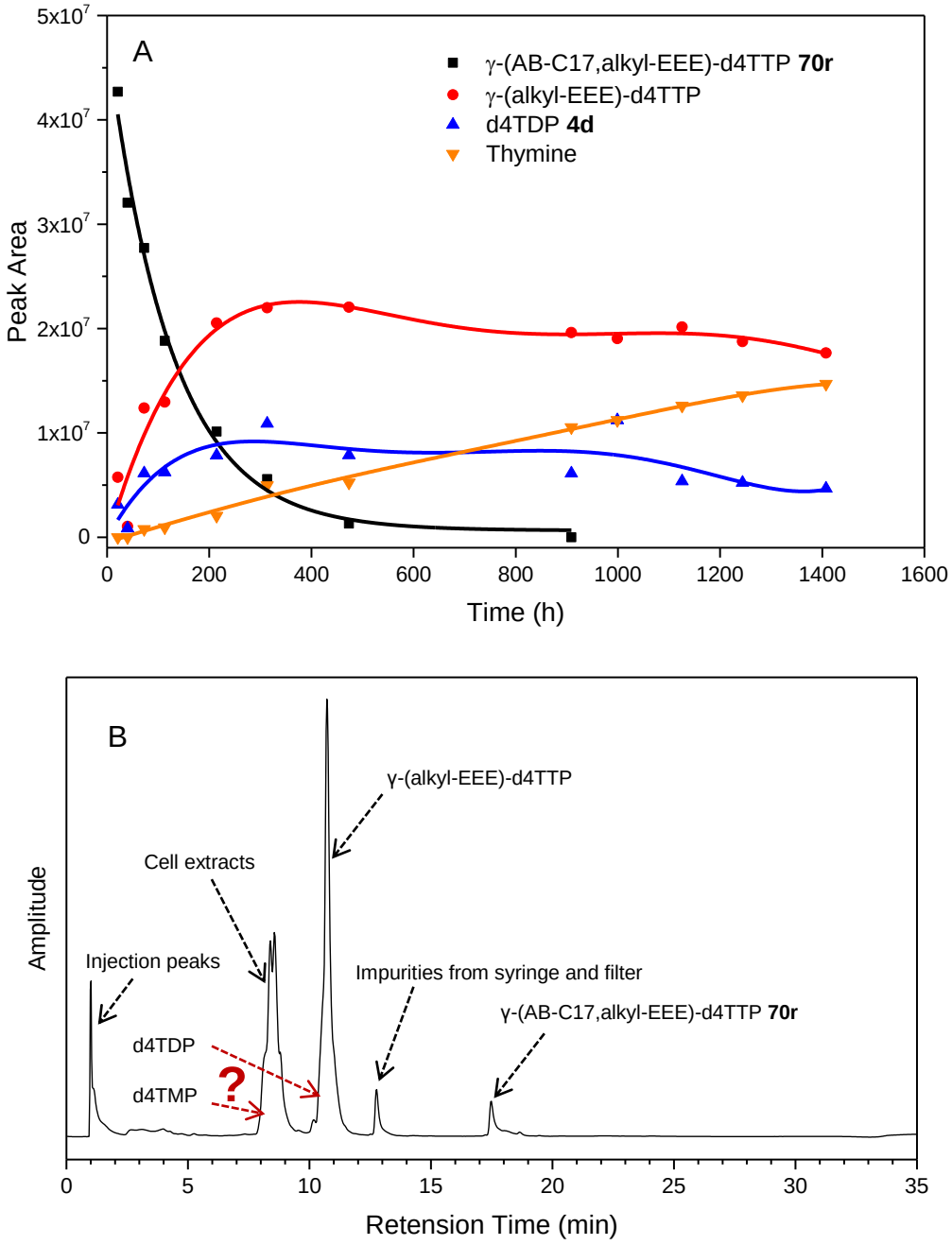


Figure 4-31: (A) Chemical hydrolysis of γ-(AB-C17,alkyl-EEE)-d4TTP **70r** (black square) in **PBS**. γ-(alkyl-EEE)-d4TTP is in red dots. D4TDP **4d**, and thymine are in blue and orange triangle, respectively. (B) Chromatogram of γ-(AB-C17,alkyl-EEE)-d4TTP **70r** in CEM cell extracts at incubation time of 480 min.

Discussion

In PBS (table 4-31, A), prodrug γ-(AB-C17,alkyl-EEE)-d4TTP **70r** was totally hydrolyzed within 900 h. γ-(Alkyl-EEE)-d4TTP was formed and reached maximum concentration after 300 h of incubation. Afterwards, its concentration slightly reduced until the end of hydrolysis (1400 h). Low concentrations of d4TDP **4d** were observed. As expected, d4TTP **4t** was not detected. Thymine was also formed after long incubation times. In cell extracts, when γ-(alkyl-EEE)-d4TTP was formed in a certain concentration, it became difficult to assign the peaks of d4TDP **4d**, d4TMP **4m**, cell extracts and γ-(alkyl-EEE)-d4TTP.

Antiviral data showed that prodrug **70r** has similar activity against HIV-1 and HIV-2 in CEM/0 cells compared to d4T **4**, while its activity is 10-fold higher than d4T against HIV-2 in CEM/TK$^-$ cells. Moreover, the comparison of the antiviral data between **70r** and **58ud** which have similar structures suggested that when AB-mask containing a very long alkyl chain residue (e.g. C17) could provide enough lipophilicity for membrane permeation and showed activity in CEM/TK$^-$ cells.

	EC_{50} [µM][a]			CC_{50} [µM][b]
Compound	CEM/0		CEM/TK$^-$	CEM/0
	HIV-1	HIV-2	HIV-2	
70r (AB-C17,alkyl-EEE)	0.14 ± 0.021	0.16 ± 0.17	5.2 ± 4.4	49 ± 32
58ud (AB-C15,alkyl-C4)	0.33 ± 0.11	0.24 ± 0.01	>10	21 ± 2
d4T **4**	0.13±0.035	0.20±0.10	>50	>50

Table 4-17: Antiviral activity and cytotoxicity of γ-(AB-C17,alkyl-EEE)-d4TTP **70r** in comparison to the parent nucleoside d4T **4**, and γ-(AB-C15,alkyl-C4)-d4TTP **58ud**. [a] Antiviral activity in $CD4^+$ T-lymphocytes: 50% effective concentration; values are the mean ±SD of n=2-3 independent experiments. [b] Cytotoxicity: 50% cytostatic concentration or compound concentration required to inhibit $CD4^+$ T-cell (CEM) proliferation by 50%; values are the mean ±SD of n=2-3 independent experiments.

4.7 Primer Extension Assays

In the antiviral assays, all compounds described here showed significant antiviral activity against HIV-1 and HIV-2-infected CEM cells. This activity was also mainly retained in CEM/TK-deficient cells. Taken the results from the hydrolysis studies that no d4TTP was formed in all the hydrolysis studies, it can be concluded that the delivered γ-(alkyl)-d4TTPs **60** are responsible for the inhibitory effect of these compounds. This is a clear difference to the formerly reported Tri*PPP*ro-compounds **41** and **56**. To shed more light into these results, primer extension assays were performed investigating the substrate properties of the γ-modified triphosphates to three different DNA-polymerases: HIV-1 reverse transcriptase (HIV-1 RT) which is an RNA-dependent-DNA-polymerase, human DNA-polymerase β (Pol β) and human DNA-polymerase γ (Pol γ), which is a mitochondrial polymerase and often responsible for toxic side effects caused by nucleoside analogue triphosphates. A 25nt DNA primer with FITC as fluorescent label and a 30nt DNA template was used.[153,154] The exact sequence of primer and template is listed in the experimental section.

In each experiment a primer extension without added polymerase (negative control (- lane)) and an experiment in which all four natural NTP were added (positive control (+ lane)) were used as controls. Two different set-ups have been used. First, a mixture of dATP, dCTP, dGTP and γ-(alkyl)-d4TTP **60** (**T***) or –TTP **62** (**N***) was used as a mixture. The mixture of dATP, dCTP, dGTP and d4TTP **4t** was summarized as **d4TTP***. Then the results obtained from these experiments were compared to those in which just γ-(alkyl)-d4TTP (**C_n-d4T**) or -TTP (**C_n-dT**) was used.

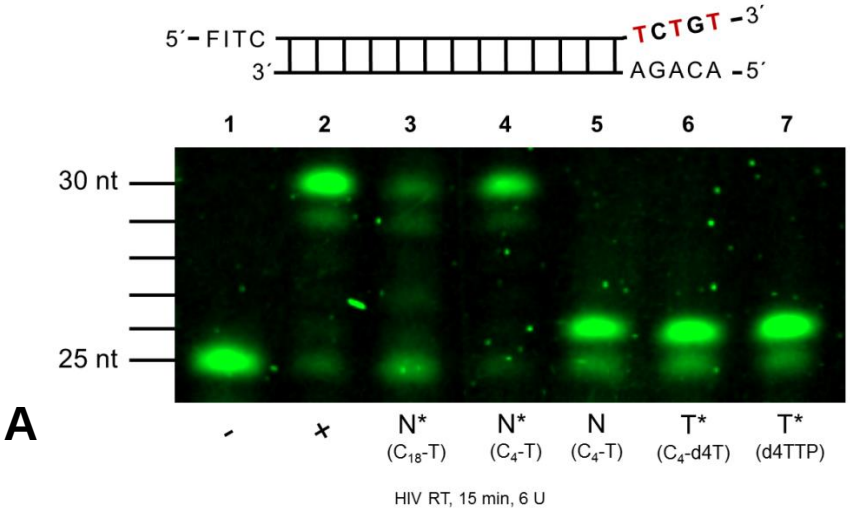
The fluorescent photograms of the primer extension assay are shown below, in which the viral enzyme HIV-RT was used. The 25nt primer must be fully elongated by the polymerase to a 30mer by addition of the sequence TCTGT (Figure 4-30). As can be seen, in the case of a missing RT or POL, no elongation proceeded (- lane). However, with enzyme and the mixture of all four canonical triphosphates, full extension to the 30mer occurred (+ lane). For HIV RT, 1 unit is the amount of enzyme required to incorporate 1 nmol of labeled TTP into acid-insoluble material in 10 min at 37 °C. Thus, for 3.2 nmol hybridization of 25nt primer and 30nt template and 6U HIV RT were added and the 25mer can be fully extended to form the 30mer oligonucleotide only after 26 min incubation time, theoretically.

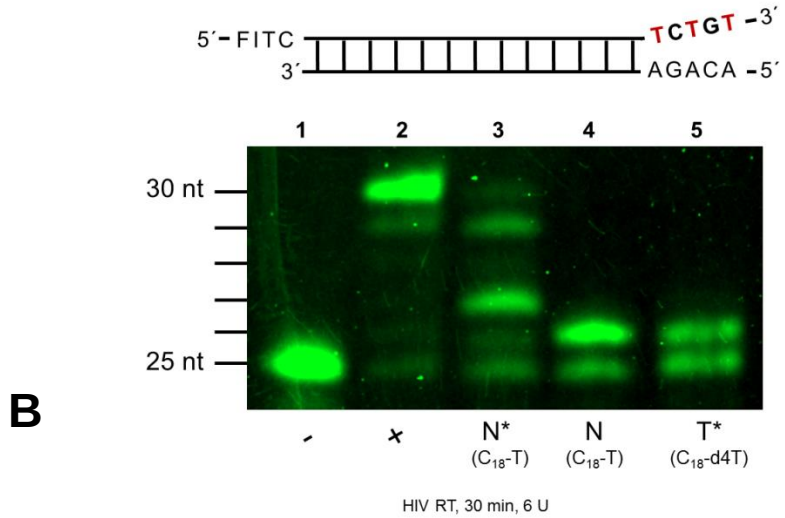
4.7.1 Primer Extension Assay with HIV-RT

4.7.1.1 Assay with 6U HIV RT in different incubation time

As can be seen, in the case of the mixture labeled "N* (C_4-dT)" (dCTP, dATP, dGTP and γ-(alkyl-C4)-TTP **62d**), again a full extension of the primer to n+5 (30 nt) was observed for the incubation time of 15 min (Figure 4-32, A). However, when the mixture labeled "N* (C_{18}-dT)" (dCTP, dATP, dGTP and γ-(alkyl-C18)-TTP **62r**) was used and incubated at the same condition and time, only 28% of the 25nt primer was extended to the 30mer (Figure 4-32, A). After the incubation time was prolonged to 60 min, then the "N* (C_{18}-dT)" lane was proved full elongation to the 30mer (Figure 4-32, C). Similar phenomenon was also observed by comparison of lane T* (C_4-d4T) in Figure 4-32 A and lane T* (C_{18}-d4T) in Figure 4-32 B and C. This complete strand extension implies that HIV-RT recognizes the γ-(alkyl)-NTP **60**, **62** as a substrate and TMP was incorporated three times more efficiently into the growing DNA strand. It also revealed that with a long alkyl moiety, the incorporation efficacy of γ-(alkyl)-NTPs by HIV RT was decreased. In other words, γ-(alkyl)-NTPs with longer alkyl group need more time to get fully incorporated by HIV RT than those with shorter alkyl group.

The comparison of lane T* (C_4-d4T) and lane d4TTP* in Figure 4-32 A showed that the use of γ-(alkyl-C4)-d4TTP **60d** by HIV RT is less efficient than that of d4TTP **4t**. An identical conclusion can be taken that γ-(alkyl-C18)-d4TTP **60r** also lost some of the incorporation efficacy compared to d4TTP **4t** (Figure 4-32 C). Furthermore, in Figure 4-32 B, for lane "N* (C_{18}-dT)", the most intensive band is n+2 (27nt). It means after the first γ-(C18)-TTP **62r** was utilized, the 26nt primer was fast extended to 27mer with incorporation of dCMP from dCTP and then the elongation the primer slowed down while a second TMP from γ-(alkyl-C18)-TTP **62r** has to be incorporated The phenomenon of delayed chain termination occurred. This observation again implied that the use of γ-(alkyl-C18)-TTP **62r** by HIV RT is much slower than that of γ-(alkyl-C4)-TTP **62d** and d4TTP **4t**. In full agreement with this result, a single incorporation of TMP starting from γ-(alkyl-C4)-TTP **62d** (lane C_4-dT) and γ-(alkyl-C18)-TTP **62r** (lane C_{18}-dT) was observed. Consequently, both γ-(alkyl)-d4TTP **60** and γ-(alkyl)-TTP **62** are substrates of HIV-RT.





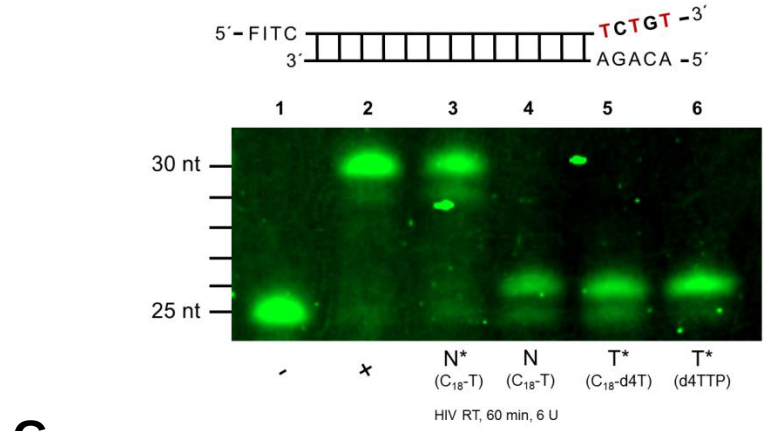


Figure 4-32: Primer extension assay with **6 U** HIV RT. Assay condition: 50 mM Tris-HCl (pH 8.6 at 22 °C), 10 mM $MgCl_2$, 40mM KCl, dNTPs 66 µM, HIV RT 6 U, hybrid 0.32 µM in a reaction volume of 10 µL, incubated 37 °C for 15 min, 30 min or 60 min, 80 °C for 3 min; 50 mA, 45w for 4 h.

Lane 1 (-): dATP, dGTP, dCTP and TTP without HIV RT; lane 2 (+):dATP, dGTP, dCTP and TTP with HIV RT; (A) with 15 min incubation time; lane 3 (N*, C_{18}-T): dATP, dGTP, dCTP and γ-(alkyl-C18)-TTP **62r**; lane 4 (N*, C_4-T): dATP, dGTP, dCTP and γ-(alkyl-C4)-TTP **62d**; lane 5 (N, C_4-T): γ-(alkyl-C4)-

TTP **62d**; lane 6 (T*, C_4-d4T): dATP, dGTP, dCTP and γ-(alkyl-C4)-d4TTP **60d**; lane 7 (T*, d4TTP): dATP, dGTP, dCTP and d4TTP **4t**; (B) with 30 min incubation time; lane 3 (N*, C_{18}-T): dATP, dGTP, dCTP and γ-(alkyl-C18)-TTP **62r**; lane 4 (N, C_{18}-T): γ-(alkyl-C18)-TTP **62r**; lane 5 (T*, C_{18}-d4T): dATP, dGTP, dCTP and γ-(alkyl-C18)-d4TTP **60r**; (C) with 60 min incubation time; lane 3 (N*, C_{18}-T): A, G, C and γ-(alkyl-C18)-TTP **62r**; lane 4 (N, C_{18}-T): γ-(alkyl-C18)-TTP **62r**; lane 5 (T*, C_{18}-d4T): dATP, dGTP, dCTP and γ-(alkyl-C18)-d4TTP **60r**; lane 6 (T*, d4TTP): dATP, dGTP, dCTP and d4TTP **4t**.

4.7.1.2 Incubation efficacy of γ-(alkyl)-d4TTPs 60d, 60k and 60r by HIV RT

To gain more insight into the utilization efficacy of γ-(alkyl)-d4TTPs **60**, an experiment was conducted using γ-(alkyl-C4)-d4TTP **60d** (lane T* (C_4-d4T)), γ-(alkyl-C11)-d4TTP **60k** (lane T* (C_{11}-d4T)) and γ-(alkyl-C18)-d4TTP **60r** (lane T* (C_{18}-d4T)) with different incubation times (10-60 min). The amount of the spot on the fluorescent photogram can be quantified by using appropriate computer softwares.

As shown in Figure 4-33, γ-(alkyl-C4)-d4TTP **60d** and γ-(alkyl-C11)-d4TTP **60k** were utilized by HIV RT at similar efficacy (in the conversion from the primer 25mer to 26mer is only 1-2% difference). When the alkyl group was increased from C11 to C18 (1-octadecyl), the conversion of the primer to the 26mer oligonucleotide was reduced with the amount of 13% at 10 min, 14% at 20 min, 10% at 40 min and 7% at 60 min. This observation clearly showed that γ-(alkyl-C18)-d4TTP **60r** is not efficiently utilized by HIV RT compared to γ-(alkyl-C4)-d4TTP **60d** and γ-(alkyl-C11)-d4TTP **60k**. However, the loss of efficacy by HIV RT did not correlate with a loss of antiviral activity against HIV-1 in CEM/0 (Table 4-12).

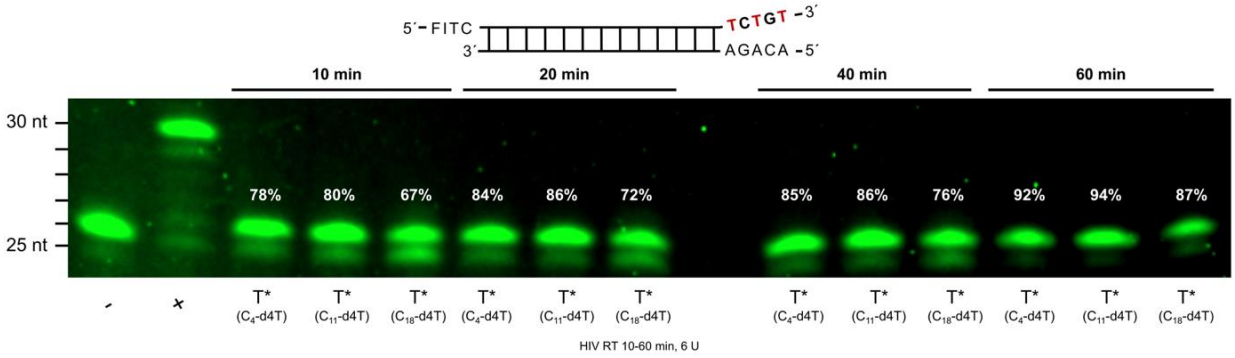


Figure 4-33: Primer extension assay with **6 U** HIV RT and different incubation time using γ-(alkyl-C4)-d4TTP **60d** (lane T* (C_4-d4T)), γ-(alkyl-C11)-d4TTP **60k** (lane T* (C_{11}-d4T)) and γ-(alkyl-C4)-d4TTP **60r** (lane T* (C_{18}-d4T)) as substrates. Assay condition: 50 mM Tris-HCl (pH 8.6 at 22 °C), 10 mM $MgCl_2$, 40 mM KCl, dNTPs 66 µM, HIV RT 6 U, Hybrid 0.32 µM in a reaction volume of 10 µL, incubated 37 °C for 10 min, 20 min, 40 min and 60 min, 80 °C for 3 min; 50 mA, 45w for 4 h.

Lane (-): dATP, dGTP, dCTP and TTP without HIV RT; lane (+): dATP, dGTP, dCTP and TTP with HIV RT; lane (T*, C_4-d4T): dATP, dGTP, dCTP and γ-(alkyl-C4)-d4TTP **60d**; lane (T*, C_{11}-d4T): dATP,

dGTP, dCTP and γ-(alkyl-C11)-d4TTP **60k**; lane (T*, C_{18}-d4T): dATP, dGTP, dCTP and γ-(alkyl-C18)-d4TTP **60r**.

4.7.1.3 Incubation efficacy competition: TTP vs γ-(alkyl-C18)-d4TTP 60r

Next, we studied the competition between thymidine triphosphate (TTP) and γ-(alkyl-C18)-d4TTP **60r**. As shown in Table 4-18, 66 µM of dATP, dGTP and dCTP was kept identical in every incubation. The total amount of TTP and γ-(alkyl-C18)-d4TTP **60r** was also identical. From lane 1 to lane 10, the ratio of TTP/ γ-(alkyl-C18)-d4TTP **60r** differed from 10:1 to 1:10. The result is shown in Figure 4-34, the 25nt primer was elongated and showed full extension to 30mer in the presence of TTP. Unfortunately, when the ratio of TTP / γ-(alkyl-C18)-d4TTP **60r** was changed from 10:1 to 1:10, the competition relationship it is hard to observe from the diagram. After calculation of the band by software, it can be concluded that when the ratio of TTP/**60r** was changed from 10:1 to 1:10, the conversion from 25mer to 30mer was reduced from 83% to 71%. It means that HIV RT prefers to use TTP as a substrate instead of γ-(alkyl-C18)-d4TTP **60r**. When excess of γ-(alkyl-C18)-d4TTP **60r** are existent (TTP:**60r** =1:10), only a slight competition phenomenon can be observed. Thus, a new experiment was needed to clarify the competition between TTP and γ-(alkyl-C18)-d4TTP **60r**.

Unit: µM	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
dATP, dGTP, dCTP	66									
TTP	60	54	48	42	36	30	24	18	12	6
γ-(alkyl-C18)-d4TTP **60r**	6	12	18	24	30	36	42	48	54	60
The ratio of TTP:**60r**	10:1	9:2	8:3	7:4	6:5	5:6	4:7	3:8	2:9	1:10

Table 4-18: The condition of primer extension assay for the competition between TTP and γ-(alkyl-C18)-d4TTP **60r** with 6 U HIV RT.

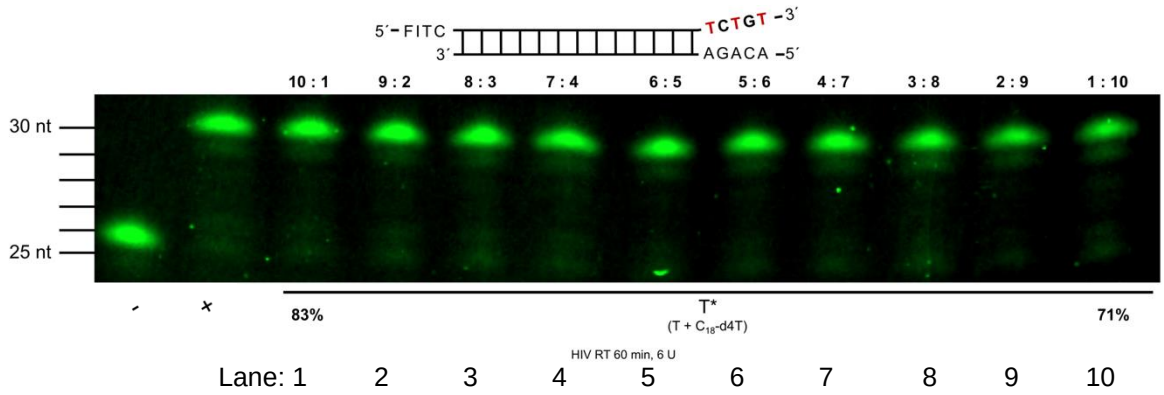


Figure 4-34: Primer extension assay for the competition between TTP and γ-(alkyl-C18)-d4TTP **60r** with 6U HIV RT. Assay condition: 50 mM Tris-HCl (pH 8.6 at 22 °C), 10 mM $MgCl_2$, 40mM KCl, dNTPs 66 µM, TTP or γ-(alkyl-C18)-d4TTP **60r** 6-60 µM, HIV RT 6 U, Hybrid 0.32 µM in a reaction volume of 10 µL, incubated 37 °C for 60 min, 80 °C for 3 min; 50 mA, 45w for 4 h.
Lane (-): dATP, dGTP, dCTP and TTP without HIV RT; lane (+): dATP, dGTP, dCTP and TTP with HIV RT; lane 1-10 (T*, T + C_{18}-d4T): dATP, dGTP, dCTP, TTP and γ-(alkyl-C18)-d4TTP **60r** (exact amounts are shown in Table 4-18).

As the weak competition phenomenon of TTP:**60r** is probably because of the large amount of the HIV RT (6U), we conducted another experiment by using just 1.2 U of HIV RT and raised the ratio of TTP:**60r** up to 1:20 and 1:40 (condition can be seen in Table 4-19). The assay is summarized in Figure 4-35. Interestingly, when the ratio of TTP:**60r** is 1:10 (lane 4), which is the same ratio as in Figure 4-34 lane 10, only trace amounts of the n+2 band (27nt) can be observed and the most intensive band is still the 25mer band (25nt) at the baseline. Thus, from this result, we assume that when excess γ-(alkyl-C18)-d4TTPs **60r** tried to bind to the active site of HIV-RT, they probably blocked the reaction center and prevent TTPs approaching.

Unit: µM	**1**	**2**	**3**	**4**	**5**	**6**
dATP, dGTP, dCTP	66	66	66	66	63	123
TTP	60	54	48	6	3	3
γ-(alkyl-C18)-d4TTP **60r**	6	12	18	60	60	120
The ratio of TTP:**60r**	10:1	7:4	4:7	1:10	0.5:10	0.5:20

Table 4-19: The condition of primer extension assay for the competition between TTP and γ-(alkyl-C18)-d4TTP **60r** under 1.2 U HIV RT.

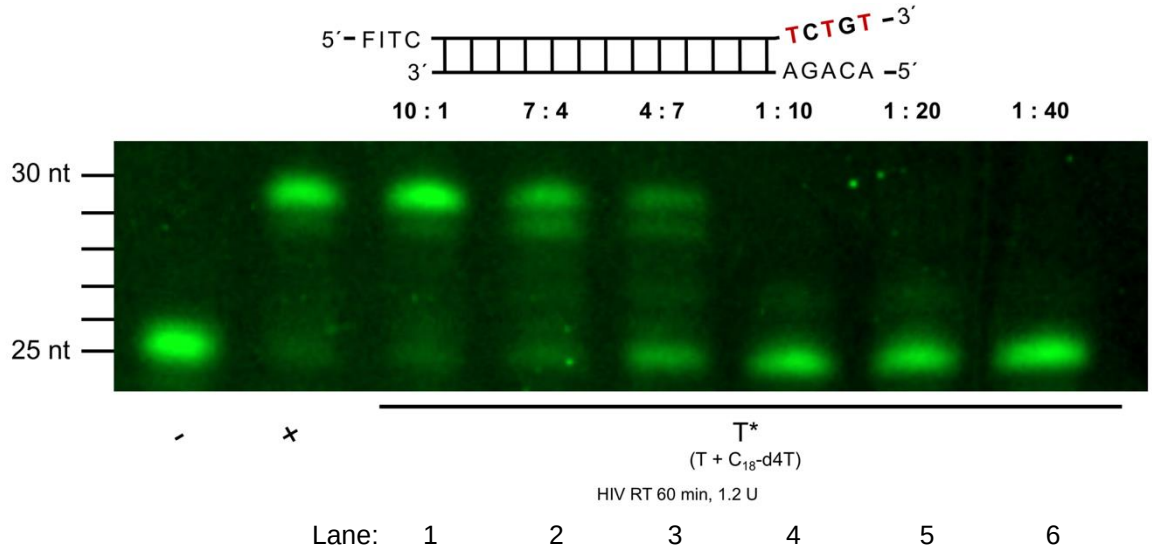


Figure 4-35: Primer extension assay for the competition between TTP and γ-(alkyl-C18)-d4TTP **60r** with 1.2 U HIV RT. Assay condition: 50 mM Tris-HCl (pH 8.6 at 22 °C), 10 mM $MgCl_2$, 40 mM KCl, dNTPs 66-123 µM, TTP or γ-(alkyl-C18)-d4TTP **60r** 3-120 µM, HIV RT 1.2 U, Hybrid 0.32 µM in a reaction volume of 10 µL, incubated 37 °C for 60 min, 80 °C for 3 min; 50 mA, 45w for 4 h.
Lane (-): dATP, dGTP, dCTP and TTP without HIV RT; lane (+): dATP, dGTP, dCTP and TTP with HIV RT; lane 1-6 (T*, T + C_{18}-d4T): dATP, dGTP, dCTP, TTP and γ-(alkyl-C18)-d4TTP **60r** (exact amounts are shown in Table 4-19).

The total amount of TTP and γ-(alkyl-C18)-d4TTP **60r** was fixed at 66 µM for a reaction volume of 10 µL in the two experiments above. Then we started another experiment to see what will happen when the TTP concentration fixed at 15 µM while the concentration of γ-(alkyl-C18)-d4TTP **60r** was varied. At this time, we used 6 U HIV RT again at the beginning and the incubation time lasted 60 min. The ratio of TTP/γ-(alkyl-C18)-d4TTP **60r** was from 2:1 to 1:16 (Table 4-20). Results can be seen in Figure 4-36. When the ratio of TTP:**60r** was up to 1:16 (lane 6), extension to 30mer was observed but in a very low level. Additionally, the 25mer was found to be the most intensive band.

Unit: µM	**1**	**2**	**3**	**4**	**5**	**6**
dATP, dGTP, dCTP	45	30	45	75	135	255
TTP	30	15	15	15	15	15
γ-(alkyl-C18)-d4TTP **60r**	15	15	30	60	120	240
The ratio of TTP:**60r**	2:1	1:1	1:2	1:4	1:8	1:16

Table 4-20: The condition of primer extension assay for the competition between TTP and γ-(alkyl-C18)-d4TTP **60r** with 6 U HIV RT.

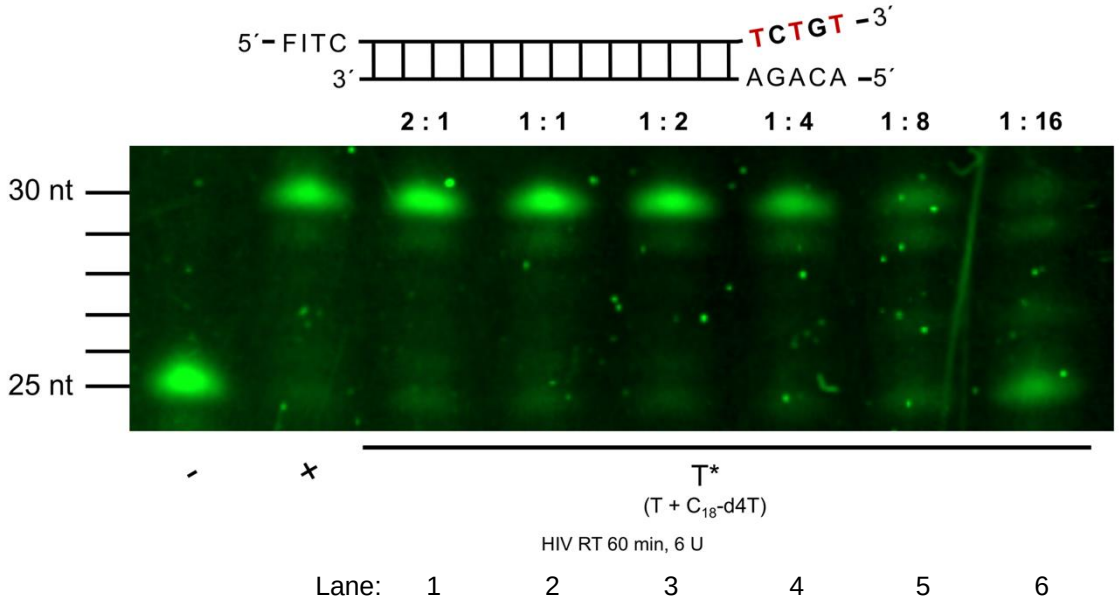


Figure 4-36: Primer extension assay for the competition between TTP and γ-(alkyl-C18)-d4TTP **60r** with 6 U HIV RT. Assay condition: 50 mM Tris-HCl (pH 8.6 at 22 °C), 10 mM $MgCl_2$, 40 mM KCl, dNTPs 45-255 µM, TTP or γ-(alkyl-C18)-d4TTP **60r** 15-240 µM, HIV RT 6 U, Hybrid 0.32 µM in a reaction volume of 10 µL, incubated 37 °C for 60 min, 80 °C for 3 min; 50 mA, 45w for 4 h.
Lane (-): dATP, dGTP, dCTP and TTP without HIV RT; lane (+):dATP, dGTP, dCTP and TTP with HIV RT; lane 1-6: dATP, dGTP, dCTP, TTP and γ-(alkyl-C18)-d4TTP **60r** (exact amounts are shown in Table 4-20).

According to the results which are shown above, the competition of TTP and γ-(alkyl-C18)-d4TTP **60r** was observed. When the amount of enzyme was reduced or the concentration ratio of TTP/γ-(alkyl-C18)-d4TTP **60r** was decreased to 1:10 (1.2 U HIV RT) and 1:16 (6U HIV RT), an obvious competition phenomenon was observed. As the

4.7.1.4 Assay with 6U HIV RT for 30 min, compared to γ-(kb-C9)-TTP 71j.

Lastly, we used γ-modified NTPs **60**, **62** alone without dATP, dGTP, dCTP in the reaction. γ-(kb-C9)-TTP **71j** was also used for comparison. This compound was synthesized by T. Nack in our group and its structure is shown below. Unlike the mask of Tri*PPP*ro-compound **41** and **56**, the ester group in the mask moiety was changed into an enzymatically non-cleavable ketone group.

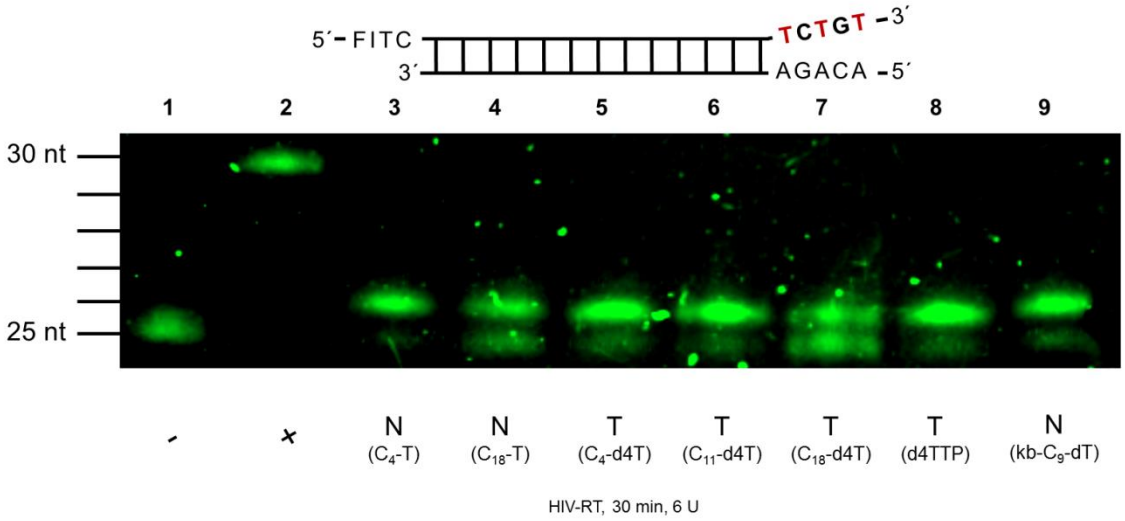
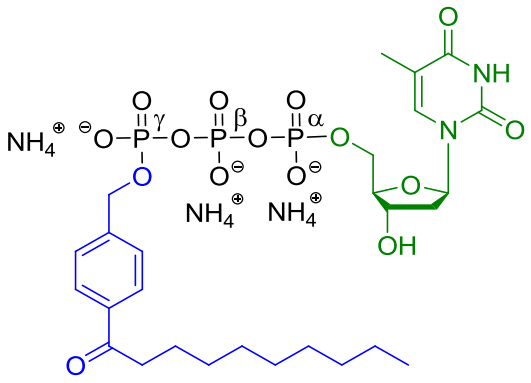


Figure 4-37: Primer extension assay with 6 U HIV RT for 30 min incubation. Assay condition: 50 mM Tris-HCl (pH 8.6 at 22 °C), 10 mM $MgCl_2$, 40 mM KCl, dNTPs 66 µM, HIV RT 6 U, Hybrid 0.32 µM in a reaction volume of 10 µL, incubated 37 °C for 30 min, 80 °C for 3 min; 50 mA, 45w for 4 h.
Lane 1 (-): dATP, dGTP, dCTP and TTP without HIV RT; lane 2 (+):dATP, dGTP, dCTP and TTP with HIV RT; lane 3 (N, C_4-T): γ-(alkyl-C4)-TTP **62d**; lane 4 (N, C_{18}-T): γ-(alkyl-C18)-TTP **62r**; lane 5 (T, C_4-d4T): γ-(alkyl-C4)-d4TTP **60d**; lane 6 (T, C_{11}-d4T): γ-(alkyl-C11)-d4TTP **60k**; lane 7 (T, C_{18}-d4T): γ-(alkyl-C18)-d4TTP **60r**; lane 8(T, d4TTP): d4TTP **4t**; lane 9 (N, kb-C_9-T): γ- (kb-C9)-TTP **71j**.



Scheme 4-15: The chemical structure of γ-(kb-C9)-TTP **71j**.

From the fluorescent gel in Figure 4-34, the incubation solution alone with γ-(alkyl)-TTPs **62** or γ-(kb)-TTPs **71j** were labeled with "N (C_n-T) or N (kb-C_n-T)". The mixture using γ-(alkyl)-d4TTPs **60** was labeled with "T (C_n-d4T)" and the mixture of d4TTP **4t** was labeled with "d4TTP". Results showed that the efficacy relationship for the utilization by HIV RT was as follows: γ-(alkyl-C4)-TTPs **62d** (lane 3) > γ-(kb-C9)-TTP **71j** (lane 9) > d4TTP **4t** (lane 8) ≥ γ-(alkyl-C4)-d4TTPs **60d** (lane5). When γ-(alkyl)-dNTP was modified with long alkyl group such as 1-octadecyl group, its efficacy for the utilization by HIV RT greatly reduced.

4.7.2 Primer Extension Assay with Human DNA Pol β

As is the simplest DNA polymerase in both size and catalysis, DNA Pol β plays an important role in base excision repair.[83] It can also fill gaps and nicks.[84] One unit of DNA Pol β is the amount of enzyme required to incorporate 1 nmole of labeled TTP into acid-insoluble material in 10 min at 37 °C. Thus, primer extension experiment under the catalysis of DNA Pol β was then conducted.

The control experiments proved that the experimental set-up was functional (lanes -, +). As there are no dATPs, dCTPs and dGTPs in the system (without star label), the primer was terminated at 26mer (n+1) if γ-(alkyl)-dNTP **60**, **62** was utilized by human DNA Pol β. D4TTP **4t** was efficiently incorporated into the primer by DNA Pol β (lane 8). However, neither γ-(alkyl-C18)-d4TTP **60r** nor γ-(alkyl-C11)-d4TTP **60k** were efficient substrates for the enzyme while incorporation was again detected with compound γ-(alkyl-C4)-d4TTP **60d** (lanes 5-7). The same was observed with γ-(alkyl-C18)-TTP **62r** (lane 4, no incorporation) and γ-(alkyl-C4)-TTP (lane 3, about 60% incorporation). Interestingly, γ-(alkyl-C4)-d4TTP **60d** seems to be a more effective substrate as compared to γ-(alkyl-C4)-TTP **62d** (lanes 5 and 3).

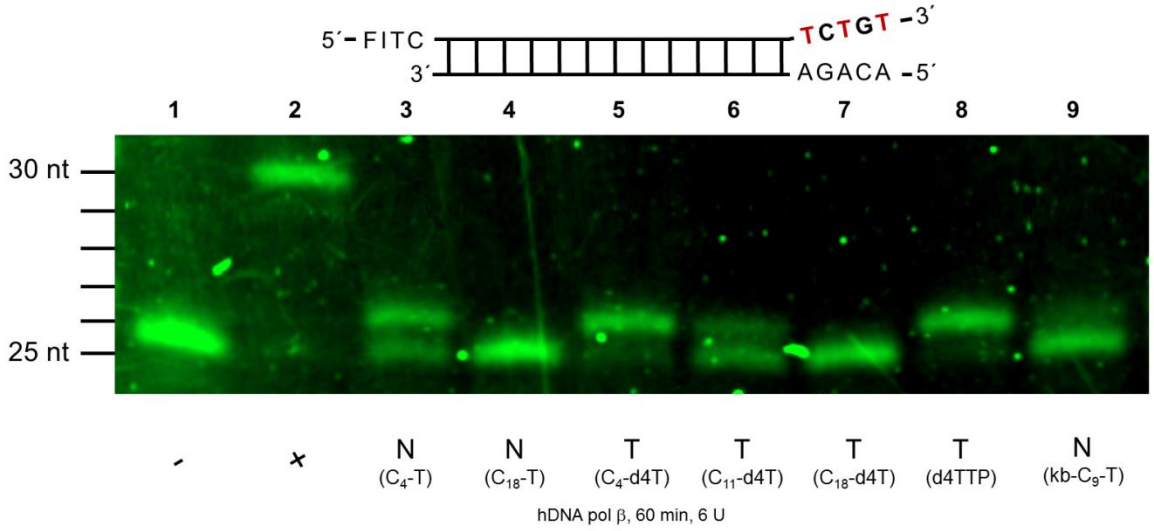


Figure 4-38: Primer extension assay with 6 U hDNA Pol β for 60 min incubation time. Assay condition: 50 mM Tris-HCl (pH 8.7), 10 mM $MgCl_2$, 100 mM KCl, 1.0 mM dithiothreitol, 0.4 mg/ml of bovine serum albumin, 15% glycerol, dNTPs 66 µM, DNA Polymerase β 6 U, Hybrid 0.32 µM in a reaction volume of 10 µL, incubated 37 °C for 60 min, 80 °C for 3 min; 50 mA, 45w for 4 h.
lane 1 (-): dATP, dGTP, dCTP and TTP without DNA Pol β (Human); lane 2 (+):dATP, dGTP, dCTP and TTP with DNA Pol β (Human); lane 3 (N, C_4-T): γ-(alkyl-C4)-TTP **62d**; lane 4 (N, C_{18}-T): γ-(alkyl-C18)-TTP **62r**; lane 5 (T, C_4-d4T): γ-(alkyl-C4)-d4TTP **60d**; lane 6 (T, C_{11}-d4T): γ-(alkyl-C11)-d4TTP **60k**; lane 7 (T, C_{18}-d4T): γ-(alkyl-C18)-d4TTP **60r**; lane 8 (T, d4TTP): d4TTP **4t**; lane 9 (N, kb-C_9-T): γ-(kb-C9)-TTP **71j**.

Consequently, triphosphates modified at the γ-position are substrates for HIV-RT while they fail to act as substrates for Pol β when the modification has an appropriate size/length. Thus, by these structural changes d4TTP which is a substrate for DNA polymerase β can be converted into a non-substrate for this enzyme while still being a substrate for HIV-RT. The ability of γ-(kb-C9)-TTP **71j** to act as a non-substrate for DNA polymerase β is similar to that of γ-(alkyl-C18)-dNTP **60r**, **62r** (lane 9, 7 and 4).

4.7.3 Primer Extension Assay with Human DNA Pol γ

Human DNA polymerase γ (hDNA Pol γ) is a human mitochondrial DNA (mtDNA) polymerase which can be used for drug toxicity testing. The enzyme is known to be slow in the catalytic reaction. Incorporation of dNTPs is several orders of magnitude lower as compared to other human DNA polymerases. One unit is the amount of enzyme required to incorporate 1 pmole of TTP in 60 min at 37 °C using polyrA:dT as template, which is 1000-fold slower than HIV-RT and hDNA Pol β.

The primer extension assay using hDNA Pol γ was conducted using the same condition of the hDNA Pol β. However, as a result, the prolongation of primer was not observed when four natural dNTPs were used. Under these conditions, the concentrations of primer and template were 10 times higher than the radioactive labeling primer extension experiment. However, it is not possible to further reduce the concentration of the fluorescent-labeled primer for the photo imaging process. Thus, for the primer extension of hDNA Pol γ, it is necessary to use ^{32}P-labeled primer/template to conduct the experiment at lower concentrations. The primer extension assay using hDNA Pol γ will be performed in the near future.

4.8 An Attempt to Explanation the Selectivity through Computational Chemistry

To explain the selectivity phenomenon observed in the primer extension experiments, a work by looking at suitable crystal structures was carried out in cooperation with Nora Constanze Fohrmann from our research group. This work focused on looking for a probable explanation of the phenomenon that γ-(alkyl-C18)-modified NTPs were incorporated into the DNA strand by HIV-RT but not in human polymerase β.

A crystal structure with cocrystallized d4TTP and HIV reverse transcriptase (HIV RT) was published recently. It has the PDB-code 6AN2 (Structure of HIV-1 RT Ternary Complex with a double stranded DNA and an incoming d4TTP at pH 7.5).[155] However, for human DNA polymerase β (hDNA Pol β), no crystal structure with cocrystallized d4TTP has been published. So, a crystal structure with a similar cocrystallized ligand was found with the PDB-code 2FMS (DNA Pol β with a gapped DNA Substrate and dUMPNPP with magnesium in the catalytic site) as the most representative crystal structure.[156]

For the HIV-RT (6AN2), the γ-phosphate is located close to the surface of the protein without being extremely covered by the protein. Therefore, an alkyl chain at the γ-phosphate might not impede binding of the triphosphate to the active site.

For hDNA Pol β (2FMS), the triphosphate must dive much deeper into the polymerase where the γ-phosphate is shielded by the protein surface. Thus, a long alkyl chain modification at the γ-phosphate will make it difficult for the triphosphate to enter the active site.

Thus, the alkyl chain can stay outside of HIV-RT whereas for human Pol β it must be dragged into the "channel" (a hole in Figure 4-39 B) for binding of the triphosphate to the active site. This may explained the result that γ-(alkyl-C4)-d4TTP **60d** and γ-(alkyl-C4)-TTP **62d** are used as substrates in primer extension using human DNA Pol β, while γ-(alkyl-C18)-d4TTP **60r** and γ-(alkyl-C18)-TTP **62r** are not substrate at same conditions.

To more clearly explain why γ-modified triphosphates are incorporated into the DNA strand n HIV-RT but not in hDNA Pol β, more works with molecular dynamic simulations need to be done.

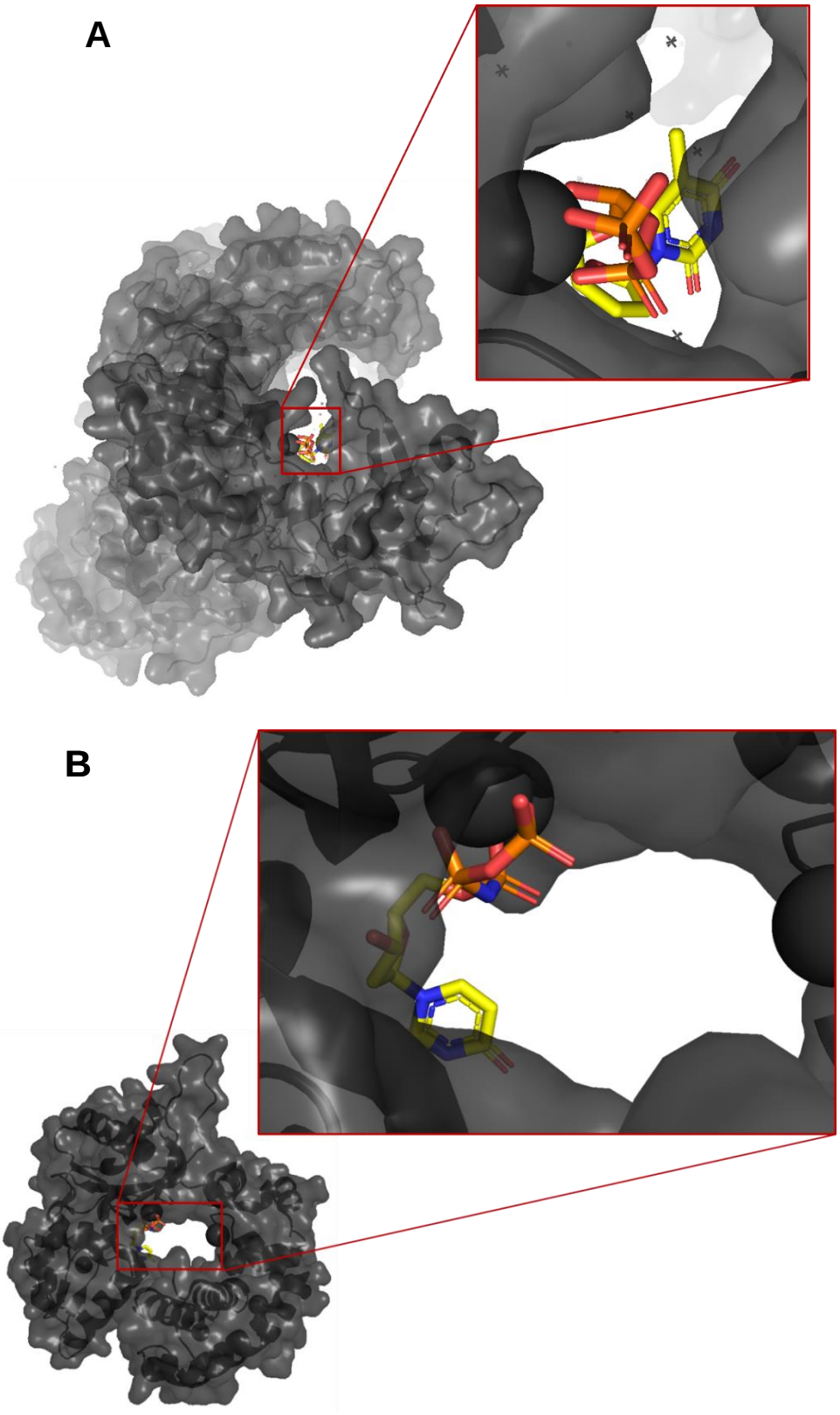


Figure 4-39: (A) A crystal structure of HIV-1 RT Ternary Complex with a double stranded DNA and an incoming d4TTP at pH 7.5.[155] (B) The hDNA Pol β with a gapped DNA substrate and dUMPNPP with magnesium in the catalytic site.[156] HIV-1 RT and hDNA Pol β are in dark grey. Phosphates are in red and orange.

5 Conclusion

In summary, a series of Tri*PPP*ro-compounds bearing two different AB-masks attached to the γ-phosphate was firstly synthesized by using *H*-phosphonate route. Hydrolysis in PBS, CEM cell extracts and pig liver esterase proved the delivery mechanism that the hydrolysis is triggered by enzymes. However, compared to the symmetric Tri*PPP*ro-compounds, hydrolysis study showed that no obvious selective cleavage occurred between short acetyl ester and long acetyl ester. Antiviral data suggested that longer acetyl ester exhibited higher activity against HIV-1 and HIV-2 in cultures of infected wild-type human $CD4^+$ T-lymphocyte (CEM/0) cells and more importantly in thymidine kinase-deficient $CD4^+$ T-cells (CEM/TK^-). Interestingly, when one AB-mask was modified with methoxytriglycol group with a diester linker, the other AB-mask with alkanoate was cleaved predominately. However, these compounds are less potent than the Tri*PPP*ro-compounds with two alkanoate AB-masks.

Then a second generation of lipophilic nucleoside triphosphate prodrugs **58** was disclosed, which they were differ from the previously described Tri*PPP*ro-derivatives **41** by comprising a non-cleavable moiety in addition to a biodegradable prodrug moiety at the γ-phosphate group. As stable groups, lipophilic alkyl residues of different lengths were attached in addition to the previously used acyloxybenzyl-groups as masks. The synthesis was achieved in good chemical yields using *H*-phosphonate chemistry. We have proven that the prodrug group was selectively cleaved to give γ-alkyl-modified nucleoside triphosphates **60** by chemical hydrolysis (slow process) or by enzymes present in cell extracts (fast process). The γ-alkyl-nucleoside triphosphates **60**, **62** proved to be stable in cell extracts while the corresponding nucleoside triphosphates d4TTP or TTP were rapidly enzymatically dephosphorylated.

In contrast to d4T itself, all these compounds showed marked antiviral activity against HIV-2 in thymidine kinase deficient cells (CEM/TK^- cells) indicating a successful cell membrane passage of these compounds and subsequent intracellular enzymatic delivery of γ-alkyl-d4TTP. Thus, although the second-generation nucleoside triphosphate prodrugs are still charged at the phosphate groups, obviously the modification at the γ-phosphate group by one lipophilic, bio-reversible moiety and the stabile γ-alkyl group gives the molecule sufficient lipophilicity to penetrate the cell membrane. γ-modified-TTPs were substrates for HIV-RT in primer extension as while

they proved to be no substrates for the cellular DNA-polymerases used here. The corresponding d4TTP derivatives were also found to be substrates for HIV-RT but were still no substrates for DNA-polymerases β. Thus, these results point to an increased selectivity of the compounds to different polymerases with a marked preference to the viral enzyme HIV-RT.

We are currently working on the question if this increased selectivity towards the viral enzyme is a general property of these γ-modified compounds by using different nucleoside analogues and different viral polymerases. The second improvement compared to the first generation Tri*PPP*ro-derivatives is that the new compounds deliver intracellularly metabolic stable nucleoside triphosphate derivatives which showed no dephosphorylation for at least 30 h. This should lead to a significant increase in bioactive metabolites inside cells.

Thus, here we disclose the development of an advanced prodrug concept for NTP derivatives that deliver γ-alkyl-NTPs with high selectivity by an enzyme-triggered mechanism which then allows i) the bypass of all phosphorylation steps usually needed for the activation of a nucleoside analogue, ii) the delivery of compounds which act as substrates for a viral polymerase (RT) but not substrates for cellular DNA-polymerases β and iii) which show very high stability against dephosphorylation of the triphosphate unit as compared to natural NTPs.

6 Experiment Section

6.1 Chemicals and Instruments

General: Without further noticed, all manipulations were carried out under an atmosphere of nitrogen using standard Schlenk techniques and all solvents were dried by using standard procedures. Triethylamine (Et_3N) were dried by being heated under reflux over calcium hydride for 5 days and followed by distillation. Trifluoroacetic anhydride (TFAA) was dried over phosphorus pentoxide for one hour and distilled under nitrogen. Anhydrous acetonitrile (CH_3CN), tetrahydrofuran (THF), dichloromethane (CH_2Cl_2), methanol (CH_3OH) and pyridine were obtained by the MBraun solvent purification system (MB SPS-800). Other dry solvents were purchased from Acros Organics (extra dry over molecular sieves). For eluent of column chromatography, technique grade Ethyl acetate (EA), petroleum ether (PE) 50-70, CH_2Cl_2, and CH_3OH were distilled before use. Tetra-*n*-butylammonium phosphate monobasic solution (0.4 M in acetonitrile) was purchased from Acros Organics directly.

Column chromatography: Normal phase column chromatography were performed with Macherey-Nagel silica gel 60 M (0.040-0.063 mm). For automatic reversed-phase chromatography an Interchim Puriflash 430 in combination with Chromabond® Flash C^{18}ec was used.

Analytical thin-layer chromatography (TLC): For thin layer chromatography Macherey-Nagel pre-coated TLC sheets Alugram® Xtra SIL G/UV254 were used. Phosphomolybdic Acid (PMA) Stain was used and prepared by dissolving 10 g of phosphomolybdic acid in 100 mL of ethanol. Potassium Permanganate Stain was used and prepared by 1.5 g of $KMnO_4$, 10 g K_2CO_3, and 1.25 mL 10% NaOH in 200 mL water.

Nuclear Magnetic Resonance (NMR): NMR spectra were recorded at room temperature in automation mode on either of a Varian Gemini 2000BB, Bruker Fourier 300, Bruker AMX 400, Bruker DRX 500 or Bruker AVIII 600 spectrometer. Chloroform-d_1 ($CDCl_3$), methanol-d_4 (MeOD), dimethyl sulfoxide-d6 (DMSO) and deuterium oxide (D_2O) were purchased form Euriso-Top. All ^1H, ^{31}P, and ^{13}C NMR chemical shifts are quoted in parts per million (ppm). Reference peaks for chloroform-*d* in ^1H NMR and ^{13}C NMR spectra were set at 7.26 ppm and 77.0 ppm, respectively.

For methanol-*d4* reference peaks in ^{1}H NMR and ^{13}C NMR spectra were set at 3.33 ppm and 49.0 ppm respectively.

Mass Spectrometry (MS): HRMS (ESI) mass spectra were recorded on an Agilent 6224 ESI-TOF spectrometer. MALDI measurements (matrix: 9-aminoacridine [9AA] or 2,5-dihydroxybenzoic acid [DHB]) were performed with a Bruker UltrafelXtreme spectrometer.

Infrared spectroscopy (IR): IR spectra were recorded on a Bruker Alpha P FT-IR at room temperature in the range of 400-4000 cm^{-1}.

Freeze dryer: Alpha 2-4 LD_{Plus} freeze dryer from Christ Co. and chemistry hydrid pump RC6 from Vacuumbrand Co. was used

Ultrapure water: Arium®pro ultrapure water system was used.

pH meter: pH value was tested with ProLab 300 from Schott Co..

Thermomixer: Eppendorf Thermomixer 5436 and CellMedia Thermoschüttler basic were used.

Ultrasonic cleaning device: Sonorex RK512H from Bandelin Co. was used.

Centrifuges: Heraeus Biofuge Pico (13000 u/min) and Biofuge Pimo R (8000 u/min) was used.

Fluorescent Imager: ChemiDoc™ MP Imaging System (170-8280) from Bio-Rad Co. was used for primer extension study.

Electrophoresis: PAGE experiment was conduct under Thermo Fisher Owl™ S4S Aluminum-Backed Sequencer System. Power supply: Consort EV2230 Gel Electrophoresis (1500 V, 300 mA, 150 W).

6.2 General Synthetic Procedures

General Procedure A: Preparation of 4-(hydroxymethyl)phenylalkanoate 44.

4-hydroxybenzylalcohol **43** and triethylamine in CH_2Cl_2 were cooled down to 0 °C or at room temperature. The corresponding acyl chloride **42** in CH_2Cl_2 was added dropwise and the mixture was stirred at room temperature. The precipitate was filtered, and the solvent was removed in vacuum. The residue was diluted with CH_2Cl_2 and washed once with water. The organic layer was dried with Na_2SO_4 and

the solvent was removed. The crude was purified by SiO_2 column chromatography to give compound **44**.

General Procedure B: Preparation of non-symmetric (AB,ab)-H-phosphonate 55

Under dry conditions, diphenyl phosphonate (1.2 equiv.) was dissolved in 3 mL pyridine and cooled to 0 °C. 4-(Hydroxymethyl)phenylalkanoate **a** (1.0 equiv.) was added and stirred at 0 °C for 30 min and then heated up to 38 °C. Following, 4-(hydroxymethyl)phenylalkanoate **b** (1.4 equiv.) was added and the mixture was stirred for 3 h. Then the solvent was removed in vacuo. The crude product was purified by flash column chromatography (silica) with EtOAc/petroleum ether/0.5% acetic acid as eluent.

General Procedure C: Preparation of non-symmetric Tri*PPP*ro-compounds 56 and γ-modified-d4TTP 58

The reactions were performed under dry conditions. a) *H*-phosphonate (1.0 eq.) was dissolved in 6 mL acetonitrile and *N*-chlorosuccinimide (2.0 eq.) was added. After stirring for 2 h at room temperature, tetra-*n*-butylammonium phosphate monobasic solution (0.4 M in acetonitrile) (3.0 eq.) was added dropwise. The mixture was stirred for 1 h and the solvent was removed in vacuum. The residue was extracted with CH_2Cl_2/H_2O. The organic phase was dried over sodium sulfate and the solvent was removed by evaporation to afford corresponding pyrophosphate in nearly quantitative yield. B) The corresponding pyrophosphate was dissolved in acetonitrile and cooled down to 0 °C. A mixture of trifluoroacetic anhydride (TFAA, 5.0 eq.) and trimethylamine (Et_3N, 8.0 eq.) in 3 mL acetonitrile was cooled to 0 °C and added to the mixture. After stirring for 10 min, all volatile components were removed in vacuum. The residue was once again co-evaporated with 3 mL acetonitrile and subsequently dissolved in 3 mL acetonitrile at 0 °C. 1-methylimidazole (3.0 eq.) and trimethylamine (Et_3N, 8.0 eq.) was added. The suspension was warmed up to room temperature and stirred for 10 min. The resulting activated imidazolidate formed and the corresponding NMP (0.7-1.0 eq.) in 3 mL acetonitrile was added. The reaction was stirred at room temperature for 2-3 h and dried in vacuum. The crude product was purified by automatic RP18 flash chromatography, and then followed by ion-exchange to the ammonium form with Dowex 50WX8 cation-exchange resin and a second RP18 chromatography purification step. Product-containing fractions were

collected, and the organic solvent evaporated. The remaining aqueous solutions were freeze-dried, and the desired product obtained as a colorless cotton.

6.3 Hydrolysis Study

6.3.1 HPLC Method

A VWR-Hitachi LaChromElite HPLC system (L-2130, L-2200, L-2455) with an EzChromElite software and a Nucleodur 100-5 C^{18}ec or Nucleodur 100-5 C8ec (Macherey-Nagel) were available. HPLC grade acetonitrile was obtained from VWR and ultrapure water (Mili-Q water) from a Sartorius Aurium® pro apparatus (Sartopore 0.2 µm, UV). 2 mM tetra-*n*-butylammonium acetate solution (TBAA, pH 6.3) was used as buffer. Method: Nucleodur 100-5 C^{18}ec; 0-20 min: TBAA buffer/acetonitrile gradient (5-80%); 20-30 min: buffer/acetonitrile (80%); 30-33 min: buffer/acetonitrile (80-5%); 33-38 min: buffer/acetonitrile (5%); flow: 1 mL/min. TBAA buffer (2 mM): 4.05 mL Tetra-n-butylammonium hydroxide in water (ca. 40%) was diluted with 3000 mL ultrapure water. Then glacial acetic acid was added to adjust the buffer to pH 6.3.

6.3.2 Chemical Hydrolysis in PBS

Stock solutions (50mM in DMSO) of Tri*PPP*ro-dNTP and γ-modified-dNTPs were prepared. After dilution of 11 µL stock solution with 100 µL ultrapure water and 189 µL DMSO to 1.83 mM hydrolysis solutions the reaction was started by addition of 300 µL phosphate buffer saline (PBS, 50 mM, pH 7.3). The solution was incubated with 800 rpm and at 37 °C in a thermomixer. An initial aliquot (25 µL) was taken directly and analyzed by analytical HPLC with UV detector. For compound containing d4T, λ = 265 nm. For compound containing d4U, λ = 260 nm. Further aliquots were taken for monitoring the kinetic hydrolysis.

6.3.3 Enzyme-catalyzed Hydrolysis in CEM cell extracts

10 µL 50 mM DMSO stock solution of Tri*PPP*ro-dNTP or γ-modified-dNTPs was diluted to 6.0 mM hydrolysis solution by addition of 73.3 µL DMSO. 7 different samples including 10 µL water and 10 µL hydrolysis solution were prepared. The reaction was started by addition of 50 µL human CEM cell extract and the mixture incubated with 800 rpm at 37 °C for different time periods of hydrolysis. The reactions were stopped by addition of 150 µL MeOH. The solution was kept on ice for 5 min

followed by centrifugation for 5 min (13000 rpm). The supernatants were filtered (Chromafil® RC-20/15 MS, 0.2 µm) and stored in liquid nitrogen. When testing, the samples were defrosted and injection volume with 80 µL was used for HPLC analysis. The calculation of $t_{1/2}$ was performed analogously to that for the chemical hydrolysis studies.

6.3.4 Enzyme-catalyzed Hydrolysis in Pig Liver Esterase (PLE)

10 µL 50 mM DMSO stock solution of Tri*PPP*ro-dNTP or γ-modified-dNTPs was diluted to 6.0 mM hydrolysis solution by addition of 31.7 mL DMSO and 41.7 mL ultrapure water. Then 83.3 ml of the 6.0mM solution was diluted with 125 µL DMSO and 833 µl 50 mM PBS buffer (pH 7.3). The reaction was started by addition of 62.5 ml of PLE in PBS buffer (3 mg/mL) and the mixture was incubated with 800 rpm at 37 °C in a thermomixer. At different times, aliquots (100 ml) were taken and the reaction was stopped by addition to 106 mL MeOH. The mixture was kept for 5min on ice followed by centrifugation for 5min (13000 rpm). The mixture was filtered (Chromafil RC-20/15 MS, 0.2 mm) and stored in liquid nitrogen. When testing, the samples were defrosted and injection volume with 80 µL was used for HPLC analysis.

6.3.5 Preparation of Cell Extracts:

Human CD_4^+ T-lymphocyte CEM cells were grown in RPMI-1640-based cell culture medium to a final density of ~3×10^6 cells/mL. Then, cells were centrifuged for 10 min at 1,250 rpm at 4 C, washed twice with cold PBS, and the pellet was resuspended at 10^8 cells/mL and sonicated (Hielscher Ultrasound Techn., 100% amplitude, 3·times for 10 sec) to destroy cell integrity. The resulting cell suspension was then centrifuged at 10000 rpm to remove cell debris, and the supernatant divided in aliquots before being frozen at -80 °C and used.

6.3.6 Data Analysis

The exponential decay curves (pseudo-first order) based on absolute integral values were calculated with commercially available software (OriginPro 9.0G) and the half-lives $t_{1/2}$ of the Tri*PPP*ro-compounds and γ-modified-dNTPs were calculated via one determination.

6.4 Antiviral Assay against HIV

Inhibition of HIV-1(III_B)- and HIV-2(ROD)-induced cytopathicity in wild-type CEM/0 and thymidine kinase-deficient CEM/TK^- cell cultures was measured in microtiter 96-well plates containing ~3×10^5 CEM cells/mL infected with 100 $CCID_{50}$ of HIV per milliliter and containing appropriate dilutions of the test compounds. After 4−5 days of incubation at 37 °C in a CO_2-controlled humidified atmosphere, CEM giant (syncytium) cell formation was examined microscopically. The EC_{50} (50% effective concentration) was defined as the compound concentration required to inhibit HIV-induced giant cell formation by 50%.

6.5 Chemicals and solutions for primer extension experiment

6.5.1 Enzyme

HIV-RT and human polymerase α, β and γ were obtained from Roboklon. The primer and template was purchased from Life Technologies. The fluorescent labeled primer was purchased from Metabion.

HIV Reverse Transcriptase (HIV RT): Human Immunodeficiency Virus (HIV) Reverse Transcriptase is RNA directed DNA polymerase which can synthesize a complementary DNA strand initiating from a primer using either RNA or single-stranded DNA as a template. One unit is the amount of enzyme required to incorporate 1 nmole of labeled TTP into acid-insoluble material in 10 min at 37 °C.

Assay Conditions: 50 mM Tris-HCl (pH 8.6 at 22 °C), 10 mM MgCl2, 40 mM KCl, 0.5 mM [^{3}H]TTP and 0.4 mM poly(A)·(dT)12-18. Incubation is at 37oC for 10 min in a reaction volume of 50 µL.

Human DNA Polymerase Beta (DNA Pol β): It is the simplest DNA polymerase known in both size and catalysis. It is also a repair polymerase able to synthesize DNA beyond the end of gap or nick with simultaneous displacement of the non-replicated strand. It also fills gaps or nicks. Need to be stored at -20 °C. Reaction buffer is supplied as: **10 x DNA Polymerase Beta - core**: 500 mM Tris-HCl (pH 8.7), 100 mM MgCl2, 10 mM dithiothreitol, 1 M KCl. **24 mg/ml bovine serum albumin**. **100 % glycerol**. One unit is the amount of enzyme required to incorporate 1 nmole of total nucleotide into acid-insoluble form in 60 min at 37 °C.

Assay condition provided by the supplier: 50 mM Tris-HCl, pH 8.7, 10 mM MgCl2, 0.4 mg/ml of bovine serum albumin, 1.0 mM dithiothreitol, 100 mM KCl, 15% glycerol, 0.05 mM each dCTP, dGTP, TTP, ^{79} dATP and 10 µg of activated DNA. Incubation is at 37°C for 15 min in a reaction volume of 50 µL.

Human DNA Polymerase Beta (DNA Pol γ): Human mitochondrial DNA polymerase. Used for drug toxicity testing. Note: The enzyme is known to be slow. Incorporation of dNTPs is several orders of magnitude lower as compared to other human DNA polymerases. Store at -80 °C. Avoid repeated freeze-thaw.

Reaction buffer is supplied as: **10 x DNA Polymerase Gamma - core**: 250 mM HEPES-KOH (pH 8.0), 25 mM ß-Mercaptoethanol, 1 M NaCl. **10 mM MnCl2**. **24 mg/ml bovine serum albumin**. Note: To avoid MnCl2 hydrolysis, 10 x Reaction Buffer needs to be always prepared fresh, just before assembling the reaction.

Assay condition provided by the supplier: 25 mM HEPES-KOH (pH 8.0), 0.5 mM MnCl2, 2.5 mM ß-Mercaptoethanol, 10 µg acetylated BSA, 0.01 mM TTP (pH 7.0), 0.3 µCi (α-^{3}H)TTP at 88 Ci/mmol, 0.1 M NaCl, 1.6 µg poly (rA)•oligo (dT)50. Incubation is at 37 °C for 15 min. in a reaction volume of 15 µL.

One unit is the amount of enzyme required to incorporate 1 pmole of TTP in 60 min at 37 °C using polyrA:dT as template.

6.5.2 Sequence of Primer and Template

25 nt FITC-Primer:

5´-FITC-CGTTGGTCCTGAAGGAGGATAGGTT-3´

30 nt Template:

5´-ACAGAAACCTATCCTCCTTCAGGACCAACG-3´

6.5.3 Chemicals and Solutions

10N NaOH Solution:

100 g NaOH was dissolved in mili-Q water and fill up to the volume of 250 mL.

0.5 M EDTA (pH 8.0):

93.05 g of disodium EDTA (Na_2EDTA dehydrate, MW = 372.2) was dissolved in 400 mL of deionized water. The pH value was adjusted to 8.0 with 10N NaOH (~25 mL). Then deionized water was added to bring a final volume of 500 mL. Sterilize by autoclaving and store at room temperature. (The disodium salt of EDTA will not dissolve until the pH of the solution is adjusted to 8.0 by the addition of NaOH.)

50x TAE Buffer:

	1000 mL	**500 mL**
2 M Tris (pH 7.6)	242.3 g	121.1 g
1 M acetic acid	57.1 mL	28.5 mL
50 mM EDTA	100 mL	50 mL

121.1 g of Tris base (MW = 121.14) was dissolved in 350 mL of deionized water. Carefully add 28.5 mL of glacial acid (MW = 60.05, d = 1.049 g/mL at 25 °C) and 50 mL of 0.5 M EDTA (pH 8.0). After that, the solution was adjusted to a final volume of 500 mL. This stock solution can be stored at room temperature. The pH of this buffer is not adjusted and should be about 8.5.

1x TAE Buffer:

1x TAE Buffer
40 mM Tris (pH 7.6)
20 mM acetic acid
1 mM EDTA

20 ml 50x TAE Buffer was diluted with 980 mL deionized water to make 1x TAE working solution. It is better to prepare before using.

Experiment Section

Polyacrylamide gel (PAA-Gel):

20% Acrylamide solution:

	Final Conc.	1000 mL	500 mL
19:1 Acrylamide/bis 40%	20%	500 mL	250 mL
50x TAE Buffer	1x TAE	20 mL	10 mL
Urea	8 M	480.5 g	240.2 g
Water		to 1000 mL	To 500 mL

8 M Urea Solution:

	Final Conc.	1000 mL	500 mL
50x TAE Buffer	1x TAE	20 mL	10 mL
Urea	8 M	480.5 g	240.2 g
Water		to 1000 mL	To 500 mL

10% Ammonium persulphate (10% (w/v) APS in mili-Q water) (0.1 g/mL):

Mix **1** g of APS with **10** mL of dH_2O (Aliquot and store at -20^{o}C in the dark)

15% sequencing gel in 1x TAE buffer:

Gel scale: 450mm×200mm×0.4mm		
20% Acrylamide solution	30 mL	75 mL
8 M Urea solution	10 ml	25 mL
10% APS	280 µL	700 µL
TEMED	40 µL	100 µL

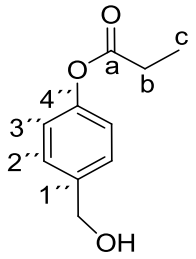
40% Acrylamide/Bis 19:1		**6%**	**10%**	**15%**	**X%**
		15 mL	25 mL	37.5 mL	2.5x**X** mL
50x TAE	1x TAE	2 mL			
Urea	8 M	48.1 g			
TEMED	3 mM 0.1 % (v/v)	100 µL			
10% APS Solution	3 mM	700 µL			
Water		Fill to 100 mL			

6.6 Primer Extension Essay Condition

For FITC labeled primer extension experiment: After 5 min incubation at 95 °C in 20 mM Tris-HCl (pH 7.6) and 50 mM NaCl, the hybridization/annealing of the primer to the template strand was achieved by a cooling phase from 95 °C to -20 °C over 2 hours. For HIV- RT assay: The final assay solution (10 µL) consists of 50 mM Tris-HCl (pH 8.6 at 22 °C), 10 mM $MgCl_2$, 40mM KCl, dNTPs 66 µM, HIV RT 6 U, Hybridization 0.32 µM in a reaction volume of 10 µL, incubated 37 °C for 15 min, 80 °C for 3 min; 50 mA, 45w for 4 h. For human DNA pol β assay: The final assay solution (10 µL) consists of 50 mM Tris-HCl (pH 8.7), 10 mM $MgCl_2$, 100mM KCl, 1.0 mM dithiothreitol, 0.4 mg/ml of bovine serum albumin, 15% glycerol, dNTPs 66 µM, DNA Polymerase Beta 6 U, Hybrid 0.32 µM in a reaction volume of 10 µL, incubated 37 °C for 60 min, 80 °C for 3 min; 50 mA, 45w for 4 h The assays were separated using a denaturating PAGE (15%). The results were visualized by phosphorimaging.

6.7 Experiment Data of Synthesized Compounds

4-(Hydroxymethyl)phenyl propionate **44c**



Yield: 60%

Chemical Formula: $C_{10}H_{12}O_3$

Molecular Weight: 180.20

According to **General Procedure A**, under atmosphere, 3.41 g 4-hydroxybenzylalcohol (27.5 mmol, 1.1 equiv.) and 3.47 mL triethylamine (25 mmol, 1.0 equiv.) was dissolved in 15 mL CH_2Cl_2 at 0 C. 2.17 mL propionyl chloride (25 mmol, 1.0 equiv.) in 25 mL CH_2Cl_2 was added dropwise. Reaction time was 2.5 h at room temperature (r.t.). Column chromatography (SiO_2, PE/EE 6:4 v/v) Yield: 60%, 2.70 g, as colorless oil.

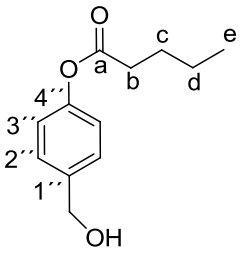
^{1}H NMR (400 MHz, Chloroform-d): δ 7.36 (d, $^3J_{HH}$ = 8.0 Hz, 2H, **H-2´´**), 7.06 (d, $^3J_{HH}$ = 8.0 Hz, 2H, **H-3´´**), 4.66 (s, 2H, **Ph-CH$_2$**), 2.59 (q, $^3J_{HH}$ = 7.5 Hz, 2H, **H-b**), 1.91 (s, 1H, **O-H**), 1.26 (t, $^3J_{HH}$ = 7.5 Hz, 3H, **H-c**).

^{13}C-NMR (101 MHz, Chloroform-d): *δ* 173.0 (**C-a**), 150.1 (**C-4´´**), 138.4 (**C-1´´**), 128.0 (**C-2´´**), 121.6 (**C-3´´**), 64.7 (s, **Ph-CH$_2$**), 27.7 (**C-b**), 9.0 (**C-c**).

HRMS (ESI-TOF) m/z: calculated for $C_{10}H_{12}NaO_3$ $[M+Na]^+$ 203.0679, found 203.0618.

IR: v = 3405, 2982, 2941, 2883, 1752, 1606, 1509, 1473, 1456, 1413, 1383, 1351, 1201, 1170, 1138, 1073, 1025, 999, 946, 891, 830, 804, 761, 727, 577, 559, 515, 423, 409.

4-(Hydroxymethyl)phenyl pentanoate **44e**



Yield: 78%

Chemical Formula: $C_{12}H_{16}O_3$

Molecular Weight: 208.25

According to **General Procedure A**, under atmosphere, 1.22 g 4-hydroxybenzylalcohol (9.79 mmol, 1.2 equiv.) and 1.36 mL triethylamine (9.79 mmol, 1.2 equiv.) was dissolved in 20 mL CH_2Cl_2 at room temperature. 1 mL pentanoyl chloride (8.16 mmol, 1.0 equiv.) in 30 mL CH_2Cl_2 was added dropwise. Reaction time was 4 h at room temperature (r.t.). Column chromatography (SiO_2, PE/EE 7:3 v/v). Yield: 78%, 1.33 g, as colorless oil.

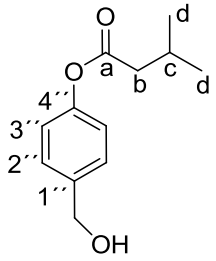
^{1}H NMR (400 MHz, Chloroform-d): δ 7.36-7.27 (d, $^3J_{HH}$ = 8.1 Hz, 2H, **H-2´´**), 7.08-6.97 (d, $^3J_{HH}$ = 8.4 Hz, 2H, **H-3´´**), 4.57 (s, 2H, **Ph-CH$_2$**), 2.54 (t, $^3J_{HH}$ = 7.5 Hz, 2H, **H-b**), 2.75-2.45 (br, s, 1H, **O-H**), 1.73 (tt, $^3J_{HH}$ = 7.5, 7.5 Hz, 2H, **H-c**), 1.44 (tq, $^3J_{HH}$ = 8.0, 7.2 Hz, 2H, **H-d**), 0.97 (t, $^3J_{HH}$ = 7.4 Hz, 3H, **H-e**).

^{13}C-NMR (101 MHz, Chloroform-d): δ 172.4 (**C-a**), 149.9 (**C-4´´**), 138.4 (**C-1´´**), 127.9 (**C-2´´**), 121.5 (**C-3´´**), 64.4 (s, **Ph-CH$_2$**), 34.0 (**C-b**), 26.9 (**C-c**), 22.1 (**C-d**), 13.6 (**C-e**).

HRMS (ESI-TOF) m/z: calculated for $C_{12}H_{16}NaO_3$ $[M+Na]^+$ 231.0992, found 231.0972.

IR: ν = 3393, 2958, 2932, 2872, 1754, 1606, 1506, 1464, 1417, 1365, 1345, 1310, 1198, 1163, 1143, 1101, 1046, 1014, 940, 919, 848, 811, 753, 733, 643, 561, 505, 458, 405, 389.

4-(Hydroxymethyl)phenyl 3-methylbutanoate **44e*i***



Yield: 64%

Chemical Formula: $C_{12}H_{16}O_3$

Molecular Weight: 208.25

According to **General Procedure A**, under atmosphere, 3.0 g 4-hydroxybenzylalcohol (24.0 mmol, 1.0 equiv.) and 3.67 mL triethylamine (26.4 mmol, 1.1 equiv.) was dissolved in 15 mL CH_2Cl_2 at 0 °C. 3.21 mL 3-methylbutanoyl chloride (25 mmol, 1.0 equiv.) in 25 mL CH_2Cl_2 was added dropwise. The mixture was stirred for 2.5 h at room temperature (r.t.). Column chromatography (SiO_2, PE/EE 7:3 v/v). Yield: 64%, 3.21 g, as colorless oil.

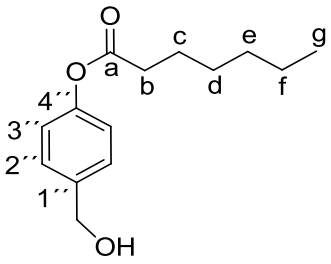
^{1}H NMR (400 MHz, Chloroform-d): δ 7.40-7.27 (m, 2H, **H-2´´**), 7.10-7.01 (m, 2H, **H-3´´**), 4.66 (s, 2H, **Ph-CH$_2$**), 2.43 (d, $^3J_{HH}$ = 7.2 Hz, 2H, **H-b**), 2.34-2.18 (m, 1H, **H-c**), 2.09-1.77 (m, 1H, **O-H**), 1.06 (d, $^3J_{HH}$ = 6.6 Hz, 6H, **H-d**).

^{13}C-NMR (101 MHz, Chloroform-d): *δ* 172.4 (**C-a**), 149.9 (**C-4´´**), 138.4 (**C-1´´**), 127.9 (**C-2´´**), 121.5 (**C-3´´**), 64.4 (s, **Ph-CH$_2$**), 34.0 (**C-b**), 26.9 (**C-c**), 22.1 (**C-d**), 13.6 (**C-e**).

HRMS (ESI-TOF) m/z: calculated for $C_{12}H_{16}NaO_3$ $[M+Na]^+$ 231.0992, found 231.0989.

IR: v = 3350, 2959, 2932, 2873, 1753, 1606, 1466, 1417, 1388, 1369, 1292, 1245, 1197, 1152, 1099, 1042, 1014, 964, 914, 890, 850, 832, 811, 628, 562, 500.

4-(Hydroxymethyl)phenyl heptanoate **44g**



Yield: 58%

Chemical Formula: $C_{14}H_{20}O_3$

Molecular Weight: 236.31

According to **General Procedure A**, under atmosphere, 3.0 g 4-hydroxybenzylalcohol (24.0 mmol, 1.0 equiv.) and 3.67 mL triethylamine (26.4 mmol, 1.1 equiv.) was dissolved in 20 mL CH_2Cl_2 at 0 °C. 4.1 mL *n*-heptanoyl chloride (26.4 mmol, 1.1 equiv.) in 30 mL CH_2Cl_2 was added dropwise. The mixture was stirred for 2.5 h at room temperature (r.t.). Column chromatography (SiO_2, PE/EE 7:3 v/v). Yield: 58%, 3.31 g, as colorless solid.

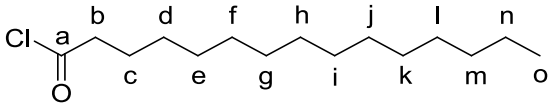
^{1}H NMR (400 MHz, Chloroform-d): δ 7.45-7.32 (m, 2H, **H-2´´**), 7.13-7.02 (m, 2H, **H-3´´**), 4.66 (s, 2H, **Ph-CH_2**), 2.55 (t, $^3J_{HH}$ = 7.5 Hz, 2H, **H-b**), 1.75 (quint, $^3J_{HH}$ = 7.0 Hz, 2H, **H-c**), 1.69 (br, s, **O-H**), 1.49-1.21 (m, 2H, **H-d, H-e, H-f**), 0.91 (t, $^3J_{HH}$ = 7.0 Hz, 3H, **H-g**).

^{13}C-NMR (101 MHz, Chloroform-d): *δ* 172.4 (**C-a**), 150.0 (**C-4´´**), 138.4 (**C-1´´**), 128.0 (**C-2´´**), 121.6 (**C-3´´**), 64.6 (s, **Ph-CH_2**), 34.3 (**C-b**), 31.4, 28.7, 22.4 (**C-d, C-e, C-f**), 24.8 (**C-c**), 14.0 (**C-g**).

HRMS (ESI-TOF) m/z: calculated for $C_{14}H_{20}NaO_3$ $[M+Na]^+$ 259.1305, found 259.1230.

IR: v = 3380, 2956, 2928, 2860, 1754, 1510, 1459, 1417, 1379, 1195, 1164, 1140, 1015, 848, 811, 503.

Pentadecanoyl chloride **42o**



Yield: calculated as quantitative

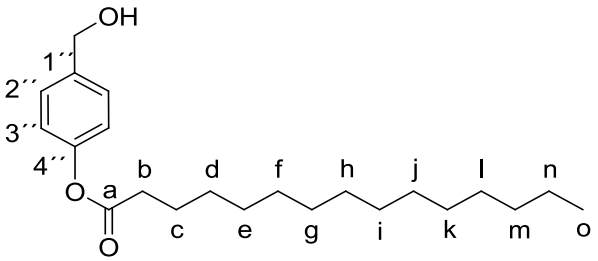
Chemical Formula: $C_{15}H_{29}ClO$

Molecular Weight: 260.19

Method 1 with thionyl chloride (b.p. 79 °C), under N_2, 10.1 mL fresh $SOCl_2$ (139.5 mmol, 3.0 equiv.) was added to a flask with 11.3 g pentadecanoic acid (46.5 mmol, 1.0 equiv.) and the mixture was refluxed for 2 h. Then $SOCl_2$ was evaporated to afford pentadecanoyl chloride as colorless liquid which yield was calculated as quantitative. The crude product was used directly without purification.

Method 2 with oxalyl chloride (b.p. 62-65 °C), under N_2, 2.0 g pentadecanoic acid (8.25 mmol, 1.0 equiv.) was dissolved in 40 mL DCM and cooled to 0 °C. 0.7 mL oxalyl chloride (9.9 mmol, 1.2 equiv.) was added to the flask and 2-3 drops of DMF was then added. Afterwards, the mixture was warm up to room temperature and stirred until no more gas generated (around 2-4 h). Then oxalyl chloride was evaporated to afford pentadecanoyl chloride as colorless liquid which yield was calculated as quantitative. The crude product was used directly without further purification.

4-(Hydroxymethyl)phenyl pentadecanoate **44o**



Yield: 63%

Chemical Formula: $C_{22}H_{36}O_3$

Molecular Weight: 348.52

According to **General Procedure A**, under atmosphere, 1.22 g 4-hydroxybenzylalcohol (9.9 mmol, 1.2 equiv.) and 1.36 mL triethylamine (9.9 mmol, 1.2 equiv.) was dissolved in 20 mL CH_2Cl_2 at room temperature. 2.14 g *n*-pentadecanoyl chloride (8.25 mmol, 1.0 equiv.) in 30 mL CH_2Cl_2 was added dropwise. The mixture was stirred for overnight at room temperature (r.t.). Column chromatography (SiO_2, PE/EE 7:3 v/v). Yield: 63%, 1.82 g, as colorless solid.

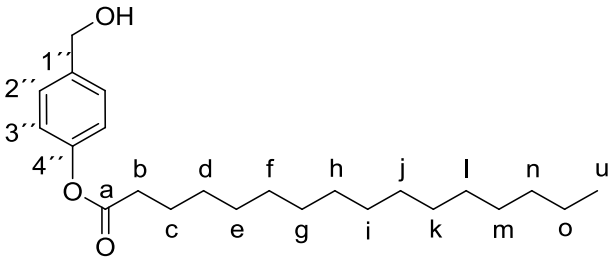
^{1}H NMR (400 MHz, Chloroform-d): δ 7.35 (dt, $^3J_{HH}$ = 8.8, 2.6 Hz, 2H, **H-2´´**), 7.06 (dt, $^3J_{HH}$ = 8.4, 2.4 Hz, 2H, **H-3´´**), 4.65 (s, 2H, **Ph-CH$_2$**), 2.55 (t, $^3J_{HH}$ = 7.6 Hz, 2H, **H-b**), 1.75 (p, $^3J_{HH}$ = 7.5 Hz, 2H, **H-c**), 1.46-1.19 (m, 22H, **H-d**, **H-e**, **H-f**, **H-g**, **H-h**, **H-I**, **H-j**, **H-k**, **H-l**, **H-m**, **H-n**), 0.89 (t, $^3J_{HH}$ = 6.8 Hz, 3H, **H-o**).

^{13}C-NMR (101 MHz, Chloroform-d): *δ* 172.4 (**C-a**), 150.1 (**C-4´´**), 138.4 (**C-1´´**), 128.0 (**C-2´´**), 121.6 (**C-3´´**), 64.7 (s, **Ph-CH$_2$**), 34.4 (**C-b**), 31.9, 29.66, 29.65, 29.57, 29.43, 29.33, 29.23, 29.08, 22.7 (**C-d**, **C-e**, **C-f**, **C-g**, **C-h**, **C-I**, **C-j**, **C-k**, **C-l**, **C-m**, **C-n**), 24.9 (**C-c**), 14.1 (**C-o**).

HRMS (ESI-TOF) m/z: calculated for $C_{22}H_{40}NO_3$ $[M+NH_4]^+$ 366.3003, found 366.3000.

IR: ν = 3321, 2955, 2914, 2847, 1748, 1605, 1509, 1463, 1411, 1386, 1318, 1294, 1270, 1246, 1218, 1166, 1150, 1094, 1037, 1014, 950, 925, 846, 817, 760, 745, 719, 580, 515.

4-(Hydroxymethyl)phenyl hexadecanoate **44u**



Yield: 80%

Chemical Formula: $C_{23}H_{38}O_3$

Molecular Weight: 362.55

According to **General Procedure A**, under atmosphere,1.22 g 4-hydroxybenzylalcohol (9.8 mmol, 1.2 equiv.) and 1.36 mL triethylamine (9.8 mmol, 1.2 equiv.) was dissolved in 20 mL CH_2Cl_2 at room temperature. 2.48 mL *n*-hexadecanoyl chloride (8.16 mmol, 1.0 equiv.) in 30 mL CH_2Cl_2 was added dropwise. The mixture was stirred for overnight at room temperature (R.T.). Column chromatography (SiO_2, PE/EE 7:3 v/v). Yield: 80%, 2.36 g, as colorless solid.

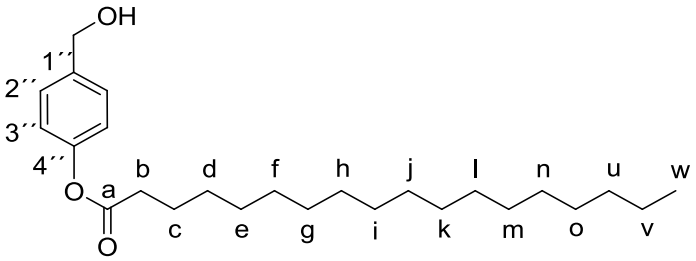
^{1}H NMR (400 MHz, Chloroform-d): δ 7.37 (d, $^3J_{HH}$ = 8.5 Hz, 2H, **H-2´´**), 7.06 (dt, $^3J_{HH}$ = 8.4, 2.4 Hz, 2H, **H-3´´**), 4.68 (s, 2H, **Ph-CH$_2$**), 2.55 (t, $^3J_{HH}$ = 7.5 Hz, 2H, **H-b**), 1.75 (p, *J* = 7.5 Hz, 2H, **H-c**), 1.48-1.18 (m, 24H, **H-d**, **H-e**, **H-f**, **H-g**, **H-h**, **H-I**, **H-j**, **H-k**, **H-l**, **H-m**, **H-n**, **H-o**), 0.88 (t, $^3J_{HH}$ = 7.0 Hz, 3H, **H-u**).

^{13}C-NMR (101 MHz, Chloroform-d): *δ* 172.4 (**C-a**), 150.1 (**C-4´´**), 138.3 (**C-1´´**), 128.0 (**C-2´´**), 121.7 (**C-3´´**), 64.8 (s, **Ph-CH$_2$**), 34.4 (**C-b**), 31.9, 29.68, 29,67, 29.64, 29.59, 29.45, 29.35, 29.25,29.10, 22.68 (**C-d**, **C-e**, **C-f**, **C-g**, **C-h**, **C-I**, **C-j**, **C-k**, **C-l**, **C-m**, **C-n**, **C-o**), 24.9 (**C-c**), 14.1 (**C-u**).

HRMS (ESI-TOF) m/z: calculated for $C_{23}H_{38}NaO_3$ $[M+Na]^+$ 385.2719, found 385.2782.

IR: v = 3322, 2955, 2914, 2847, 1748, 1606, 1509, 1471, 1463, 1410, 1387, 1348, 1330, 1308, 1286, 1263, 1241, 1218, 1166, 1150, 1097, 1039, 1014, 950, 925, 846, 817, 779, 960, 739, 727, 719, 581, 515.

4-(Hydroxymethyl)phenyl octadecanoate **44w**



Yield: 78%

Chemical Formula: $C_{25}H_{42}O_3$

Molecular Weight: 390.60

According to **General Procedure A**, under atmosphere, 2.46 g 4-hydroxybenzylalcohol (19.8 mmol, 1.2 equiv.) and 2.75 mL triethylamine (19.8 mmol, 1.2 equiv.) was added in 100 mL CH_2Cl_2. 5.57 mL octadecanoyl chloride (16.5 mmol, 1.0 equiv.) in 150 mL CH_2Cl_2 was added dropwise and the mixture was stirred at room temperature overnight. The precipitate was filtered, and the solvent was removed in vacuum. The residue was diluted with CH_2Cl_2 and washed once with water. The organic layer was dried with Na_2SO_4 and the solvent was removed. Column chromatography (SiO_2, petrol ether/ethylacetate 6:4 v/v). Yield: 78%, 5.0 g, as colorless solid.

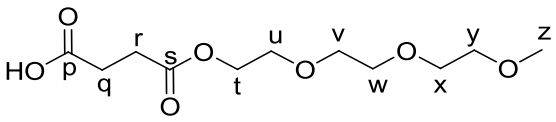
^{1}H NMR (500 MHz, Chloroform-d): δ 7.37 (d, $^3J_{HH}$ = 8.4 Hz, 2H, **H-2´´**), 7.07 (dt, $^3J_{HH}$ = 8.4 Hz, 2H, **H-3´´**), 4.68 (s, 2H, **Ph-CH$_2$**), 2.55 (t, $^3J_{HH}$ = 7.5 Hz, 2H, **H-b**), 1.74 (p, *J* = 7.5 Hz, 2H, **H-c**), 1.64 (br, s, 1H, **O-H**), 1.49-1.12 (m, 28H, **H-d**, **H-e**, **H-f**, **H-g**, **H-h**, **H-I**, **H-j**, **H-k**, **H-l**, **H-m**, **H-n**, **H-o**, **H-u**, **H-v**), 0.88 (t, $^3J_{HH}$ = 6.8 Hz, 3H, **H-w**).

^{13}C-NMR (126 MHz, Chloroform-d): *δ* 172.38 (**C-a**), 150.16 (**C-4´´**), 138.32 (**C-1´´**), 128.03 (**C-2´´**), 121.69 (**C-3´´**), 64.79 (s, **Ph-CH$_2$**), 34.39 (**C-b**), 31.92, 29.69, 29.67, 29.65, 29.64, 29.59, 29.45, 29.35, 29.25, 29.10 (**C-d**, **C-e**, **C-f**, **C-g**, **C-h**, **C-I**, **C-j**, **C-k**, **C-l**, **C-m**, **C-n**, **C-o**, **C-u**), 24.94 (**C-c**), 22.68 (**C-v**), 14.11 (**C-w**).

HRMS (ESI-TOF) m/z: calculated for $C_{25}H_{46}NO_3$ $[M+NH_4]^+$ 408.3472, found 408.3478.

IR: v = 3318, 2955, 2914, 2847, 1748, 1605, 1509, 1471, 1463, 1410, 1387, 1331, 1312, 1292, 1272, 1252, 1232, 1218, 1166, 1150, 1101, 1034, 1014, 950, 924, 846, 817, 760, 727, 719, 580, 511.

12-oxo-2,5,8,11-tetraoxapentadecan-15-oic acid **48b**



Yield: 72%

Chemical Formula: $C_{11}H_{20}O_7$

Molecular Weight: 264.27

Under atmosphere, 10 g 2-(2-(2-Methoxyethoxy)ethoxy)ethan-1-ol **46** (60.9 mmol, 1.0 equiv.) and 7.3 g succinic anhydride **47b** (73.1 mmol, 1.2 equiv.) was added in 40 mL CH_2Cl_2. Then 1.49 g DMAP (12.2 mmol, 0.2 equiv.) was added and the mixture was stirred at room temperature overnight. The reaction mixture was then quenched with 4 mL water, diluted with 20 mL DCM and extract with (3×10 mL) 10% $NaHSO_4$ and (1×10 mL) brine. The organic phase was dried with $MgSO_4$ and the solvent was removed in vacuum. The product was used directly without further purification. Yield: 72%, 11.6 g, as colorless liquid.

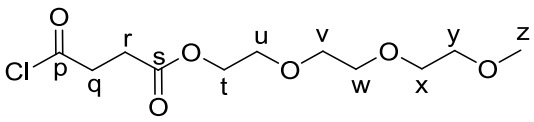
^{1}H NMR (400 MHz, DMSO-d6): δ 4.16-4.07 (m, 2H, **H-t**), 3.65-3.56 (m, 2H, **H-u**), 3.56-3.48 (m, 6H, **H-v**, **H-w**, **H-x**), 3.48-3.38 (m, 2H, **H-y**) 3.24 (s, 3H, **H-z**), 2.57 -2.42 (m, 4H, **H-q**, **H-r**).

^{13}C-NMR (101 MHz, DMSO-d6): *δ* 173.24, 172.07 (**C-p**, **C-s**), 71.23 (**C-y**), 69.76, 69.68, 69.56 (**C-v**, **C-w**, **C-x**), 68.20 (**C-u**), 63.33 (**C-t**), 58.00 (**C-z**), 28.60 (**C-q**, **C-r**).

HRMS (ESI-TOF): calculated for $C_{11}H_{20}NaO_7$ $[M+Na]^+$ 287.1101, found 287.1072.

IR: v = 2877, 1730, 1452, 1383, 1349, 1244, 1199, 1160, 1097, 1027, 988, 942, 849, 623, 564, 405.

2-(2-(2-methoxyethoxy)ethoxy)ethyl 4-chloro-4-oxobutanoate **49b**



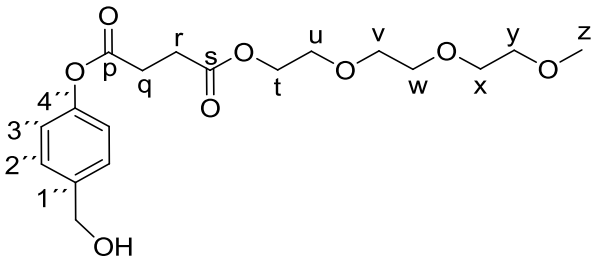
Yield: calculated as quantitative

Chemical Formula: $C_{11}H_{19}ClO_6$

Molecular Weight: 282.72

5.0 g 12-oxo-2,5,8,11-tetraoxapentadecan-15-oic acid **48b** (18.9 mmol, 1.0 equiv.) was dissolved in 100 mL DCM and cooled to 0 °C. 0.7 mL oxalyl chloride (22.7 mmol, 1.2 equiv.) was added to the flask and 3 drops of DMF was then added. Afterwards, the mixture was warm up to room temperature and stirred until no more gas generated (around 2-4 h). Then oxalyl chloride was evaporated to afford target acyl chloride as colorless liquid. The yield was calculated as quantitative. The crude product was used directly without further purification.

4-(Hydroxymethyl)phenyl (2-(2-(2-methoxyethoxy)ethoxy)ethyl) succsinate **50b**



Yield: 50%

Chemical Formula: $C_{18}H_{26}O_8$

Molecular Weight: 370.40

According to **General Procedure A**, under atmosphere, 2.25 g 4-hydroxybenzylalcohol (18.1 mmol, 1.2 equiv.) and 2.52 mL triethylamine (18.1 mmol, 1.2 equiv.) was added in 20 mL CH_2Cl_2. 4.27 g 2-(2-(2-methoxyethoxy)ethoxy)ethyl 4-chloro-4-oxobutanoate **49b** (15.1 mmol, 1.0 equiv.) in 30 mL CH_2Cl_2 was added dropwise and the mixture was stirred at room temperature overnight. The precipitate was filtered and the solvent was removed in vacuum. The residue was diluted with CH_2Cl_2 and washed once more with water. The organic layer was dried with Na_2SO_4 and the solvent was removed. Column chromatography (SiO_2, petrol ether/ethylacetate 2:8 v/v). Yield: 50%, 3.0 g, as colorless oil.

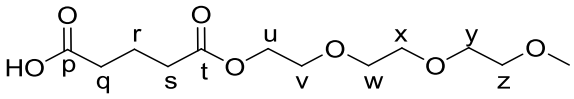
^{1}H NMR (400 MHz, Chloroform-d) δ 7.36 (d, $^3J_{HH}$ = 8.5 Hz, 2H, **H-2´´**), 7.07 (d, $^3J_{HH}$ = 8.5 Hz, 2H, **H-3´´**), 4.66 (s, 2H, **Ph-CH_2**), 4.32-4.22 (m, 2H, **H-t**), 3.74-3.67 (m, 2H, **H-u**), 3.67-3.58 (m, 6H, **H-v, H-w, H-x**), 3.56-3.50 (m, 2H, **H-y**) 3.37 (s, 3H, **-OCH_3**), 2.87 (t, $^3J_{HH}$ = 6.8 Hz, 2H, **H-q**), 2.76 (t, $^3J_{HH}$ = 6.8 Hz, 2H, **H-r**), 1.97 (s, 1H, **O-H**).

^{13}C-NMR (101 MHz, Chloroform-d): δ 172.07 (**C-s**), 170.89 (**C-p**), 149.97 (**C-4´´**), 138.58 (**C-1´´**), 128.00 (**C-2´´**), 121.54 (**C-3´´**), 71.88 (**C-y**), 70.53, 70.50 (**C-v, C-w, C-x**), 69.02 (**C-u**), 64.66 (s, **Ph-CH_2**), 63.96 (**C-t**), 58.98 (**-OCH_3**), 29.26 (**C-q**), 29.04 (**C-r**).

HRMS (ESI-TOF): calculated for $C_{18}H_{26}NaO_8$ $[M+Na]^+$ 393.1520, found 393.1514.

IR: v = 3437, 2875, 1856, 1732, 1607, 1507, 1453, 1411, 1350, 1244, 1196, 1164, 1131, 1099, 1015, 997, 944, 888, 847, 811, 550, 506.

12-oxo-2,5,8,11-tetraoxahexadecan-16-oic acid **48c**



Yield:59%

Chemical Formula: $C_{12}H_{22}O_7$

Molecular Weight: 278.30

Under atmosphere, 10 g 2-(2-(2-Methoxyethoxy)ethoxy)ethan-1-ol **46** (60.9 mmol, 1.0 equiv.) and 8.4 g glutaric anhydride **47c** (73.1 mmol, 1.2 equiv.) was added in 50 mL CH_2Cl_2. Then 1.49 g DMAP (12.2 mmol, 0.2 equiv.) was added and the mixture was stirred at room temperature overnight. The reaction mixture was then quenched with 4 mL water, diluted with 20 mL DCM and extract with (3×10 mL) 10% $NaHSO_4$ and (1×10 mL) brine. The organic phase was dried with $MgSO_4$ and the solvent was removed in vacuum. The product was used directly without further purification. Yield: 59%, 9.8 g, as colorless liquid.

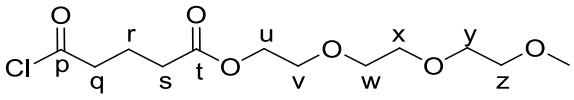
^{1}H NMR (400 MHz, Chloroform-d) δ 4.31-4.20 (m, 2H, **H-u**), 3.75-3.61 (m, 8H, **H-v, H-w, H-x, H-y**), 3.60-3.52 (m, 2H, **H-z**), 3.38 (s, 3H, **-OCH$_3$**), 2.44 (td, $^3J_{HH}$ = 7.1, 3.5 Hz, 4H, **H-q, H-s**), 1.98 (quint, $^3J_{HH}$ = 7.2 Hz, 2H, **H-r**).

^{13}C-NMR (101 MHz, Chloroform-d): *δ* 172.9 (**C-p, C-t**), 71.9 (**C-z**), 70.61, 70.49, 70.46 (**C-w**, **C-x**, **C-y**), 69.10 (**C-v**), 63.57 (**C-u**), 58.95 (**-OCH$_3$**), 33.10, 32.74 (**C-q, C-s**), 19.88 (**C-r**).

HRMS (ESI-TOF): calculated for $C_{12}H_{22}NaO_7$ $[M+Na]^+$ 301.1258, found 301.1263.

IR: v = 2880, 1731, 1451, 1385, 1344, 1240, 1189, 1155, 1098, 1024, 978, 951, 832, 611, 542, 410.

2-(2-(2-methoxyethoxy)ethoxy)ethyl 5-chloro-5-oxopentanoate **49c**



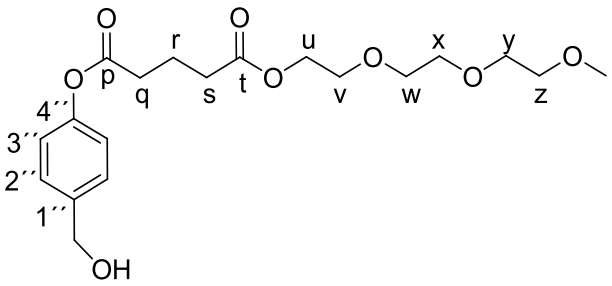
Yield: 77%

Chemical Formula: $C_{12}H_{21}ClO_6$

Molecular Weight: 296.74

4.3 g 12-oxo-2,5,8,11-tetraoxahexadecan-16-oic acid **48c** (15.6 mmol, 1.0 equiv.) was dissolved in 50 mL DCM and cooled to 0 °C. 1.58 mL oxalyl chloride (18.7 mmol, 1.2 equiv.) was added to the flask and 3 drops of DMF was then added. Afterwards, the mixture was warm up to room temperature and stirred until no more gas generated (around 2-4 h). Then oxalyl chloride was evaporated to afford 4.3 g target acyl chloride as colorless liquid. The yield was calculated as 77%. The crude product was used directly without further purification.

4-(Hydroxymethyl)phenyl (2-(2-(2-methoxyethoxy)ethoxy)ethyl) glutarate **50c**



Yield: 50%

Chemical Formula: $C_{19}H_{28}O_8$

Molecular Weight: 384.42

According to **General Procedure A**, under atmosphere, 2.32 g 4-hydroxybenzylalcohol (18.7 mmol, 1.2 equiv.) and 2.59 mL triethylamine (18.7 mmol, 1.2 equiv.) was added in 20 mL CH_2Cl_2. 4.3 g 2-(2-(2-methoxyethoxy)ethoxy)ethyl 5-chloro-5-oxopentanoate **49c** (15.6 mmol, 1.0 equiv.) in 30 mL CH_2Cl_2 was added dropwise and the mixture was stirred at room temperature overnight. The precipitate was filtered, and the solvent was removed in vacuum. The residue was diluted with CH_2Cl_2 and washed once more with water. The organic layer was dried with Na_2SO_4 and the solvent was removed. Column chromatography (SiO_2, petrol ether/ethylacetate 2:8 v/v). Yield: 50%, 3.0 g, as colorless oil.

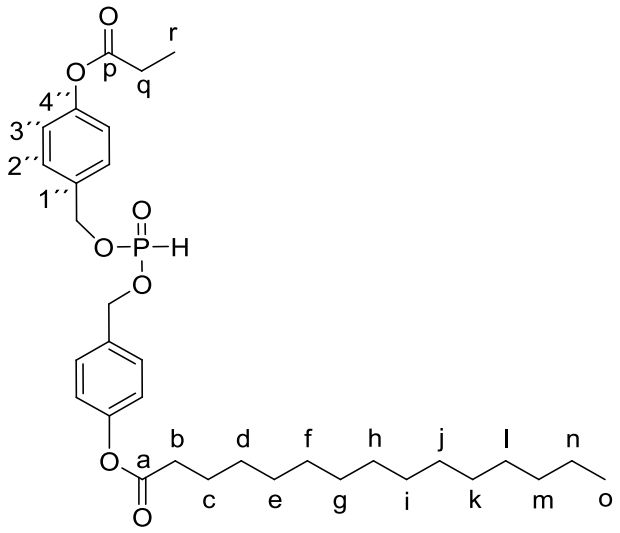
^{1}H NMR (400 MHz, Chloroform-d) δ 7.37 (d, $^3J_{HH}$ = 8.5 Hz, 2H, **H-2´´**), 7.07 (d, $^3J_{HH}$ = 9.2 Hz, 2H, **H-3´´**), 4.68 (d, $^3J_{HH}$ = 4.3 Hz, 2H, **Ph-CH$_2$**), 4.32-4.20 (m, 2H, **H-u**), 3.75-3.67 (m, 2H, **H-v**), 3.67-3.60 (m, 6H, **H-w, H-x, H-y**), 3.57-3.51 (m, 2H, **H-z**) 3.37 (s, 3H, **-OCH$_3$**), 2.64 (t, $^3J_{HH}$ = 7.4 Hz, 2H, **H-q**), 2.49 (t, $^3J_{HH}$ = 7.3 Hz, 2H, **H-s**), 2.07 (quint, $^3J_{HH}$ = 7.3 Hz, 2H, **H-r**), 1.80 (s, 1H, **O-H**).

^{13}C-NMR (101 MHz, Chloroform-d): *δ* 172.8 (**C-t**), 171.5 (**C-p**), 150.0 (**C-4´´**), 138.5 (**C-1´´**), 128.0 (**C-2´´**), 121.6 (**C-3´´**), 71.93 (**C-z**), 70.60, 70.57 (**C-w, C-x, C-y**), 69.11 (**C-v**), 64.73 (s, **Ph-CH$_2$**), 63.63 (**C-u**), 59.0 (**-OCH$_3$**), 33.30 (**C-q**), 33.06 (**C-s**), 20.03 (**C-r**).

HRMS (ESI-TOF) m/z: calculated for $C_{19}H_{28}NaO_8$ $[M+Na]^+$ 407.1676, found 407.1703.

IR: v = 3439, 2874, 1731, 1601, 1507, 1452, 1417, 1381, 1352, 1195, 1163, 1127, 1016, 944, 849, 562, 507, 385.

(AB-C_2H_5,ab-$C_{14}H_{29}$)-*H*-phosphonate **55co**



Yield: 50%

Chemical Formula: $C_{32}H_{47}O_7P$

Molecular Weight: 574.69

According to **General Procedure B**, 0.23 mL diphenyl phosphonate (1.2 mmol, 1.2 equiv.) was added to 5 ml pyridine at 0 °C. Then 0.35 g 4-(Hydroxymethyl)phenyl pentadecanoate **44o** (1.0 mmol, 1.0 equiv.) was added and followed with 0.25 g 4-(Hydroxymethyl)phenyl propionate **44c** (1.4 mmol, 1.4 equiv.). The mixture was stirred for 3 h at room temperature. Column chromatography (SiO_2, petrol ether/ethylacetate/CH_3COOH 7:3:0.005 v/v/v). Yield: 50%, 0.288 g, as colorless solid.

^{1}H NMR (400 MHz, Chloroform-d): δ 7.39-7.30 (m, 4H, **H-2´´**), 7.17-7.02 (m, 4H, **H-3´´**), 6.94 (d, $^1J_{PH}$ = 708 Hz, 1H, **P-H**), 5.13-4.93 (m, 4H, **Ph-CH_2**), 2.59 (q, $^3J_{HH}$ = 8.0 Hz, 2H, **H-q**), 2.55 (t, $^3J_{HH}$ = 6.3 Hz, 2H, **H-b**), 1.75 (p, $^3J_{HH}$ = 7.5 Hz, 2H, **H-c**), 1.47-1.25 (m, 25H, **H-d**, **H-e**, **H-f**, **H-g**, **H-h**, **H-i**, **H-j**, **H-k**, **H-l**, **H-m**, **H-n**, **H-r**), 0.88 (t, $^3J_{HH}$ = 6.7 Hz, 3H, **H-o**).

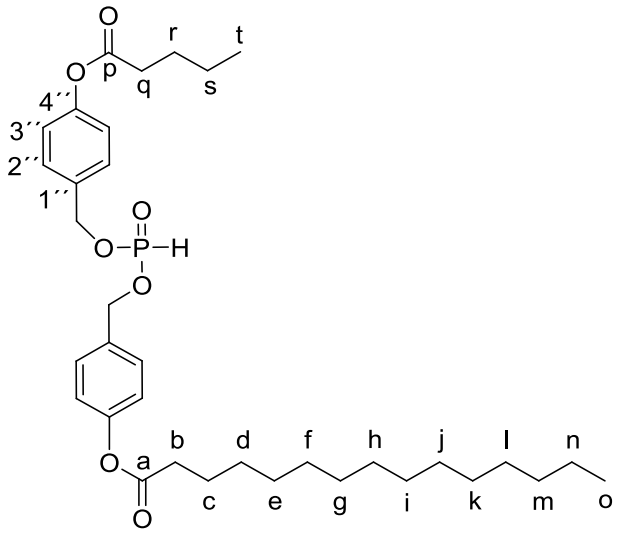
^{13}C-NMR (101 MHz, Chloroform-d): *δ* 172.7 (**C-p**),172.1 (**C-a**), 151.0 (**2×C-4´´**), 132.98, 132.92 (**2×C-1´´**), 129.2 (**4×C-2´´**), 121.90, 121.87 (**4×C-3´´**), 66.65 (d, $^3J_{CP}$ = 6.1 Hz, **Ph-CH_2**), 34.4 (**C-b**), 31.9, 29.65, 29,64, 29.61, 29.57, 29.43, 29.32, 29.22, 29.08 (**C-d**, **C-e**, **C-f**, **C-g**, **C-h**, **C-i**, **C-j**, **C-k**, **C-l**, **C-m**), 27.7 (**C-q**), 24.9 (**C-c**), 22.7 (**C-n**), 14.1 (**C-o**), 9.0 (**C-r**).

^{31}P-NMR (162 MHz, Chloroform-d): *δ* 7.71.

HRMS (ESI-TOF) m/z: calculated for $C_{32}H_{47}NaO_7P$ $[M+Na]^+$ 597.2952, found 597.2952.

IR: v = 2956, 2915, 2848, 1754, 1607, 1510, 1463, 1412, 1386, 1359, 1318, 1295, 1269, 1249, 1219, 1167, 1149, 1061, 996, 925, 896, 876, 827, 806, 769, 720, 806, 769, 720, 581, 538, 515, 455, 422, 410.

(AB-C_4H_9,ab-$C_{14}H_{29}$)-*H*-phosphonate **55eo**



Yield: 48%

Chemical Formula: $C_{34}H_{51}O_7P$

Molecular Weight: 602.74

According to **General Procedure B**, 0.23 mL diphenyl phosphonate (1.2 mmol, 1.2 equiv.) was added to 5 ml pyridine at 0 °C. Then 0.35 g 4-(Hydroxymethyl)phenyl pentadecanoate **44o** (1.0 mmol, 1.0 equiv.) was added and followed with 0.29 g 4-(Hydroxymethyl)phenyl pentanoate **44e** (1.4 mmol, 1.4 equiv.). The mixture was stirred for 3 h at room temperature. Column chromatography (SiO_2, petrol ether/ethylacetate/CH_3COOH 7:3:0.005 v/v/v). Yield: 48%, 0.292 g, as colorless solid.

^{1}H NMR (400 MHz, Chloroform-d): δ 7.44-7.31 (m, 4H, **H-2´´**), 7.17-7.01 (m, 4H, **H-3´´**), 6.94 (d, $^1J_{PH}$ = 708 Hz, 1H, **P-H**), 5.16-4.93 (m, 4H, **Ph-CH$_2$**), 2.56 (t, $^3J_{HH}$ = 7.5 Hz, 2H, **H-q**), 2.55 (t, $^3J_{HH}$ = 7.5 Hz, 2H, **H-b**), 1.85-1.65 (m, 4H, **H-c**, **H-r**), 1.53-1.17 (m, 24H, **H-d**, **H-e**, **H-f**, **H-g**, **H-h**, **H-i**, **H-j**, **H-k**, **H-l**, **H-m**, **H-n**, **H-s**), 0.97 (t, $^3J_{HH}$ = 7.3 Hz, 3H, **H-t**), 0.88 (t, $^3J_{HH}$ = 6.7 Hz, 3H, **H-o**).

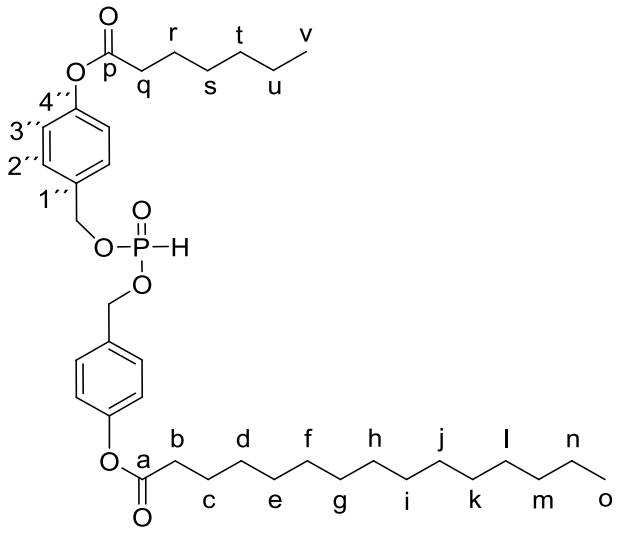
^{13}C-NMR (101 MHz, Chloroform-d): *δ* 172.08 (**C-p**),172.06 **(C-a**), 151.0 (**2×C-4´´**), 132.98, 132.92 (**2×C-1´´**), 129.2 (**4×C-2´´**), 121.90 (**4×C-3´´**), 66.69, 66.63 (**2×Ph-CH$_2$**), 34.4 (**C-b**), 34.1 (**C-q**), 31.9, 29.66, 29,65, 29.62, 29.57, 29.43, 29.33, 29.23, 29.08 (**C-d**, **C-e**, **C-f**, **C-g**, **C-h**, **C-i**, **C-j**, **C-k**, **C-l**, **C-m**), 26.9 (**C-r**), 24.9 (**C-c**), 22.7 (**C-n**), 22.2 (**C-s**), 14.1 (**C-o**), 13.7 (**C-t**)

^{31}P-NMR (162 MHz, Chloroform-d): *δ* 7.70.

HRMS (ESI-TOF) m/z: calculated for $C_{34}H_{55}NO_7P$ $[M+NH_4]^+$ 620.3711, found 620.3709.

IR: ν = 2956, 2915, 2848, 1748, 1608, 1510, 1465, 1413, 1383, 1350, 1317, 1295, 1268, 1250, 1219, 1167, 1150, 1105, 1061, 1009, 996, 925, 897, 878, 834, 769, 720, 692, 580, 559, 538, 515, 456, 420, 408.

(AB-C_6H_{13},ab-$C_{14}H_{29}$)-*H*-phosphonate **55go**



Yield: 52%

Chemical Formula: $C_{36}H_{55}O_7P$

Molecular Weight: 630.79

According to **General Procedure B**, 0.23 mL diphenyl phosphonate (1.2 mmol, 1.2 equiv.) was added to 5 ml pyridine at 0 °C. Then 0.35 g 4-(Hydroxymethyl)phenyl pentadecanoate **44o** (1.0 mmol, 1.0 equiv.) was added and followed with 0.33 g 4-(Hydroxymethyl)phenyl heptanoate **44g** (1.4 mmol, 1.4 equiv.). The mixture was stirred for 3 h at room temperature. Column chromatography (SiO_2, petrol ether/ethylacetate/CH_3COOH 7:3:0.005 v/v/v). Yield: 52%, 0.329 g, as colorless solid.

^{1}H NMR (400 MHz, Chloroform-d): δ 7.51-7.32 (m, 4H, **H-2´´**), 7.18-6.97 (m, 4H, **H-3´´**), 6.93 (d, $^1J_{PH}$ = 708 Hz, 1H, **P-H**), 5.20-4.85 (m, 4H, **Ph-CH_2**), 2.55 (m, 4H, **H-q, H-b**), 1.75 (m, 4H, **H-c**, **H-r**), 1.55-1.18 (m, 28H, **H-d**, **H-e**, **H-f**, **H-g**, **H-h**, **H-i**, **H-j**, **H-k**, **H-l**, **H-m**, **H-n**, **H-s**, **H-t**, **H-u**), 0.940.84 (m, 6H, **H-v**, **H-o**).

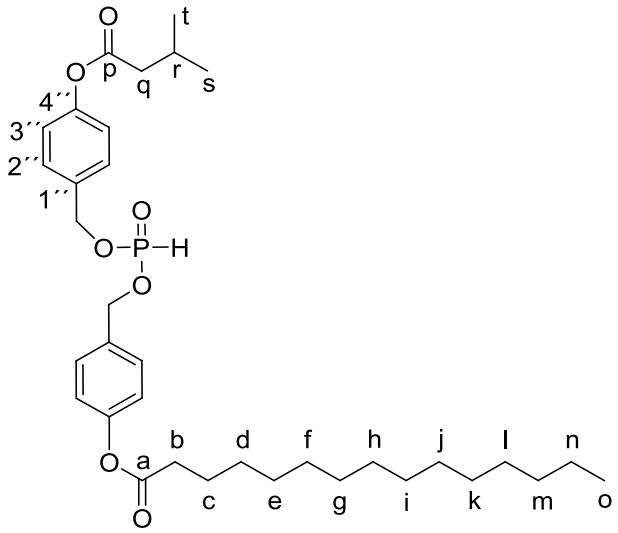
^{13}C-NMR (101 MHz, Chloroform-d): *δ* 172.05 (**C-p**, **C-a**), 151.0 (**2×C-4´´**), 132.97, 132.91 (**2×C-1´´**), 129.2 (**4×C-2´´**), 121.90 (**4×C-3´´**), 66.67, 66.61 (**2×Ph-CH_2**), 34.3 (**C-b**, **C-q**), 31.88, 31.38, 29.64, 29,63, 29.60, 29.56, 29.42, 29.31, 29.21, 29.07, 28.72 (**C-d**, **C-e**, **C-f**, **C-g**, **C-h**, **C-i**, **C-j**, **C-k**, **C-l**, **C-m, C-s**, **C-t**), 24.87,24,82 (**C-r**, **C-c**), 22.6 (**C-n**), 22.4 (**C-u**), 14.1 (**C-o**), 14.0 (**C-v**)

^{31}P-NMR (162 MHz, Chloroform-d): *δ* 7.70.

HRMS (ESI-TOF) m/z: calculated for $C_{36}H_{59}NO_7P$ $[M+NH_4]^+$ 648.4024, found 648.4037.

IR: v =2956, 2915, 2848, 1748, 1608, 1510, 1465, 1411, 1384, 1293, 1270, 1250, 1236, 1218, 1167, 1149, 1116, 1061, 996, 925, 878, 834, 769, 739, 721, 581, 539, 515, 453, 427.

(AB-*iso*-C_4H_9,ab-$C_{14}H_{29}$)-*H*-phosphonate **55e*io***



Yield: 46%

Chemical Formula: $C_{34}H_{51}O_7P$

Molecular Weight: 602.74

According to **General Procedure B**, 0.23 mL diphenyl phosphonate (1.2 mmol, 1.2 equiv.) was added to 5 ml pyridine at 0 °C. Then 0.35 g 4-(Hydroxymethyl)phenyl pentadecanoate **44o** (1.0 mmol, 1.0 equiv.) was added and followed with 0.29 g 4-(Hydroxymethyl)phenyl 3-methylbutanoate **44e*i*** (1.4 mmol, 1.4 equiv.). The mixture was stirred for 3 h at room temperature. Column chromatography (SiO_2, petrol ether/ethylacetate/CH_3COOH 7:3:0.005 v/v/v). Yield: 48%, 0.277 g, as colorless solid.

^{1}H NMR (400 MHz, Chloroform-d): δ 7.33-7.23 (m, 4H, **H-2´´**), 7.08-6.95 (m, 4H, **H-3´´**), 6.87 (d, $^1J_{PH}$ = 708 Hz, 1H, **P-H**), 5.16-4.89 (m, 4H, **Ph-CH$_2$**), 2.48 (t, $^3J_{HH}$ = 7.5 Hz, 2H, **H-b**), 2.36 (d, $^3J_{HH}$ = 7.2 Hz, 2H, **H-q**), 2.17 (tq, $^3J_{HH}$ = 6.8 Hz, 1H, **H-r**), 1.67 (p, $^3J_{HH}$ = 7.5 Hz, 2H, **H-c**), 1.40-1.11 (m, 22H, **H-d**, **H-e**, **H-f**, **H-g**, **H-h**, **H-i**, **H-j**, **H-k**, **H-l**, **H-m**, **H-n**), 0.99 (d, $^3J_{HH}$ = 6.7 Hz, 6H, **H-t**), 0.81 (t, $^3J_{HH}$ = 6.8 Hz, 3H, **H-o**).

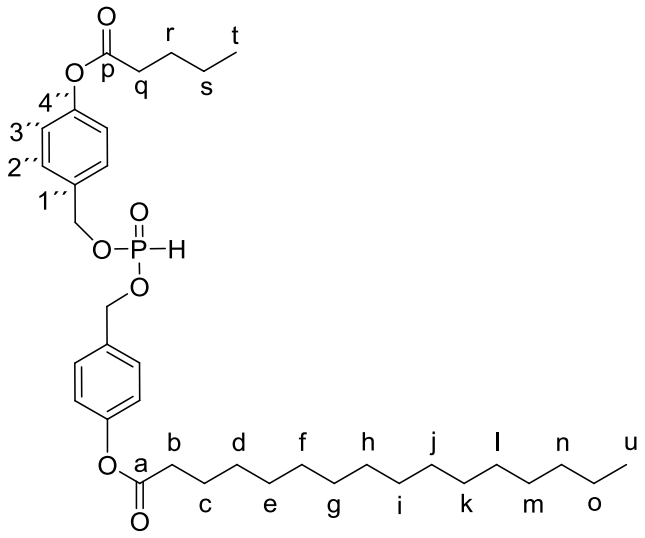
^{13}C-NMR (101 MHz, Chloroform-d): *δ* 172.08 (**C-a**), 171.31 **(C-p**), 150.99, 150.94 (**2×C-4´´**), 133.01, 132.95 (**2×C-1´´**), 129.22, 129.21 (**4×C-2´´**), 121.94, 121.92 (**4×C-3´´**), 66.70, 66.65 (**2×Ph-CH$_2$**), 43.30 (**C-q**), 34.36 (**C-b**), 31.90, 29.66, 29.65, 29.62, 29.58, 29.44, 29.33, 29.23, 29.09 (**C-d**, **C-e**, **C-f**, **C-g**, **C-h**, **C-i**, **C-j**, **C-k**, **C-l**, **C-m**), 25.83 (**C-r**), 24.89 (**C-c**), 22.66 (**C-n)**, 22.37 (**C-s**, **C-t**), 14.09 (**C-o**).

^{31}P-NMR (162 MHz, Chloroform-d): *δ* 7.70.

HRMS (ESI-TOF) m/z: calculated for $C_{34}H_{51}NaO_7P$ $[M+Na]^+$ 625.3265, found 625.3262.

IR: v = 2956, 2915, 2848, 1748, 1608, 1510, 1464, 1413, 1385, 1317, 1295, 1250, 1218, 1166, 1150, 1106, 1060, 996, 924, 877, 832, 768, 719, 693, 580, 558, 538, 515, 456, 423.

(AB-C_4H_9,ab-$C_{15}H_{31}$)-*H*-phosphonate **55eu**



Yield: 44%

Chemical Formula: $C_{35}H_{53}O_7P$

Molecular Weight: 616.76

According to **General Procedure B**, 0.23 mL diphenyl phosphonate (1.2 mmol, 1.2 equiv.) was added to 5 ml pyridine at 0 °C. Then 0.36 g 4-(Hydroxymethyl)phenyl hexadecanoate **44u** (1.0 mmol, 1.0 equiv.) was added and followed with 0.29 g 4-(Hydroxymethyl)phenyl pentanoate **44e** (1.4 mmol, 1.4 equiv.). The mixture was stirred for 3 h at room temperature. Column chromatography (SiO_2, petrol ether/ethylacetate/CH_3COOH 7:3:0.005 v/v/v). Yield: 44%, 0.270 g, as colorless solid.

^{1}H NMR (600 MHz, Chloroform-d): δ 7.39-7.34 (m, 4H, **H-2´´**), 7.10-7.04 (m, 4H, **H-3´´**), 6.93 (d, $^1J_{PH}$ = 708 Hz, 1H, **P-H**), 5.12-4.95 (m, 4H, **Ph-CH$_2$**), 2.56 (t, $^3J_{HH}$ = 7.6 Hz, 2H, **H-q**), 2.55 (t, $^3J_{HH}$ = 7.5 Hz, 2H, **H-b**), 1.74 (m, 4H, **H-c**, **H-r**), 1.54-1.19 (m, 26H, **H-d**, **H-e**, **H-f**, **H-g**, **H-h**, **H-i**, **H-j**, **H-k**, **H-l**, **H-m**, **H-n**, **H-o, H-s**), 0.97 (t, $^3J_{HH}$ = 7.4 Hz, 3H, **H-t**), 0.88 (t, $^3J_{HH}$ = 7.0 Hz, 3H, **H-u**).

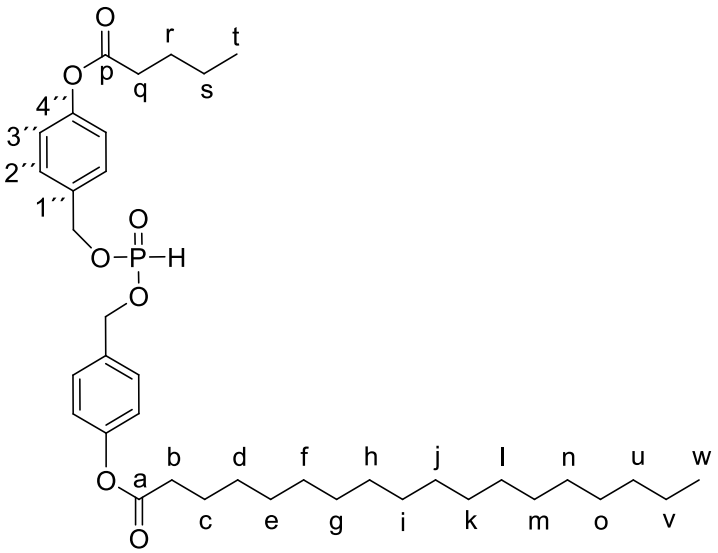
^{13}C-NMR (151 MHz, Chloroform-d): *δ* 172.09 (**C-p**),172.08 **(C-a**), 151.0 (**2×C-4´´**), 132.97, 132.93 (**2×C-1´´**), 129.2 (**4×C-2´´**), 121.90 (**4×C-3´´**), 66.68, 66.64 (**2×Ph-CH$_2$**), 34.4 (**C-b**), 34.1 (**C-q**), 31.9, 29.66, 29,65, 29.63, 29.62, 29.58, 29.44, 29.33, 29.23, 29.09 (**C-d**, **C-e**, **C-f**, **C-g**, **C-h**, **C-i**, **C-j**, **C-k**, **C-l**, **C-m**, **C-n**), 26.9 (**C-r**), 24.9 (**C-c**), 22.7 (**C-o**), 22.2 (**C-s**), 14.1 (**C-u**), 13.7 (**C-t**).

^{31}P-NMR (243 MHz, Chloroform-d): *δ* 7.70.

HRMS (ESI-TOF) m/z: calculated for $C_{35}H_{57}NO_7P$ $[M+NH_4]^+$ 634.3867, found 634.3861.

IR: v =2956, 2915, 2848, 1748, 1608, 1510, 1465, 1413, 1383, 1348, 1250, 1240, 1219, 1167, 1150, 1105, 1061, 1008, 996, 925, 897, 879, 833, 769, 720, 580, 538, 514, 451, 421, 403.

(AB-C_4H_9,ab-$C_{17}H_{35}$)-*H*-phosphonate **55ew**



Yield: 43%

Chemical Formula: $C_{37}H_{57}O_7P$

Molecular Weight: 644.82

According to **General Procedure B**, 0.23 mL diphenyl phosphonate (1.2 mmol, 1.2 equiv.) was added to 5 ml pyridine at 0 °C. Then 0.39 g 4-(Hydroxymethyl)phenyl octadecanoate **44w** (1.0 mmol, 1.0 equiv.) was added and followed with 0.29 g 4-(Hydroxymethyl)phenyl pentanoate **44e** (1.4 mmol, 1.4 equiv.). The mixture was stirred for 3 h at room temperature. Column chromatography (SiO_2, petrol ether/ethylacetate/CH_3COOH 8:2:0.005 v/v/v). Yield: 43%, 0.280 g, as colorless solid.

^{1}H NMR (600 MHz, Chloroform-d): δ 7.39-7.33 (m, 4H, **H-2´´**), 7.11-7.05 (m, 4H, **H-3´´**), 6.93 (d, $^1J_{PH}$ = 708 Hz, 1H, **P-H**), 5.16-4.91 (m, 4H, **Ph-CH$_2$**), 2.56 (t, $^3J_{HH}$ = 7.5 Hz, 2H, **H-q**), 2.55 (t, $^3J_{HH}$ = 7.5 Hz, 2H, **H-b**), 1.74 (m, 4H, **H-c**, **H-r**), 1.49-1.20 (m, 30H, **H-d**, **H-e**, **H-f**, **H-g**, **H-h**, **H-i**, **H-j**, **H-k**, **H-l**, **H-m**, **H-n**, **H-o**, **H-u**, **H-v**, **H-s**), 0.97 (t, $^3J_{HH}$ = 7.3 Hz, 3H, **H-t**), 0.88 (t, $^3J_{HH}$ = 7.0 Hz, 3H, **H-w**).

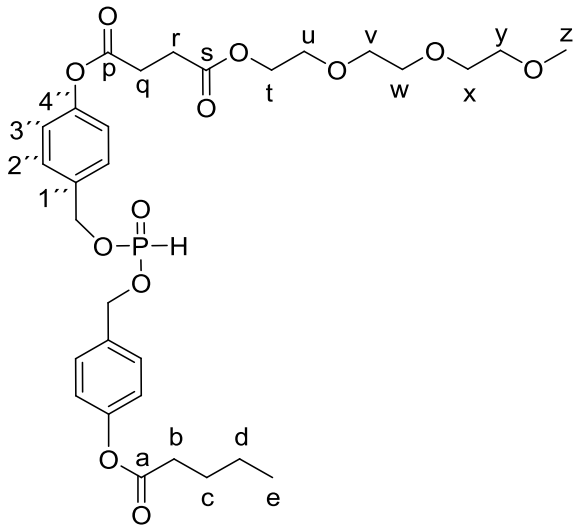
^{13}C-NMR (151 MHz, Chloroform-d): *δ* 172.08 (**C-p**),172.06 **(C-a**), 151.0 (**2×C-4´´**), 132.95, 132.91 (**2×C-1´´**), 129.2 (**4×C-2´´**), 121.90 (**4×C-3´´**), 66.67, 66.63 (**2×Ph-CH$_2$**), 34.3 (**C-b**), 34.1 (**C-q**), 31.9, 29.66, 29,64, 29.62, 29.61, 29.57, 29.43, 29.33, 29.22, 29.08 (**C-d**, **C-e**, **C-f**, **C-g**, **C-h**, **C-i**, **C-j**, **C-k**, **C-l**, **C-m**, **C-n**, **C-o**, **C-u**), 26.9 (**C-r**), 24.9 (**C-c**), 22.7 (**C-v**), 22.2 (**C-s**), 14.1 (**C-w**), 13.7 (**C-t**).

^{31}P-NMR (243 MHz, Chloroform-d): *δ* 7.71.

HRMS (ESI-TOF) m/z: calculated for $C_{37}H_{61}NO_7P$ $[M+NH_4]^+$ 662.4180, found 662.4200.

IR: v =2956, 2915, 2848, 1748, 1608, 1510, 1465, 1383, 1251, 1235, 1220, 1167, 1150, 1104, 1062, 1010, 997, 925, 878, 834, 769, 720, 510.

(AB-C_4H_9,ab-MEEES)-*H*-phosphonate **55e-MEEES**



Yield: 52%

Chemical Formula: $C_{30}H_{41}O_{12}P$

Molecular Weight: 624.61

According to **General Procedure B**, 0.23 mL diphenyl phosphonate (1.2 mmol, 1.2 equiv.) was added to 5 ml pyridine at 0 °C. Then 0.28 g 4-(Hydroxymethyl)phenyl pentanoate **44e** (1.0 mmol, 1.0 equiv.) was added and followed with 0.52 g 4-(Hydroxymethyl)phenyl (2-(2-(2-methoxyethoxy)ethoxy)ethyl) succsinate **50b** (1.4 mmol, 1.4 equiv.). The mixture was stirred for 3 h at room temperature. Column chromatography (SiO_2, petrol ether/ethylacetate/CH_3COOH 2:8:0.005 v/v/v). Yield: 52%, 0.325 g, as colorless solid.

^{1}H NMR (400 MHz, Chloroform-d): δ 7.40-7.32 (m, 4H, **H-2´´**), 7.09 (m, 4H, **H-3´´**), 6.93 (d, $^1J_{PH}$ = 708 Hz, 1H, **P-H**), 5.13-4.93 (m, 4H, **Ph-CH$_2$**), 4.27 (t, $^3J_{HH}$ = 4.8 Hz, 2H, **H-t**), 3.74-3.68 (m, 2H, **H-u**), 3.68-3.60 (m, 6H, **H-v**, **H-w, H-x**), 3.57-3.51 (m, 2H, **H-y**), 3.37 (s, 3H, **H-z**), 2.88 (t, $^3J_{HH}$ = 6.9 Hz, 2H, **H-q**), 2.77 (t, $^3J_{HH}$ = 6.9 Hz, 2H, **H-r**), 2.56 (t, $^3J_{HH}$ = 7.6 Hz, 2H, **H-b**), 1.73 (quint, $^3J_{HH}$ = 7.5 Hz, 2H, **H-c**), 1.44 (tq, $^3J_{HH}$ = 7.6, 7.5 Hz, 2H, **H-d**), 0.97 (t, $^3J_{HH}$ = 7.3 Hz, 3H, **H-e**).

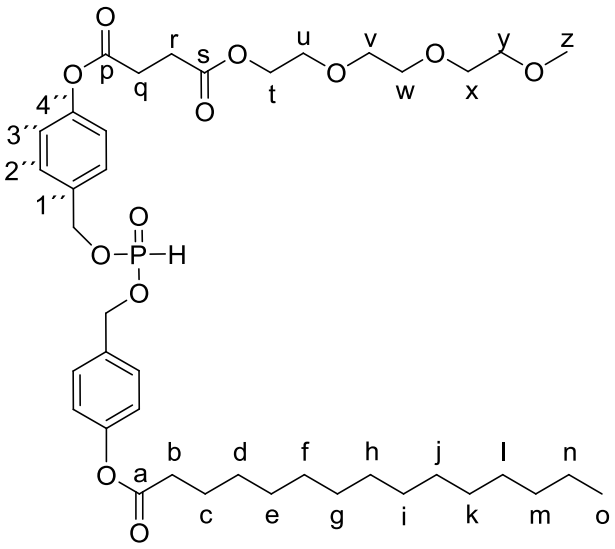
^{13}C-NMR (101 MHz, Chloroform-d): *δ* 172.06, 171.99 (**C-p, C-s**), 170.67 (**C-a**), 150.97, 150.81 (**2×C-4´´**), 133.13 (d, $^3J_{CP}$ = 6.1 Hz, **C-1´´**), 132.93 (d, $^3J_{CP}$ = 6.2 Hz, **C-1´´**), 129.20 (**2×C-2´´**), 121.86 (d, J_{CP} = 8.5 Hz, **C-3´´**), 71.90 (**C-z**), 70.56, 70.54 (**C-v**, **C-w**, **C-x**), 69.02 (**C-u**), 66.70, 66.64, 66.58 (**Ph-CH$_2$**), 63.98 (**C-t**), 58.99 (**C-z**), 34.05 (**C-b**), 29.25 (**C-q**), 28.99 (**C-r**), 26.92 (**C-c**), 22.20 (**C-d**), 13.67 (**C-e**)

^{31}P-NMR (162 MHz, Chloroform-d): *δ* 7.71.

HRMS (ESI-TOF) m/z: calculated for $C_{30}H_{45}NO_{12}P$ $[M+NH_4]^+$ 642.2674, found 642.2687.

IR: v = 3435, 2874, 1756, 1733, 1607, 1508, 1456, 1417, 1350, 1248, 1198, 1165, 1131, 1101, 1029, 1016, 947, 888, 849, 811, 553, 504, 429.

(AB-$C_{14}H_{29}$,ab-MEEES)-*H*-phosphonate **55o-MEEES**



Yield: 52%

Chemical Formula: $C_{40}H_{61}O_{12}P$

Molecular Weight: 764.88

According to **General Procedure B**, 0.23 mL diphenyl phosphonate (1.2 mmol, 1.2 equiv.) was added to 5 ml pyridine at 0 °C. Then 0.35 g 4-(Hydroxymethyl)phenyl pentadecanoate **44o** (1.0 mmol, 1.0 equiv.) was added and followed with 0.52 g 4-(Hydroxymethyl)phenyl (2-(2-(2-methoxyethoxy)ethoxy)ethyl) succsinate **50b** (1.4 mmol, 1.4 equiv.). The mixture was stirred for 3 h at room temperature. Column chromatography (SiO_2, petrol ether/ethylacetate/CH_3COOH 2:8:0.005 v/v/v). Yield: 52%, 0.394 g, as colorless solid.

^{1}H NMR (600 MHz, Chloroform-d): δ 7.47-7.29 (m, 4H, **H-2´´**), 7.15-7.00 (m, 4H, **H-3´´**), 6.92 (d, $^{1}J_{PH}$ = 708 Hz, 1H, **P-H**), 5.11-4.93 (m, 4H, **Ph-CH$_2$**), 4.26 (t, $^{3}J_{HH}$ = 5.7 Hz, 2H, **H-t**), 3.76-3.67 (m, 2H, **H-u**), 3.68-3.57 (m, 6H, **H-v**, **H-w, H-x**), 3.57-3.49 (m, 2H, **H-y**), 3.36 (s, 3H, **H-z**), 2.87 (t, $^{3}J_{HH}$ = 6.4 Hz, 2H, **H-q**), 2.76 (t, $^{3}J_{HH}$ = 6.7 Hz, 2H, **H-r**), 2.54 (t, $^{3}J_{HH}$ = 7.5 Hz, 2H, **H-b**), 1.73 (quint, $^{3}J_{HH}$ = 7.5 Hz, 2H, **H-c**), 1.43-1.36 (m, 2H, **H-d**), 1.35-1.16 (m, 20H, **H-e**, **H-f**, **H-g**, **H-h**, **H-i**, **H-j**, **H-k**, **H-l**, **H-m**, **H-n**), 0.87 (t, $^{3}J_{HH}$ = 7.0 Hz, 3H, **H-o**).

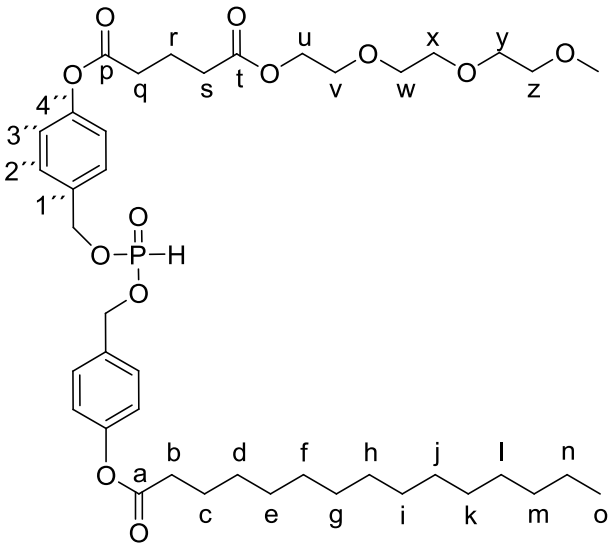
^{13}C-NMR (151 MHz, Chloroform-d): *δ* 172.07, 171.98 (**C-p, C-s**), 170.66 (**C-a**), 150.95, 150.85 (**2×C-4´´**), 133.14 (d, $^{3}J_{CP}$ = 6.1 Hz, **C-1´´**), 132.96 (d, $^{3}J_{CP}$ = 6.2 Hz, **C-1´´**), 129.21 (**2×C-2´´**), 121.88 (d, J_{CP} = 8.5 Hz, **C-3´´**), 71.91 (**C-y**), 70.55, 70.54 (**C-v**, **C-w**, **C-x**), 69.03 (**C-u**), 66.70, 66.66, 66.60 (**Ph-CH$_2$**), 63.98 (**C-t**), 58.99 (**C-z**), 34.40 (**C-b**), 31.90, 29.64, 29.64, 29.62, 29.52, 29.44, 29.35, 29.27, 29.11 (**C-d**, **C-e**, **C-f**, **C-g**, **C-h**, **C-i**, **C-j**, **C-k**, **C-l**, **C-m**, **C-q**), 28.99 (**C-r**), 24.91 (**C-c**), 22.68 (**C-n**), 14.15 (**C-o**)

^{31}P-NMR (243 MHz, Chloroform-d): *δ* 7.69.

HRMS (ESI-TOF) m/z: calculated for $C_{40}H_{65}NO_{12}P$ $[M+NH_4]^+$ 782.4239, found 782.4233.

IR: v = 2955, 2915, 2848, 1747, 1607, 1510, 1464, 1423, 1412, 1387, 1340, 1317, 1296, 1270, 1250, 1222, 1200, 1166, 1138, 1062, 1009, 995, 925, 835, 770, 727, 720, 657, 580, 554, 538, 516, 455, 441, 421.

(AB-$C_{14}H_{29}$,ab-MEEEG)-*H*-phosphonate **55o-MEEEG**



Yield:46%

Chemical Formula: $C_{41}H_{63}O_{12}P$

Molecular Weight: 778.91

According to **General Procedure B**, 0.23 mL diphenyl phosphonate (1.2 mmol, 1.2 equiv.) was added to 5 ml pyridine at 0 °C. Then 0.35 g 4-(Hydroxymethyl)phenyl pentadecanoate **44o** (1.0 mmol, 1.0 equiv.) was added and followed with 0.54 g 4-(Hydroxymethyl)phenyl (2-(2-(2-methoxyethoxy)ethoxy)ethyl) glutarate **50c** (1.4 mmol, 1.4 equiv.). The mixture was stirred for 3 h at room temperature. Column chromatography (SiO_2, petrol ether/ethylacetate/CH_3COOH 2:8:0.005 v/v/v). Yield: 46%, 0.358 g, as colorless solid.

^{1}H NMR (400 MHz, Chloroform-d): δ 7.48-7.30 (m, 4H, **H-2´´**), 7.17-7.00 (m, 4H, **H-3´´**), 6.92 (d, $^1J_{PH}$ = 712 Hz, 1H, **P-H**), 5.15-4.90 (m, 4H, **Ph-CH$_2$**), 4.25 (t, $^3J_{HH}$ = 4.9 Hz, 2H, **H-u**), 3.75-3.67 (m, 2H, **H-v**), 3.67-3.60 (m, 6H, **H-w, H-x, H-y**), 3.57-3.51 (m, 2H, **H-z**), 3.36 (s, 3H, **-OCH$_3$**), 2.64 (t, *J* = 7.3 Hz, 2H, **H-q**), 2.54 (t, $^3J_{HH}$ = 7.5 Hz, 2H, **H-b**), 2.49 (t, *J* = 7.2 Hz, 2H, **H-s**), 2.06 (quint, $^3J_{HH}$ = 7.0 Hz, 2H, **H-r**), 1.74 (quint, $^3J_{HH}$ = 7.4 Hz, 2H, **H-c**), 1.46-1.19 (m, 22H, **H-d**, **H-e**, **H-f**, **H-g**, **H-h**, **H-i**, **H-j**, **H-k**, **H-l**, **H-m**, **H-n**), 0.87 (t, $^3J_{HH}$ = 6.5 Hz, 3H, **H-o**).

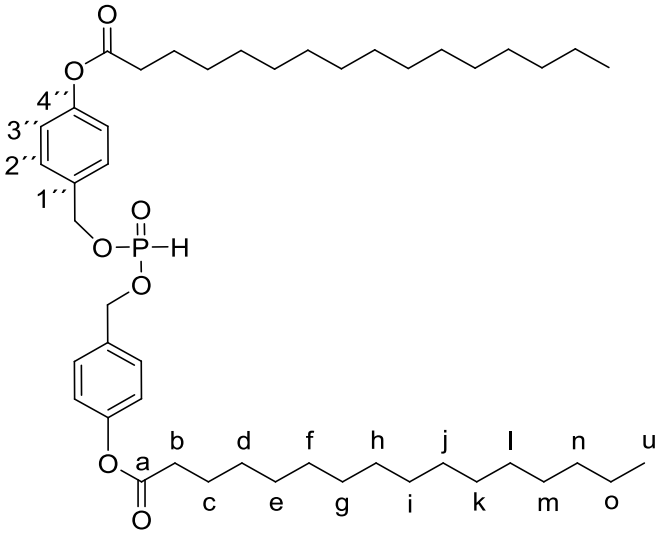
^{13}C-NMR (151 MHz, Chloroform-d): *δ* 172.7 (**C-t**), 172.1 (**C-a**), 171.2 (**C-p**), 151.0, 150.8 (**2×C-4´´**), 133.07 (d, $^4J_{CP}$ = 6.0 Hz, **C-1´´**), 133.92 (d, $^3J_{CP}$ = 6.0 Hz, **C-1´´**), 129.2 (**2×C-2´´**), 121.92, 121.86 (**2×C-3´´**), 71.9 (**C-z**), 70.60, 70.54 (**C-w, C-x, C-y**), 69.1 (**C-v**), 66.69, 66.66 (**Ph-CH$_2$**), 64.7 (**Ph´´-CH$_2$**), 63.6 (**C-u**), 59.0 (**-OCH$_3$**), 34.4 (**C-b**), 33.3 (**C-q**), 33.0 (**C-s**), 31.89, 29.65, 29.64, 29.61, 29.56, 29.43, 29.32, 29.22, 29.07 (**C-d**, **C-e**, **C-f**, **C-g**, **C-h**, **C-i**, **C-j**, **C-k**, **C-l**, **C-m**), 24.9 (**C-c**), 22.7 (**C-n**), 20.0 (**C-r**), 14.1 (**C-o**).

^{31}P-NMR (162 MHz, Chloroform-d): *δ* 7.71.

HRMS (ESI-TOF) m/z: calculated for $C_{41}H_{67}NO_{12}P$ $[M+NH_4]^+$ 796.4395, found 796.4416.

IR: v = 2915, 2849, 1756, 1747, 1607, 1510, 1464, 1412, 1390, 1270, 1250, 1223, 1196, 1167, 1136, 1063, 1021, 1011, 997, 925, 877, 835, 770, 726, 582, 540, 516, 455.

(AB-$C_{15}H_{31}$,ab-$C_{15}H_{31}$)-*H*-phosphonate **55uu**



Yield: 34%

Chemical Formula: $C_{46}H_{75}O_7P$

Molecular Weight: 771.06

0.08 mL diphenyl phosphonate (0.42 mmol, 1.0 equiv.) was added to 12 ml pyridine at 0 °C. Then 0.33 g 4-(Hydroxymethyl)phenyl hexadecanoate **44u** (0.92 mmol, 2.2 equiv.) was added. The mixture was stirred overnight at room temperature. The pure product was afforded after recrystallization with hexane and methanol. Yield: 34%, 0.113 g, as colorless solid.

^{1}H NMR (400 MHz, Chloroform-d): δ 7.36 (d, $^3J_{HH}$ = 8.5 Hz, 4H, **H-2´´**), 7.08 (d, $^3J_{HH}$ = 8.5 Hz, 4H, **H-3´´**), 6.94 (d, $^1J_{PH}$ = 708 Hz, 1H, **P-H**), 5.04 (quint, $^3J_{HH}$ = 9.8 Hz, 4H, **Ph-CH$_2$**), 2.55 (t, $^3J_{HH}$ = 7.5 Hz, 4H, **H-b**), 1.75 (quint, $^3J_{HH}$ = 7.5 Hz, 4H, **H-c**), 1.47-1.17 (m, 48H, **H-d**, **H-e**, **H-f**, **H-g**, **H-h**, **H-i**, **H-j**, **H-k**, **H-l**, **H-m**, **H-n**, **H-o**), 0.88 (t, $^3J_{HH}$ = 6.8 Hz, 6H, **H-u**).

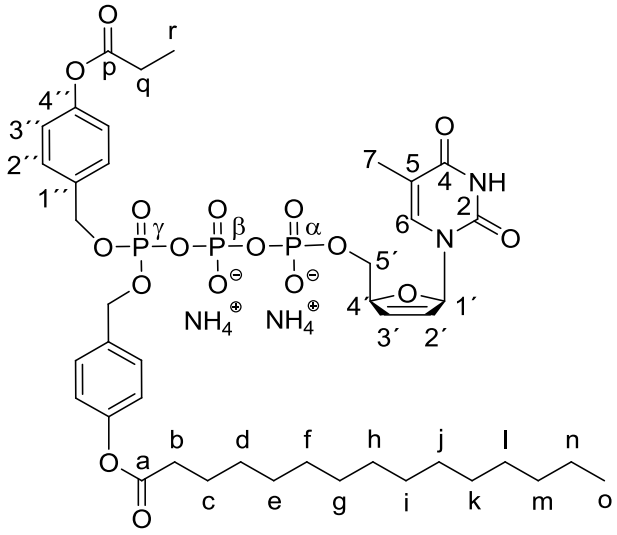
^{13}C-NMR (151 MHz, Chloroform-d): *δ* 172.12 **(C-a**), 150.99 (**2×C-4´´**), 132.98, 132.94 (**2×C-1´´**), 129.24 (**4×C-2´´**), 121.94 (**4×C-3´´**), 66.71, 66.67 (**2×Ph-CH$_2$**), 34.38 (**C-b**), 31.92, 29.69, 29.67, 29.65, 29.60, 29.46, 29.35, 29.26, 29.11 (**C-d**, **C-e**, **C-f**, **C-g**, **C-h**, **C-i**, **C-j**, **C-k**, **C-l**, **C-m**, **C-n**), 24.91 (**C-c**), 22.69 (**C-o**), 14.11 (**C-u**).

^{31}P-NMR (162 MHz, Chloroform-d): *δ* 7.69.

HRMS (ESI-TOF) m/z: calculated for $C_{46}H_{75}NaO_7P$ $[M+Na]^+$ 793.5143, found 793.5151.

IR: ν = 2955, 2914, 2847, 1754, 1607, 1510, 1463, 1410, 1385, 1348, 1330, 1308, 1285, 1262, 1240, 1220, 1197, 1167, 1149, 1097, 1061, 997, 924, 888, 835, 770, 739, 719, 693, 582, 540, 517, 502, 450.

Tri*PPP*ro γ-(AB-C_2H_5,ab-$C_{14}H_{29}$)-d4TTP (ammonium salt) **56co**



Yield: 64%

Chemical Formula: $C_{42}H_{65}N_4O_{17}P_3$

Molecular Weight: 990.90

According to **General Procedure C**, the reactions were performed under dry conditions using 100 mg *H*-phosphonate **55co** (0.174 mmol, 1.0 equiv.) and 137 mg d4TMP 2×nBu_4N^+ salt (0.174 mmol, 1.0 equiv.). Yield: 64%, 111 mg, as colorless cotton.

^{1}H-NMR (600 MHz, Methanol-d4): *δ* 7.69-7.62 (m, 1H, **H_{het}-6**), 7.40 (dt, $^3J_{HH}$ = 8.6, 2.5 Hz, 4H, **H-2´´**), 7.08-7.03 (m, 4H, **H-3´´**), 6.92 (m, 1H, **H-1´**), 6.45 (dt, $^3J_{HH}$ = 5.9, $^4J_{HH}$ = 1.8 Hz, 1H, **H-3´**), 5.80 (dt, $^3J_{HH}$ = 5.8 Hz, $^4J_{HH}$ = 1.8 Hz, 1H, **H-2´**), 5.15 (d, $^3J_{HP}$ = 8.1Hz, 4H, **Ph-CH_2**), 4.96-4.91 (m, 1H, **H-4´**), 4.30-4.15 (m, 2H, **H-5´**), 2.60 (q, $^3J_{HH}$ = 7.8 Hz, 2H, **H-q**), 2.57 (t, $^3J_{HH}$ = 7.2 Hz, 2H, **H-b**), 1.89 (s, 3H, **H_{het}-7**), 1.76-1.69 (m, 2H, **H-c**), 1.47-1.25 (m, 22H, **H-d**, **H-e**, **H-f**, **H-g**, **H-h**, **H-i**, **H-j**, **H-k**, **H-l**, **H-m**, **H-n**), 1.23 (t, $^3J_{HH}$ = 7.6 Hz, 3H, **H-r**), 0.90 (t, $^3J_{HH}$ = 6.9 Hz, 3H, **H-o**).

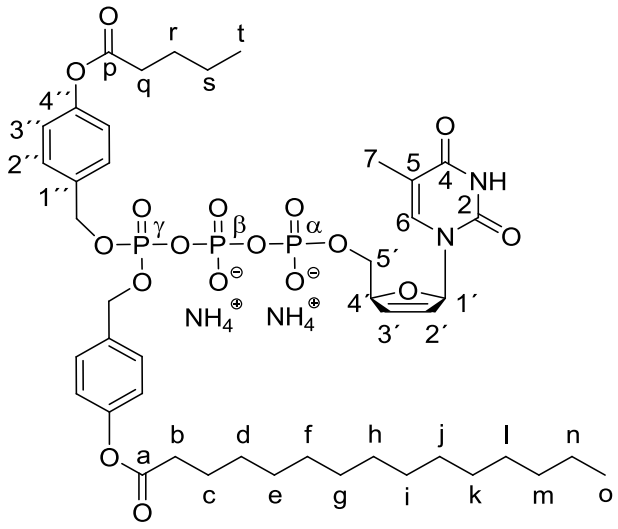
^{13}C-NMR (151 MHz, Methanol-d4): *δ* 174.5 (**C-p**), 173.8 **(C-a**), 166.5.3 (**C_{het}-4**), 152.8 (**C_{het}-2**), 152.4 (**2×C-4´´**), 138.6 (**C_{het}-6**), 135.7 (**C-3´**), 134.9 (d, $^3J_{CP}$ = 7.6 Hz, **2×C-1´´**), 130.5 (d, $^4J_{CP}$ = 2.9 Hz, **4×C-2´´**), 127.2 (**C-2´**), 122.89, 122.86, 122.82 (**4×C-3´´**), 112.1 (**C_{het}-5**), 90.8 (**C-1´**), 87.2 (d, $^3J_{CP}$ = 9.1 Hz, **C-4´**), 70.39 (d, $^3J_{CP}$ = 5.7 Hz, **Ph-CH_2**), 67.9 (d, $^2J_{CP}$ = 5.8 Hz, **C-5´**), 35.0 (**C-b**), 33.0 (**C-q**), 30.78, 30.77, 30.75, 30.71, 30.60, 30.46, 30.40, 30.16, 28.4 (**C-d**, **C-e**, **C-f**, **C-g**, **C-h**, **C-i**, **C-j**, **C-k**, **C-l**, **C-m**), 26.0 (**C-c**), 23.7 (**C-n**), 14.4 (**C-o**), 12.5 (**C_{het}-7**), 9.3 (**C-r**).

^{31}P-NMR (243 MHz, Methanol-d4): *δ* -11.81 (d, $^2J_{PP}$ = 19.6 Hz, **P-α**), -13.23 (d, $^2J_{PP}$ = 17.2 Hz, **P-γ**), -23.74 (br, s, **P-β**).

HRMS (ESI-TOF) m/z: calculated for $C_{42}H_{58}N_2O_{17}P_3$ $[M-H]^-$ 955.2954, found 955.2911.

IR: v = 2922, 2852, 1757, 1689, 1509, 1460, 1422, 1248, 1218, 1168, 1128, 1079, 1008, 903, 837, 806, 784, 768, 721, 697, 645, 577, 488, 426, 401.

Tri*PPP*ro γ-(AB-C_4H_9,ab-$C_{14}H_{29}$)-d4TTP (ammonium salt) **56eo**



Yield: 28%

Chemical Formula: $C_{44}H_{69}N_4O_{17}P_3$

Molecular Weight: 1018.96

According to **General Procedure C**, the reactions were performed under dry conditions using 276 mg *H*-phosphonate **55eo** (0.458 mmol, 1.0 equiv.) and 360 mg d4TMP 2×nBu_4N^+ salt (0.458 mmol, 1.0 equiv.). Yield: 28%, 131 mg, as colorless cotton.

^{1}H-NMR (400 MHz, Methanol-d4): *δ* 7.68 (d, $^4J_{HH}$ = 1.2 Hz, 1H, **H_{het}-6**), 7.55-7.25 (m, 4H, **H-2´´**), 7.12-6.99 (m, 4H, **H-3´´**), 6.92 (dt, $^3J_{HH}$ = 3.5 Hz, $^4J_{HH}$ = 1.6 Hz, 1H, **H-1´**), 6.46 (dt, $^3J_{HH}$ = 6.0, $^4J_{HH}$ = 1.8 Hz, 1H, **H-3´**), 5.83-5.76 (m, 1H, **H-2´**), 5.16 (d, $^3J_{HP}$ = 8.0 Hz, 4H, **Ph-CH_2**), 4.99-4.93 (m, 1H, **H-4´**), 4.36-4.16 (m, 2H, **H-5´**), 2.58 (t, $^3J_{HH}$ = 7.2 Hz, 2H, **H-q**), 2.57 (t, $^3J_{HH}$ = 7.2 Hz, 2H, **H-b**), 1.90 (d, $^4J_{HH}$ = 1.2 Hz, 3H, **H_{het}-7**), 1.84-1.66 (m, 4H, **H-c**, **H-r**), 1.57-1.23 (m, 24H, **H-d**, **H-e**, **H-f**, **H-g**, **H-h**, **H-i**, **H-j**, **H-k**, **H-l**, **H-m**, **H-n**, **H-s**), 0.99 (t, $^3J_{HH}$ = 7.4 Hz, 3H, **H-t**), 0.90 (t, $^3J_{HH}$ = 6.8 Hz, 3H, **H-o**).

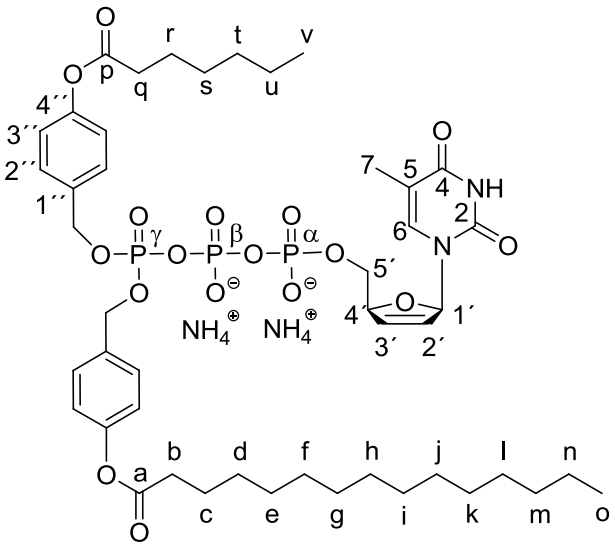
^{13}C-NMR (101 MHz, Methanol-d4): *δ* 174.6 (**C-p, C-a**), 167.3 (**C_{het}-4**), 153.6 (**C_{het}-2**), 153.2 (**2×C-4´´**), 139.5 (**C_{het}-6**), 136.6 (**C-3´**), 135.8 (d, $^3J_{CP}$ = 7.5 Hz, **2×C-1´´**), 131.4 (d, $^4J_{CP}$ = 2.9 Hz, **4×C-2´´**), 128,0 (**C-2´**), 123.73, 123.72 (**4×C-3´´**), 112.9 (**C_{het}-5**), 91.7 (**C-1´**), 88.1 (d, $^3J_{CP}$ = 9.1 Hz, **C-4´**), 71.3 (d, J_{CP} = 6.1 Hz, **Ph-CH_2**), 71.2 (d, J_{CP} = 5.3 Hz, **Ph-CH_2**), 68.9 (d, $^2J_{CP}$ = 5.8 Hz, **C-5´**), 35.9 (**C-b**), 35.6 (**C-q**), 33.9, 31.64, 31.63, 31.61, 31.57, 31.46, 31.32, 31.26, 31.03 (**C-d**, **C-e**, **C-f**, **C-g**, **C-h**, **C-i**, **C-j**, **C-k**, **C-l**, **C-m**), 28.9 (**C-r**), 26.9 (**C-c**), 24.6 (**C-n**), 24.1 (**C-s**), 15.3 (**C-o**), 15.0 (**C-t**), 12.5 (**C_{het}-7**).

^{31}P-NMR (81 MHz, Methanol-d4): *δ* -11.81 (br, s, **P-α**), -13.24 (d, *J* = 17.2 Hz, **P-γ**), -23.77 (br, s, **P-β**).

HRMS (ESI-TOF) m/z: calculated for $C_{44}H_{62}N_2O_{17}P_3$ $[M-H]^-$ 983.3267, found 983.3229.

IR: ν = 3183, 3042, 2923, 2853, 1756, 1689, 1509, 1462, 1380, 1248, 1218, 1202, 1167, 1128, 1112, 1082, 1008, 908, 837, 784, 723, 697, 644, 488, 421, 401.

Tri*PPP*ro γ-(AB-C_6H_{13},ab-$C_{14}H_{29}$)-d4TTP (ammonium salt) **56go**



Yield: 52%

Chemical Formula: $C_{46}H_{73}N_4O_{17}P_3$

Molecular Weight: 1047.01

According to **General Procedure C**, the reactions were performed under dry conditions using 100 mg *H*-phosphonate **55go** (0.159 mmol, 1.0 equiv.) and 125 mg d4TMP 2×nBu_4N^+ salt (0.159 mmol, 1.0 equiv.). Yield: 52%, 87 mg, as colorless cotton.

^{1}H-NMR (600 MHz, Methanol-d4): *δ* 7.70 -7.62 (m, 1H, **H_{het}-6**), 7.44-7.37 (m, 4H, **H-2´´**), 7.12-7.01 (m, 4H, **H-3´´**), 6.94 (dt, $^3J_{HH}$ = 3.5 Hz, $^4J_{HH}$ = 1.7 Hz, 1H, **H-1´**), 6.47 (dt, $^3J_{HH}$ = 5.9 Hz, $^4J_{HH}$ = 1.8 Hz, 1H, **H-3´**), 5.84-5.79 (m, 1H, **H-2´**), 5.17 (d, $^3J_{HP}$ = 8.2 Hz, 4H, **Ph-CH_2**), 4.99-4.93 (m, 1H, **H-4´**), 4.33-4.17 (m, 2H, **H-5´**), 2.59 (t, $^3J_{HH}$ = 7.4 Hz, 4H, **H-q**, **H-b**), 1.91 (d, $^4J_{HH}$ = 1.3 Hz, 3H, **H_{het}-7**), 1.82-1.69 (m, 4H, **H-c**, **H-r**), 1.52-1.23 (m, 28H, **H-d**, **H-e**, **H-f**, **H-g**, **H-h**, **H-i**, **H-j**, **H-k**, **H-l**, **H-m**, **H-n**, **H-s**, **H-t**, **H-u**), 0.95 (t, $^3J_{HH}$ = 7.2 Hz, 3H, **H-v**), 0.92 (t, $^3J_{HH}$ = 7.0 Hz, 3H, **H-o**).

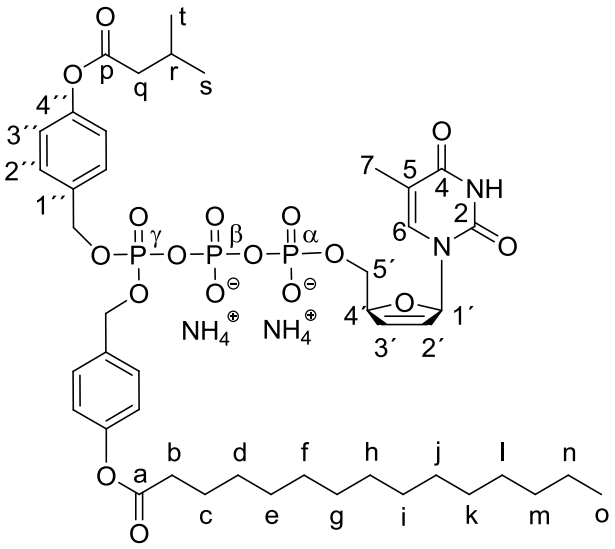
^{13}C-NMR (151 MHz, Methanol-d4): *δ* 173.76 (**C-a, C-p**), 166.5 (**C_{het}-4**), 152.8 (**C_{het}-2**), 152.4 (**2×C-4´´**), 138.6 (**C_{het}-6**), 135.7 (**C-3´**), 134.9 (d, $^3J_{CP}$ = 7.6 Hz, **2×C-1´´**), 130.49 (d, $^4J_{CP}$ = 4.5 Hz, **4×C-2´´**), 127.2 (**C-2´**), 122.88, 122.87 (**4×C-3´´**), 112.1 (**C_{het}-5**), 90.9 (**C-1´**), 87.12 (d, $^3J_{CP}$ = 9.1 Hz, **C-4´**), 70.42 (d, J_{CP} = 3.8 Hz, **Ph-CH_2**), 70.39 (d, J_{CP} = 4.4 Hz, **Ph-CH_2**), 67.96 (d, $^2J_{CP}$ = 5.3 Hz, **C-5´**), 35.0 (**C-b**, **C-q**), 33.07, 32.66, 30.79, 30.77, 30.75, 30.72, 30.60, 30.47, 30.41, 30.17, 29.8 (**C-d**, **C-e**, **C-f**, **C-g**, **C-h**, **C-i**, **C-j**, **C-k**, **C-l**, **C-m**, **C-s**, **C-t**), 25.96 (**C-r**), 25.93 (**C-c**), 23.73 (**C-n**), 23.58 (**C-u**), 14.44 (**C-o**), 14.39 (**C-v**), 12.5 (**C_{het}-7**).

^{31}P-NMR (243 MHz, Methanol-d4): *δ* -11.84 (d, $^2J_{PP}$ = 18.2 Hz, **P-α**), -13.18 (d, $^2J_{PP}$ = 16.8 Hz, **P-γ**), -23.82 (t, $^2J_{PP}$ = 16.3 Hz, **P-β**).

HRMS (ESI-TOF) m/z: calculated for $C_{46}H_{66}N_2O_{17}P_3$ $[M-H]^-$ 1011.3580, found .1011.3472.

IR: v = 3184, 3045, 2955, 2922, 2852, 1754, 1690, 1509, 1464, 1380, 1249, 1220, 1168, 1128, 1113, 1084, 1007, 910, 838, 784, 768, 722, 696, 643, 577, 494, 421, 400.

Tri*PPP*ro γ-(AB-*iso*-C_4H_9,ab-$C_{14}H_{29}$)-d4TTP (ammonium salt) **56e*io***



Yield: 39%

Chemical Formula: $C_{44}H_{69}N_4O_{17}P_3$

Molecular Weight: 1018.96

According to **General Procedure C**, the reactions were performed under dry conditions using 90.8 mg *H*-phosphonate **55e*io*** (0.151 mmol, 1.0 equiv.) and 106 mg d4TMP 2×nBu_4N^+ salt (0.151 mmol, 1.0 equiv.). Yield: 39%, 60 mg, as colorless cotton.

^{1}H-NMR (600 MHz, Methanol-d4): *δ* 7.68 (d, $^4J_{HH}$ = 1.2 Hz, 1H, **H_{het}-6**), 7.47-7.35 (m, 4H, **H-2´´**), 7.09-7.04 (m, 4H, **H-3´´**), 6.94 (ddd, $^3J_{HH}$ = 3.5 Hz, $^4J_{HH}$ = 1.6, 1.3 Hz, 1H, **H-1´**), 6.48 (dt, $^3J_{HH}$ = 6.0 Hz, $^4J_{HH}$ = 1.7 Hz, 1H, **H-3´**), 5.81 (ddd, $^3J_{HH}$ = 6.1 Hz, $^3J_{HH}$ = 2.4 Hz, $^4J_{HH}$ = 1.4 Hz, 1H, **H-2´**), 5.17 (d, $^3J_{HP}$ = 8.1 Hz, 4H, **Ph-CH_2**), 4.98-4.93 (m, 1H, **H-4´**), 4.29 (ddd, $^2J_{HH}$ = 11.6 Hz, $^3J_{HH}$ = 6.8 Hz, $^4J_{HH}$ = 3.3 Hz, 1H, **H-5´**), 4.21 (ddd, $^2J_{HH}$ = 11.6 Hz, $^3J_{HH}$ = 5.4 Hz, $^4J_{HH}$ = 3.1 Hz, 1H, **H-5´**), 2.59 (td, $^3J_{HH}$ = 7.4 Hz, $^4J_{HH}$ = 1.3 Hz 2H, **H-b**), 2.47 (dd, $^2J_{HH}$ = 7.2 Hz, $^4J_{HH}$ = 1.3 Hz, 2H, **H-q**), 2.28-2.17 (m, 1H, **H-r**), 1.91 (d, $^4J_{HH}$ = 1.2 Hz, 3H, **H_{het}-7**), 1.80-1.69 (m, 2H, **H-c**), 1.51-1.22 (m, 22H, **H-d**, **H-e**, **H-f**, **H-g**, **H-h**, **H-i**, **H-j**, **H-k**, **H-l**, **H-m**, **H-n**), 1.08 (dd, $^3J_{HH}$ = 6.7 Hz, $^4J_{HH}$ = 0.8 Hz, 6H, **H-t**, **H-s**), 0.92 (t, $^3J_{HH}$ = 7.0 Hz, 3H, **H-o**).

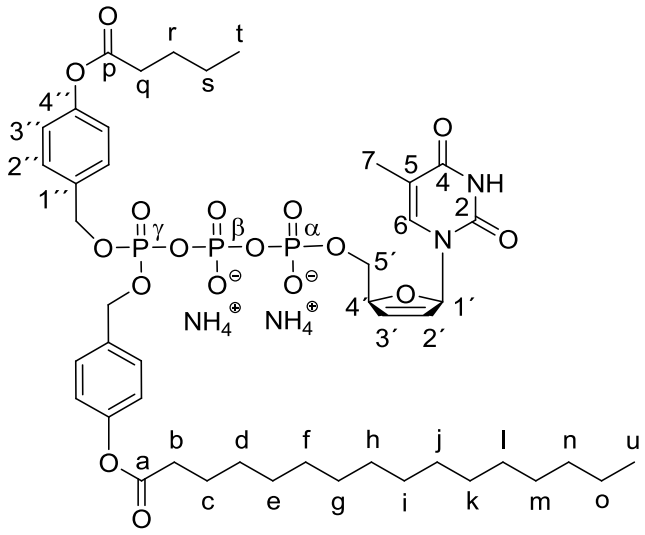
^{13}C-NMR (151 MHz, Methanol-d4): *δ* 173.8 (**C-p**), 173.0 (**C-a**), 166.5 (**C_{het}-4**), 152.8 (**C_{het}-2**), 152.4, 152.3 (**2×C-4´´**), 138.7 (**C_{het}-6**), 135.8 (**C-3´**), 135.8 (**2×C-1´´**), 130.5 (d, $^4J_{CP}$ = 4.5 Hz, **4×C-2´´**), 127.2 (**C-2´**), 122.9 (**4×C-3´´**), 112.1 (**C_{het}-5**), 90.9 (**C-1´**), 87.2 (d, $^3J_{CP}$ = 9.6 Hz, **C-4´**), 70.4 (**2×Ph-CH_2**), 67.88 (d, $^2J_{CP}$ = 5.6 Hz, **C-5´**), 44.0 (**C-q**), 35.0 (**C-b**), 33.1, 30.79, 30.78, 30.76, 30.72, 30.61, 30.48, 30.41, 30.17 (**C-d**, **C-e**, **C-f**, **C-g**, **C-h**, **C-i**, **C-j**, **C-k**, **C-l**, **C-m**), 27.0 (**C-r**), 26.0 (**C-c**), 23.7 (**C-n**), 22.7 (**C-s**, **C-t**), 14.4 (**C-o**), 12.5 (**C_{het}-7**)

^{31}P-NMR (243 MHz, Methanol-d4): *δ* -11.80 (d, *J* = 19.6 Hz, **P-α**), -13.21 (d, *J* = 17.3 Hz, **P-γ**), -23.72 (t, *J* = 19.3 Hz, **P-β**).

HRMS (ESI-TOF) m/z: calculated for $C_{44}H_{62}N_2O_{17}P_3$ $[M-H]^-$ 983.3267, found 983.3160.

IR: v = 3046, 2957, 2923, 2853, 1756, 1690, 1509, 1464, 1369, 1247, 1218, 1202, 1167, 1128, 1112, 1083, 1009, 909, 837, 784, 769, 722, 696, 644, 573, 490, 425.

Tri*PPP*ro γ-(AB-C_4H_9,ab-$C_{15}H_{31}$)-d4TTP (ammonium salt) **56eu**



Yield: 47%

Chemical Formula: $C_{45}H_{71}N_4O_{17}P_3$

Molecular Weight: 1032.98

According to **General Procedure C**, the reactions were performed under dry conditions using 100 mg *H*-phosphonate **55eu** (0.162 mmol, 1.0 equiv.) and 128 mg d4TMP 2×nBu_4N^+ salt (0.162 mmol, 1.0 equiv.). Yield: 47%, 78 mg, as colorless cotton.

^{1}H-NMR (600 MHz, Methanol-d4): *δ* 7.68 (d, $^4J_{HH}$ = 1.2 Hz,, 1H, **H_{het}-6**), 7.44-7.38 (m, 4H, **H-2´´**), 7.14-7.01 (m, 4H, **H-3´´**), 6.94 (dt, $^3J_{HH}$ = 3.5, $^4J_{HH}$ = 1.6 Hz, 1H, **H-1´**), 6.48 (dt, $^3J_{HH}$ = 6.1 Hz, $^4J_{HH}$ = 1.7 Hz, 1H, **H-3´**), 5.81 (ddd, $^3J_{HH}$ = 6.1, $^3J_{HH}$ = 2.4 Hz, $^4J_{HH}$ = 1.3 Hz, 1H, **H-2´**), 5.17 (d, $^3J_{HP}$ = 8.4 Hz, 4H, **Ph-CH_2**), 4.99-4.92 (m, 1H, **H-4´**), 4.32-4.16 (m, 2H, **H-5´**), 2.63-2.56 (m, 4H, **H-b**, **H-q**), 1.91 (d, $^4J_{HH}$ = 1.2 Hz, 3H, **H_{het}-7**), 1.79-1.69 (m, 4H, **H-c**, **H-r**), 1.52-1.23 (m, 26H, **H-d**, **H-e**, **H-f**, **H-g**, **H-h**, **H-i**, **H-j**, **H-k**, **H-l**, **H-m**, **H-n**, **H-o**, **H-s**), 1.01 (t, $^3J_{HH}$ = 7.4 Hz, 3H, **H-t**), 0.92 (t, $^3J_{HH}$ = 7.0 Hz, 3H, **H-u**).

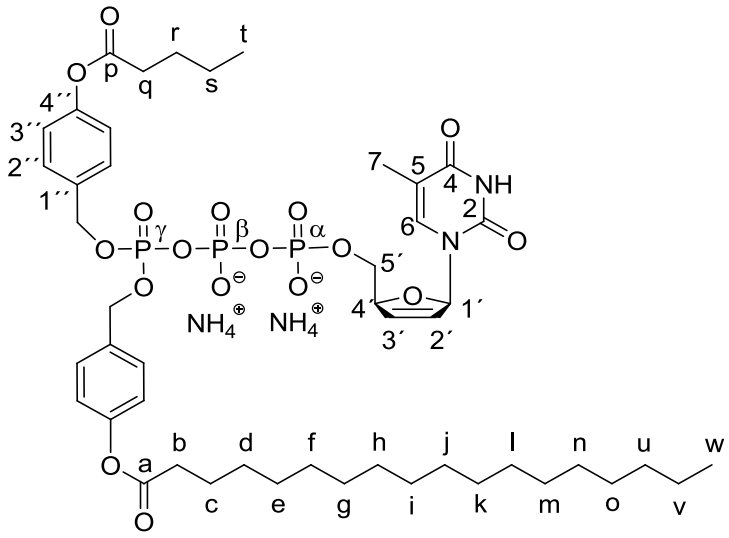
^{13}C-NMR (151 MHz, Methanol-d4): *δ* 173.78 (**C-p, C-a**), 166.52 (**C_{het}-4**), 152.76 (**C_{het}-2**), 152.35 (**2×C-4´´**), 138.66 (**C_{het}-6**), 135.76 (**C-3´**), 134.93 (d, $^3J_{CP}$ = 7.4 Hz, **2×C-1´´**), 130.48 (d, $^4J_{CP}$ = 2.9 Hz, **4×C-2´´**), 127.16 (**C-2´**), 122.87 (d, $^3J_{CP}$ = 2.1 Hz, **4×C-3´´**), 112.06 (**C_{het}-5**), 90.84 (**C-1´**), 87.20 (d, $^3J_{CP}$ = 9.1 Hz, **C-4´**), 70.41, 70.38, 70.36 (**2×Ph-CH_2**), 67.88 (d, $^2J_{CP}$ = 5.6 Hz, **C-5´**), 35.03, 34.76 (**C-q**, **C-b**), 33.07, 30.78, 30.77, 30.75, 30.71, 30.60, 30.46, 30.40, 30.17(**C-d**, **C-e**, **C-f**, **C-g**, **C-h**, **C-i**, **C-j**, **C-k**, **C-l**, **C-m**, **C-n**), 28.07 (**C-r**), 25.96 (**C-c**), 23.73 (**C-o**), 23.25 (**C-s**), 14.44 (**C-u**), 14.10 (**C-t**), 12.48 (**C_{het}-7**).

^{31}P-NMR (243 MHz, Methanol-d4): *δ* -11.73 (br, s, **P-α**), -13.18 (d, *J* = 17.2 Hz, **P-γ**), -23.68 (br, s, **P-β**).

HRMS (ESI-TOF) m/z: calculated for $C_{45}H_{64}N_2O_{17}P_3$ $[M-H]^-$ 997.3423, found 997.3389.

IR: v = 3191, 3045, 2956, 2921, 2852, 1755, 1689, 1509, 1464, 1380, 1249, 1219, 1168, 1128, 1082, 1008, 908, 838, 784, 769, 721, 696, 644, 492, 422, 401.

Tri*PPP*ro γ-(AB-C_4H_9,ab-$C_{17}H_{35}$)-d4TTP (ammonium salt) **56ew**



Yield: 40%

Chemical Formula: $C_{47}H_{75}N_4O_{17}P_3$

Molecular Weight: 1061.04

According to **General Procedure C**, the reactions were performed under dry conditions using 97 mg *H*-phosphonate **55ew** (0.150 mmol, 1.0 equiv.) and 118 mg d4TMP 2×nBu_4N^+ salt (0.150 mmol, 1.0 equiv.). Yield: 40%, 64 mg, as colorless cotton.

^{1}H-NMR (600 MHz, Methanol-d4): *δ* 7.71-7.66 (m, 1H, **H_{het}-6**), 7.44-7.38 (m, 4H, **H-2´´**), 7.09-7.04 (m, 4H, **H-3´´**), 6.94 (dt, $^3J_{HH}$ = 3.5 Hz, $^4J_{HH}$ = 1.6 Hz, 1H, **H-1´**), 6.48 (dt, $^3J_{HH}$ = 6.0 Hz, $^4J_{HH}$ = 1.8 Hz, 1H, **H-3´**), 5.81 (ddd, $^3J_{HH}$ = 6.1 Hz, $^3J_{HH}$ = 2.4 Hz, $^4J_{HH}$ = 1.3 Hz, 1H, **H-2´**), 5.17 (d, $^3J_{HP}$ = 8.2 Hz, 4H, **Ph-CH_2**), 4.98-4.93 (m, 1H, **H-4´**), 4.33-4.15 (m, 2H, **H-5´**), 2.63-2.56 (m, 4H, **H-b**, **H-q**), 1.91 (d, $^4J_{HH}$ = 1.2 Hz, 3H, **H_{het}-7**), 1.79-1.69 (m, 4H, **H-c**, **H-r**), 1.52-1.23 (m, 30H, **H-d**, **H-e**, **H-f**, **H-g**, **H-h**, **H-i**, **H-j**, **H-k**, **H-l**, **H-m**, **H-n**, **H-o**, **H-u**, **H-v**, **H-s**), 1.01 (t, $^3J_{HH}$ = 7.4 Hz, 3H, **H-t**), 0.92 (t, $^3J_{HH}$ = 7.0 Hz, 3H, **H-w**).

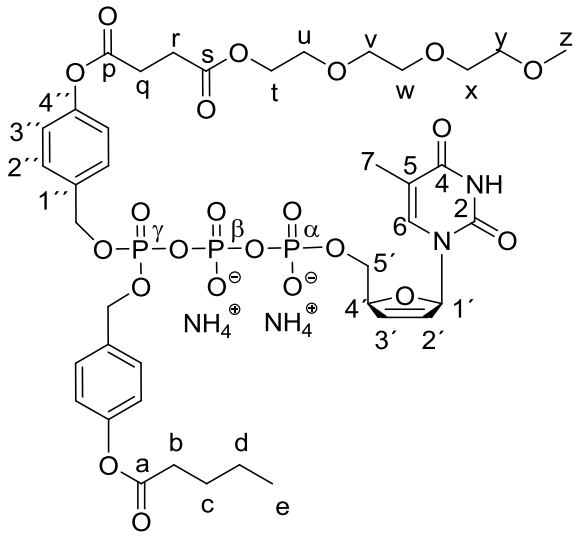
^{13}C-NMR (151 MHz, Methanol-d4): *δ* 173.77 (**C-p, C-a**), 166.53 (**C_{het}-4**), 152.76 (**C_{het}-2**), 152.35 (**2×C-4´´**), 138.67 (**C_{het}-6**), 135.76 (**C-3´**), 134.93 (d, $^3J_{CP}$ = 7.5 Hz, **2×C-1´´**), 130.48 (d, $^4J_{CP}$ = 2.9 Hz, **4×C-2´´**), 127.16 (**C-2´**), 122.88 (**4×C-3´´**), 112.05 (**C_{het}-5**), 90.83 (**C-1´**), 87.20 (d, $^3J_{CP}$ = 9.1 Hz, **C-4´**), 70.38 (**2×Ph-CH_2**), 67.87 (d, $^2J_{CP}$ = 5.6 Hz, **C-5´**), 35.03, 34.76 (**C-q**, **C-b**), 33.07, 30.79, 30.77, 30.75, 30.72, 30.61, 30.47, 30.42, 30.18 (**C-d**, **C-e**, **C-f**, **C-g**, **C-h**, **C-i**, **C-j**, **C-k**, **C-l**, **C-m**, **C-n**, **C-o**, **C-u**), 28.07 (**C-r**), 25.96 (**C-c**), 23.74 (**C-v**), 23.26 (**C-s**), 14.45 (**C-w**), 14.11 (**C-t**), 12.49 (**C_{het}-7**).

^{31}P-NMR (243 MHz, Methanol-d4): *δ* -11.77 (d, *J* = 19.6 Hz, **P-α**), -13.20 (d, *J* = 17.3 Hz, **P-γ**), -23.70 (t, *J* = 18.1 Hz, **P-β**).

HRMS (ESI-TOF) m/z: calculated for $C_{47}H_{68}N_2O_{17}P_3$ $[M-H]^-$ 1025.3736, found 1025.3703.

IR: v = 3040, 2920, 2851, 1755, 1690, 1509, 1465, 1380, 1249, 1219, 1168, 1128, 1082, 1007, 910, 838, 784, 768, 721, 644, 491, 420.

Tri*PPP*ro γ-(AB-C_4H_9,ab-MEEES)-d4TTP (ammonium salt) **56e-MEEES**



Yield: 45%

Chemical Formula: $C_{40}H_{59}N_4O_{22}P_3$

Molecular Weight: 1040.83

According to **General Procedure C**, the reactions were performed under dry conditions using 144 mg *H*-phosphonate **55e-MEEES** (0.231 mmol, 1.0 equiv.) and 187 mg d4TMP 2×nBu_4N^+ salt (0.231 mmol, 1.0 equiv.). Yield: 45%, 101 mg, as colorless solid.

^{1}H-NMR (600 MHz, Methanol-d4): *δ* 7.69 (d, $^4J_{HH}$ = 1.3 Hz, 1H, **H_{het}-6**), 7.46-7.39 (m, 4H, **H-2´´**), 7.12-7.04 (m, 4H, **H-3´´**), 6.94 (dt, $^3J_{HH}$ = 3.5 Hz, $^4J_{HH}$ = 1.7 Hz, 1H, **H-1´**), 6.48 (dt, $^3J_{HH}$ = 6.0 Hz, $^4J_{HH}$ = 1.8 Hz, 1H, **H-3´**), 5.83-5.76 (m, 1H, **H-2´**), 5.17 (dd, $^3J_{HP}$ = 8.1, 4.8 Hz, 4H, **Ph-CH_2**), 4.98-4.93 (m, 1H, **H-4´**), 4.32-4.17 (m, 4H, **H-5´**, **H-t**), 3.74-3.51 (m, 10H, **H-u**, **H-v**, **H-w**, **H-x**, **H-y**), 3.26 (s, 3H, **H-z**), 2.93-2.87 (m, 2H, **H-q**), 2.78 (t, $^3J_{HH}$ = 6.6 Hz, 2H, **H-r**) 2.61 (td, $^3J_{HH}$ = 7.4, 1.3 Hz, 2H, **H-b**), 1.91 (d, $^4J_{HH}$ = 1.2 Hz, 3H, **H_{het}-7**), 1.78-1.70 (m, 2H, **H-c**), 1.52-1.44 (m, 2H, **H-d**), 1.01 (t, $^3J_{HH}$ = 7.4 Hz, 3H, **H-e**).

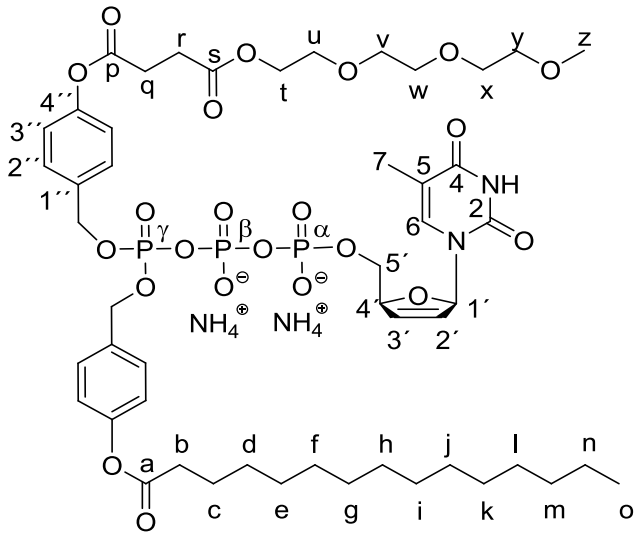
^{13}C-NMR (151 MHz, Methanol-d4): *δ* 173.9,173.8 (**C-p, C-s**), 172.6 (**C-a**), 166.53 (**C_{het}-4**), 152.8 (**C_{het}-2**), 152.4, 152.3 (**2×C-4´´**), 138.7 (**C_{het}-6**), 135.8 (**C-3´**), 135.8 (**2×C-1´´**), 130.50,130.46 (**4×C-2´´**), 127.1 (**C-2´**), 122.88, 122.86, 122.84, 122.83 (**4×C-3´´**), 112.1 (**C_{het}-5**), 90.8 (**C-1´**), 87.2 (d, $^3J_{CP}$ = 9.5 Hz, **C-4´**), 72.9, 71,54, 71.50, 71,3, 70.07 (**C-u**, **C-v**, **C-w**, **C-x**, **C-y**), 70.38, 70.35, 70.35, 70.32 (**2×Ph-CH_2**), 67.85 (d, $^2J_{CP}$ = 6.0 Hz, **C-5´**), 65.02 (**C-t**), 59.08 (**C-z**), 34.8 (**C-b**), 30.1, 29.9 (**C-q**, **C-r**), 28.1 (**H-c**), 23.3 (**C-d**), 14.1 (**C-e**), 12.5 (**C_{het}-7**)

^{31}P-NMR (243 MHz, Methanol-d4): *δ* -11.77 (d, *J* = 19.8 Hz, **P-α**), -13.24 (d, *J* = 17.0 Hz, **P-γ**), -23.71 (t, *J* = 19.0 Hz, **P-β**).

HRMS (ESI-TOF) m/z: calculated for $C_{40}H_{52}N_2O_{22}P_3$ $[M-H]^-$ 1005.2230, found 1005.2268.

IR: ν = 3187, 2932, 2874, 1755, 1736, 1688, 1509, 1453, 1422, 1247, 1218, 1200, 1167, 1127, 1082, 1007, 903, 837, 806, 783, 731, 696, 644, 480, 422, 401.

Tri*PPP*ro γ-(AB-$C_{14}H_{29}$,ab-MEEES)-d4TTP (ammonium salt) **56o-MEEES**



Yield: 39%

Chemical Formula: $C_{50}H_{79}N_4O_{22}P_3$

Molecular Weight: 1181.10

According to **General Procedure C**, the reactions were performed under dry conditions using 100 mg *H*-phosphonate **55o-MEEES** (0.162 mmol, 1.0 equiv.) and 127 mg d4TMP 2×nBu_4N^+ salt (0.162 mmol, 1.0 equiv.). Yield: 39%, 75 mg, as colorless solid.

^{1}H-NMR (600 MHz, Methanol-d4): *δ* 7.69 (m, 1H, **H_{het}-6**), 7.46-7.38 (m, 4H, **H-2´´**), 7.13-7.03 (m, 4H, **H-3´´**), 6.94 (dt, $^3J_{HH}$ = 3.5 Hz, $^4J_{HH}$ = 1.6 Hz, 1H, **H-1´**), 6.49 (dt, $^3J_{HH}$ = 5.6 Hz, $^4J_{HH}$ = 1.7 Hz, 1H, **H-3´**), 5.81 (ddd, $^3J_{HH}$ = 5.6 Hz, $^3J_{HH}$ = 1.9 Hz, $^4J_{HH}$ = 1.8 Hz, 1H, **H-2´**), 5.17 (d, $^3J_{HP}$ = 7.3 Hz, 4H, **Ph-CH_2**), 4.98-4.93 (m, 1H, **H-4´**), 4.33-4.17 (m, 4H, **H-5´**, **H-t**), 3.75-3.69 (m, 2H, **H-u**), 3.67-3.59 (m, 6H, **H-v**, **H-w**, **H-x**), 3.56-3.51 (m, 2H, **H-y**), 3.36 (m, 3H, **H-z**), 2.91 (t, $^3J_{HH}$ = 6.6 Hz, 2H, **H-q**), 2.78 (t, $^3J_{HH}$ = 6.3 Hz, 2H, **H-r**), 2.60 (t, $^3J_{HH}$ = 7.5 Hz, 2H, **H-b**), 1.91 (d, $^4J_{HH}$ = 2.9 Hz, 3H, **H_{het}-7**), 1.75 (quint, $^3J_{HH}$ = 7.5 Hz, 2H, **H-c**), 1.49-1.24 (m, 22H, **H-d**, **H-e**, **H-f**, **H-g**, **H-h**, **H-i**, **H-j**, **H-k**, **H-l**, **H-m**, **H-n**), 0.92 (t, $^3J_{HH}$ = 5.7 Hz, 3H, **H-o**).

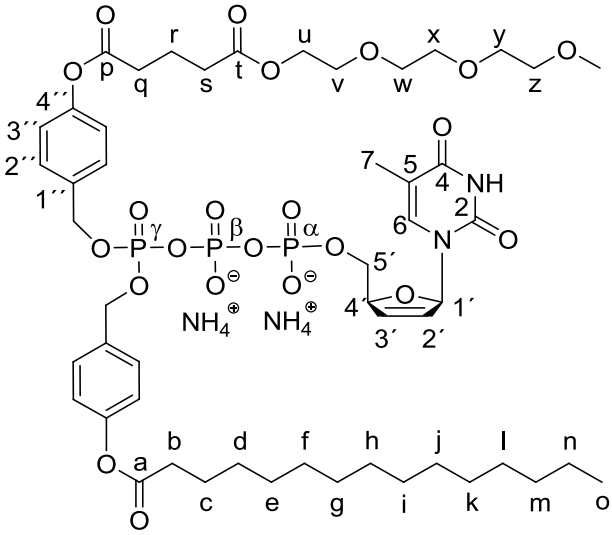
^{13}C-NMR (151 MHz, Methanol-d4): *δ* 173.88, 173.79, 172.59 (**C-p, C-s**, **C-a**), 166.53 (**C_{het}-4**), 152.77 (**C_{het}-2**), 152.36, 152.29 (**C-4´´**), 138.70 (**C_{het}-6**), 135.83 (**C-3´**), 135.10 (d, $^4J_{CP}$ = 7.4 Hz, **C-1´´**), 134.97 (d, $^3J_{CP}$ = 7.7 Hz, **C-1´´**), 130.50, 130.47 (**4×C-2´´**), 127.12 (**C-2´**), 122.88, 122.86, 122.84, 122.82 (**4×C-3´´**), 112.08 (**C_{het}-5**), 90.83 (**C-1´**), 87.25 (d, $^3J_{CP}$ = 8.9 Hz, **C-4´**), 72.95 (**C-y**), 71.55, 71.51, 71.38 (**C-w, C-x, C-y**), 70.39, 70.36, 70.33 (**2×Ph-CH_2**), 70.08 (**C-u**), 67.84 (d, $^2J_{CP}$ = 5.5 Hz, **C-5´**), 65.03 (**C-t**), 59.09 (**C-z**), 35.03 (**C-b**), 33.07, 30.79, 30.78, 30.76, 30.72, 30.61, 30.47, 30.42, 30.18, 30.12, 29.92 (**C-d**, **C-e**, **C-f**, **C-g**, **C-h**, **C-i**, **C-j**, **C-k**, **C-l**, **C-m**, **C-q**, **C-r**), 25.97 (**C-c**), 23.74 (**C-n**), 14.44 (**C-o**), 12.49 (**C_{het}-7**).

^{31}P-NMR (243 MHz, Methanol-d4): *δ* -11.71 (d, *J* = 19.6 Hz, **P-α**), -13.16 (d, *J* = 17.1 Hz, **P-γ**), -23.60 (t, *J* = 17.1 Hz, **P-β**).

HRMS (ESI-TOF) m/z: calculated for $C_{50}H_{72}N_2O_{22}P_3$ $[M-H]^-$ 1145.3795, found 1145.3786.

IR: ν **=** 3184, 2923, 2853,1756, 1738, 1689, 1509, 1456, 1367, 1248, 1219, 1200, 1167, 1128, 1084, 1009, 906, 838, 807, 784, 722, 645, 486, 423, 399.

Tri*PPP*ro γ-(AB-$C_{14}H_{29}$,ab-MEEEG)-d4TTP (ammonium salt) **56o-MEEEG**



Yield: 35%

Chemical Formula: $C_{51}H_{81}N_4O_{22}P_3$

Molecular Weight: 1195.12

According to **General Procedure C**, the reactions were performed under dry conditions using 117 mg *H*-phosphonate **55o-MEEEG** (0.150 mmol, 1.0 equiv.) and 118 mg d4TMP 2×nBu_4N^+ salt (0.150 mmol, 1.0 equiv.). Yield: 35%, 62 mg, as colorless solid.

^{1}H-NMR (600 MHz, Methanol-d4): δ 7.69 (d, $^4J_{HH}$ = 1.4 Hz, 1H, **H_{het}-6**), 7.47-7.38 (m, 4H, **H-2´´**), 7.13-7.03 (m, 4H, **H-3´´**), 6.94 (dt, $^3J_{HH}$ = 3.5 Hz, $^4J_{HH}$ = 1.6 Hz, 1H, **H-1´**), 6.48 (dt, $^3J_{HH}$ = 6.0 Hz, $^4J_{HH}$ = 1.7 Hz, 1H, **H-3´**), 5.81 (ddd, $^3J_{HH}$ = 5.9 Hz, $^3J_{HH}$ = 2.4 Hz, $^4J_{HH}$ = 1.9 Hz, 1H, **H-2´**), 5.17 (d, $^3J_{HP}$ = 8.1 Hz, 4H, **Ph-CH_2**), 4.98-4.93 (m, 1H, **H-4´**), 4.32-4.17 (m, 4H, **H-5´**, **H-u**), 3.74-3.70 (m, 2H, **H-v**), 3.67-3.61 (m, 6H, **H-w**, **H-x**, **H-y**), 3.56-3.52 (m, 2H, **H-z**), 3.36 (s, 3H, **-OCH_3**), 2.69 (td, $^3J_{HH}$ = 7.4 Hz, $^4J_{HH}$ = 1.3 Hz, 2H, **H-q**), 2.60 (td, $^3J_{HH}$ = 7.4 Hz, $^4J_{HH}$ = 1.3 Hz, 2H, **H-b**), 2.52 (t, $^3J_{HH}$ = 7.2 Hz, 2H, **H-s**), 2.04 (quint, $^3J_{HH}$ = 7.2 Hz, 2H, **H-r**), 1.91 (d, $^4J_{HH}$ = 1.3 Hz, 3H, **H_{het}-7**), 1.75 (quint, $^3J_{HH}$ = 7.4 Hz, 2H, **H-c**), 1.49-1.25 (m, 22H, **H-d**, **H-e**, **H-f**, **H-g**, **H-h**, **H-i**, **H-j**, **H-k**, **H-l**, **H-m**, **H-n**), 0.92 (t, $^3J_{HH}$ = 7.0 Hz, 3H, **H-o**).

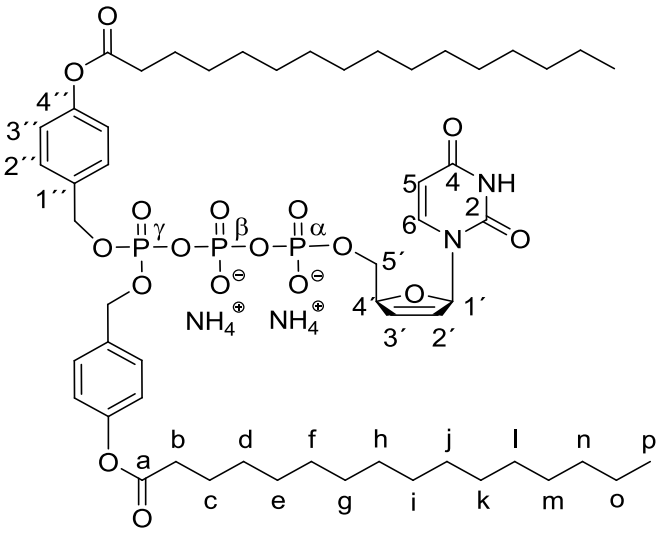
^{13}C-NMR (151 MHz, Methanol-d4): δ 174.6 (**C-t**), 173.8 (**C-a**), 173.1 (**C-p**), 166.5 (**C_{het}-4**), 152.8 (**C_{het}-2**), 152.36, 152.27 (**C-4´´**), 138.7 (**C_{het}-6**), 135.80 (**C-3´**), 135.03 (d, $^4J_{CP}$ = 7.6 Hz, **C-1´´**), 134.96 (d, $^3J_{CP}$ = 7.7 Hz, **C-1´´**), 130.50, 130.47 (**4×C-2´´**), 127.2 (**C-2´**), 122.89, 122.87 (**4×C-3´´**), 112.1 (**C_{het}-5**), 90.8 (**C-1´**), 87.23 (d, $^3J_{CP}$ = 9.0 Hz, **C-4´**), 73.0 (**C-z**), 71.53, 71.39 (**C-w, C-x, C-y**), 70.39, 70.36, 70.33 (**2×Ph-CH_2**), 70.12 (**C-v**), 67.86 (d, $^2J_{CP}$ = 6.0 Hz, **C-5´**), 64.7 (**C-u**), 59.1 (**-OCH_3**), 35.03 (**C-b**), 33.98, 33.91 (**C-q**, **C-s**), 33.08, 30.80, 30.79, 30.76, 30.73, 30.62, 30.48, 30.42, 30.18 (**C-d**, **C-e**, **C-f**, **C-g**, **C-h**, **C-i**, **C-j**, **C-k**, **C-l**, **C-m**), 25.97 (**C-c**), 23.74 (**C-n**), 21.19 (**C-r**), 14.45 (**C-o**), 12.5 (**C_{het}-7**).

^{31}P-NMR (243 MHz, Methanol-d4): δ -11.80 (d, *J* = 19.9 Hz, **P-α**), -13.23 (d, *J* = 17.2 Hz, **P-γ**), -23.75 (t, *J* = 18.6 Hz, **P-β**).

HRMS (ESI-TOF) m/z: calculated for $C_{51}H_{74}N_2O_{22}P_3$ $[M-H]^-$ 1159.3952, found 1159.3885.

IR: v = 3184, 2922, 2853, 1755, 1736, 1689, 1509, 1455, 1247, 1219, 1200, 1167, 1127, 1083, 1009, 905, 838, 784, 722, 643, 489, 420, 399.

Tri*PPP*ro γ-(AB-$C_{15}H_{31}$,ab-$C_{15}H_{31}$)-d4UTP (ammonium salt) **56uu**



Yield: 50%

Chemical Formula: $C_{55}H_{91}N_4O_{17}P_3$

Molecular Weight: 1173.25

According to **General Procedure C**, the reactions were performed under dry conditions using 114 mg *H*-phosphonate **55uu** (0.150 mmol, 1.0 equiv.) and 118 mg d4TMP 2×nBu_4N^+ salt (0.150 mmol, 1.0 equiv.). Yield: 50%, 88 mg, as colorless cotton.

^{1}H-NMR (500 MHz, THF-d8): δ 10.18 (s, 1H, **N-H**), 7.88 (d, $^4J_{HH}$ = 1.3 Hz, 1H, **H_{het}-6**), 7.48-7.42 (m, 4H, **H-2´´**), 7.08-7.01 (m, 4H, **H-3´´**), 6.93 (dt, $^3J_{HH}$ = 3.6 Hz, $^4J_{HH}$ = 1.5 Hz, 1H, **H-1´**), 6.55 (dt, $^3J_{HH}$ = 5.8 Hz, $^4J_{HH}$ = 1.6 Hz, 1H, **H-3´**), 5.67-5.61 (m, 1H, **H-2´**), 5.52 (dd, $^3J_{HH}$ = 8.2 Hz, $^4J_{HH}$ = 3.7 Hz, 1H, **H-5**), 5.22-5.13 (m, 4H, **Ph-CH_2**), 4.87-4.82 (m, 1H, **H-4´**), 4.38-4.06 (m, 2H, **H-5´**), 2.53 (t, $^3J_{HH}$ = 7.5 Hz, 4H, **H-b**), 1.70-1.64 (m, 4H, **H-c**), 1.49-1.18 (m, 48H, H-d, H-e, H-f, H-g, H-h, H-i, H-j, H-k, H-l, H-m, H-n, H-o), 0.89 (t, 6H, $^3J_{HH}$ = 6.7 Hz, 6H, **H-p**).

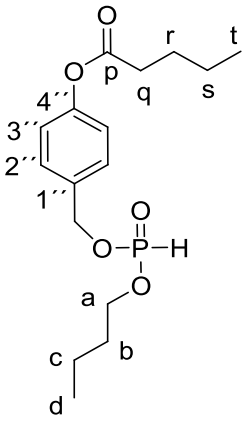
^{13}C-NMR (126 MHz, THF-d8): δ 172.2 (**C-a**), 164.8 (**C_{het}-4**), 152.1 (**C_{het}-2**), 152.0 (**C-4´´**), 137.9 (**C_{het}-6**), 136.5 (**C-3´**), 135.4 (d, $^3J_{CP}$ = 8.5 Hz, **C-1´´**), 130.2 (**4×C-2´´**), 126.4 (**C-2´**), 122.5 (**4×C-3´´**), 103.1 (**C_{het}-5**), 90.3 (**C-1´**), 87.2 (d, $^3J_{CP}$ = 7.6 Hz, **C-4´**), 69.4 (d, $^2J_{CP}$ = 7.1 Hz, **Ph-CH_2**), 66.9 (**C-5´**), 34.9 (**C-b**), 32.9, 30.9, 30.8, 30.8, 30.7, 30.7, 30.5, 30.4, 30.2, 23.7 (**C-d**, **C-e**, **C-f**, **C-g**, **C-h**, **C-i**, **C-j**, **C-k**, **C-l**, **C-m**, **C-n**, **C-o**), 26.1 (**C-c**), 14.6 (**C-p**).

^{31}P-NMR (162 MHz, THF-d8): δ -12.55 (d, $^2J_{CP}$ = 19.4 Hz, **P-α**), -13.36 (d, $^2J_{CP}$ = 17.6 Hz, **P-γ**), -24.15 (t, $^2J_{CP}$ = 18.4 Hz, **P-β**).

HRMS (ESI-TOF) m/z: calculated for $C_{55}H_{84}N_2O_{17}P_3$ $[M-H]^-$ 1137.4988, found 1137.4958.

IR: ν = 3198, 3054, 2956, 2916, 2848, 1753, 1687, 1510, 1464, 1421, 1381, 1346, 1329, 1241, 1224, 1169, 1154, 1128, 1083, 1006, 951, 924, 839, 816, 767, 719, 653, 625, 517.

(AB-C_4H_9,alkyl-C_4H_9)-*H*-phosphonate **57ed**



Yield: 31%

Chemical Formula: $C_{16}H_{25}O_5P$

Molecular Weight: 328.34

According to **General Procedure D**, 0.23 mL diphenyl phosphonate (1.2 mmol, 1.2 equiv.) was dissolved in pyridine at 0 °C. 0.21 g 4-(hydroxymethyl)phenyl pentanoate **44e** (1.0 mmol, 1.0 equiv.) was added and followed by 0.10 g 1-butanol (1.4 mmol, 1.4 equiv.). The mixture was stirred overnight at room temperature. Column chromatography (SiO_2, petrol ether/ethylacetate/CH_3COOH 2:8:0.005 v/v/v). Yield: 31%, 0.103 g, as colorless oil.

^{1}H-NMR (500 MHz, Chloroform-d): δ 7.41 (d, $^3J_{HH}$ = 8.5 Hz, 2H, **H-2´´**), 7.09 (d, $^3J_{HH}$ = 8.5 Hz, 2H, **H-3´´**), 6.87 (d, $^1J_{PH}$ = 700 Hz, 1H, **P-H**), 5.09 (d, $^3J_{HH}$ = 9.6 Hz, 2H, **Ph-CH$_2$**), 4.11-3.96 (m, 2H, **H-a**), 2.56 (t, $^3J_{HH}$ = 7.5 Hz, 2H, **H-q**), 1.74 (quint, $^3J_{HH}$ = 7.6 Hz, 2H, **H-r**), 1.64 (quint, $^3J_{HH}$ = 6.7 Hz, 2H, **H-b**), 1.50-1.33 (m, 4H, **H-c**, **H-s**), 0.97 (t, $^3J_{HH}$ = 7.4 Hz, 3H, **H-t**), 0.92 (t, $^3J_{HH}$ = 7.4 Hz, 3H, **H-d**).

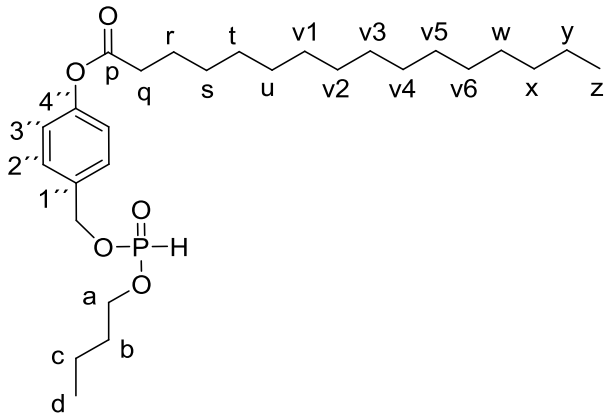
^{13}C-NMR (126 MHz, Chloroform-d): *δ* 172.12 (**C-p**), 150.92 (**C-4´´**), 133.20 (**C-1´´**), 129.15 (**C-2´´**), 121.89 (**C-2´´**), 66.53 (d, $^2J_{CP}$ = 5.5 Hz, **Ph-CH$_2$**), 65.67 (d, $^2J_{CP}$ = 6.2 Hz, **C-a**), 34.07 (**C-q**), 32.30 (d, $^3J_{CP}$ = 6.3 Hz, **C-b**), 26.94 (**C-r**), 22.22 (**C-s**), 18.66 (**C-c**), 13.70, 13.50 (**C-d**, **C-t**).

^{31}P-NMR (162 MHz, Chloroform-d): *δ* 7.70.

HRMS (ESI-TOF) m/z: calculated for $C_{16}H_{25}NaO_5P$ $[M+Na]^+$ 351.1332, found 351.1338.

IR: ν = 2959, 2933, 2872, 1756, 1612, 1508, 1464, 1418, 1379, 1200, 1165, 1141, 1078, 974, 920, 850, 827, 510, 434.

(AB-$C_{15}H_{31}$,alkyl-C_4H_9)-*H*-phosphonate **57ud**



Yield: 39%

Chemical Formula: $C_{27}H_{47}O_5P$

Molecular Weight: 482.63

According to **General Procedure D**, 0.23 mL diphenyl phosphonate (1.2 mmol, 1.2 equiv.) was dissolved in pyridine at 0 °C. 0.36 g 4-(hydroxymethyl)phenylhexadecanoate **44u** (1.0 mmol, 1.0 equiv.) was added and followed by 0.10 g 1-butanol (1.4 mmol, 1.4 equiv.). The mixture was stirred overnight at room temperature. Column chromatography (SiO_2, petrol ether/ethylacetate/CH_3COOH 8:2:0.005 v/v/v). Yield: 39%, 0.188 g, as colorless solid.

^{1}H-NMR (400 MHz, Chloroform-d): δ 7.41 (d, $^3J_{HH}$ = 8.5 Hz, 2H, **H-2´´**), 7.09 (d, $^3J_{HH}$ = 8.5 Hz, 2H, **H-3´´**), 6.87 (d, $^1J_{PH}$ = 704 Hz, 1H, **P-H**), 5.09 (d, $^3J_{HH}$ = 9.5 Hz, 2H, **Ph-CH$_2$**), 4.12-3.93 (m, 2H, **H-a**), 2.55 (t, $^3J_{HH}$ = 7.5 Hz, 2H, **H-q**), 1.75 (quint, $^3J_{HH}$ = 7.4 Hz, 2H, **H-r**), 1.64 (quint, $^3J_{HH}$ = 8.4 Hz, 2H, **H-b**), 1.48-1.17 (m, 26H, **H-c**, **H-s**, **H-t**, **H-u**, **H-v1**, **H-v2**, **H-v3**, **H-v4**, **H-v5**, **H-v6**, **H-w**, **H-x**, **H-y**), 0.92 (t, $^3J_{HH}$ = 7.3 Hz, 3H, **H-d**), 0.88 (t, $^3J_{HH}$ = 7.0 Hz, 3H, **H-z**).

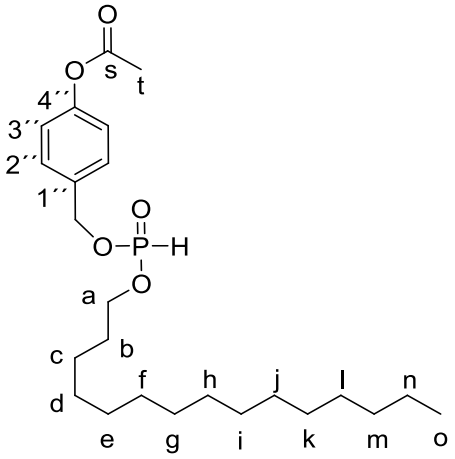
^{13}C-NMR (101 MHz, Chloroform-d): *δ* 172.16 (**C-p**), 150.94 (**C-4´´**), 133.12 (**C-1´´**), 129.17 (**C-2´´**), 121.91 (**C-3´´**), 66.58 (d, $^2J_{CP}$ = 5.6 Hz, **Ph-CH$_2$**), 65.71 (d, $^2J_{CP}$ = 6.2 Hz, **C-a**), 34.38 (**C-q**), 32.30 (d, $^3J_{CP}$ = 6.3 Hz, **C-b**), 31.91, 29.68, 29.67, 29.64, 29.59, 29.45, 29.35, 29.24, 29.09 (**C-s**, **C-t**, **C-u**, **C-v1**, **C-v2**, **C-v3**, **C-v4**, **C-v5**, **C-v6**, **C-w**, **C-x**), 24.90 (**C-r**), 22.68 (**C-y**), 18.66 (**C-c**), 14.11, 13.51 (**C-d**, **C-z**).

^{31}P-NMR (243 MHz, Chloroform-d): *δ* 7.72.

HRMS (ESI-TOF) m/z: calculated for $C_{27}H_{47}NaO_5P$ $[M+Na]^+$ 505.3053, found 505.3054.

IR: v = 2956, 2914, 2872, 2848, 1748, 1606, 1510, 1464, 1411, 1385, 1348, 1330, 1308, 1285, 1262, 1241, 1221, 1167, 1150, 1072, 987, 925, 819, 770, 719, 693, 580, 524, 446, 416.

(AB-CH_3,alkyl-$C_{15}H_{31}$)-*H*-phosphonate **57bo**



Yield: 65%

Chemical Formula: $C_{24}H_{41}O_5P$

Molecular Weight: 440.55

According to **General Procedure D**, 0.23 mL diphenyl phosphonate (1.2 mmol, 1.2 equiv.) was dissolved in pyridine at 0 °C. 0.23 g 1-pentadecanol (1.0 mmol, 1.0 equiv.) was added and followed by 0.23 g 4-(hydroxymethyl)phenyl acetate **44b** (1.4 mmol, 1.4 equiv.). The mixture was stirred overnight at room temperature. Column chromatography (SiO_2, petrol ether/ethylacetate/CH_3COOH 7:3:0.005 v/v/v). Yield: 65%, 0.284 g, as colorless solid.

^{1}H-NMR (600 MHz, Chloroform-d): δ 7.43-7.35 (m, 2H, **H-2´´**), 7.16-7.04 (m, 2H, **H-3´´**), 6.87 (d, $^1J_{PH}$ = 696 Hz, 1H, **P-H**), 5.09 (d, $^3J_{HH}$ = 9.4 Hz, 2H, **Ph-CH_2**), 4.09-3.96 (m, 2H, **H-a**), 2.30 (s, 3H, **H-t**), 1.65 (quint, $^3J_{HH}$ = 6.8 Hz, 2H, **H-b**), 1.40-1.19 (m, 24H**, H-c**, **H-d**, **H-e**, **H-f**, **H-g**, **H-h**, **H-i**, **H-j**, **H-k**, **H-l**, **H-m**, **H-n**), 0.88 (t, $^3J_{HH}$ = 7.0 Hz, 3H, **H-o**).

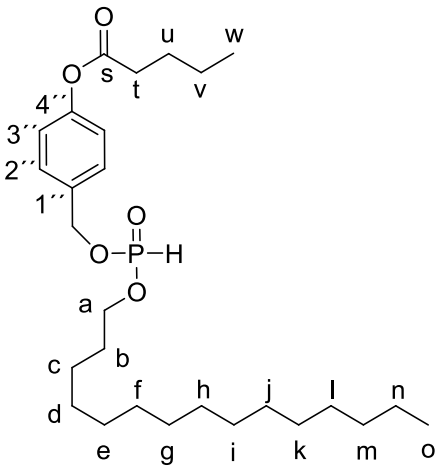
^{13}C-NMR (151 MHz, Chloroform-d): *δ* 169.25 (**C-s**), 150.81 (**C-4´´**), 133.32 (d, $^2J_{CP}$ = 6.0 Hz, **C-1´´**), 129.15 (**C-2´´**), 121.88 (**C-3´´**), 66.48 (d, $^2J_{CP}$ = 5.5 Hz, **Ph-CH_2**), 66.01 (d, $^2J_{CP}$ = 6.2 Hz, **C-a**), 30.33 (d, $^3J_{CP}$ = 6.3 Hz, **C-b**), 31.90, 29.67, 29.66, 29.65, 29.63, 29.61, 29.53, 29.46, 29.34, 29.07, 22.67 (**C-d**, **C-e**, **C-f**, **C-g**, **C-h**, **C-i**, **C-j**, **C-k**, **C-l**, **C-m**, **C-n**), 25.44 (**C-c**), 21.08 (**C-t**), 14.10 (**C-o**).

^{31}P-NMR (243 MHz, Chloroform-d): *δ* 7.72.

HRMS (ESI-TOF) m/z: calculated for $C_{24}H_{41}NaO_5P$ $[M+Na]^+$ 463.2584, found 463.2582.

IR: v = 2955, 2918, 2848, 1754, 1509, 1460, 1373, 1232, 1199, 1167, 1104, 1084, 990, 918, 877, 853, 816, 784, 724, 659, 596, 554, 490, 463, 441, 420.

(AB-C_4H_9,alkyl-$C_{15}H_{31}$)-*H*-phosphonate **57eo**



Yield: 43%

Chemical Formula: $C_{27}H_{47}O_5P$

Molecular Weight: 482.63

According to **General Procedure D**, 0.23 mL diphenyl phosphonate (1.2 mmol, 1.2 equiv.) was dissolved in pyridine at 0 °C. 0.23 g 1-pentadecanol (1.0 mmol, 1.0 equiv.) was added and followed by 0.29 g 4-(hydroxymethyl)phenyl pentanoate **44e** (1.4 mmol, 1.4 equiv.). The mixture was stirred overnight at room temperature. Column chromatography (SiO_2, petrol ether/ethylacetate/CH_3COOH 7:3:0.005 v/v/v). Yield: 43%, 0.208 g, as colorless solid.

^{1}H-NMR (600 MHz, Chloroform-d): δ 7.41 (d, $^3J_{HH}$ = 8.5 Hz, 2H, **H-2´´**), 7.12-7.06 (m, 2H, **H-3´´**), 6.87 (d, $^1J_{PH}$ = 702 Hz, 1H, **P-H**), 5.09 (d, $^3J_{HH}$ = 9.5 Hz, 2H, **Ph-CH_2**), 4.09-3.95 (m, 2H, **H-a**), 2.56 (t, $^3J_{HH}$ = 7.5 Hz, 3H, **H-t**), 1.74 (quint, $^3J_{HH}$ = 7.6 Hz, 2H, **H-u**), 1.65 (quint, $^3J_{HH}$ = 6.6 Hz, 2H, **H-b**), 1.44 (tq, $^3J_{HH}$ = 7.6, 7.5 Hz, 2H, **H-v**), 1.40-1.19 (m, 24H**, H-c**, **H-d**, **H-e**, **H-f**, **H-g**, **H-h**, **H-i**, **H-j**, **H-k**, **H-l**, **H-m**, **H-n**), 0.97 (t, $^3J_{HH}$ = 7.4 Hz, 3H, **H-w**), 0.88 (t, $^3J_{HH}$ = 7.0 Hz, 3H, **H-o**).

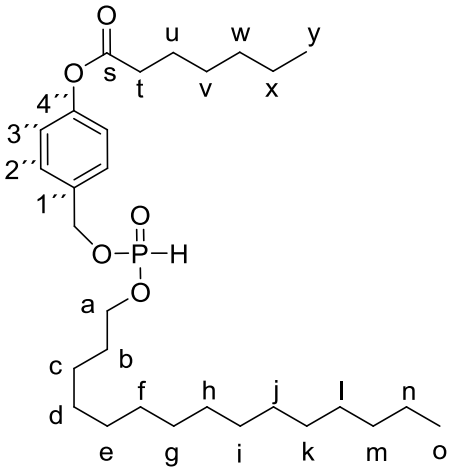
^{13}C-NMR (151 MHz, Chloroform-d): *δ* 172.14 (**C-s**), 150.92 (**C-4´´**), 133.16 (**C-1´´**), 129.16 (**C-2´´**), 121.90 (**C-3´´**), 66.54 (d, $^2J_{CP}$ = 5.5 Hz, **Ph-CH_2**), 66.02 (d, $^2J_{CP}$ = 6.2 Hz, **C-a**), 34.08 (**C-t**), 30.34 (d, $^3J_{CP}$ = 6.2 Hz, **C-b**), 31.91, 29.69, 29.68, 29.66, 29.65, 29.63, 29.55, 29.48, 29.35, 29.09, 22.23 (**C-d**, **C-e**, **C-f**, **C-g**, **C-h**, **C-i**, **C-j**, **C-k**, **C-l**, **C-m**, **C-n**), 26.95 (**C-u**), 25.45 (**C-c**), 22.68 (**C-v**), 14.11(**C-o**), 13.71 (**C-w**).

^{31}P-NMR (243 MHz, Chloroform-d): *δ* 7.73.

HRMS (ESI-TOF) m/z: calculated for $C_{27}H_{51}NO_5P$ $[M+NH_4]^+$ 500.3499, found 500.3510.

IR: v **=** 2958, 2915, 2847, 1751, 1511, 1471, 1462, 1249, 1218, 1203, 1170, 1141, 1098, 1065, 1032, 1010, 992, 957, 949, 925, 859, 848, 823, 727, 720, 551, 492, 420.

(AB-C_6H_{13},alkyl-$C_{15}H_{31}$)-*H*-phosphonate **57go**



Yield: 31%

Chemical Formula: $C_{29}H_{51}O_5P$

Molecular Weight: 510.69

According to **General Procedure D**, 0.23 mL diphenyl phosphonate (1.2 mmol, 1.2 equiv.) was dissolved in pyridine at 0 °C. 0.23 g 1-pentadecanol (1.0 mmol, 1.0 equiv.) was added and followed by 0.33 g 4-(hydroxymethyl)phenyl heptanoate **44g** (1.4 mmol, 1.4 equiv.). The mixture was stirred overnight at room temperature. Column chromatography (SiO_2, petrol ether/ethylacetate/CH_3COOH 7:3:0.005 v/v/v) and then recrystallized in DCM/hexane at -26 °C. Yield: 31%, 0.158 g, as colorless solid.

^{1}H-NMR (600 MHz, Chloroform-d): δ 7.42 (d, $^{3}J_{HH}$ = 8.4 Hz, 2H, **H-2´´**), 7.13-7.06 (m, 2H, **H-3´´**), 6.86 (d, $^{1}J_{PH}$ = 708 Hz, 1H, **P-H**), 5.09 (d, $^{3}J_{HH}$ = 9.6 Hz, 2H, **Ph-CH$_2$**), 4.09-3.95 (m, 2H, **H-a**), 2.55 (t, $^{3}J_{HH}$ = 7.5 Hz, 3H, **H-t**), 1.75 (quint, $^{3}J_{HH}$ = 7.6 Hz, 2H, **H-u**), 1.64 (quint, $^{3}J_{HH}$ = 6.6 Hz, 2H, **H-b**), 1.45-1.36 (m, 2H, **H-v**), 1.36-1.18 (m, 28H**, H-c**, **H-d**, **H-e**, **H-f**, **H-g**, **H-h**, **H-i**, **H-j**, **H-k**, **H-l**, **H-m**, **H-n**, **H-w**, **H-x**), 0.91 (t, $^{3}J_{HH}$ = 7.0 Hz, 3H, **H-y**), 0.89 (t, $^{3}J_{HH}$ = 7.1 Hz, 3H, **H-o**).

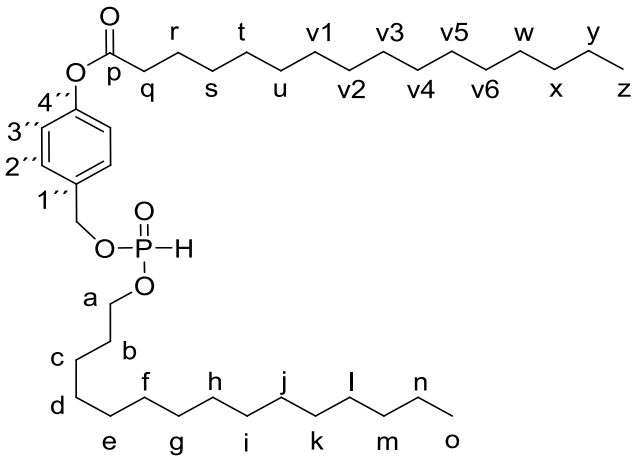
^{13}C-NMR (151 MHz, Chloroform-d): *δ* 172.14 (**C-s**), 150.91 (**C-4´´**), 133.20 (**C-1´´**), 129.15 (**C-2´´**), 121.92 (**C-3´´**), 66.53 (d, $^{2}J_{CP}$ = 5.5 Hz, **Ph-CH$_2$**), 66.01 (d, $^{2}J_{CP}$ = 6.2 Hz, **C-a**), 34.35 (**C-t**), 30.33 (d, $^{3}J_{CP}$ = 6.3 Hz, **C-b**), 31.90, 29.65, 29.64, 29.62, 29.53, 29.46, 29.34, 29.08, 22.67, 22.45 (**C-d**, **C-e**, **C-f**, **C-g**, **C-h**, **C-i**, **C-j**, **C-k**, **C-l**, **C-m**, **C-n**, **C-w**, **C-x**), 28.74 (**C-v**), 25.44 (**C-c**), 24.82 (**C-u**), 14.12 (**C-o**), 14.01 (**C-y**).

^{31}P-NMR (243 MHz, Chloroform-d): *δ* 7.72.

HRMS (ESI-TOF) m/z: calculated for $C_{29}H_{55}NO_5P$ $[M+NH_4]^+$ 528.3812, found 528.3825.

IR: ν = 2955, 2916, 2871, 2849, 1753, 1509, 1466, 1386, 1291, 1236, 1218, 1167, 1153, 1104, 1076, 1039, 985, 947, 925, 866, 821, 721.

(AB-$C_{15}H_{31}$,alkyl-$C_{15}H_{31}$)-*H*-phosphonate **57uo**



Yield:46%

Chemical Formula: $C_{38}H_{69}O_5P$

Molecular Weight: 636.93

According to **General Procedure D**, 0.23 mL diphenyl phosphonate (1.2 mmol, 1.2 equiv.) was dissolved in pyridine at 0 °C. 0.23 g 1-pentadecanol (1.0 mmol, 1.0 equiv.) was added and followed by 0.51 g 4-(hydroxymethyl)phenyl hexadecanoate **44u** (1.4 mmol, 1.4 equiv.). The mixture was stirred overnight at room temperature. Column chromatography (SiO_2, petrol ether/ethylacetate/CH_3COOH 8:2:0.005 v/v/v). Yield: 46%, 0.293 g, as colorless solid.

^{1}H-NMR (600 MHz, Chloroform-d): δ 7.41 (d, $^3J_{HH}$ = 8.2 Hz, 2H, **H-2´´**), 7.15-7.05 (m, 2H, **H-3´´**), 6.87 (d, $^1J_{PH}$ = 702 Hz, 1H, **P-H**), 5.09 (d, $^3J_{HH}$ = 9.6 Hz, 2H, **Ph-CH$_2$**), 4.10-3.93 (m, 2H, **H-a**), 2.55 (t, $^3J_{HH}$ = 7.5 Hz, 3H, **H-q**), 1.74 (quint, $^3J_{HH}$ = 7.5 Hz, 2H, **H-r**), 1.65 (quint, $^3J_{HH}$ = 6.9 Hz, 2H, **H-b**), 1.40 (quint, $^3J_{HH}$ = 6.9 Hz, 2H, **H-s**), 1.38-1.18 (m, 46H**, H-c**, **H-d**, **H-e**, **H-f**, **H-g**, **H-h**, **H-i**, **H-j**, **H-k**, **H-l**, **H-m**, **H-n**, **H-t**, **H-u**, **H-v1**, **H-v2**, **H-v3**, **H-v4**, **H-v5**, **H-v6**, **H-w**, **H-x**, **H-y**), 0.88 (t, $^3J_{HH}$ = 7.0 Hz, 6H, **H-o, H-z**).

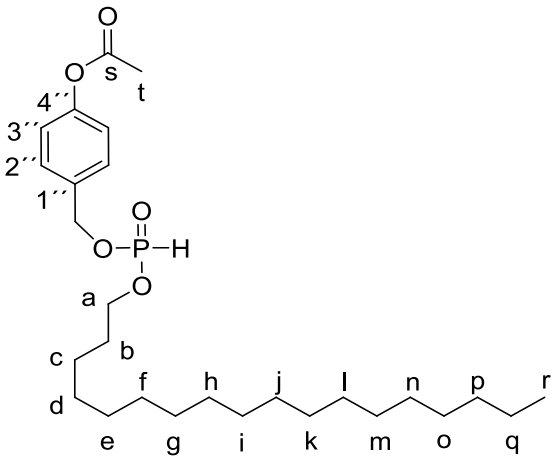
^{13}C-NMR (151 MHz, Chloroform-d): *δ* 172.14 (**C-p**), 150.92 (**C-4´´**), 133.16 (d, $^3J_{CP}$ = 6.1 Hz, **C-1´´**), 129.15 (**C-2´´**), 121.90 (**C-3´´**), 66.54 (d, $^2J_{CP}$ = 5.5 Hz, **Ph-CH$_2$**), 66.02 (d, $^2J_{CP}$ = 6.2 Hz, **C-a**), 34.37 (**C-q**), 30.33 (d, $^3J_{CP}$ = 6.3 Hz, **C-b**), 31.91, 29.68, 29.66, 29.64, 29.63, 29.59, 29.54, 29.47, 29.45, 29.35, 29.24, 29.09, 29.08, 22.68 (**C-d**, **C-e**, **C-f**, **C-g**, **C-h**, **C-i**, **C-j**, **C-k**, **C-l**, **C-m**, **C-n**, **C-s**, **C-t**, **C-u**, **C-v1**, **C-v2**, **C-v3**, **C-v4**, **C-v5**, **C-v6**, **C-w**, **C-x**, **C-y**), 25.44 (**C-c**), 24.90 (**C-r**), 14.11 (**C-o**, **C-z**).

^{31}P-NMR (243 MHz, Chloroform-d): *δ* 7.72.

HRMS (ESI-TOF) m/z: calculated for $C_{38}H_{73}NO_5P$ $[M+NH_4]^+$ 654.5221, found 654.5215.

IR: ν = 2961, 2915, 2847, 1750, 1511, 1472, 1463, 1382, 1263, 1249, 1218, 1203, 1010, 992, 957, 949, 925, 859, 848, 823, 728, 720, 551, 492, 421.

(AB-CH_3,alkyl-$C_{18}H_{37}$)-*H*-phosphonate **57br**



Yield: 48%

Chemical Formula: $C_{27}H_{47}O_5P$

Molecular Weight: 482.63

According to **General Procedure D**, 0.23 mL diphenyl phosphonate (1.2 mmol, 1.2 equiv.) was dissolved in pyridine at 0 °C. 0.27 g 1-octadecanol (1.0 mmol, 1.0 equiv.) was added and followed by 0.23 g 4-(hydroxymethyl)phenyl acetate **44b** (1.4 mmol, 1.4 equiv.). The mixture was stirred overnight at room temperature. Column chromatography (SiO_2, petrol ether/ethylacetate/CH_3COOH 8:2:0.005 v/v/v). Yield: 48%, 0.231 g, as colorless solid.

^{1}H-NMR (600 MHz, Chloroform-d): δ 7.41 (d, $^3J_{HH}$ = 8.4 Hz, 2H, **H-2´´**), 7.10 (d, $^3J_{HH}$ = 8.5 Hz, 2H, **H-3´´**), 6.87 (d, $^1J_{PH}$ = 696 Hz, 1H, **P-H**), 5.09 (d, $^3J_{HH}$ = 9.4 Hz, 2H, **Ph-CH_2**), 4.10-3.93 (m, 2H, **H-a**), 2.30 (s, 3H, **H-t**), 1.64 (quint, $^3J_{HH}$ = 6.9 Hz, 2H, **H-b**), 1.42-1.18 (m, 30H**, H-c**, **H-d**, **H-e**, **H-f**, **H-g**, **H-h**, **H-i**, **H-j**, **H-k**, **H-l**, **H-m**, **H-n**, **H-o**, **H-p**, **H-q**), 0.88 (t, $^3J_{HH}$ = 7.0 Hz, 3H, **H-r**).

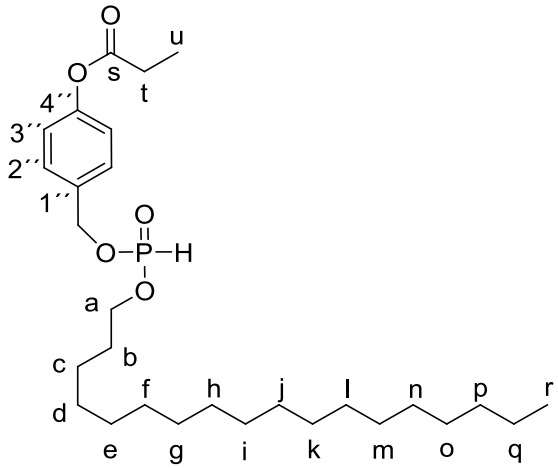
^{13}C-NMR (151 MHz, Chloroform-d): *δ* 169.27 (**C-s**), 150.81 (**C-4´´**), 133.32 (d, $^2J_{CP}$ = 6.0 Hz, **C-1´´**), 129.16 (**C-2´´**), 121.88 (**C-3´´**), 66.49 (d, $^2J_{CP}$ = 5.5 Hz, **Ph-CH_2**), 66.02 (d, $^2J_{CP}$ = 6.1 Hz, **C-a**), 30.34 (d, $^3J_{CP}$ = 6.3 Hz, **C-b**), 31.91, 29.68, 29.66, 29.64, 29.62, 29.54, 29.47, 29.34, 29.08 (**C-d**, **C-e**, **C-f**, **C-g**, **C-h**, **C-i**, **C-j**, **C-k**, **C-l**, **C-m, C-n**, **C-o**, **C-p**), 25.44 (**C-c**), 22.68 (**C-q**), 21.09 (**C-t**), 14.10 (**C-r**).

^{31}P-NMR (243 MHz, Chloroform-d): *δ* 7.73.

HRMS (ESI-TOF) m/z: calculated for $C_{27}H_{47}NaO_5P$ $[M+Na]^+$ 505.3053, found 505.3049.

IR: v =.2955, 2918, 2870, 2847, 1754, 1609, 1510, 1460, 1420, 1373, 1232, 1200, 1110, 1085, 1052, 1021, 989, 918, 877, 853, 816, 784, 752, 724, 704, 659, 597, 555, 515, 502, 493, 479, 451, 427.

(AB-C_2H_5,alkyl-$C_{18}H_{37}$)-*H*-phosphonate **57cr**



Yield: 52%

Chemical Formula: $C_{28}H_{49}O_5P$

Molecular Weight: 496.66

According to **General Procedure D**, 0.23 mL diphenyl phosphonate (1.2 mmol, 1.2 equiv.) was dissolved in pyridine at 0 °C. 0.27 g 1-octadecanol (1.0 mmol, 1.0 equiv.) was added and followed by 0.25 g 4-(hydroxymethyl)phenyl propionate **44c** (1.4 mmol, 1.4 equiv.). The mixture was stirred overnight at room temperature. Column chromatography (SiO_2, petrol ether/ethylacetate/CH_3COOH 8:2:0.005 v/v/v). Yield: 52%, 0.260 g, as colorless solid.

^{1}H-NMR (600 MHz, Chloroform-d): δ 7.40 (d, $^3J_{HH}$ = 8.3 Hz, 2H, **H-2´´**), 7.18-7.02 (m, 2H, **H-3´´**), 6.87 (d, $^1J_{PH}$ = 702 Hz, 1H, **P-H**), 5.09 (d, $^3J_{HH}$ = 9.5 Hz, 2H, **Ph-CH$_2$**), 4.11-3.92 (m, 2H, **H-a**), 2.59 (q, $^3J_{HH}$ = 7.5 Hz, 2H, **H-t**), 1.64 (quint, $^3J_{HH}$ = 6.7 Hz, 2H, **H-b**), 1.45-1.13 (m, 33H**, H-c**, **H-d**, **H-e**, **H-f**, **H-g**, **H-h**, **H-i**, **H-j**, **H-k**, **H-l**, **H-m**, **H-n**, **H-o**, **H-p**, **H-q, H-u**), 0.87 (t, $^3J_{HH}$ = 6.9 Hz, 3H, **H-r**).

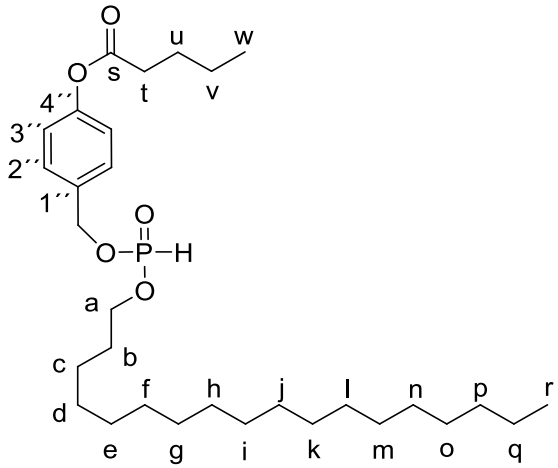
^{13}C-NMR (151 MHz, Chloroform-d): *δ* 172.7 (**C-s**), 150.9 (**C-4´´**), 133.2 (**C-1´´**), 129.1 (**C-2´´**), 121.9 (**C-3´´**), 66.50 (d, $^2J_{CP}$ = 5.5 Hz, **Ph-CH$_2$**), 65.98 (d, $^2J_{CP}$ = 6.1 Hz, **C-a**), 30.32 (d, $^3J_{CP}$ = 6.2 Hz, **C-b**), 31.89, 29.67, 29.64, 29.63, 29.61, 29.52, 29.45, 29.33, 29.06 (**C-d**, **C-e**, **C-f**, **C-g**, **C-h**, **C-i**, **C-j**, **C-k**, **C-l**, **C-m, C-n**, **C-o**, **C-p**), 27.69 (**C-t**), 25.43 (**C-c**), 22.66 (**C-q**), 14.08 (**C-r**), 8.99 (**C-u**).

^{31}P-NMR (243 MHz, Chloroform-d): *δ* 7.70.

HRMS (ESI-TOF) m/z: calculated for $C_{28}H_{53}NO_5P$ $[M+NH_4]^+$ 514.3656, found 514.3663.

IR: v = 2955, 2919, 2847, 1753, 1598, 1509, 1460, 1422, 1384, 1358, 1254, 1241, 1222, 1202, 1173, 1158, 1109, 1087, 992, 903, 870, 830, 803, 770, 753, 725, 567, 551, 531, 515, 478, 446, 426, 419.

(AB-C_4H_9,alkyl-$C_{18}H_{37}$)-*H*-phosphonate **57er**



Yield: 57%

Chemical Formula: $C_{30}H_{53}O_5P$

Molecular Weight: 524.71

According to **General Procedure D**, 0.23 mL diphenyl phosphonate (1.2 mmol, 1.2 equiv.) was dissolved in pyridine at 0 °C. 0.27 g 1-octadecanol (1.0 mmol, 1.0 equiv.) was added and followed by 0.29 g 4-(hydroxymethyl)phenyl pentanoate **44e** (1.4 mmol, 1.4 equiv.). The mixture was stirred overnight at room temperature. Column chromatography (SiO_2, petrol ether/ethylacetate/CH_3COOH 8:2:0.005 v/v/v). Yield: 57%, 0.300 g, as colorless solid.

^{1}H-NMR (600 MHz, Chloroform-d): δ 7.43-7.36 (m, 2H, **H-2´´**), 7.17-7.04 (m, 2H, **H-3´´**), 6.87 (d, $^1J_{PH}$ = 702 Hz, 1H, **P-H**), 5.09 (d, $^3J_{HH}$ = 9.5 Hz, 2H, **Ph-CH$_2$**), 4.10-3.95 (m, 2H, **H-a**), 2.56 (t, $^3J_{HH}$ = 7.5 Hz, 2H, **H-t**), 1.74 (quint, $^3J_{HH}$ = 7.5 Hz, 2H, **H-u**), 1.64 (quint, $^3J_{HH}$ = 7.2 Hz, 2H, **H-b**),1.44 (tq, $^3J_{HH}$ = 7.6, 7.5 Hz, 2H, **H-v**), 1.38-1.17 (m, 30H**, H-c**, **H-d**, **H-e**, **H-f**, **H-g**, **H-h**, **H-i**, **H-j**, **H-k**, **H-l**, **H-m**, **H-n**, **H-o**, **H-p**, **H-q**), 0.97 (t, $^3J_{HH}$ = 7.4 Hz, 3H, **H-w**), 0.87 (t, $^3J_{HH}$ = 7.0 Hz, 3H, **H-r**).

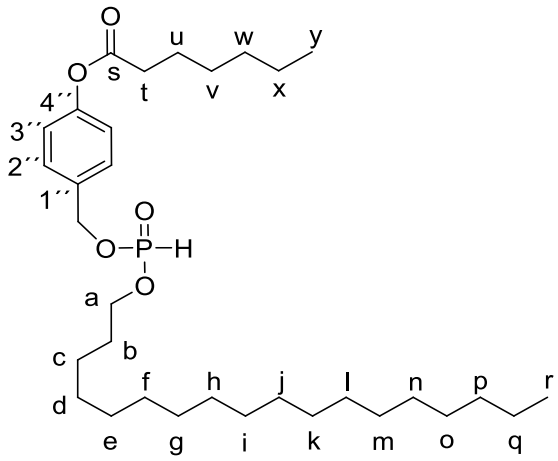
^{13}C-NMR (151 MHz, Chloroform-d): *δ* 172.1 (**C-s**), 150.9 (**C-4´´**), 133.19 (**C-1´´**), 129.1 (**C-2´´**), 121.9 (**C-3´´**), 66.52 (d, $^2J_{CP}$ = 6.0 Hz, **Ph-CH$_2$**), 65.98 (d, $^2J_{CP}$ = 6.0 Hz, **C-a**), 34.1 (**C-t**), 30.33 (d, $^3J_{CP}$ = 6.0 Hz, **C-b**), 31.89, 30.35, 30.31, 29.67, 29.65, 29.63, 29.62, 29.53, 29.46, 29.33, 29.07 (**C-d**, **C-e**, **C-f**, **C-g**, **C-h**, **C-i**, **C-j**, **C-k**, **C-l**, **C-m**, **C-n, C-o, C-p**), 26.93 (**C-u**), 25.44 (**C-c**), 22.67 (**C-q**), 22.21 (**C-v**), 14.09 (**C-r**), 13.69 (**C-w**).

^{31}P-NMR (243 MHz, Chloroform-d): *δ* 7.70.

HRMS (ESI-TOF) m/z: calculated for $C_{30}H_{53}NaO_5P$ $[M+Na]^+$ 547.3523, found 547.3525.

IR: ν **=** 2955, 2918, 2872, 2848, 1753, 1509, 1460, 1414, 1382, 1352, 1248, 1234, 1220, 1204, 1171, 1147, 1106, 1086, 1023, 1003, 991, 971, 952, 930, 896, 876, 852, 822, 723, 552, 514, 454, 426, 415.

(AB-C_6H_{13},alkyl-$C_{18}H_{37}$)-*H*-phosphonate **57gr**



Yield: 63%

Chemical Formula: $C_{32}H_{57}O_5P$

Molecular Weight: 552.77

According to **General Procedure D**, 0.23 mL diphenyl phosphonate (1.2 mmol, 1.2 equiv.) was dissolved in pyridine at 0 °C. 0.27 g 1-octadecanol (1.0 mmol, 1.0 equiv.) was added and followed by 0.33 g 4-(hydroxymethyl)phenylheptanoate **44g** (1.4 mmol, 1.4 equiv.). The mixture was stirred overnight at room temperature. Column chromatography (SiO_2, petrol ether/ethylacetate/CH_3COOH 8:2:0.005 v/v/v). Yield: 63%%, 0.352 g, as colorless solid.

^{1}H-NMR (600 MHz, Chloroform-d): δ 7.43-7.36 (m, 2H, **H-2´´**), 7.12-7.04 (m, 2H, **H-3´´**), 6.87 (d, $^1J_{PH}$ = 702 Hz, 1H, **P-H**), 5.09 (d, $^3J_{HH}$ = 9.6 Hz, 2H, **Ph-CH$_2$**), 4.10-3.94 (m, 2H, **H-a**), 2.55 (t, $^3J_{HH}$ = 7.5 Hz, 2H, **H-t**), 1.75 (quint, $^3J_{HH}$ = 7.5 Hz, 2H, **H-u**), 1.64 (quint, $^3J_{HH}$ = 7.2 Hz, 2H, **H-b**), 1.46-1.37 (m, 2H, **H-v**), 1.37-1.18 (m, 34H**, H-c**, **H-d**, **H-e**, **H-f**, **H-g**, **H-h**, **H-i**, **H-j**, **H-k**, **H-l**, **H-m**, **H-n**, **H-o**, **H-p**, **H-q, H-w, H-x**), 0.90 (t, $^3J_{HH}$ = 6.9 Hz, 3H, **H-y**), 0.87 (t, $^3J_{HH}$ = 7.0 Hz, 3H, **H-r**).

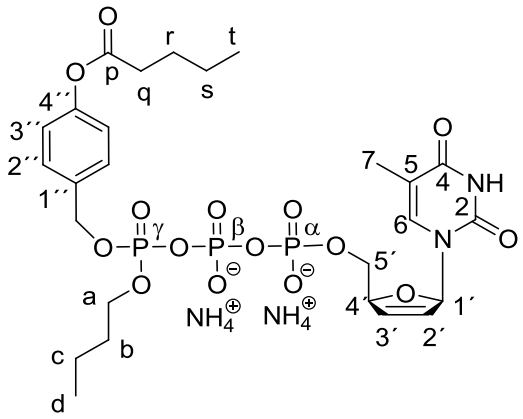
^{13}C-NMR (151 MHz, Chloroform-d): *δ* 172.1 (**C-s**), 150.9 (**C-4´´**), 133.2 (**C-1´´**), 129.1 (**C-2´´**), 121.9 (**C-3´´**), 66.52 (d, $^2J_{CP}$ = 5.5 Hz, **Ph-CH$_2$**), 65.99 (d, $^2J_{CP}$ = 6.2 Hz, **C-a**), 34.35 (**C-t**), 30.33 (d, $^3J_{CP}$ = 6.3 Hz, **C-b**), 31.90, 31.40, 29.68, 29.65, 29.64, 29.62, 29.53, 29.46, 29.34, 29.08, 22.67, 22.45 (**C-d**, **C-e**, **C-f**, **C-g**, **C-h**, **C-i**, **C-j**, **C-k**, **C-l**, **C-m**, **C-n**, **C-o**, **C-p**, **C-q**, **C-w**, **C-x**), 28.74 (**C-v**), 25.44 (**C-c**), 24.84 (**C-u**), 14.09 (**C-r**), 13.99 (**C-y**).

^{31}P-NMR (243 MHz, Chloroform-d): *δ* 7.70.

HRMS (ESI-TOF) m/z: calculated for $C_{32}H_{57}NaO_5P$ $[M+Na]^+$ 575.3836, found 575.3831.

IR: ν = 2956, 2919, 2870, 2847, 1754, 1509, 1460, 1384, 1249, 1235, 1219, 1204, 1171, 1146, 1110, 1086, 1023, 1003, 991, 971, 952, 927, 877, 853, 823, 724, 552, 514, 452, 428, 413.

γ-(AB-C_4H_9,alkyl-C_4H_9)-d4TTP (ammonium salt) **58ed**



Yield: 59%

Chemical Formula: $C_{26}H_{43}N_4O_{15}P_3$

Molecular Weight: 744.56

According to **General Procedure C**, the reactions were performed under dry conditions using 49 mg *H*-phosphonate **57ed** (0.150 mmol, 1.0 equiv.) and 94 mg d4TMP 2×nBu_4N^+ salt (0.12 mmol, 0.8 equiv.). Yield: 59%, 53 mg, as colorless cotton.

^{1}H-NMR (500 MHz, Methanol-d4): δ 7.74-7.65 (m, 1H, **H_{het}-6**), 7.56-7.46 (m, 2H, **H-2´´**), 7.12-7.07 (m, 2H, **H-3´´**), 6.98-6.93 (m, 1H, **H-1´**), 6.51 (dt, $^3J_{HH}$ = 5.9 Hz, $^4J_{HH}$ = 1.8 Hz, 1H, **H-3´**), 5.85 (dt, $^3J_{HH}$ = 6.0 Hz, $^4J_{HH}$ = 1.7 Hz, 1H, **H-2´**), 5.27-5.16 (m, 2H, **Ph-CH_2**), 5.03-4.95 (m, 1H, **H-4´**), 4.35-4.18 (m, 2H, **H-5´**), 4.18-4.10 (m, 2H, **H-a**), 2.60 (t, $^3J_{HH}$ = 7.4 Hz, 2H, **H-q**), 1.92 (dd, $^4J_{HH}$ = 3.7, 1.2 Hz, 3H, **H_{het}-7**), 1.73 (quint, $^3J_{HH}$ = 7.5 Hz, 2H, **H-r**), 1.64 (quint, $^3J_{HH}$ = 6.7 Hz, 2H, **H-b**), 1.52-1.44 (m, 2H, **H-s**), 1.43-1.34 (m, 2H, **H-c**), 1.00 (t, $^3J_{HH}$ = 7.4 Hz, 3H, **H-t**), 0.92 (t, $^3J_{HH}$ = 7.4 Hz, 3H, **H-d**).

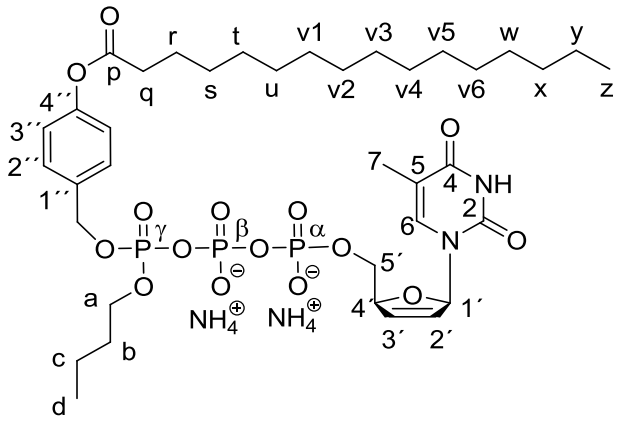
^{13}C-NMR (151 MHz, Methanol-d4): *δ* 173.82 (**C-p**), 166.58 (**C_{het}-4**), 152.81 (**C_{het}-2**), 152.35 (**C-4´´**), 138.67 (**C_{het}-6**), 135.78 (**C-3´**), 135.15 (d, $^3J_{CP}$ = 7.6 Hz, **C-1´´**), 130.39 (d, $^4J_{CP}$ = 4.2 Hz, **2×C-2´´**), 127.15 (**C-2´**), 122.87 (**2×C-3´´**), 112.06 (**C_{het}-5**), 90.88 (**C-1´**), 87.22 (d, $^3J_{CP}$ = 9.2 Hz, **C-4´**), 70.23 (d, $^2J_{CP}$ = 6.9 Hz, **Ph-CH_2**), 69.52 (d, $^2J_{CP}$ = 6.3 Hz, **C-a**), 67.85 (d, $^2J_{CP}$ = 5.7 Hz, **C-5´**), 34.74 (**C-q**), 33.26 (d, $^3J_{CP}$ = 7.3 Hz, **C-b**), 28.05 (**C-r**), 23.23 (**C-s**), 19.68 (**C-c**), 14.06, 13.92 (**C-d**, **C-t**), 12.47 (**C_{het}-7**).

^{31}P-NMR (162 MHz, Methanol-d4): *δ* -11.73 (d, $^2J_{PP}$ = 19.1 Hz, **P-α**), -12.97 (d, $^2J_{PP}$ = 16.8 Hz, **P-γ**), -23.60 (t, $^2J_{PP}$ = 18.0 Hz, **P-β**).

MALDI-MS (m/z): calculated $C_{26}H_{37}N_2NaO_{15}P_3$ $[M+Na]^+$ 733.130, found 733.090; calculated $C_{26}H_{37}KN_2O_{15}P_3$ $[M+K]^+$ 749.104, found 749.061; calculated $C_{26}H_{36}N_2O_{15}P_3$ $[M-H]^-$ 709.133, found 709.198.

IR: ν = 3167, 3030, 2961, 2873, 1757, 1689, 1453, 1245, 1201, 1168, 1126, 1080, 1008, 903, 835, 805, 783, 721, 695, 642, 520, 480, 452, 430, 423, 416, 402.

γ-(AB-$C_{15}H_{31}$,alkyl-C_4H_9)-d4TTP (ammonium salt) **58ud**



Yield: 33%

Chemical Formula: $C_{37}H_{65}N_4O_{15}P_3$

Molecular Weight: 898.85

According to **General Procedure C**, the reactions were performed under dry conditions using 73 mg *H*-phosphonate **57ud** (0.150 mmol, 1.0 equiv.) and 88 mg d4TMP 2×nBu_4N^+ salt (0.11 mmol, 0.75 equiv.). Yield: 33%, 29 mg, as colorless cotton.

^{1}H-NMR (600 MHz, Methanol-d4): *δ* 7.75-7.68 (m, 1H, **H_{het}-6**), 7.53-7.47 (m, 2H, **H-2´´**), 7.12-7.07 (m, 2H, **H-3´´**), 6.98-6.94 (m, 1H, **H-1´**), 6.53 (dt, $^3J_{HH}$ = 6.0 Hz, $^4J_{HH}$ = 1.8 Hz, 1H, **H-3´**), 5.85 (dt, $^3J_{HH}$ = 6.0 Hz, $^4J_{HH}$ = 1.4 Hz, 1H, **H-2´**), 5.28-5.17 (m, 2H, **Ph-CH_2**), 5.03-4.96 (m, 1H, **H-4´**), 4.37-4.18 (m, 2H, **H-5´**), 4.17-4.11 (m, 2H, **H-a**), 2.60 (t, $^3J_{HH}$ = 7.4 Hz, 2H, **H-q**), 1.93 (dd, $^4J_{HH}$ = 3.9, 1.2 Hz, 3H, **H_{het}-7**), 1.75 (quint, $^3J_{HH}$ = 7.4 Hz, 2H, **H-r**), 1.64 (quint, $^3J_{HH}$ = 6.6 Hz, 2H, **H-b**), 1.49-1.23 (m, 26H, **H-c**, **H-s**, **H-t**, **H-u**, **H-v1**, **H-v2**, **H-v3**, **H-v4**, **H-v5**, **H-v6**, **H-w**, **H-x**, **H-y**), 0.95-0.89 (m, 6H, **H-d, H-z**).

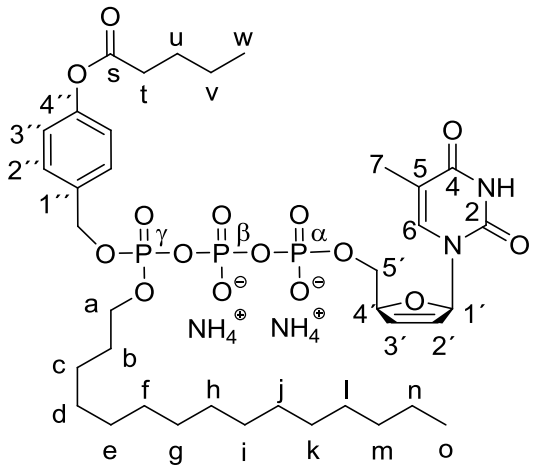
^{13}C-NMR (151 MHz, Methanol-d4): *δ* 173.81 (**C-p**), 166.60 (**C_{het}-4**), 152.81 (**C_{het}-2**), 152.34 (**C-4´´**), 138.74 (**C_{het}-6**), 135.87 (**C-3´**), 135.22 (d, $^3J_{CP}$ = 3.1 Hz, **C-1´´**), 130.40 (d, $^4J_{CP}$ = 4.3 Hz, **2×C-2´´**), 127.12 (**C-2´**), 122.86 (**2×C-3´´**), 112.08 (**C_{het}-5**), 90.85 (**C-1´**), 87.27 (d, $^3J_{CP}$ = 9.2 Hz, **C-4´**), 70.23 (d, $^2J_{CP}$ = 4.1 Hz, **Ph-CH_2**), 69.51 (d, $^2J_{CP}$ = 6.3 Hz, **C-a**), 67.85 (d, $^2J_{CP}$ = 5.7 Hz, **C-5´**), 35.02 (**C-q**), 33.28 (d, $^3J_{CP}$ = 7.6 Hz, **C-b**), 33.08, 30.79, 30.78, 30.76, 30.71, 30.60, 30.48, 30.40, (**C-t**, **C-u**, **C-v1**, **C-v2**, **C-v3**, **C-v4**, **C-v5**, **C-v6**, **C-w**, **C-x**), 30.16 (**C-s**), 25.96 (**C-r**), 23.74 (**C-y**), 19.70 (**C-c**), 14.44, 13.95 (**C-d**, **C-z**), 12.48 (**C_{het}-7**).

^{31}P-NMR (243 MHz, Methanol-d4): *δ* -11.81 (d, $^2J_{PP}$ = 19.4 Hz, **P-α**), -13.02 (d, $^2J_{PP}$ = 16.9 Hz, **P-γ**), -23.76 (t, $^2J_{PP}$ = 18.3 Hz, **P-β**).

HRMS (ESI-TOF) m/z: calculated for $C_{37}H_{58}N_2O_{15}P_3$ $[M-H]^-$ 863.3056, found 863.3045.

IR: ν = 3184, 3049, 2958, 2850, 1759, 1692, 1508, 1465, 1249, 1167, 1127, 1082, 1012, 908, 838, 784, 768, 720, 646, 517, 492, 419, 401.

γ-(AB-C_4H_9,alkyl-$C_{15}H_{31}$)-d4TTP (ammonium salt) **58eo**



Yield: 33%

Chemical Formula: $C_{37}H_{65}N_4O_{15}P_3$

Molecular Weight: 898.85

According to **General Procedure C**, the reactions were performed under dry conditions using 114 mg *H*-phosphonate **57eo** (0.150 mmol, 1.0 equiv.) and 118 mg d4TMP 2×nBu_4N^+ salt (0.150 mmol, 1.0 equiv.). Yield: 33%, 44 mg, as colorless cotton.

^{1}H-NMR (600 MHz, Methanol-d4): δ 7.73 -7.66 (m, 1H, **H_{het}-6**), 7.54-7.46 (m, 2H, **H-2´´**), 7.13-7.07 (m, 2H, **H-3´´**), 6.99-6.93 (m, 1H, **H-1´**), 6.56-6.49 (m, 1H, **H-3´**), 5.85 (dt, $^3J_{HH}$ = 6.1 Hz, $^4J_{HH}$ = 2.4 Hz, 1H, **H-2´**), 5.26-5.16 (m, 2H, **Ph-CH_2**), 4.99 (s, 1H, **H-4´**), 4.36-4.17 (m, 2H, **H-5´**), 4.17-4.07 (m, 2H, **H-a**), 2.60 (t, $^3J_{HH}$ = 7.4 Hz, 2H, **H-t**), 1.92 (d, $^4J_{HH}$ = 3.3 Hz, 3H, **H_{het}-7**), 1.74 (quint, $^3J_{HH}$ = 7.4 Hz, 2H, **H-u**), 1.64 (quint, $^3J_{HH}$ = 6.5 Hz, 2H, **H-b**), 1.48 (tq, $^3J_{HH}$ = 8.6, 8.0 Hz, 2H, **H-v**), 1.38-1.23 (m, 24H**, H-c**, **H-d**, **H-e**, **H-f**, **H-g**, **H-h**, **H-i**, **H-j**, **H-k**, **H-l**, **H-m**, **H-n**), 1.00 (t, $^3J_{HH}$ = 8.0 Hz, 3H, **H-w**), 0.92 (t, $^3J_{HH}$ = 6.4 Hz, 3H, **H-o**).

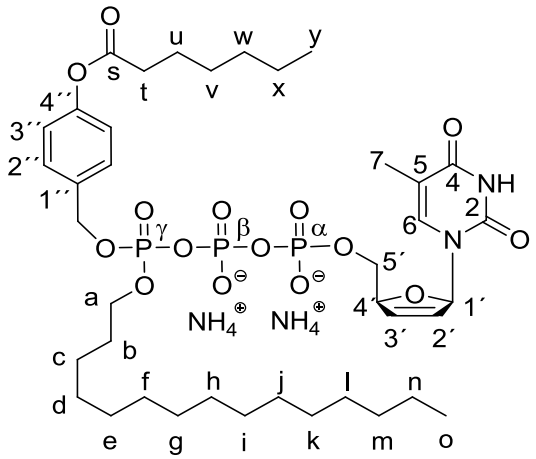
^{13}C-NMR (151 MHz, Methanol-d4): δ 173.73 (**C-s**), 166.59 (**C_{het}-4**), 152.79 (**C_{het}-2**), 152.34 (**C-4´´**), 138.69 (**C_{het}-6**), 135.78 (**C-3´**), 135.17 (d, $^3J_{CP}$ = 3.5 Hz, **C-1´´**), 130.39 (d, $^4J_{CP}$ = 4.2 Hz, **2×C-2´´**), 127.18 (**C-2´**), 122.88, 122.87 (**2×C-3´´**), 112.07 (**C_{het}-5**), 90.85 (**C-1´**), 87.21 (d, $^3J_{CP}$ = 9.0 Hz, **C-4´**), 70.25 (d, $^2J_{CP}$ = 5.5 Hz, **Ph-CH_2**), 69.85 (d, $^2J_{CP}$ = 6.3 Hz, **C-a**), 67.87 (d, $^2J_{CP}$ = 5.7 Hz, **C-5´**), 34.76 (**C-t**), 33.08 (**C-d**), 31.23 (d, $^3J_{CP}$ = 7.2 Hz, **C-b**), 30.82, 30.80, 30.78, 30.73, 30.68, 30.49, 30.30 (**C-e**, **C-f**, **C-g**, **C-h**, **C-i**, **C-j**, **C-k**, **C-l**, **C-m**), 28.07 (**C-u**), 26.55 (**C-c**), 23.74 (**C-n**), 23.25 (**C-v**), 14.45 (**C-o**), 14.10 (**C-w**), 12.49 (**C_{het}-7**).

^{31}P-NMR (243 MHz, Methanol-d4): δ -11.81 (d, $^2J_{PP}$ = 18.7 Hz, **P-α**), -12.98 (d, $^2J_{PP}$ = 16.7 Hz, **P-γ**), -23.71 (t, $^2J_{PP}$ = 17.3 Hz, **P-β**).

HRMS (ESI-TOF) m/z: calculated for $C_{37}H_{58}N_2O_{15}P_3$ $[M-H]^-$ 863.3056, found 863.2956.

IR: v = 3182, 3040, 2957, 2923, 2853, 1760, 1690, 1509, 1463, 1248, 1202, 1167, 1128, 1082, 1010, 906, 838, 807, 783, 768, 721, 696, 644, 485, 425, 401.

γ-(AB-C_6H_{13},alkyl-$C_{15}H_{31}$)-d4TTP (ammonium salt) **58go**



Yield: 59%

Chemical Formula: $C_{39}H_{69}N_4O_{15}P_3$

Molecular Weight: 926.90

According to **General Procedure C**, the reactions were performed under dry conditions using 57 mg *H*-phosphonate **57go** (0.11 mmol, 1.0 equiv.) and 66 mg d4TMP 2×nBu_4N^+ salt (0.08 mmol, 0.75 equiv.). Yield: 59%, 46 mg, as colorless cotton.

^{1}H-NMR (600 MHz, Methanol-d4): *δ* 7.73 -7.65 (m, 1H, **H_{het}-6**), 7.54-7.46 (m, 2H, **H-2´´**), 7.10 (d, $^3J_{HH}$ = 8.3 Hz, 2H, **H-3´´**), 6.98-6.93 (m, 1H, **H-1´**), 6.52 (dt, $^3J_{HH}$ = 6.0 Hz, $^4J_{HH}$ = 1.7 Hz, 1H, **H-3´**), 5.85 (dt, $^3J_{HH}$ = 6.1 Hz, $^4J_{HH}$ = 2.5 Hz, 1H, **H-2´**), 5.26-5.16 (m, 2H, **Ph-CH_2**), 5.02-4.96 (m, 1H, **H-4´**), 4.33-4.17 (m, 2H, **H-5´**), 4.17-4.07 (m, 2H, **H-a**), 2.59 (t, $^3J_{HH}$ = 7.4 Hz, 2H, **H-t**), 1.93 (dd, $^4J_{HH}$ = 3.5, 1.2 Hz, 3H, **H_{het}-7**), 1.75 (quint, $^3J_{HH}$ = 7.4 Hz, 2H, **H-u**), 1.64 (quint, $^3J_{HH}$ = 6.7 Hz, 2H, **H-b**),1.52-1.42 (m, 2H, **H-v**), 1.42-1.22 (m, 28H**, H-c**, **H-d**, **H-e**, **H-f**, **H-g**, **H-h**, **H-i**, **H-j**, **H-k**, **H-l**, **H-m**, **H-n, H-w**, **H-x**), 0.95 (t, $^3J_{HH}$ = 7.1 Hz, 3H, **H-y**), 0.92 (t, $^3J_{HH}$ = 7.0 Hz, 3H, **H-o**).

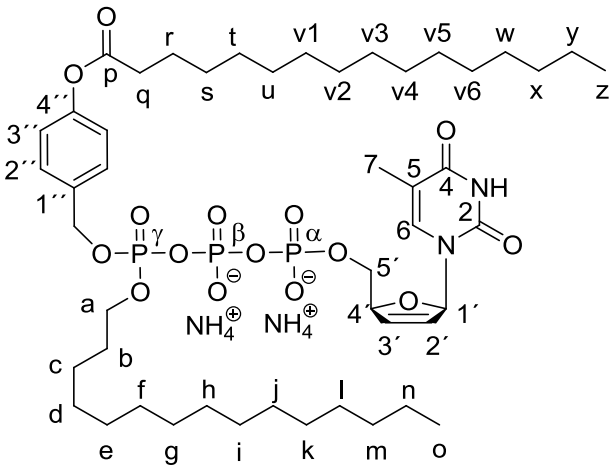
^{13}C-NMR (151 MHz, Methanol-d4): *δ* 173.74 (**C-s**), 166.56 (**C_{het}-4**), 152.81 (**C_{het}-2**), 152.37 (**C-4´´**), 138.63 (**C_{het}-6**), 135.81 (**C-3´**), 135.19 (dd, $^3J_{CP}$ = 7.2, 2.7 Hz, **C-1´´**), 130.37 (d, $^4J_{CP}$ = 3.7 Hz, **2×C-2´´**), 127.15 (**C-2´**), 122.84, 122.83 (**2×C-3´´**), 112.10 (**C_{het}-5**), 90.93 (**C-1´**), 87.23 (d, $^3J_{CP}$ = 9.1 Hz, **C-4´**), 70.25 (d, $^2J_{CP}$ = 7.4 Hz, **Ph-CH_2**), 69.86 (d, $^2J_{CP}$ = 6.4 Hz, **C-a**), 67.90 (d, $^2J_{CP}$ = 5.9 Hz, **C-5´**), 35.08 (**C-t**), 33.05 (**C-d**), 31.23 (d, $^3J_{CP}$ = 7.3 Hz, **C-b**), 30.79, 30.77, 30.74, 30.70, 30.64, 30.45, 30.27 (**C-e**, **C-f**, **C-g**, **C-h**, **C-i**, **C-j**, **C-k**, **C-l**, **C-m**), 32.62 (**C-w**), 29.84 (**C-v**), 26.54 (**C-c**), 25.94 (**C-u**), 23.70 (**C-n**), 23.54 (**C-x**), 14.40 (**C-o**), 14.34 (**C-y**), 12.45 (**C_{het}-7**).

^{31}P-NMR (243 MHz, Methanol-d4): *δ* -11.72 (d, $^2J_{PP}$ = 18.9 Hz, **P-α**), -12.94 (d, $^2J_{PP}$ = 16.8 Hz, **P-γ**), -23.62 (t, $^2J_{PP}$ = 17.7 Hz, **P-β**).

HRMS (ESI-TOF) m/z: calculated for $C_{39}H_{62}N_2O_{15}P_3$ $[M-H]^-$ 891.3369, found 891.3350.

IR: v = 3181, 2956, 2921, 2852, 1759, 1690, 1509, 1466, 1379, 1250, 1167, 1128, 1113, 1083, 1009, 910, 838, 807, 784, 768, 722, 696, 644, 492, 423, 403.

γ-(AB-$C_{15}H_{31}$,alkyl-$C_{15}H_{31}$)-d4TTP (ammonium salt) **58uo**



Yield: 44%

Chemical Formula: $C_{48}H_{87}N_4O_{15}P_3$

Molecular Weight: 1053.14

According to **General Procedure C**, the reactions were performed under dry conditions using 97 mg *H*-phosphonate **57uo** (0.15 mmol, 1.0 equiv.) and 89 mg d4TMP 2×nBu_4N^+ salt (0.11 mmol, 0.75 equiv.). Yield: 44%, 52 mg, as colorless cotton.

^{1}H-NMR (600 MHz, Methanol-d4): *δ* 7.71 (d, $^3J_{HH}$ = 2.7 Hz, 1H, **H_{het}-6**), 7.56-7.45 (m, 2H, **H-2´´**), 7.15-7.06 (m, 2H, **H-3´´**), 6.98-6.94 (m, 1H, **H-1´**), 6.53 (dt, $^3J_{HH}$ = 6.0 Hz, $^4J_{HH}$ = 1.7 Hz, 1H, **H-3´**), 5.85 (dt, $^3J_{HH}$ = 5.8 Hz, $^4J_{HH}$ = 1.8 Hz, 1H, **H-2´**), 5.29-5.17 (m, 2H, **Ph-CH_2**), 5.01-4.97 (m, 1H, **H-4´**), 4.34-4.17 (m, 2H, **H-5´**), 4.16-4.06 (m, 2H, **H-a**), 2.59 (t, $^3J_{HH}$ = 7.4 Hz, 2H, **H-q**), 1.93 (d, $^4J_{HH}$ = 3.8 Hz, 3H, **H_{het}-7**), 1.75 (quint, $^3J_{HH}$ = 7.5 Hz, 2H, **H-r**), 1.62 (quint, $^3J_{HH}$ = 7.2 Hz, 2H, **H-b**),1.49-1.42 (m, 2H, **H-s**), 1.39-1.19 (m, 46H**, H-c**, **H-d**, **H-e**, **H-f**, **H-g**, **H-h**, **H-i**, **H-j**, **H-k**, **H-l**, **H-m**, **H-n**, **H-t**, **H-u**, **H-v1, H-v2, H-v3**, **H-v4, H-v5, H-v6**, **H-w, H-x, H-y**), 0.92 (t, $^3J_{HH}$ = 7.0 Hz, 6H, **H-o**, **H-z**).

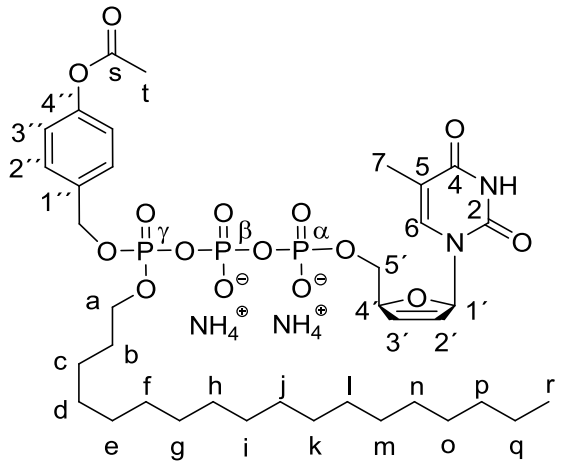
^{13}C-NMR (151 MHz, Methanol-d4): *δ* 173.72 (**C-p**), 166.57 (**C_{het}-4**),152.80 (**C_{het}-2**), 152.35 (**C-4´´**), 138.70 (**C_{het}-6**), 135.83 (**C-3´**), 135.15 (dd, $^3J_{CP}$ = 7.1, 3.6 Hz, **C-1´´**), 130.42 (d, $^4J_{CP}$ = 4.1 Hz, **2×C-2´´**), 127.16 (**C-2´**), 122.87, 122.86 (**2×C-3´´**), 112.09 (**C_{het}-5**), 90.85 (**C-1´**), 87.24 (d, $^3J_{CP}$ = 9.0 Hz, **C-4´**), 70.27 (d, $^2J_{CP}$ = 7.5 Hz, **Ph-CH_2**), 69.83 (d, $^2J_{CP}$ = 6.2 Hz, **C-a**), 67.85 (d, $^2J_{CP}$ = 5.7 Hz, **C-5´**), 35.05 (**C-q**), 33.10, 33.08 (**C-d**, **C-t**), 31.23 (d, $^3J_{CP}$ = 7.3 Hz, **C-b**), 30.85, 30.83, 30.80, 30.78, 30.76, 30.72, 30.69, 30.61, 30.52, 30.48, 30.42, 30.31 (**C-e**, **C-f**, **C-g**, **C-h**, **C-i**, **C-j**, **C-k**, **C-l**, **C-m**, **C-t**, **C-u**, **C-v1**, **C-v2**, **C-v3**, **C-v4**, **C-v5**, **C-v6**, **C-w**, **C-x**), 30.18 (**C-s**), 26.56 (**C-c**), 25.98 (**C-r**), 23.76, 23.74 (**C-n**, **C-y**), 14.47, 14.45 (**C-o**, **C-z**), 12.49 (**C_{het}-7**).

^{31}P-NMR (243 MHz, Methanol-d4): *δ* -11.76 (d, $^2J_{PP}$ = 19.2 Hz, **P-α**), -12.92 (d, $^2J_{PP}$ = 16.6 Hz, **P-γ**), -23.66 (t, $^2J_{PP}$ = 18.0 Hz, **P-β**).

HRMS (ESI-TOF) m/z: calculated for $C_{48}H_{80}N_2O_{15}P_3$ $[M-H]^-$ 1017.4777, found 1017.4731.

IR: v = 3183, 2918, 2850, 1759, 1692, 1509, 1467, 1379, 1331, 1250, 1219, 1168, 1127, 1083, 1009, 913, 839, 784, 768, 721, 644, 515, 493, 427.

γ-(AB-CH_3,alkyl-$C_{18}H_{37}$)-d4TTP (ammonium salt) **58br**



Yield:47%

Chemical Formula: $C_{37}H_{65}N_4O_{15}P_3$

Molecular Weight: 898.85

According to **General Procedure C**, the reactions were performed under dry conditions using 72 mg *H*-phosphonate **57br** (0.15 mmol, 1.0 equiv.) and 89 mg d4TMP 2×nBu_4N^+ salt (0.11 mmol, 0.75 equiv.). Yield: 47%, 48 mg, as colorless cotton.

^{1}H-NMR (600 MHz, Methanol-d4): *δ* 7.73-7.68 (m, 1H, **H_{het}-6**), 7.52-7.48 (m, 2H, **H-2´´**), 7.13-7.09 (m, 2H, **H-3´´**), 6.96 (dt, $^3J_{HH}$ = 3.3 Hz, $^4J_{HH}$ = 1.6 Hz, 1H, **H-1´**), 6.52 (dt, $^3J_{HH}$ = 6.0 Hz, $^4J_{HH}$ = 1.8 Hz, 1H, **H-3´**), 5.85 (d, $^3J_{HH}$ = 6.1 Hz, 1H, **H-2´**), 5.24-5.20 (m, 2H, **Ph-CH_2**), 5.02-4.96 (m, 1H, **H-4´**), 4.35-4.17 (m, 2H, **H-5´**), 4.17-4.08 (m, 2H, **H-a**), 2.29 (s, 3H, **H-t**), 1.93 (d, $^4J_{HH}$ = 4.1 Hz, 3H, **H_{het}-7**), 1.65 (quint, $^3J_{HH}$ = 6.5 Hz, 2H, **H-b**), 1.40-1.23 (m, 30H, **H-c**, **H-d**, **H-e**, **H-f**, **H-g**, **H-h**, **H-i**, **H-j**, **H-k**, **H-l**, **H-m**, **H-n**, **H-o**, **H-p**, **H-q**), 0.91 (t, $^3J_{HH}$ = 7.1 Hz, 3H, **H-r**).

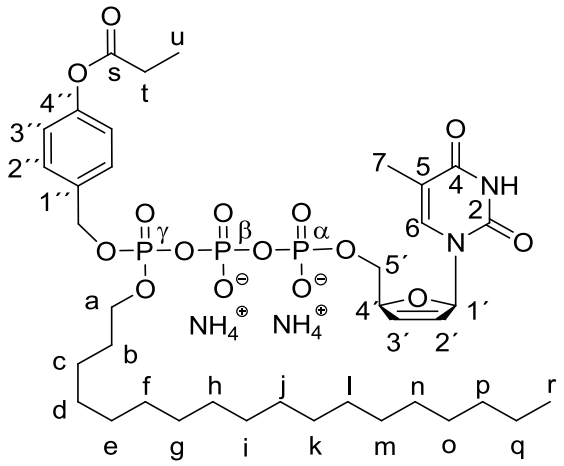
^{13}C-NMR (151 MHz, Methanol-d4): *δ* 171.00 (**C-s**), 166.60 (**C_{het}-4**), 152.80 (**C_{het}-2**), 152.32 (**C-4´´**), 138.72 (**C_{het}-6**), 135.84 (**C-3´**), 134.05 (**C-1´´**), 130.35 (d, $^4J_{CP}$ = 4.4 Hz, **2×C-2´´**), 127.15 (**C-2´**), 122.88, 122.87 (**2×C-3´´**), 112.09 (**C_{het}-5**), 90.85 (**C-1´**), 87.26 (d, $^3J_{CP}$ = 7.7 Hz, **C-4´**), 70.22 (d, $^2J_{CP}$ = 5.8 Hz, **Ph-CH_2**), 69.84 (d, $^2J_{CP}$ = 6.2 Hz, **C-a**), 67.84 (d, $^2J_{CP}$ = 6.3 Hz, **C-5´**), 33.08 (**C-d**), 31.25 (d, $^3J_{CP}$ = 7.8 Hz, **C-b**), 30.80, 30.76, 30.73, 30.68, 30.48, 30.31 (**C-e**, **C-f**, **C-g**, **C-h**, **C-i**, **C-j**, **C-k**, **C-l**, **C-m**, **C-n**, **C-o**, **C-p**), 26.56 (**C-c**), 23.74 (**C-q**), 20.93 (**C-t**), 14.44 (**C-r**), 12.49 (**C_{het}-7**).

^{31}P-NMR (243 MHz, Methanol-d4): *δ* -11.76 (d, $^2J_{PP}$ = 17.9 Hz, **P-α**), -12.98 (d, $^2J_{PP}$ = 17.3 Hz, **P-γ**), -23.67 (t, $^2J_{PP}$ = 18.6 Hz, **P-β**).

HRMS (ESI-TOF) m/z: calculated for $C_{37}H_{58}N_2O_{15}P_3$ $[M-H]^-$ 863.3056, found 863.3169.

IR: ν = 3037, 2921, 2851, 1762, 1690, 1508, 1456, 1368, 1248, 1216, 1195, 1167, 1126, 1080, 1007, 905, 837, 808, 783, 767, 720, 647, 484.

γ-(AB-C_2H_5,alkyl-$C_{18}H_{37}$)-d4TTP (ammonium salt) **58cr**



Yield:63%

Chemical Formula: $C_{38}H_{67}N_4O_{15}P_3$

Molecular Weight: 912.88

According to **General Procedure C**, the reactions were performed under dry conditions using 75 mg *H*-phosphonate **57cr** (0.15 mmol, 1.0 equiv.) and 89 mg d4TMP 2×nBu_4N^+ salt (0.11 mmol, 0.75 equiv.). Yield: 63%, 64 mg, as colorless cotton.

^{1}H-NMR (600 MHz, Methanol-d4): *δ* 7.74-7.67 (m, 1H, **H_{het}-6**), 7.54-7.46 (m, 2H, **H-2´´**), 7.11 (d, $^3J_{HH}$ = 8.1 Hz, 2H, **H-3´´**), 6.96 (dt, $^3J_{HH}$ = 3.6 Hz, $^4J_{HH}$ = 1.7 Hz, 1H, **H-1´**), 6.52 (dt, $^3J_{HH}$ = 5.9 Hz, $^4J_{HH}$ = 1.8 Hz, 1H, **H-3´**), 5.85 (d, $^3J_{HH}$ = 6.0 Hz, 1H, **H-2´**), 5.28-5.16 (m, 2H, **Ph-CH_2**), 5.02-4.96 (m, 1H, **H-4´**), 4.35-4.17 (m, 2H, **H-5´**), 4.17-4.08 (m, 2H, **H-a**), 2.62 (q, $^3J_{HH}$ = 7.5 Hz, 2H, **H-t**), 1.93 (dd, $^4J_{HH}$ = 3.7, 1.3 Hz, 3H, **H_{het}-7**), 1.64 (quint, $^3J_{HH}$ = 7.2 Hz, 2H, **H-b**), 1.39-1.27 (m, 30H**, H-c**, **H-d**, **H-e**, **H-f**, **H-g**, **H-h**, **H-i**, **H-j**, **H-k**, **H-l**, **H-m**, **H-n**, **H-o**, **H-p**, **H-q**), 1.25 (t, $^3J_{HH}$ = 7.5 Hz, 3H, **H-u**), 0.92 (t, $^3J_{HH}$ = 7.0 Hz, 3H, **H-r**).

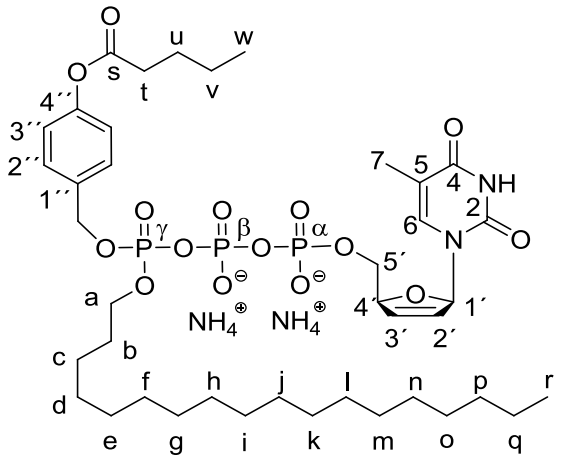
^{13}C-NMR (151 MHz, Methanol-d4): *δ* 174.45 (**C-s**), 166.55 (**C_{het}-4**), 152.79 (**C_{het}-2**), 152.38 (**C-4´´**), 138.67 (**C_{het}-6**), 135.77 (**C-3´**), 135.14 (**C-1´´**), 130.36 (d, $^4J_{CP}$ = 4.2 Hz, **2×C-2´´**), 127.18 (**C-2´**), 122.87, 122.86 (**2×C-3´´**), 112.06 (**C_{het}-5**), 90.85 (**C-1´**), 87.22 (d, $^3J_{CP}$ = 9.1 Hz, **C-4´**), 70.24 (d, $^2J_{CP}$ = 5.7 Hz, **Ph-CH_2**), 69.85 (d, $^2J_{CP}$ = 6.2 Hz, **C-a**), 67.85 (d, $^2J_{CP}$ = 5.8 Hz, **C-5´**), 31.24 (d, $^3J_{CP}$ = 7.4 Hz, **C-b**), 33.07 (**C-d**), 30.80, 30.76, 30.73, 30.68, 30.47, 30.30 (**C-e**, **C-f**, **C-g**, **C-h**, **C-i**, **C-j**, **C-k**, **C-l**, **C-m**, **C-n**, **C-o**, **C-p**), 28.37 (**C-t**), 26.56 (**C-c**), 23.74 (**C-q**), 14.46 (**C-r**), 12.50 (**C_{het}-7**), 9.32 (**C-u**),.

^{31}P-NMR (243 MHz, Methanol-d4): *δ* -11.84 (d, $^2J_{PP}$ = 19.7 Hz, **P-α**), -13.05 (d, $^2J_{PP}$ = 17.3 Hz, **P-γ**), -23.84 (s, **P-β**).

HRMS (ESI-TOF) m/z: calculated for $C_{38}H_{60}N_2O_{15}P_3$ $[M-H]^-$ 877.3212, found 877.3171.

IR: v = 2922, 2852, 1762, 1689, 1509, 1462, 1356, 1248, 1167, 1128, 1079, 1009, 904, 838, 806, 784, 768, 721, 697, 645, 576, 489, 401.

γ-(AB-C_4H_9,alkyl-$C_{18}H_{37}$)-d4TTP (ammonium salt) **58er**



Yield: 30%

Chemical Formula: $C_{40}H_{71}N_4O_{15}P_3$

Molecular Weight: 940.93

According to **General Procedure C**, the reactions were performed under dry conditions using 79 mg *H*-phosphonate **57er** (0.15 mmol, 1.0 equiv.) and 118 mg d4TMP 2×nBu_4N^+ salt (0.15 mmol, 1.0 equiv.). Yield: 30%, 42 mg, as colorless cotton.

^{1}H-NMR (600 MHz, Methanol-d4): *δ* 7.75-7.67 (m, 1H, **H_{het}-6**), 7.50 (dd, $^3J_{HH}$ = 8.7 Hz, $^4J_{HH}$ = 2.5 Hz, 2H, **H-2´´**), 7.10 (d, $^3J_{HH}$ = 8.3 Hz, 2H, **H-3´´**), 6.96 (dt, $^3J_{HH}$ = 3.6 Hz, $^4J_{HH}$ = 1.8 Hz, 1H, **H-1´**), 6.56 - 6.48 (m, 1H, **H-3´**), 5.88-5.83 (m, 1H, **H-2´**), 5.28-5.16 (m, 2H, **Ph-CH_2**), 5.02-4.96 (m, 1H, **H-4´**), 4.35-4.17 (m, 2H, **H-5´**), 4.17-4.06 (m, 2H, **H-a**), 2.60 (t, $^3J_{HH}$ = 7.4 Hz, 2H, **H-t**), 1.93 (dd, $^4J_{HH}$ = 3.8, 1.2 Hz, 3H, **H_{het}-7**), 1.73 (quint, $^3J_{HH}$ = 7.6 Hz, 2H, **H-u**), 1.64 (quint, $^3J_{HH}$ = 7.3 Hz, 2H, **H-b**), 1.48 (tq, $^3J_{HH}$ = 7.7, 7.4 Hz, 2H, **H-v**), 1.38-1.24 (m, 30H**, H-c**, **H-d**, **H-e**, **H-f**, **H-g**, **H-h**, **H-i**, **H-j**, **H-k**, **H-l**, **H-m**, **H-n**, **H-o**, **H-p**, **H-q**), 1.01 (t, $^3J_{HH}$ = 7.4 Hz, 3H, **H-w**), 0.92 (t, $^3J_{HH}$ = 7.0 Hz, 3H, **H-r**).

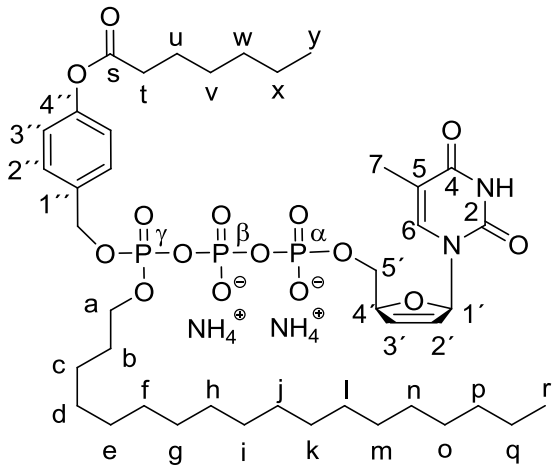
^{13}C-NMR (151 MHz, Methanol-d4): *δ* 173.74 (**C-s**), 166.56 (**C_{het}-4**), 152.79 (**C_{het}-2**), 152.35 (**C-4´´**), 138.68 (**C_{het}-6**), 135.83 (**C-3´**), 135.16 (dd, $^3J_{CP}$ = 7.3, 3.5 Hz, **C-1´´**), 130.38 (d, $^4J_{CP}$ = 4.2 Hz, **2×C-2´´**), 127.16 (**C-2´**), 122.87, 122.86 (**2×C-3´´**), 112.08 (**C_{het}-5**), 90.86 (**C-1´**), 87.23 (d, $^3J_{CP}$ = 9.0 Hz, **C-4´**), 70.25 (d, $^2J_{CP}$ = 6.0 Hz, **Ph-CH_2**), 69.84 (d, $^2J_{CP}$ = 6.3 Hz, **C-a**), 67.85 (d, $^2J_{CP}$ = 5.8 Hz, **C-5´**), 34.77 (**C-t**), 31.24 (d, $^3J_{CP}$ = 7.1 Hz, **C-b**), 33.07 (**C-d**), 30.80, 30.75, 30.73, 30.67, 30.47, 30.29 (**C-e**, **C-f**, **C-g**, **C-h**, **C-i**, **C-j**, **C-k**, **C-l**, **C-m**, **C-n**, **C-o**, **C-p**), 28.07 (**C-u**), 26.55 (**C-c**), 23.73 (**C-q**), 23.25 (**C-v**), 14.45 (**C-r**), 14.10 (**C-w**), 12.49 (**C_{het}-7**).

^{31}P-NMR (243 MHz, Methanol-d4): *δ* -11.76 (d, $^2J_{PP}$ = 18.0 Hz, **P-α**), -12.96 (d, $^2J_{PP}$ = 16.9 Hz, **P-γ**), -23.61-23.70 (m, **P-β**).

HRMS (ESI-TOF) m/z: calculated for $C_{40}H_{64}N_2O_{15}P_3$ $[M-H]^-$ 905.3525, found 905.3530.

IR: v = 2957, 2922, 2852, 1760, 1690, 1509, 1465, 1249, 1167, 1128, 1082, 1010, 908, 838, 807, 784, 768, 721, 696, 643, 576, 491, 422.

γ-(AB-C_6H_{13},alkyl-$C_{18}H_{37}$)-d4TTP (ammonium salt) **58gr**



Yield: 36%

Chemical Formula: $C_{42}H_{75}N_4O_{15}P_3$

Molecular Weight: 968.98

According to **General Procedure C**, the reactions were performed under dry conditions using 83 mg *H*-phosphonate **57gr** (0.15 mmol, 1.0 equiv.) and 118 mg d4TMP 2×nBu_4N^+ salt (0.15 mmol, 1.0 equiv.). Yield: 36%, 52 mg, as colorless cotton.

^{1}H-NMR (600 MHz, Methanol-d4): *δ* 7.73-7.63 (m, 1H, **H_{het}-6**), 7.47 (dd, $^3J_{HH}$ = 8.6 Hz, $^4J_{HH}$ = 2.6 Hz, 2H, **H-2´´**), 7.07 (d, $^3J_{HH}$ = 8.1 Hz, 2H, **H-3´´**), 6.93 (dt, $^3J_{HH}$ = 3.3 Hz, $^4J_{HH}$ = 1.5 Hz, 1H, **H-1´**), 6.49 (dt, $^3J_{HH}$ = 5.9 Hz, $^4J_{HH}$ = 1.8 Hz, 1H, **H-3´**), 5.82 (dt, $^3J_{HH}$ = 6.1 Hz, $^4J_{HH}$ = 1.8 Hz, 1H, **H-2´**), 5.25-5.13 (m, 2H, **Ph-CH_2**), 4.99-4.93 (m, 1H, **H-4´**), 4.32-4.15 (m, 2H, **H-5´**), 4.15-4.03 (m, 2H, **H-a**), 2.57 (t, $^3J_{HH}$ = 7.4 Hz, 2H, **H-t**), 1.90 (d, $^4J_{HH}$ = 3.8 Hz, 3H, **H_{het}-7**), 1.72 (quint, $^3J_{HH}$ = 7.5 Hz, 2H, **H-u**), 1.60 (quint, $^3J_{HH}$ = 6.9 Hz, 2H, **H-b**),1.46-1.39 (m, 2H, **H-v**), 1.39-1.19 (m, 34H**, H-c**, **H-d**, **H-e**, **H-f**, **H-g**, **H-h**, **H-i**, **H-j**, **H-k**, **H-l**, **H-m**, **H-n**, **H-o**, **H-p**, **H-q, H-w, H-x**), 0.92 (t, $^3J_{HH}$ = 6.9 Hz, 3H, **H-y**), 0.89 (t, $^3J_{HH}$ = 7.0 Hz, 3H, **H-r**).

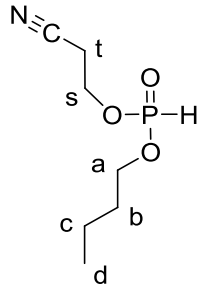
^{13}C-NMR (151 MHz, Methanol-d4): *δ* 173.72 (**C-s**), 166.58 (**C_{het}-4**), 152.79 (**C_{het}-2**), 152.35 (**C-4´´**), 138.69 (**C_{het}-6**), 135.80 (**C-3´**), 135.15 (dd, $^3J_{CP}$ = 7.2, 3.6 Hz, **C-1´´**), 130.40 (d, $^4J_{CP}$ = 4.2 Hz, **2×C-2´´**), 127.16 (**C-2´**), 122.87, 122.86 (**2×C-3´´**), 112.07 (**C_{het}-5**), 90.86 (**C-1´**), 87.23 (d, $^3J_{CP}$ = 9.1 Hz, **C-4´**), 70.25 (d, $^2J_{CP}$ = 7.3 Hz, **Ph-CH_2**), 69.84 (d, $^2J_{CP}$ = 6.3 Hz, **C-a**), 67.85 (d, $^2J_{CP}$ = 5.7 Hz, **C-5´**), 35.05 (**C-t**), 31.23 (d, $^3J_{CP}$ = 7.3 Hz, **C-b**), 33.08 (**C-d**), 30.82, 30.81, 30.77, 30.74, 30.68, 30.48, 30.30 (**C-e**, **C-f**, **C-g**, **C-h**, **C-i**, **C-j**, **C-k**, **C-l**, **C-m**, **C-n**, **C-o**, **C-p**), 32.67 (**C-w**), 29.87 (**C-v**), 26.55 (**C-c**), 25.94 (**C-u**), 23.74 (**C-q**), 23.59 (**C-x**), 14.46 (**C-r**), 14.40 (**C-y**), 12.50 (**C_{het}-7**).

^{31}P-NMR (243 MHz, Methanol-d4): *δ* -11.75 (d, $^2J_{PP}$ = 19.0 Hz, **P-α**), -12.94 (d, $^2J_{PP}$ = 17.0 Hz, **P-γ**), -23.63 (t, $^2J_{PP}$ = 17.9 Hz, **P-β**).

HRMS (ESI-TOF) m/z: calculated for $C_{42}H_{68}N_2O_{15}P_3$ $[M-H]^-$ 933.3838, found 933.3800.

IR: v = 3184, 2956, 2921, 2852, 1759, 1691, 1509, 1466, 1379, 1250, 1168, 1127, 1113, 1082, 1010, 911, 839, 807, 784, 767, 722, 696, 644, 576, 492, 425, 400.

(β-cyanoethyl,alkyl-C_4H_9)-*H*-phosphonate **59d**



Yield: 38%

Chemical Formula: $C_7H_{14}NO_3P$

Molecular Weight: 191.16

According to **General Procedure D**, 0.40 mL diphenyl phosphonate (2.1 mmol, 1.05 equiv.) was dissolved in pyridine at 0 °C. 0.14 g 3-hydroxypropionitrile (2.0 mmol, 1.0 equiv.) was added and followed by 0.16 g 1-butanol (2.2 mmol, 1.1 equiv.). The mixture was stirred overnight at room temperature. Column chromatography (SiO_2, petrol ether/ethylacetate/CH_3COOH 2:8:0.005 v/v/v). Yield: 38%, 0.145 g, as colorless oil.

^{1}H-NMR (400 MHz, Chloroform-d): δ 6.90 (d, $^1J_{PH}$ = 712 Hz, 1H, **P-H**), 4.30 (dt, $^3J_{HH}$ = 8.9 Hz, $^4J_{HH}$ = 6.2 Hz, 2H, **H-s**), 4.22-4.05 (m, 2H, **H-a**), 2.77 (t, $^3J_{HH}$ = 6.6 Hz, 2H, **H-t**), 1.70 (quint, $^3J_{HH}$ = 7.9 Hz, 2H, **H-b**), 1.49-1.1.36 (m, 2H, **H-c**), 0.95 (t, $^3J_{HH}$ = 7.4 Hz, 3H, **H-d**).

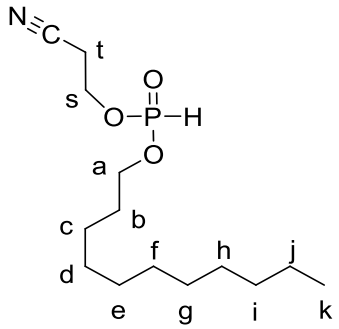
^{13}C-NMR (151 MHz, Chloroform-d): *δ* 116.27 (**C-CN**), 66.24 (d, $^2J_{CP}$ = 6.2 Hz, **C-a**), 59.90 (d, $^2J_{CP}$ = 5.3 Hz, **C-s**), 32.26 (d, $^3J_{CP}$ = 6.2 Hz, **C-b**), 20.61 (**C-c**), 19.97 (d, $^3J_{CP}$ = 6.5 Hz, **C-t**), 13.46 (**C-d**).

^{31}P-NMR (243 MHz, Chloroform-d): *δ* 7.73.

HRMS (ESI-TOF) m/z: calculated for $C_7H_{14}NNaO_3P$ $[M+Na]^+$ 214.0604, found 214.0608.

IR: v = 2963, 2875, 2254, 1751, 1720, 1467, 1389, 1252, 1214, 1036, 969, 833, 762, 607, 579, 545, 449, 399.

(β-cyanoethyl,alkyl-$C_{11}H_{23}$)-*H*-phosphonate **59k**



Yield: 54%

Chemical Formula: $C_{14}H_{28}NO_3P$

Molecular Weight: 289.35

According to **General Procedure D**, 0.40 mL diphenyl phosphonate (2.1 mmol, 1.05 equiv.) was dissolved in pyridine at 0 °C. 134 µL 3-hydroxypropionitrile (2.0 mmol, 1.0 equiv.) was added and followed by 0.38 g 1-undecanol (2.2 mmol, 1.1 equiv.). The mixture was stirred overnight at room temperature. Column chromatography (SiO_2, petrol ether/ethylacetate/CH_3COOH 2:8:0.005 v/v/v). Yield: 54%, 0.313 g, as colorless oil.

^{1}H-NMR (400 MHz, Chloroform-d): δ 6.89 (d, $^{1}J_{PH}$ = 708 Hz, 1H, **P-H**), 4.29 (dt, $^{2}J_{HH}$ = 9.0 Hz, $^{3}J_{HH}$ = 6.2 Hz, 2H, **H-s**), 4.18-4.03 (m, 2H, **H-a**), 2.77 (t, $^{3}J_{HH}$ = 6.2 Hz, 2H, **H-t**), 1.71 (quint, $^{3}J_{HH}$ = 6.5 Hz, 2H, **H-b**), 1.45-1.18 (m, 16H, **H-c**, **H-d**, **H-e**, **H-f**, **H-g**, **H-h**, **H-i**, **H-j**), 0.88 (t, $^{3}J_{HH}$ = 6.6 Hz, 3H, **H-k**).

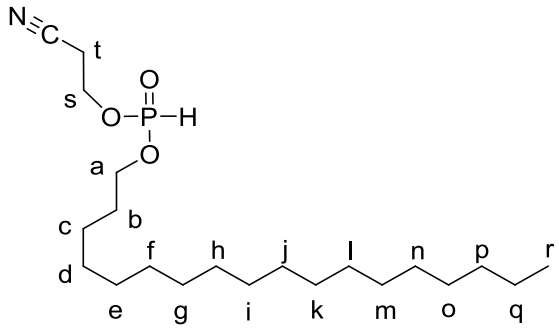
^{13}C-NMR (101 MHz, Chloroform-d): *δ* 116.24 (**C-CN**), 66.49 (d, $^{2}J_{CP}$ = 6.2 Hz, **C-a**), 59.80 (d, $^{2}J_{CP}$ = 5.3 Hz, **C-s**), 30.33 (d, $^{3}J_{CP}$ = 6.3 Hz, **C-b**), 31.88, 29.55, 29.52, 29.45, 29.29, 29.06 (**C-d**, **C-e**, **C-f**, **C-g**, **C-h**, **C-i**), 25.42 (**C-c**), 22.66 (**C-j**), 20.00 (d, $^{3}J_{CP}$ = 6.5 Hz, **C-t**), 14.09 (**C-k**).

^{31}P-NMR (162 MHz, Chloroform-d): *δ* 7.63.

HRMS (ESI-TOF) m/z: calculated for $C_{14}H_{28}NNaO_3P$ $[M+Na]^+$ 312.1699, found 312.1706.

IR: ν =2922, 2853, 1750, 1720, 1466, 1415, 1378, 1338, 1249, 1052, 969, 832, 764, 721, 543, 420, 395.

(β-cyanoethyl,alkyl-$C_{18}H_{37}$)-*H*-phosphonate **59r**



Yield: 66%

Chemical Formula: $C_{21}H_{42}NO_3P$

Molecular Weight: 387.54

According to **General Procedure D**, 0.23 mL diphenyl phosphonate (1.2 mmol, 1.2 equiv.) was dissolved in pyridine at 0 °C. 0.27 g 1-octadecanol (1.0 mmol, 1.0 equiv.) was added and followed by 94 µL 3-hydroxypropionitrile (1.4 mmol, 1.4 equiv.). The mixture was stirred overnight at room temperature. Column chromatography (SiO_2, ethylacetate/CH_3COOH 1:0.005 v/v). Yield: 66%, 0.261 g, as colorless solid.

^{1}H-NMR (600 MHz, Chloroform-d): δ 6.89 (d, $^1J_{PH}$ = 690 Hz, 1H, **P-H**), 4.36-4.20 (m, 2H, **H-s**), 4.18-4.05 (m, 2H, **H-a**), 2.77 (t, $^3J_{HH}$ = 6.2 Hz, 2H, **H-t**), 1.64 (quint, $^3J_{HH}$ = 6.8 Hz, 2H, **H-b**), 1.45-1.18 (m, 30H, **H-c**, **H-d**, **H-e**, **H-f**, **H-g**, **H-h**, **H-i**, **H-j**, **H-k**, **H-l**, **H-m**, **H-n**, **H-o**, **H-p**, **H-q**), 0.87 (t, $^3J_{HH}$ = 7.1 Hz, 3H, **H-r**).

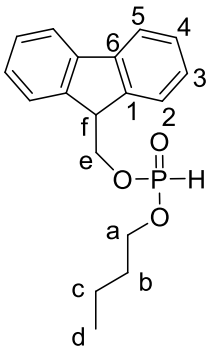
^{13}C-NMR (151 MHz, Chloroform-d): *δ* 116.26 (**C-CN**), 66.53 (d, $^2J_{CP}$ = 6.2 Hz, **C-a**), 59.84 (d, $^2J_{CP}$ = 5.3 Hz, **C-s**), 30.31 (d, $^3J_{CP}$ = 6.5 Hz, **C-b**), 31.90, 29.67, 29.63, 29.60, 29.52, 29.45, 29.33, 29.06 (**C-d**, **C-e**, **C-f**, **C-g**, **C-h**, **C-i**, **C-j**, **C-k**, **C-l**, **C-m**, **C-n**, **C-o**, **C-p**), 25.42 (**C-c**), 22.66 (**C-q**), 19.99 (d, $^3J_{CP}$ = 6.5 Hz, **C-t**), 14.09 (**C-r**).

^{31}P-NMR (243 MHz, Chloroform-d): *δ* 7.63.

HRMS (ESI-TOF) m/z: calculated for $C_{21}H_{46}N_2O_3P$ $[M+NH_4]^+$ 405.3241, found 405.3244.

IR: v = 3317, 2956, 2916, 2848, 1472, 1462, 1423, 1372, 1332, 1300, 1186, 1124, 1061, 1039, 1022, 1004, 983, 967, 935, 905, 889, 730, 719, 525, 493, 422, 389.

(β-fluorenemethyl,alkyl-C_4H_9)-*H*-phosphonate **63d**



Yield: 52%

Chemical Formula: $C_{18}H_{21}NO_3P$

Molecular Weight: 316.33

According to **General Procedure D**, 0.46 mL diphenyl phosphonate (2.4 mmol, 1.2 equiv.) was dissolved in pyridine at 0 °C. 0.39 g 9-fluorenemethanol (2.0 mmol, 1.0 equiv.) was added and followed by 188 µL 3-hydroxypropionitrile (2.8 mmol, 1.4 equiv.). The mixture was stirred overnight at room temperature. Column chromatography (SiO_2, petrol ether/ethylacetate/CH_3COOH 4:6:0.005 v/v/v). Yield: 52%, 0.327 g, as colorless oil.

^{1}H-NMR (400 MHz, Chloroform-d): δ 7.77 (d, $^3J_{HH}$ = 7.6 Hz, 2H, **H-5**), 7.62 (d, $^3J_{HH}$ = 7.5 Hz, 2H, **H-2**), 7.42 (t, $^3J_{HH}$ = 6.5 Hz, 2H, **H-4**), 7.33 (t, $^3J_{HH}$ = 6.5 Hz, 2H, **H-3**), 6.76 (d, $^1J_{PH}$ = 700 Hz, 1H, **P-H**), 4.42 (t, $^3J_{HH}$ = 6.6 Hz, 2H, **H-a**),4.24 (t, $^3J_{HH}$ = 6.6 Hz, 2H, **H-f**), 4.05-3.86 (m, 2H, **H-e**), 1.60 (quint, $^3J_{HH}$ = 6.6 Hz, 2H, **H-b**), 1.42-1.28 (m, 2H, **H-c**), 0.90 (t, $^3J_{HH}$ = 7.4 Hz, 3H, **H-d**).

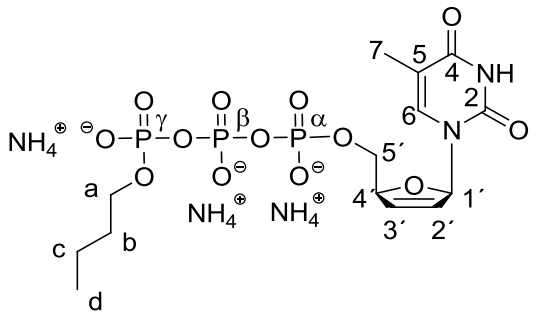
^{13}C-NMR (101 MHz, Chloroform-d): *δ* 143.07, 143.03 (**C-1**), 141.42 (**C-6**), 127.99 (**C-3**), 127.20 (**C-4**), 125.04 (**C-2**), 120.07 (**C-5**), 67.21 (d, $^2J_{CP}$ = 6.1 Hz, **C-a**), 65.60 (d, $^2J_{CP}$ = 6.2 Hz, **C-e**), 48.14 (d, $^3J_{CP}$ = 6.2 Hz, **C-f**), 32.29 (d, $^3J_{CP}$ = 6.2 Hz, **C-b**), 18.63 (**C-c**), 13.49 (**C-d**).

^{31}P-NMR (162 MHz, Chloroform-d): *δ* 7.89.

HRMS (ESI-TOF) m/z: calculated for $C_{18}H_{21}NNaO_3P$ $[M+Na]^+$ 339.1121, found 339.1124.

IR: v = 2958, 2872, 1720, 1449, 1381, 1253, 1103, 1066, 1030, 964, 901, 822, 785, 757, 739, 666, 644, 620, 608, 589, 548, 426.

γ-(alkyl-C_4H_9)-d4TTP **60d**



Yield: 15-31%

Chemical Formula: $C_{14}H_{32}N_5O_{13}P_3$

Molecular Weight: 571.35

According to **General Procedure C** the reactions were performed under dry conditions using 29 mg *H*-phosphonate **59d** (0.15 mmol, 1.0 equiv.), 94 mg d4TMP 2×nBu_4N^+ salt (0.12 mmol, 0.80 equiv.). Yield: 15%, 10 mg, as colorless solid.

Another synthesis method was starting from **63d** (9-fluorenemethoxy as protection group). According to **General Procedure C** the reactions were performed under dry conditions using 47 mg *H*-phosphonate **63d** (0.15 mmol, 1.0 equiv.), 94 mg d4TMP 2×nBu_4N^+ salt (0.12 mmol, 0.80 equiv.). Yield: 31%, 21 mg, as colorless solid.

After the crude product was concentrated in vacuum, the cleavage of the protection group was achieved in a mixture of 5 mL CH_3CN and 0.97 mL 40% $nBu_4N^+OH^-$ in H_2O (1.50 mmol, 10 equiv.) and then stirred for 8 h at room temperature followed by automatic RP18 flash chromatography. The counter ion was exchanged to the ammonium-form with Dowex 50WX8 ion-exchange resin and then purified with RP18 chromatography. Product-containing fractions were collected and the organic solvent evaporated. The remaining aqueous solutions were freeze-dried and the desired product obtained.

^{1}H-NMR (600 MHz, Methanol-d4): *δ* 7.73 (d, $^4J_{HH}$ = 1.3 Hz, 1H, **H_{het}-6**), 6.98 (dt, $^3J_{HH}$ = 3.1 Hz, $^4J_{HH}$ = 1.8 Hz, 1H, **H-1´**), 6.58 (dt, $^3J_{HH}$ = 6.2 Hz, $^4J_{HH}$ = 1.7 Hz, 1H, **H-3´**), 5.89 (dt, $^3J_{HH}$ = 6.0Hz, $^4J_{HH}$ = 2.4 Hz, 1H, **H-2´**), 5.06-4.99 (m, 1H, **H-4´**), 4.33-4.14 (m, 2H, **H-5´**), 3.91-3.80 (m, 2H, **H-a**), 1.92 (d, $^4J_{HH}$ = 1.4 Hz, 3H, **H_{het}-7**), 1.66 (quint, $^3J_{HH}$ = 6.9 Hz, 2H, **H-b**), 1.50-1.36 (m, 2H, **H-c**), 0.96 (t, $^3J_{HH}$ = 7.1 Hz, 3H, **H-d**).

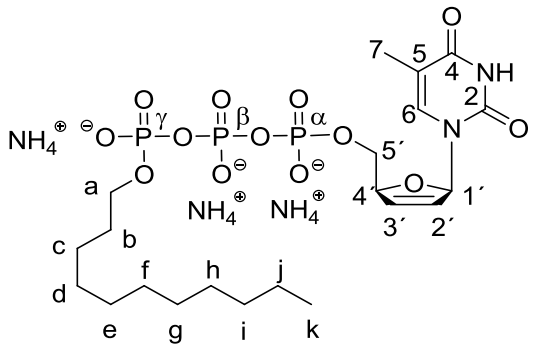
^{13}C-NMR (151 MHz, Methanol-d4): *δ* 166.62 (**C_{het}-4**), 152.85 (**C_{het}-2**), 138.67 (**C_{het}-6**), 135.92 (**C-3´**), 127.05 (**C-2´**), 112.01 (**C_{het}-5**), 90.94 (**C-1´**), 87.26 (d, $^3J_{CP}$ = 9.1 Hz, **C-4´**), 67.41 (d, $^2J_{CP}$ = 5.8 Hz, **C-5´**), 67.01 (d, $^2J_{CP}$ = 6.0 Hz, **C-a**) 33.82 (d, $^3J_{CP}$ = 8.1 Hz, **C-b**), 19.98 (**C-c**), 14.16 (**C-d**), 12.47 (**C_{het}-7**).

^{31}P-NMR (162 MHz, Methanol-d4): *δ* -10.99 (d, $^2J_{PP}$ = 17.8 Hz, **P-α**), -11.36 (d, $^2J_{PP}$ = 17.1 Hz, **P-γ**), -22.18 (t, $^2J_{PP}$ = 19.4 Hz, **P-β**).

MALDI-MS (m/z): calculated $C_{14}H_{23}N_2NaO_{13}P_3$ $[M+Na]^+$ 543.031, found 542.986; calculated $C_{14}H_{23}KN_2O_{13}P_3$ $[M+K]^+$ 559.005, found 558.960; calculated $C_{14}H_{22}N_2O_{13}P_3$ $[M-H]^-$ 519.034, found 519.219.

IR: v = 3185, 2958, 1661, 1428, 1212, 1114, 1062, 985, 891, 835, 784, 736, 643, 482.

γ-(alkyl-$C_{11}H_{23}$)-d4TTP **60k**



Yield:28%

Chemical Formula: $C_{21}H_{46}N_5O_{13}P_3$

Molecular Weight: 669.54

According to **General Procedure C** the reactions were performed under dry conditions using 43 mg *H*-phosphonate **59k** (0.15 mmol, 1.0 equiv.), 94 mg d4TMP 2×nBu_4N^+ salt (0.12 mmol, 0.80 equiv.). After the crude product was concentrated in vacuum, the cleavage of the β-cyanoethyl-moiety was achieved in a mixture of 5 mL CH_3CN and 0.97 mL 40% n$Bu_4N^+OH^-$ in H_2O (1.50 mmol, 10 equiv.) and then stirred for 8 h at room temperature followed by automatic RP18 flash chromatography. The counterion was exchanged to the ammonium-form with Dowex 50WX8 ion-exchange resin and then purified with RP18 chromatography. Product-containing fractions were collected and the organic solvent evaporated. The remaining aqueous solutions were freeze-dried and the desired product obtained. Yield: 28%, 23 mg, as colorless cotton.

^{1}H-NMR (600 MHz, Methanol-d4): *δ* 7.73 (d, $^4J_{HH}$ = 1.3 Hz, 1H, **H_{het}-6**), 6.97 (dt, $^3J_{HH}$ = 3.4 Hz, $^4J_{HH}$ = 1.7 Hz, 1H, **H-1´**), 6.59 (dt, $^3J_{HH}$ = 6.1 Hz, $^4J_{HH}$ = 1.7 Hz, 1H, **H-3´**), 5.88 (dt, $^3J_{HH}$ = 6.1 Hz, $^4J_{HH}$ = 2.4 Hz, 1H, **H-2´**), 5.07-4.98 (m, 1H, **H-4´**), 4.31 (ddd, $^2J_{HH}$ = 11.6 Hz, $^3J_{HP}$ = 6.8 Hz, $^3J_{HH}$ = 3.4 Hz, 1H, **H-5´$_a$**), 4.20 (ddd, $^2J_{HH}$ = 11.6 Hz, $^3J_{HP}$ = 5.5 Hz, $^3J_{HH}$ = 3.2 Hz, 1H, **H-5´$_b$**), 4.00 (q, $^3J_{HH}$ = 6.6 Hz, 2H, **H-a**), 1.94 (d, $^4J_{HH}$ = 1.2 Hz, 3H, **H_{het}-7**), 1.66 (quint, $^3J_{HH}$ = 6.9 Hz, 2H, **H-b**), 1.48-1.26 (m, 16H, **H-c**, **H-d**, **H-e**, **H-f**, **H-g**, **H-h**, **H-i**, **H-j**), 0.92 (t, $^3J_{HH}$ = 7.0 Hz, 3H, **H-k**).

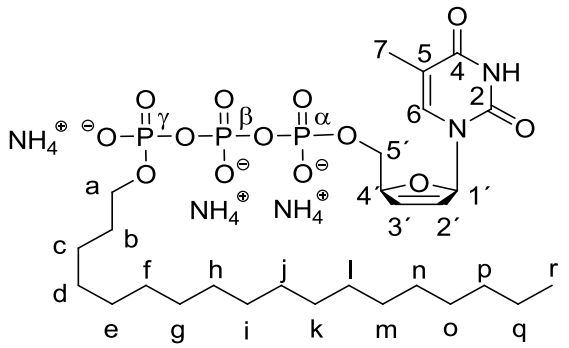
^{13}C-NMR (151 MHz, Methanol-d4): *δ* 166.61 (**C_{het}-4**), 152.85 (**C_{het}-2**), 138.67 (**C_{het}-6**), 135.91 (**C-3´**), 127.07 (**C-2´**), 112.02 (**C_{het}-5**), 90.94 (**C-1´**), 87.25 (d, $^3J_{CP}$ = 9.1 Hz, **C-4´**), 67.79 (d, $^2J_{CP}$ = 5.8 Hz, **C-a**), 67.39 (d, $^2J_{CP}$ = 5.8 Hz, **C-5´**), 33.06 (**C-d**), 31.82 (d, $^3J_{CP}$ = 8.1 Hz, **C-b**), 30.78, 30.76, 30.58, 30.47 (**C-e**, **C-f**, **C-g**, **C-h**, **C-i**, **C-j**), 26.90 (**C-c**), 23.73 (**C-q**), 14.43 (**C-k**), 12.47 (**C_{het}-7**).

^{31}P-NMR (243 MHz, Methanol-d4): *δ* -10.76 (d, $^2J_{PP}$ = 19.3 Hz, **P-α**), -11.48 (d, $^2J_{PP}$ = 19.1 Hz, **P-γ**), -22.48 (t, $^2J_{PP}$ = 19.2 Hz, **P-β**).

MALDI-MS (m/z): calculated $C_{21}H_{37}N_2NaO_{13}P_3$ $[M+Na]^+$ 641.140, found 641.105; calculated $C_{21}H_{37}KN_2O_{13}P_3$ $[M+K]^+$ 657.114, found 657.080; calculated $C_{21}H_{36}N_2O_{13}P_3$ $[M-H]^-$ 617.144, found 617.432.

IR: ν = 2922, 2852, 1688, 1661, 1429, 1217, 1126, 1064, 1045, 991, 900, 836, 783, 768, 736, 644, 488, 427, 400.

γ-(alkyl-$C_{18}H_{37}$)-d4TTP **60r**



Yield: 46%

Chemical Formula: $C_{28}H_{60}N_5O_{13}P_3$

Molecular Weight: 767.72

According to **General Procedure C** the reactions were performed under dry conditions using 58 mg *H*-phosphonate **59r** (0.15 mmol, 1.0 equiv.), 94 mg d4TMP 2×nBu_4N^+ salt (0.12 mmol, 0.80 equiv.). After the crude product was purified by automatic RP18 flash chromatography, the cleavage of the β-cyanoethyl-moiety was achieved in a mixture of 5 mL CH_3CN and 0.97 mL 40% $nBu_4N^+OH^-$ in H_2O (1.50 mmol, 10 equiv.) and then stirred for 20 h at room temperature followed by ion-exchange to the ammonium-form with Dowex 50WX8 ion-exchange resin and a second RP18 chromatography purification step. Product-containing fractions were collected and the organic solvent evaporated. The remaining aqueous solutions were freeze-dried and the desired product obtained. Yield: 46%, 43 mg, as colorless cotton.

^{1}H-NMR (600 MHz, Methanol-d4): *δ* 7.75-7.66 (m, 1H, **H_{het}-6**), 7.02-6.93 (m, 1H, **H-1´**), 6.62-6.53 (m, 1H, **H-3´**), 5.85 (dt, $^3J_{HH}$ = 5.9 Hz, $^4J_{HH}$ = 2.8 Hz, 1H, **H-2´**), 5.06-5.00 (m, 1H, **H-4´**), 4.35-4.14 (m, 2H, **H-5´**), 4.00 (q, $^3J_{HH}$ = 6.7 Hz, 2H, **H-a**), 1.93 (d, $^4J_{HH}$ = 1.2 Hz, 3H, **H_{het}-7**), 1.66 (quint, $^3J_{HH}$ = 6.8 Hz, 2H, **H-b**), 1.48-1.23 (m, 30H, **H-c**, **H-d**, **H-e**, **H-f**, **H-g**, **H-h**, **H-i**, **H-j**, **H-k**, **H-l**, **H-m**, **H-n**, **H-o**, **H-p**, **H-q**), 0.92 (t, $^3J_{HH}$ = 7.0 Hz, 3H, **H-r**).

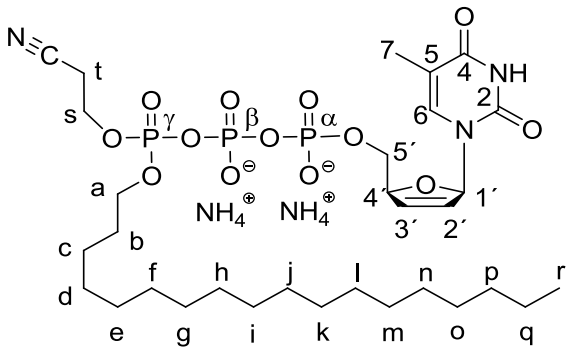
^{13}C-NMR (101 MHz, Methanol-d4): *δ* 166.64 (**C_{het}-4**), 152.86 (**C_{het}-2**), 138.69 (**C_{het}-6**), 135.86 (**C-3´**), 127.13 (**C-2´**), 112.04 (**C_{het}-5**), 90.91 (**C-1´**), 87.22 (d, $^3J_{CP}$ = 9.2 Hz, **C-4´**), 67.80 (d, $^2J_{CP}$ = 6.0 Hz, **C-a**), 67.54 (d, $^2J_{CP}$ = 5.8 Hz, **C-5´**), 31.77 (d, $^3J_{CP}$ = 8.1 Hz, **C-b**), 33.08 (**C-d**), 30.80, 30.76, 30.58, 30.48 (**C-e**, **C-f**, **C-g**, **C-h**, **C-i**, **C-j**, **C-k**, **C-l**, **C-m**, **C-n**, **C-o**, **C-p**), 26.89 (**C-c**), 23.74 (**C-q**), 14.45 (**C-r**), 12.48 (**C_{het}-7**).

^{31}P-NMR (243 MHz, Methanol-d4): *δ* -10.84 (d, $^2J_{PP}$ = 18.9 Hz, **P-α**), -11.46 (d, $^2J_{PP}$ = 18.6 Hz, **P-γ**), -22.57 (t, $^2J_{PP}$ = 18.9 Hz, **P-β**).

HRMS (ESI-TOF) m/z: calculated for $C_{28}H_{50}N_2O_{13}P_3$ $[M-H]^-$ 715.2531, found 715.2518.

IR: ν = 3190, 3025, 2921, 2851, 1689, 1455, 1221, 1128, 1067, 1045, 994, 904, 838, 784, 768, 737, 721, 644, 489, 424, 402.

γ-(β-cyanoethyl,alkyl-$C_{18}H_{37}$)-d4TTP **61r**



Yield: 65%

Chemical Formula: $C_{31}H_{60}N_5O_{13}P_3$

Molecular Weight: 803.76

According to **General Procedure C** the reactions were performed under dry conditions using 58 mg *H*-phosphonate **59r** (0.15 mmol, 1.0 equiv.), 94 mg d4TMP 2×nBu_4N^+ salt (0.12 mmol, 0.80 equiv.). Yield: 65%, 63 mg, as colorless cotton.

^{1}H-NMR (400 MHz, Methanol-d4): *δ* 7.75-7.66 (m, 1H, **H_{het}-6**), 7.01-6.95 (m, 1H, **H-1´**), 6.57 (dt, $^3J_{HH}$ = 6.0 Hz, $^4J_{HH}$ = 1.8 Hz, 1H, **H-3´**), 5.89 (ddd, $^3J_{HH}$ = 5.9 Hz, $^4J_{HH}$ = 2.4, 1.5 Hz, 1H, **H-2´**), 5.06-4.96 (m, 1H, **H-4´**), 4.39 (dt, $^3J_{HH}$ = 7.8 Hz, $^4J_{HH}$ = 1.8 Hz, 2H, **H-s**), 4.35-4.14 (m, 4H, **H-5´**, **H-a**), 2.94 (t, $^3J_{HH}$ = 4.8 Hz, 2H, **H-t**), 1.95 (d, $^4J_{HH}$ = 1.2 Hz, 3H, **H_{het}-7**), 1.73 (quint, $^3J_{HH}$ = 6.8 Hz, 2H, **H-b**), 1.50-1.22 (m, 30H, **H-c**, **H-d**, **H-e**, **H-f**, **H-g**, **H-h**, **H-i**, **H-j**, **H-k**, **H-l**, **H-m**, **H-n**, **H-o**, **H-p**, **H-q**), 0.92 (t, $^3J_{HH}$ = 7.0 Hz, 3H, **H-r**).

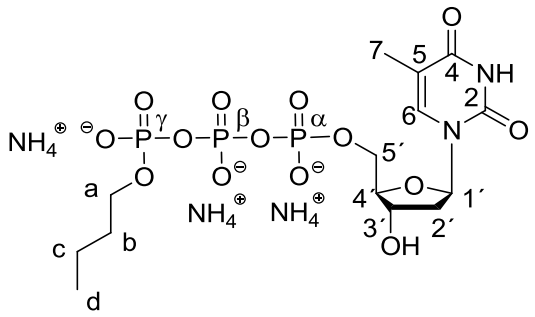
^{13}C-NMR (101 MHz, Methanol-d4): *δ* 166.66 (**C_{het}-4**), 152.87 (**C_{het}-2**), 138.73 (**C_{het}-6**), 135.90 (**C-3´**), 127.12 (**C-2´**), 118.63 (**C-CN**), 112.11 (**C_{het}-5**), 90.90 (**C-1´**), 87.22 (d, $^3J_{CP}$ = 9.7 Hz, **C-4´**), 70.10 (d, $^2J_{CP}$ = 6.4 Hz, **C-a**), 67.85 (d, $^2J_{CP}$ = 5.1 Hz, **C-5´**), 64.22 (d, $^2J_{CP}$ = 5.5 Hz, **C-s**),31.30 (d, $^3J_{CP}$ = 7.4 Hz, **C-b**), 33.08 (**C-d**), 31.33, 31.26, 30.80, 30.76, 30.75, 30.70, 30.48, 30.35 (**C-e**, **C-f**, **C-g**, **C-h**, **C-i**, **C-j**, **C-k**, **C-l**, **C-m**, **C-n**, **C-o**, **C-p**), 26.57 (**C-c**), 23.74 (**C-q**), 20.0 (d, $^3J_{CP}$ = 8.2 Hz, **C-t**), 14.44 (**C-r**), 12.48 (**C_{het}-7**).

^{31}P-NMR (243 MHz, Methanol-d4): *δ* -11.81 (d, $^2J_{PP}$ = 19.9 Hz, **P-α**), -13.59 (d, $^2J_{PP}$ = 16.7 Hz, **P-γ**), -23.78 (t, $^2J_{PP}$ = 18.5 Hz, **P-β**).

HRMS (ESI-TOF) m/z: calculated for $C_{31}H_{53}N_3O_{13}P_3$ $[M-H]^-$ 768.2797, found 768.2898.

IR: ν = 3181, 2921, 2851, 1691, 1464, 1247, 1128, 1114, 1079, 1011, 908, 837, 806, 783, 720, 697, 644, 578, 519, 488, 423, 401.

γ-(alkyl-C_4H_9)-TTP **62d**



Yield: 27%

Chemical Formula: $C_{14}H_{34}N_5O_{14}P_3$

Molecular Weight: 589.37

According to **General Procedure C** the reactions were performed under dry conditions using 47 mg *H*-phosphonate **59d** (0.15 mmol, 1.0 equiv.), 97 mg dTMP 2×nBu_4N^+ salt (0.12 mmol, 0.80 equiv.). After the crude product was concentrated in vacuum, the cleavage of the β-cyanoethyl-moiety was achieved in a mixture of 5 mL CH_3CN and 0.97 mL 40% $nBu_4N^+OH^-$ in H_2O (1.50 mmol, 10 equiv.) and then stirred for 8 h at room temperature followed by automatic RP18 flash chromatography. The counterion was exchanged to the ammonium-form with Dowex 50WX8 ion-exchange resin and then purified with RP18 chromatography. Product-containing fractions were collected and the organic solvent evaporated. The remaining aqueous solutions were freeze-dried and the desired product obtained. Yield: 27%, 19 mg, as colorless solid.

^{1}H-NMR (600 MHz, Methanol-d4): δ 7.83 (s, 1H, **H_{het}-6**), 6.32 (t, $^3J_{HH}$ = 6.7 Hz, 1H, **H-1´**), 4.61 (dt, $^3J_{HH}$ = 6.7 Hz, $^4J_{HH}$ = 3.4 Hz, **H-3´**), 4.31-4.16 (m, 2H, **H-5´**), 4.08-4.03 (m, 1H, **H-4´**), 4.06 (q, $^3J_{HH}$ = 6.6 Hz, 2H, **H-a**), 2.35-2.20 (m, 2H, **H-2´**), 1.96 (d, $^4J_{HH}$ = 1.3 Hz, 3H, **H_{het}-7**), 1.64 (quint, $^3J_{HH}$ = 6.7 Hz, 2H, **H-b**), 1.44 (m, 2H, **H-c**), 0.95 (t, $^3J_{HH}$ = 7.4 Hz, 3H, **H-d**).

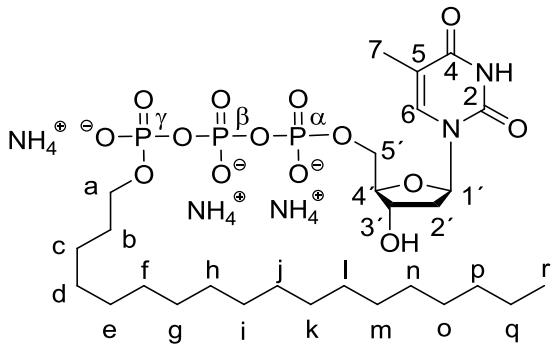
^{13}C-NMR (151 MHz, Methanol-d4): δ 166.52 (**C_{het}-4**), 152.43 (**C_{het}-2**), 138.22 (**C_{het}-6**), 111.93 (**C_{het}-5**), 87.30 (d, $^3J_{CP}$ = 8.9 Hz, **C-4´**), 86.15 (**C-1´**), 72.08 (**C-3´**), 67.04 (d, $^2J_{CP}$ = 6.2 Hz, **C-a**), 66.63 (d, $^2J_{CP}$ = 5.4 Hz, **C-5´**), 40.61 (**C-2´**), 33.85 (d, $^3J_{CP}$ = 8.0 Hz, **C-b**), 19.99 (**C-c**), 14.19 (**C-d**), 12.61 (**C_{het}-7**).

^{31}P-NMR (243 MHz, Methanol-d4): δ -10.54 (d, $^2J_{PP}$ = 18.2 Hz, **P-α**), -11.20 (d, $^2J_{PP}$ = 18.0 Hz, **P-γ**), -21.89 (t, $^2J_{PP}$ = 18.1 Hz, **P-β**).

MALDI-MS (m/z): calculated $C_{14}H_{25}N_2NaO_{14}P_3$ $[M+Na]^+$ 561.041, found 560.988; calculated $C_{14}H_{25}KN_2O_{14}P_3$ $[M+K]^+$ 577.015, found 576.962; calculated $C_{14}H_{24}N_2O_{14}P_3$ $[M-H]^-$ 537.045, found 537.211.

IR: v = 3170, 2958, 1659, 1427, 1209, 1124, 1060, 995, 915, 890, 824, 728, 616, 489, 414.

γ-(alkyl-$C_{18}H_{37}$)-TTP **62r**



Yield: 43%

Chemical Formula: $C_{28}H_{62}N_5O_{14}P_3$

Molecular Weight: 785.74

According to **General Procedure C** the reactions were performed under dry conditions using 58 mg *H*-phosphonate **59r** (0.15 mmol, 1.0 equiv.), 97 mg dTMP 2×nBu_4N^+ salt (0.12 mmol, 0.80 equiv.). After the crude product was concentrated in vacuum, the cleavage of the β-cyanoethyl-moiety was achieved in a mixture of 5 mL CH_3CN and 0.97 mL 40% n$Bu_4N^+OH^-$ in H_2O (1.50 mmol, 10 equiv.) and then stirred for 8 h at room temperature followed by automatic RP18 flash chromatography. The counterion was exchanged to the ammonium-form with Dowex 50WX8 ion-exchange resin and then purified with RP18 chromatography. Product-containing fractions were collected and the organic solvent evaporated. The remaining aqueous solutions were freeze-dried and the desired product obtained. Yield: 43%, 41 mg, as colorless cotton.

^{1}H-NMR (600 MHz, Methanol-d4): *δ* 7.85 (d, $^4J_{HH}$ = 1.2 Hz, 1H, **H_{het}-6**), 6.33 (t, $^3J_{HH}$ = 6.2 Hz, 1H, **H-1´**), 4.63 (dt, $^3J_{HH}$ = 6.2 Hz, $^4J_{HH}$ = 3.2 Hz, **H-3´**), 4.34-4.18 (m, 2H, **H-5´**), 4.08-3.98 (m, 3H, **H-4´**, **H-a**), 2.33-2.21 (m, 2H, **H-2´**), 1.96 (d, $^4J_{HH}$ = 1.3 Hz, 3H, **H_{het}-7**), 1.67 (quint, $^3J_{HH}$ = 6.8 Hz, 2H, **H-b**), 1.46-1.26 (m, 30H, **H-c**, **H-d**, **H-e**, **H-f**, **H-g**, **H-h**, **H-i**, **H-j**, **H-k**, **H-l**, **H-m**, **H-n**, **H-o**, **H-p**, **H-q**), 0.92 (t, $^3J_{HH}$ = 7.0 Hz, 3H, **H-r**).

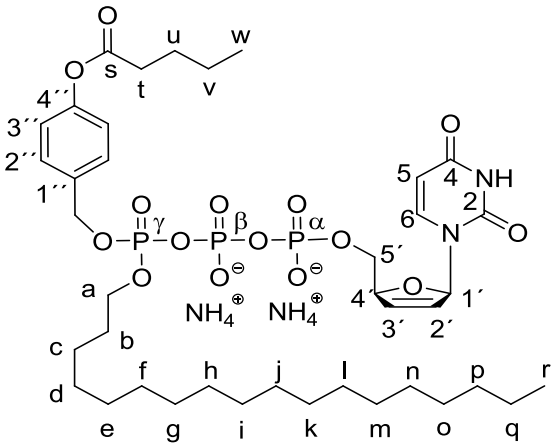
^{13}C-NMR (151 MHz, Methanol-d4): *δ* 166.54 (**C_{het}-4**), 152.43 (**C_{het}-2**), 138.17 (**C_{het}-6**), 111.96 (**C_{het}-5**), 87.39 (d, $^3J_{CP}$ = 9.1 Hz, **C-4´**), 86.03 (**C-1´**), 72.05 (**C-3´**), 67.58 (d, $^2J_{CP}$ = 6.0 Hz, **C-a**), 66.69 (d, $^2J_{CP}$ = 5.4 Hz, **C-5´**), 40.70 (**C-2´**), 33.07 (**C-d**), 31.77 (d, $^3J_{CP}$ = 7.8 Hz, **C-b**), 30.81, 30.79, 30.75, 30.57, 30.46 (**C-e**, **C-f**, **C-g**, **C-h**, **C-i**, **C-j**, **C-k**, **C-l**, **C-m**, **C-n**, **C-o**, **C-p**), 26.88 (**C-c**), 23.73 (**C-q**), 14.43 (**C-r**), 12.60 (**C_{het}-7**).

^{31}P-NMR (243 MHz, Methanol-d4): *δ* -10.97 (d, $^2J_{PP}$ = 19.3 Hz, **P-α**), -11.56 (d, $^2J_{PP}$ = 19.5 Hz, **P-γ**), -22.82 (t, $^2J_{PP}$ = 19.4 Hz, **P-β**).

MALDI-MS (m/z): calculated $C_{28}H_{53}N_2NaO_{14}P_3$ $[M+Na]^+$ 757.260, found 757.216; calculated $C_{28}H_{53}KN_2O_{14}P_3$ $[M+K]^+$ 773.234, found 773.190.

IR: v = 3185, 3027, 2918, 2850, 1681, 1465, 1432, 1222, 1124, 1066, 996, 921, 854, 822, 720, 494, 423, 380.

γ-(AB-C_4H_9,alkyl-$C_{18}H_{37}$)-d4UTP (ammonium salt) **64er**



Yield: 33%

Chemical Formula: $C_{39}H_{69}N_4O_{15}P_3$

Molecular Weight: 926.90

According to **General Procedure C** the reactions were performed under dry conditions using using 79 mg *H*-phosphonate **57er** (0.15 mmol, 1.0 equiv.) and 118 mg d4TMP 2×nBu_4N^+ salt (0.15 mmol, 1.0 equiv.). Yield: 33%, 46 mg, as colorless cotton.

^{1}H-NMR (600 MHz, Methanol-d4): δ 7.91 (dd, $^3J_{HH}$ = 8.1 Hz, $^4J_{HH}$ = 3.1 Hz, 1H, **H_{het}-6**), 7.50 (dt, $^3J_{HH}$ = 8. Hz, $^4J_{HH}$ = 2.1 Hz, 2H, **H-2´´**), 7.11 (dt, $^3J_{HH}$ = 8.5 Hz, $^4J_{HH}$ = 2.0 Hz, 2H, **H-3´´**), 6.96 (dt, $^3J_{HH}$ = 3.3 Hz, $^4J_{HH}$ = 1.5 Hz, 1H, **H-1´**), 6.53 (dt, $^3J_{HH}$ = 6.1 Hz, $^4J_{HH}$ = 1.7 Hz, 1H, **H-3´**), 5.86 (ddd, $^3J_{HH}$ = 7.1 Hz, $^4J_{HH}$ = 2.4, 1.2 Hz, 1H, **H-2´**), 5.80 (dd, $^3J_{HH}$ = 8.1 Hz, $^4J_{HH}$ = 3.8 Hz, 2H, **H-5**), 5.22 (d, $^3J_{HH}$ = 8.2 Hz 1H, **Ph-CH_2**), 4.99 (dt, $^3J_{HH}$ = 3.8 Hz, $^4J_{HH}$ = 1.9 Hz, 1H, **H-4´**), 4.33-4.17 (m, 2H, **H-5´**), 4.17-4.07 (m, 2H, **H-a**), 2.60 (t, $^3J_{HH}$ = 7.4 Hz, 2H, **H-t**), 1.74 (quint, $^3J_{HH}$ = 7.6 Hz, 2H, **H-u**), 1.64 (quint, $^3J_{HH}$ = 6.9 Hz, 2H, **H-b**), 1.48 (tq, $^3J_{HH}$ = 7.5, 7.4 Hz, 2H, **H-v**), 1.40-1.23 (m, 30H**, H-c**, **H-d**, **H-e**, **H-f**, **H-g**, **H-h**, **H-i**, **H-j**, **H-k**, **H-l**, **H-m**, **H-n**, **H-o**, **H-p**, **H-q**), 1.01 (t, $^3J_{HH}$ = 7.4 Hz, 3H, **H-w**), 0.92 (t, $^3J_{HH}$ = 7.0 Hz, 3H, **H-r**).

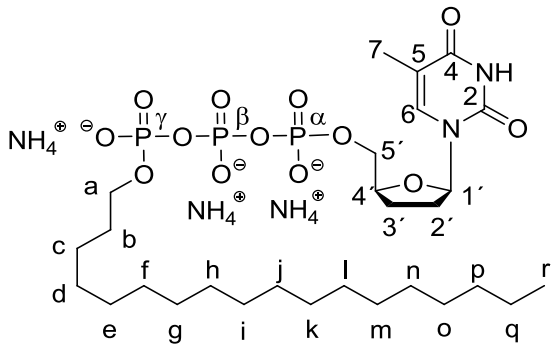
^{13}C-NMR (151 MHz, Methanol-d4): δ 173.72 (**C-s**), 166.29 (**C_{het}-4**), 152.71 (**C_{het}-2**), 152.33 (**C-4´´**), 143.50 (**C_{het}-6**), 136.11 (**C-3´**), 135.25 (d, $^3J_{CP}$ = 7.5 Hz, **C-1´´**), 130.42 (d, $^4J_{CP}$ = 4.4 Hz, **2×C-2´´**), 126.91 (**C-2´**), 122.86 (**2×C-3´´**), 103.28 (**C_{het}-5**), 91.04 (**C-1´**), 87.39 (d, $^3J_{CP}$ = 9.4 Hz, **C-4´**), 70.24 (d, $^2J_{CP}$ = 8.0 Hz, **Ph-CH_2**), 69.84 (d, $^2J_{CP}$ = 6.8 Hz, **C-a**), 67.66 (d, $^2J_{CP}$ = 4.9 Hz, **C-5´**), 34.77 (**C-t**), 33.08 (**C-d**), 31.25 (d, $^3J_{CP}$ = 7.4 Hz, **C-b**), 30.82, 30.81, 30.77, 30.75, 30.69, 30.48, 30.32 (**C-e**, **C-f**, **C-g**, **C-h**, **C-i**, **C-j**, **C-k**, **C-l**, **C-m**, **C-n**, **C-o**, **C-p**), 28.08 (**C-u**), 26.57 (**C-c**), 23.74 (**C-q**), 23.26 (**C-v**), 14.45 (**C-r**), 14.10 (**C-w**).

^{31}P-NMR (243 MHz, Methanol-d4): δ -11.86 (d, $^2J_{PP}$ = 20.0 Hz, **P-α**), -13.04 (d, $^2J_{PP}$ = 17.0 Hz, **P-γ**), -23.81 (t, $^2J_{PP}$ = 15.9 Hz, **P-β**).

HRMS (ESI-TOF) m/z: calculated for $C_{39}H_{62}N_2O_{15}P_3$ $[M-H]^-$ 891.3369, found 891.3359.

IR: ν = 3191, 3053, 2956, 2917, 2849, 1755, 1688, 1509, 1463, 1423, 1380, 1242, 1224, 1168, 1127, 1082, 1006, 910, 838, 768, 719, 697, 653, 625, 517, 403.

γ-(alkyl-$C_{18}H_{37}$)-ddTTP (ammonium salt) **67r**



Yield: 35%

Chemical Formula: $C_{28}H_{62}N_5O_{13}P_3$

Molecular Weight: 769.74

According to **General Procedure C** the reactions were performed under dry conditions using 58 mg *H*-phosphonate **59r** (0.15 mmol, 1.0 equiv.), 94 mg d4TMP 2×nBu_4N^+ salt (0.12 mmol, 0.80 equiv.). After the crude product was concentrated in vacuum, the cleavage of the β-cyanoethyl-moiety was achieved in a mixture of 5 mL CH_3CN and 0.97 mL 40% n$Bu_4N^+OH^-$ in H_2O (1.50 mmol, 10 equiv.) and then stirred for 8 h at room temperature followed by automatic RP18 flash chromatography. The counterion was exchanged to the ammonium-form with Dowex 50WX8 ion-exchange resin and then purified with RP18 chromatography. Product-containing fractions were collected and the organic solvent evaporated. The remaining aqueous solutions were freeze-dried and the desired product obtained. Yield: 35%, 32 mg, as colorless cotton.

^{1}H-NMR (600 MHz, Methanol-d4): *δ* 7.91 (q, $^4J_{HH}$ = 1.2 Hz, 1H, **H_{het}-6**), 6.08 (dd, $^3J_{HH}$ = 6.6 Hz, $^3J_{HH}$ = 4.2 Hz, 1H, **H-1´**), 4.35-4.26 (m, 2H, **H-4´**, **H-5´$_a$**), 4.25-4.17 (m, 1H, **H-5´$_b$**), 4.01 (q, $^3J_{HH}$ = 6.6 Hz, 2H, **H-a**), 2.39-2.29 (m, 1H, **H-2´$_a$**), 2.21-2.04 (m, 3H, **H-2´$_b$**, **H-3´**),1.97 (d, $^4J_{HH}$ = 1.2 Hz, 3H, **H_{het}-7**), 1.66 (quint, $^3J_{HH}$ = 6.7 Hz, 2H, **H-b**), 1.47-1.25 (m, 30H, **H-c**, **H-d**, **H-e**, **H-f**, **H-g**, **H-h**, **H-i**, **H-j**, **H-k**, **H-l**, **H-m**, **H-n**, **H-o**, **H-p**, **H-q**), 0.92 (t, $^3J_{HH}$ = 7.0 Hz, 3H, **H-r**).

^{13}C-NMR (151 MHz, Methanol-d4): *δ* 166.64 (**C_{het}-4**), 152.42 (**C_{het}-2**), 138.30 (**C_{het}-6**), 111.46 (**C_{het}-5**), 87.27 (**C-1´**), 81.28 (d, $^3J_{CP}$ = 9.5 Hz, **C-4´**), 68.05 (d, $^2J_{CP}$ = 5.5 Hz, **C-5´**), 67.46 (d, $^2J_{CP}$ = 6.0 Hz, **C-a**), 33.07 (**C-d**), 33.03 (**C-2´**), 31.80 (d, $^3J_{CP}$ = 8.0 Hz, **C-b**), 30.79, 30.75, 30.59, 30.46 (**C-e**, **C-f**, **C-g**, **C-h**, **C-i**, **C-j**, **C-k**, **C-l**, **C-m**, **C-n**, **C-o**, **C-p**), 26.90 (**C-c**), 26.49 (**C-3´**), 23.73 (**C-q**), 14.43 (**C-r**), 12.63 (**C_{het}-7**).

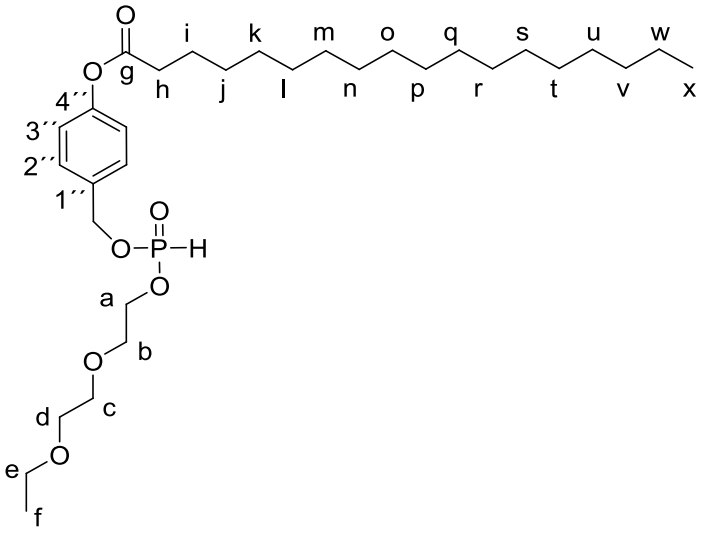
^{31}P-NMR (243 MHz, Methanol-d4): *δ* -10.77 (d, $^2J_{PP}$ = 19.0 Hz, **P-α**), -11.26 (d, $^2J_{PP}$ = 18.8 Hz, **P-γ**), -22.46 (t, $^2J_{PP}$ = 18.9 Hz, **P-β**).

HRMS (ESI-TOF) m/z: calculated for $C_{28}H_{52}N_2O_{13}P_3$ $[M-H]^-$ 717.2688, found 717.2678.

MALDI-MS (m/z): calculated $C_{28}H_{53}N_2NaO_{13}P_3$ $[M+Na]^+$ 741.265, found 741.225; calculated $C_{28}H_{53}KN_2O_{13}P_3$ $[M+K]^+$ 757.239, found 757.197; calculated $C_{28}H_{52}N_2O_{13}P_3$ $[M-H]^-$ 717.269, found 717.211.

IR: v = 2920, 2851, 1683, 1454, 1221, 1128, 1064, 991, 917, 845, 765, 721, 489, 422.

(AB-$C_{17}H_{35}$,alkyl-EEE)-*H*-phosphonate **69r**



Yield: 26%

Chemical Formula: $C_{31}H_{55}O_7P$

Molecular Weight: 570.74

According to **General Procedure D**, 0.23 mL diphenyl phosphonate (1.2 mmol, 1.2 equiv.) was dissolved in pyridine at 0 °C. 039 g 4-(Hydroxymethyl)phenyl octadecanoate **44w** (1.0 mmol, 1.0 equiv.) was added and followed by 0.19 mL 2-(2-ethoxyethoxy)ethan-1-ol (1.4 mmol, 1.4 equiv.). The mixture was stirred overnight at room temperature. Column chromatography (SiO_2, petrol ether/ethylacetate/CH_3COOH 8:2:0.005 v/v/v). Yield: 26%, 0.150 g, as colorless solid.

^{1}H-NMR (600 MHz, Chloroform-d): δ 7.41 (d, $^{3}J_{HH}$ = 8.5 Hz, 2H, **H-2´´**), 7.08 (d, $^{3}J_{HH}$ = 8.5 Hz, 2H, **H-3´´**), 6.96 (d, $^{1}J_{PH}$ = 714 Hz, 1H, **P-H**), 5.10 (d, $^{3}J_{HH}$ = 9.3 Hz, 2H, **Ph-CH_2**), 4.28-4.10 (m, 2H, **H-a**), 3.69 (t, $^{3}J_{HH}$ = 4.7 Hz, 2H, **H-b**), 3.68-3.60 (m, 2H, **H-c**), 3.57 (t, $^{3}J_{HH}$ = 4.6 Hz, 2H, **H-d**), 3.50 (q, $^{3}J_{HH}$ = 7.0 Hz, 2H, **H-e**), 2.54 (t, $^{3}J_{HH}$ = 7.5 Hz, 2H, **H-h**), 1.74 (quint, $^{3}J_{HH}$ = 7.5 Hz, 2H, **H-i**), 1.44-1.22 (m, 28H, **H-j**, **H-k**, **H-l**, **H-m**, **H-n**, **H-o**, **H-p**, **H-q**, **H-r**, **H-s**, **H-t**, **H-u**, **H-v**, **H-w**), 1.19 (t, $^{3}J_{HH}$ = 7.0 Hz, 3H, **H-f**), 0.87 (t, $^{3}J_{HH}$ = 7.0 Hz, 3H, **H-x**).

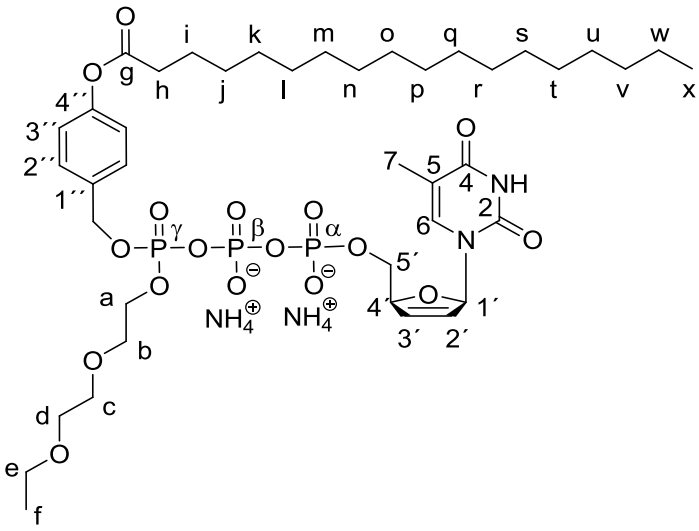
^{13}C-NMR (101 MHz, Chloroform-d): *δ* 172.14 (**C-g**), 150.89 (**C-4´´**), 133.19 (**C-1´´**), 129.17 (**C-2´´**), 121.87 (**C-3´´**), 70.63 (**C-c**), 70.10 (d, $^{3}J_{CP}$ = 5.1 Hz, **C-b**), 69.76 (**C-d**), 66.65 (**C-e**), 66.35 (d, $^{2}J_{CP}$ = 5.6 Hz, **Ph-CH_2**), 64.97 (d, $^{2}J_{CP}$ = 6.2 Hz, **C-a**), 34.36 (**C-h**), 31.90, 29.67, 29.65, 29.63, 29.63, 29.58, 29.44, 29.34, 29.24, 29.09 (**C-j**, **C-k**, **C-l**, **C-m**, **C-n**, **C-o**, **C-p**, **C-q**, **C-r**, **C-s**, **C-t**, **C-u**, **C-v**), 24.89 (**C-i**), 22.67 (**C-w**), 15.13 (**C-f**), 14.10 (**C-x**).

^{31}P-NMR (243 MHz, Chloroform-d): *δ* 8.52.

HRMS (ESI-TOF) m/z: calculated for $C_{31}H_{55}NaO_7P$ $[M+Na]^+$ 593.3578, found 593.3576.

IR: v = 3184, 2921, 2851, 1761, 1690, 1508, 1461, 1355, 1247, 1167, 1127, 1078, 1009, 904, 837, 805, 783, 767, 720, 697, 643, 483.

γ-(AB-$C_{17}H_{35}$,alkyl-EEE)-d4TTP (ammonium salt) **70r**



Yield: 46%

Chemical Formula: $C_{41}H_{73}N_4O_{17}P_3$

Molecular Weight: 986.96

According to **General Procedure C** the reactions were performed under dry conditions using using 86 mg *H*-phosphonate **69r** (0.15 mmol, 1.0 equiv.) and 94 mg d4TMP 2×nBu_4N^+ salt (0.12 mmol, 0.8 equiv.). Yield: 46%, 54 mg, as colorless cotton.

^{1}H-NMR (600 MHz, Methanol-d4): *δ* 7.74-7.66 (m, 1H, **H_{het}-6**), 7.52 (dd, $^3J_{HH}$ = 8.5 Hz, $^4J_{HH}$ = 1.9 Hz, 2H, **H-2´´**), 7.10 (d, $^3J_{HH}$ = 8.4 Hz, 2H, **H-3´´**), 6.96 (dt, $^3J_{HH}$ = 3.6 Hz, $^4J_{HH}$ = 1.2 Hz, 1H, **H-1´**), 6.58-6.47 (m, 1H, **H-3´**), 5.89-5.80 (m, 1H, **H-2´**), 5.28-5.18 (m, 2H, **Ph-CH_2**), 5.02-4.94 (m, 1H, **H-4´**), 4.36-4.25 (m, 2H, **H-5´**), 4.24-4.18 (m, 2H, **H-a**), 3.70 (td, $^3J_{HH}$ = 4.5 Hz, $^4J_{HH}$ = 1.5 Hz, 2H, **H-b**), 3.65-3.60 (m, 2H, **H-c**), 3.58-3.54 (m, 2H, **H-d**), 3.51 (qd, $^3J_{HH}$ = 7.0 Hz, $^4J_{HH}$ = 1.3 Hz, 2H, **H-e**), 2.60 (t, $^3J_{HH}$ = 7.4 Hz, 2H, **H-h**), 1.93 (dd, $^4J_{HH}$ = 2.6, 1.2 Hz, 3H, **H_{het}-7**), 1.75 (quint, $^3J_{HH}$ = 7.5 Hz, 2H, **H-i**), 1.50-1.25 (m, 28H**, H-j**, **H-k**, **H-l**, **H-m**, **H-n**, **H-o**, **H-p**, **H-q**, **H-r**, **H-s**, **H-t**, **H-u**, **H-v**, **H-w**), 1.17 (t, $^3J_{HH}$ = 7.0 Hz, 3H, **H-f**), 0.92 (t, $^3J_{HH}$ = 7.0 Hz, 3H, **H-x**).

^{13}C-NMR (151 MHz, Methanol-d4): *δ* 172.18 (**C-g**), 166.57 (**C_{het}-4**), 152.80 (**C_{het}-2**), 150.88 (**C-4´´**), 138.67 (**C_{het}-6**), 135.84 (**C-3´**), 133.17 (**C-1´´**), 129.19 (**C-2´´**), 127.20 (**C-2´**), 121.89 (**C-3´´**), 112.10 (**C_{het}-5**), 90.88 (**C-1´**), 87.25 (d, $^3J_{CP}$ = 9.0 Hz, **C-4´**), 70.64 (**C-c**), 70.22 (d, $^2J_{CP}$ = 6.0 Hz, **Ph-CH_2**), 70.11 (d, $^3J_{CP}$ = 5.1 Hz, **C-b**), 69.78 (**C-d**), 67.84 (d, $^2J_{CP}$ = 5.8 Hz, **C-5´**), 66.66 (**C-e**), 64.96 (d, $^2J_{CP}$ = 6.2 Hz, **C-a**), 34.37 (**C-h**), 31.91, 29.66, 29.66, 29.62, 29.61, 29.54, 29.47, 29.36, 29.25, 29.10 (**C-j**, **C-k**, **C-l**, **C-m**, **C-n**, **C-o**, **C-p**, **C-q**, **C-r**, **C-s**, **C-t**, **C-u**, **C-v**), 24.88 (**C-i**), 22.68 (**C-w**), 15.14 (**C-f**), 14.12 (**C-x**), 12.50 (**C_{het}-7**).

^{31}P-NMR (243 MHz, Methanol-d4): *δ* -11.93 (d, $^2J_{PP}$ = 19.9 Hz, **P-α**), -13.14 (d, $^2J_{PP}$ = 17.2 Hz, **P-γ**), -23.97 (t, $^2J_{PP}$ = 18.5 Hz, **P-β**).

HRMS (ESI-TOF) m/z: calculated for $C_{41}H_{66}N_2O_{17}P_3$ $[M-H]^-$ 951.3580, found 951.3512.

IR: v = 3186, 2917, 2850, 1758, 1691, 1509, 1467, 1380, 1331, 1251, 1168, 1127, 1113, 1083, 1013, 913, 838, 784, 768, 721, 697, 644, 576, 491, 423, 400.

7 Reference

Reference:

1 Jordheim, L. P., Durantel, D., Zoulim, F. & Dumontet, C. Advances in the development of nucleoside and nucleotide analogues for cancer and viral diseases. *Nat. Rev. Drug Discov.* **12**, 447-464 (2013).

2 Deval, J. Antimicrobial Strategies Inhibition of Viral Polymerases by 3 '-Hydroxyl Nucleosides. *Drugs* **69**, 151-166 (2009).

3 Cihlar, T. & Ray, A. S. Nucleoside and nucleotide HIV reverse transcriptase inhibitors: 25 years after zidovudine. *Antiviral Res.* **85**, 39-58 (2010).

4 El Safadi, Y., Vivet-Boudou, V. & Marquet, R. HIV-1 reverse transcriptase inhibitors. *Appl. Microbiol. Biotechnol.* **75**, 723-737 (2007).

5 Burton, J. R. & Everson, G. T. HCV NS5B Polymerase Inhibitors. *Clinics in Liver Disease* **13**, 453 (2009).

6 De Clercq, E. Antiviral drugs in current clinical use. *J. Clin. Virol.* **30**, 115-133 (2004).

7 Balzarini, J., Herdewijn, P. & De Clercq, E. Differential patterns of intracellular metabolism of 2', 3'-didehydro-2', 3'-dideoxythymidine and 3'-azido-2', 3'-dideoxythymidine, two potent anti-human immunodeficiency virus compounds. *J. Biological Chem.* **264**, 6127-6133 (1989).

8 Van Rompay, A. R., Johansson, M. & Karlsson, A. Phosphorylation of nucleosides and nucleoside analogs by mammalian nucleoside monophosphate kinases. *Pharm. Ther.* **87**, 189-198 (2000).

9 Asahchop, E. L., Wainberg, M. A., Sloan, R. D. & Tremblay, C. L. Antiviral Drug Resistance and the Need for Development of New HIV-1 Reverse Transcriptase Inhibitors. *Antimicrob. Agents Chemother.* **56**, 5000-5008 (2012).

10 Meier, C. Pro-nucleotides - Recent advances in the design of efficient tools for the delivery of biologically active nucleoside monophosphates. *Synlett*, 233-242 (1998).

11 Wagner, C. R., Iyer, V. V. & McIntee, E. J. Pronucleotides: Toward the in vivo delivery of antiviral and anticancer nucleotides. *Med. Res. Rev.* **20**, 417-451(2000).

12 Hecker, S. J. & Erion, M. D. Prodrugs of phosphates and phosphonates. *J. Med. Chem.* **51**, 2328-2345 (2008).

13 Pradere, U., Garnier-Amblard, E. C., Coats, S. J., Amblard, F. & Schinazi, R. F. Synthesis of Nucleoside Phosphate and Phosphonate Prodrugs. *Chem. Rev.* **114**, 9154-9218 (2014).

14 Cahard, D., McGuigan, C. & Balzarini, J. Aryloxy phosphoramidate triesters as pro-tides. *Mini-Rev. Med. Chem.* **4**, 371-381 (2004).

15 Li, F. J., Maag, H. & Alfredson, T. Prodrugs of nucleoside analogues for improved oral absorption and tissue targeting. *J. Pharm. Sci.* **97**, 1109-1134 (2008).

16 Zhang, Y., Gao, Y., Wen, X. & Ma, H. Current prodrug strategies for improving oral absorption of nucleoside analogues. *Asian J. Pharm. Sci.* **9**, 65-74 (2014).

17 Meier, C. cycloSal phosphates as chemical Trojan horses for intracellular nucleotide and glycosylmonophosphate delivery - Chemistry meets biology. *European J. Org. Chem.*, 1081-1102 (2006).

18 Krylov, I. S., Kashemirov, B. A., Hilfinger, J. M. & McKenna, C. E. Evolution of an Amino Acid Based Prodrug Approach: Stay Tuned. *Mol. Pharm.* **10**, 445-458 (2013).

19 Meier, C. & Balzarini, J. Application of the cycloSal-prodrug approach for improving the biological potential of phosphorylated biomolecules. *Antiviral Res.* **71**, 282-292 (2006).

20 Jessen, H. J., Schulz, T., Balzarini, J. & Meier, C. Bioreversible protection of nucleoside diphosphates. *Angew. Chem. Int. Ed.* **47**, 8719-8722 (2008).

21 Weinschenk, L., Schols, D., Balzarini, J. & Meier, C. Nucleoside Diphosphate Prodrugs: Nonsymmetric Di PP ro-Nucleotides. *J. Med. Chem.* **58**, 6114-6130 (2015).

22 Meier, C. *et al.* Rational Development of Nucleoside Diphosphate Prodrugs: DiPPro-Compounds. *Curr. Med. Chem.* **22**, 3933-3950 (2015).

23 Tan, X. L., Chu, C. K. & Boudinot, F. D. Development and optimization of anti-HIV nucleoside analogs and prodrugs: A review of their cellular pharmacology, structure-activity relationships and pharmacokinetics. *Adv. Drug Deliv. Rev.* **39**, 117-151 (1999).

24 Camarasa, M. J. Prodrugs of Nucleoside Triphosphates as a Sound and Challenging Approach: A Pioneering Work That Opens a New Era in the Direct Intracellular Delivery of Nucleoside Triphosphates. *ChemMedChem* **13**, 1885-1889 (2018).

25 Gollnest, T., De Oliveira, T. D., Schols, D., Balzarini, J. & Meier, C. Lipophilic prodrugs of nucleoside triphosphates as biochemical probes and potential antivirals. *Nat. Comm.* **6**, 8716 (2015).

26 Gollnest, T. *et al.* Membrane-permeable Triphosphate Prodrugs of Nucleoside Analogues. *Angew. Chem.* **128**, 5341-5344 (2016).

27 Meier, C. Nucleoside diphosphate and triphosphate prodrugs–An unsolvable task? *Antiv. Chem. Chemother.* **25**, 69-82 (2017).

28 McKenna, C. E., Kashemirov, B. A., Peterson, L. W. & Goodman, M. F. Modifications to the dNTP triphosphate moiety: From mechanistic probes for DNA polymerases to antiviral and anti-cancer drug design. *Biochim. Biophys. Acta-Proteins Proteom.* **1804**, 1223-1230 (2010).

29 Ono, K., Nakane, H., Herdewijn, P., Balzarini, J. & De Clercq, E. Differential inhibitory effects of several pyrimidine 2', 3'-dideoxynucleoside 5'-triphosphates on the activities of reverse transcriptase and various cellular DNA polymerases. *Mol. Pharm.* **35**, 578-583 (1989).

30 Alexandrova, L. A., Skoblov, A. Y., Jasko, M. V., Victorova, L. S. & Krayevsky, A. A. 2 '-deoxynucleoside 5 '-triphosphates modified at alpha-, beta- and gamma-phosphates as substrates for DNA polymerases. *Nucleic Acids Res.* **26**, 778-786 (1998).

31 Arzumanov, A. A., Semizarov, D. G., Victorova, L. S., Dyatkina, N. B. & Krayevsky, A. A. gamma-phosphate-substituted 2'-deoxynucleoside 5'-triphosphates as substrates for DNA polymerases. *J. Biol. Chem.* **271**, 24389-24394 (1996).

32 Shipitsin, A. V. *et al.* New modified nucleoside 5 '-triphosphates: synthesis, properties towards DNA polymerases, stability in blood serum and antiviral activity. *J. Chem. Society-Perkin Trans. 1*, 1039-1050 (1999).

33 Weiss, R. A. How does HIV cause AIDS? *Science* **260**, 1273-1279 (1993).

34 Friis, H. & Michaelsen, K. F. Micronutrients and HIV infection: a review. *European J. Clin. Nutr.* **52**, 157 (1998).

35 Brenchley, J. & Douek, D. HIV infection and the gastrointestinal immune system. *Mucosal immunol.***1**, 23 (2008).

36 Sepkowitz, K. A. AIDS—the first 20 years. *New England J. Med.* **344**, 1764-1772 (2001).

37 Doitsh, G. & Greene, W. C. Dissecting how CD4 T cells are lost during HIV infection. *Cell Host Microbe* **19**, 280-291 (2016).

38 Hazenberg, M. D., Hamann, D., Schuitemaker, H. & Miedema, F. T cell depletion in HIV-1 infection: how CD4+ T cells go out of stock. *Nat. Immunol.* **1**, 285 (2000).

39 Ammassari, A. *et al.* Self-reported symptoms and medication side effects influence adherence to highly active antiretroviral therapy in persons with HIV infection. *J. Acquir. Immune Defic. Syndr.* **28**, 445-449 (2001).

40 Celum, C. L. *et al.* Early human immunodeficiency virus (HIV) infection in the HIV network for prevention trials vaccine preparedness cohort: Risk behaviors, symptoms, and early plasma and genital tract virus load. *J. Infect. Dis.* **183**, 23-35 317658 (2001).

41 Nakamura, H. *et al.* Clinical Symptoms and Courses of Primary HIV-1 Infection in Recent Years in Japan. *Intern. Med.* **50**, 95-101 (2011).

42 Sharp, P. M. & Hahn, B. H. Origins of HIV and the AIDS pandemic. *Cold Spring Harb. Perspecti. Med.* **1**, a006841 (2011).

43 Hirsch, V. M., Olmsted, R. A., Murphey-Corb, M., Purcell, R. H. & Johnson, P. R. An African primate lentivirus (SIVsmclosely related to HIV-2. *Nature* **339**, 389 (1989).

44 Gao, F. *et al.* Human infection by genetically diverse SIVSM-related HIV-2 in west Africa. *Nature* **358**, 495 (1992).

45 Chen, Z. *et al.* Genetic characterization of new West African simian immunodeficiency virus SIVsm: geographic clustering of household-derived SIV strains with human immunodeficiency virus type 2 subtypes and genetically diverse viruses from a single feral sooty mangabey troop. *J. Virol.* **70**, 3617-3627 (1996).

46 Berman, H. M. *et al.* The Protein Data Bank. *Nucleic Acids Res.* **28**, 235–242, (2000).

47 Services, U. S. D. o. H. a. H. *Life Cycle*, (2018). <https://aidsinfo.nih.gov/understanding-hiv-aids/glossary/1596/life-cycle>

48 Gottlieb, M. S. *et al.* Pneumocystis pneumonia--Los Angeles. *MMWR.* **30**, 250-252 (1981).

49 Gottlieb, M. S. Pneumocystis Pneumonia--Los Angeles. *American journal of public health* **96**, 980-981, doi:10.2105/ajph.96.6.980 (2006).

50 Pomerantz, R. J. & Horn, D. L. Twenty years of therapy for HIV-1 infection. *Nat. Med.* **9**, 867 (2003).

51 UNAIDS. *UNAIDS data 2018*. (Joint United Nations Programme on HIV/AIDS, 2018).

52 Wiebe, L. I. Applications of nucleoside-based molecular probes for the in vivo assessment of tumour biochemistry using positron emission tomography (PET). *Braz. Arch. Biol. Technol.* **50**, 445-459 (2007).

53 Jordheim, L. P., Durantel, D., Zoulim, F. & Dumontet, C. Advances in the development of nucleoside and nucleotide analogues for cancer and viral diseases. *Nat. Rev. Drug Discov.* **12**, 447 (2013).

54 Bazzoli, C. *et al.* Intracellular pharmacokinetics of antiretroviral drugs in HIV-infected patients, and their correlation with drug action. *Clin. Pharm.* **49**, 17-45 (2010).

55 August, E. M., Birks, E. M. & Prusoff, W. H. 3'-Deoxythymidin-2'-ene permeation of human lymphocyte H9 cells by nonfacilitated diffusion. *Mol. Pharm.* **39**, 246-249 (1991).

56 Furman, P. A. *et al.* Phosphorylation of 3'-azido-3'-deoxythymidine and selective interaction of the 5'-triphosphate with human immunodeficiency virus reverse transcriptase. *Proc. Natl. Acad. Sci.* **83**, 8333-8337 (1986).

57 Lavie, A. *et al.* Structure of thymidylate kinase reveals the cause behind the limiting step in AZT activation. *Nat. Struct. Biol.* **4**, 601-604 (1997).

58 Chen, F.-F. & Wang, F. Electronic Structure of the Azide Group in 3´-Azido-3´-deoxythymidine (AZT) Compared to Small Azide Compounds. *Molecules* **14**, 2656-2668 (2009).

59 Gao, W., Agbaria, R., Driscoll, J. S. & Mitsuya, H. Divergent anti-human immunodeficiency virus activity and anabolic phosphorylation of 2', 3'-dideoxynucleoside analogs in resting and activated human cells. J*. Biol. Chem.* **269**, 12633-12638 (1994).

60 Törnevik, Y., Jacobsson, B., Britton, S. & Eriksson, S. Intracellular metabolism of 3´-azidothymidine in isolated human peripheral blood mononuclear cells. *AIDS Res. Hum. Retroviruses* **7**, 751-759 (1991).

61 Ho, H. & Hitchcock, M. Cellular pharmacology of 2', 3'-dideoxy-2', 3'-didehydrothymidine, a nucleoside analog active against human immunodeficiency virus. *Antimicrob. Agents Chemother.* **33**, 844-849 (1989).

62 Törnevik, Y., Ullman, B., Balzarini, J., Wahren, B. & Eriksson, S. Cytotoxicity of 3´azido-3´-deoxythymidine correlates with 3´-azidothymidine-5´-monophosphate (AZTMP) levels, whereas antihuman immunodeficiency virus (HIV) activity correlates with 3´-azidothymidine-5´-triphosphate (AZTTP) levels in cultured CEM T-lymphoblastoid cells. *Bioch. Pharm.* **49**, 829-837 (1995).

63 Cheng, Y. C., Gao, W. Y., Chen, C. H., Vazquez-Padua, M. & Starnes, M. C. DNA polymerases versus HIV reverse transcriptase in AIDS therapy. *Ann. New York Acad. Sci.* **616**, 217-223 (1990).

64 Kohlstaedt, L., Wang, J., Friedman, J., Rice, P. & Steitz, T. Crystal structure at 3.5 A resolution of HIV-1 reverse transcriptase complexed with an inhibitor. *Science* **256**, 1783-1790 (1992).

65 Jacobo-Molina, A. *et al.* Crystal structure of human immunodeficiency virus type 1 reverse transcriptase complexed with double-stranded DNA at 3.0 A resolution shows bent DNA. *Proc. Natl. Acad. Sci.* **90**, 6320-6324 (1993).

66 Sarafianos, S. G. *et al.* Structure and function of HIV-1 reverse transcriptase: molecular mechanisms of polymerization and inhibition. *J. Mol. Biol.* **385**, 693-713 (2009).

67 Kati, W. M., Johnson, K. A., Jerva, L. & Anderson, K. Mechanism and fidelity of HIV reverse transcriptase. *J. Biol. Chem.* **267**, 25988-25997 (1992).

68 Furfine, E. S. & Reardon, J. E. Human immunodeficiency virus reverse transcriptase ribonuclease H: specificity of tRNALys3-primer excision. *Biochemistry* **30**, 7041-7046 (1991).

69 Pullen, K. A., Ishimoto, L. K. & Champoux, J. J. Incomplete removal of the RNA primer for minus-strand DNA synthesis by human immunodeficiency virus type 1 reverse transcriptase. *J. Virol.* **66**, 367-373 (1992).

70 Nikolenko, G. N. *et al.* Mutations in the connection domain of HIV-1 reverse transcriptase increase 3′-azido-3′-deoxythymidine resistance. *Proc. Natl. Acad. Sci.* **104**, 317-322 (2007).

71 Delviks-Frankenberry, K. A., Nikolenko, G. N., Barr, R. & Pathak, V. K. Mutations in human immunodeficiency virus type 1 RNase H primer grip enhance 3′ -azido-3′ -deoxythymidine resistance. *J. Virol.* **81**, 6837-6845 (2007).

72 Arion, D., Kaushik, N., McCormick, S., Borkow, G. & Parniak, M. A. Phenotypic mechanism of HIV-1 resistance to 3 ‘-azido-3 ‘-deoxythymidine (AZT): Increased polymerization processivity and enhanced sensitivity to pyrophosphate of the mutant viral reverse transcriptase. *Biochemistry* **37**, 15908-15917 (1998).

73 Meyer, P. R., Matsuura, S. E., So, A. G. & Scott, W. A. Unblocking of chain-terminated primer by HIV-1 reverse transcriptase through a nucleotide-dependent mechanism. *Proc. Natl. Acad. of Sci.* **95**, 13471-13476 (1998).

74 Meyer, P. R., Matsuura, S. E., Mian, A. M., So, A. G. & Scott, W. A. A mechanism of AZT resistance: an increase in nucleotide-dependent primer unblocking by mutant HIV-1 reverse transcriptase. *Mol. Cell* **4**, 35-43 (1999).

75 Vivet-Boudou, V., Didierjean, J., Isel, C. & Marquet, R. Nucleoside and nucleotide inhibitors of HIV-1 replication. *Cell. Mol. Life Sci.* **63**, 163-186 (2006).

76 Steitz, T. A. DNA polymerases: structural diversity and common mechanisms. *J. Biol.Chem.* **274**, 17395-17398 (1999).

77 Graziewicz, M. A., Longley, M. J. & Copeland, W. C. DNA polymerase γ in mitochondrial DNA replication and repair. *Chem. Rev.* **106**, 383-405 (2006).

78 Lehman, I. *et al.* DNA polymerase alpha. *J. Biol. Chem.* **264** (1989).

79 Baranovskiy, A. G. *et al.* Activity and fidelity of human DNA polymerase α depend on primer structure. *J. Biol. Chem.*, jbc. RA117. 001074 (2018).

80 Bertazzoni, U. *et al.* Variations of DNA polymerase-alpha and-beta during prolonged stimulation of human lymphocytes. *Proc. Natl. Acad. Sci.* **73**, 785-789 (1976).

81 Craig, R. K., Costello, P. A. & Keir, H. M. Dexyribonucleic acid polymerases of BHK-21/C13cells. Relationship to the physiological state of the cells, and to synchronous indution of synthesis of deoxyribonuleic acid. *Biochem. J.* **145**, 233-240 (1975).

82 Wang, T. S. F. in *Cold Spring Harbor Monograph Series; DNA replication in eukaryotic cells* Vol. 31 *Cold Spring Harbor Monograph Series* (ed M. L. DePamphilis) 461-493 (Cold Spring Harbor Laboratory Press {a}, 10 Skyline Drive, Plainview, New York 11803, USA, 1996).

83 Matsumoto, Y. & Kim, K. Excision of deoxyribose phosphate residues by DNA polymerase beta during DNA repair. *Science* **269**, 699-702 (1995).

84 Kaufman, B. A. & Van Houten, B. POLB: A new role of DNA polymerase beta in mitochondrial base excision repair. *DNA Repair* **60**, A1-A5 (2017).

85 Chan, S. S. L. & Copeland, W. C. DNA polymerase gamma and mitochondrial disease: Understanding the consequence of POLG mutations. *Biochim. Biophys. Acta-Bioenergetics* **1787**, 312-319 (2009).

86 Lim, S. E. & Copeland, W. C. Differential incorporation and removal of antiviral deoxynucleotides by human DNA polymerase γ. *J. Biol. Chem.* **276**, 23616-23623 (2001).

87 Johnson, A. A. *et al.* Toxicity of antiviral nucleoside analogs and the human mitochondrial DNA polymerase. *J. Biol. Chem.* **276**, 40847-40857 (2001).

88 Becher, F. *et al.* Significant levels of intracellular stavudine triphosphate are found in HIV-infected zidovudine-treated patients. *Aids* **17**, 555-561 (2003).

89 Hudson, G. & Chinnery, P. F. Mitochondrial DNA polymerase-γ and human disease. *Hum. Mol. Genet.* **15**, R244-R252 (2006).

90 Kohler, J. J. & Lewis, W. A brief overview of mechanisms of mitochondrial toxicity from NRTIs. *Environ. Mol. Mutagen.* **48**, 166-172 (2007).

91 Lewis, W. *et al.* Antiretroviral nucleosides, deoxynucleotide carrier and mitochondrial DNA: evidence supporting the DNA pol γ hypothesis. *AIDS (London, England)* **20**, 675-684 (2006).

92 Sohl, C. D. *et al.* Probing the structural and molecular basis of nucleotide selectivity by human mitochondrial DNA polymerase γ. *Proc. Natl. Acad. Sci.* **112**, 8596-8601 (2015).

93 Sawaya, M. R., Prasad, R., Wilson, S. H., Kraut, J. & Pelletier, H. Crystal structures of human DNA polymerase β complexed with gapped and nicked DNA: evidence for an induced fit mechanism. *Biochem.* **36**, 11205-11215 (1997).

94 Rose, A. S. *et al.* in *Proceedings of the 21st international conference on Web3D technology.* 185-186 (ACM).

95 Rose, A. S. & Hildebrand, P. W. NGL Viewer: a web application for molecular visualization. *Nucleic Acids Res.* **43**, W576-W579 (2015).

96 Johnson, R. E., Klassen, R., Prakash, L. & Prakash, S. A major role of DNA polymerase δ in replication of both the leading and lagging DNA strands. *Mol. Cell* **59**, 163-175 (2015).

97 Pospiech, H. & Syväoja, J. E. DNA polymerase e-more than a polymerase. *Scient. World J.* **3**, 87-104 (2003).

98 Miyabe, I., Kunkel, T. A. & Carr, A. M. The Major Roles of DNA Polymerases Epsilon and Delta at the Eukaryotic Replication Fork Are Evolutionarily Conserved. *PLoS Genet.* **7**, e1002407 (2011).

99 Martin, J. L., Brown, C. E., Matthews-Davis, N. & Reardon, J. E. Effects of antiviral nucleoside analogs on human DNA polymerases and mitochondrial DNA synthesis. *Antimicrob. Agents ´Chemother* **38**, 2743-2749 (1994).

100 Huang, P., Farquhar, D. & Plunkett, W. Selective action of 2', 3'-didehydro-2', 3'-dideoxythymidine triphosphate on human immunodeficiency virus reverse transcriptase and human DNA polymerases. *J. Biol. Chem.* **267**, 2817-2822 (1992).

101 CHENG, Y. C., GAO, W. Y., CHEN, C. H., VAZQUEZ‐PADUA, M. & Starnes, M. C. DNA polymerases versus HIV reverse transcriptase in AIDS therapy. *Ann. New York Acad. Sci.* **616**, 217-223 (1990).

102 De Schutter, C., Ehteshami, M., Hammond, E. T., Amblard, F. & Schinazi, R. F. Metabolism of nucleosides and nucleotides prodrugs. *Curr. Pharm. Design* **23**, 6984-7002 (2017).

103 Ballatore, C., McGuigan, C., De Clercq, E. & Balzarini, J. Synthesis and evaluation of novel amidate prodrugs of PMEA and PMPA. *Bioorg. Med. Chem. Lett.* **11**, 1053-1056 (2001).

104 Dando, T. M. & Plosker, G. L. Adefovir Dipivoxil: a review of its use in chronic hepatitis B. *Drugs* **63**, 2215-2234 (2003).

105 Cundy, K. C. *et al.* Clinical pharmacokinetics of adefovir in human immunodeficiency virus type 1-infected patients. *Antimicrob. Agents Chemother.* **39**, 2401-2405 (1995).

106 Naesens, L. *et al.* HPMPC (cidofovir), PMEA (adefovir) and related acyclic nucleoside phosphonate analogues: a review of their pharmacology and clinical potential in the treatment of viral infections. *Antivir. Chem. Chemother.* **8**, 1-23 (1997).

107 Palu, G. *et al.* Cellular uptake of phosphonylmethoxyalkylpurine derivatives. *Antivir. Res.* **16**, 115-119 (1991).

108 Balzarini, J. & De Clercq, E. 5-Phosphoribosyl 1-pyrophosphate synthetase converts the acyclic nucleoside phosphonates 9-(3-hydroxy-2-phosphonylmethoxypropyl) adenine and 9-(2-phosphonylmethoxyethyl) adenine directly to their antivirally active diphosphate derivatives. *J. Biol. Chem.* **266**, 8686-8689 (1991).

109 Merta, A. *et al.* Phosphorylation of 9-(2-phosphonomethoxyethyl) adenine and 9-(S)-(3-hydroxy-2-phosphonomethoxypropyl) adenine by AMP (dAMP) kinase from L1210 cells. *Biochem. Pharm.* **44**, 2067-2077 (1992).

110 Robbins, B. L., Greenhaw, J., Connelly, M. C. & Fridland, A. Metabolic pathways for activation of the antiviral agent 9-(2-phosphonylmethoxyethyl) adenine in human lymphoid cells. *Antimicrob. Agents Chemother.* **39**, 2304-2308 (1995).

111 Balzarini, J. Metabolism and mechanism of antiretroviral action of purine and pyrimidine derivatives. *Pharm. World Sci.* **16**, 113-126 (1994).

112 Farquhar, D., Khan, S., Srivastva, D. N. & Saunders, P. P. Synthesis and antitumor evaluation of bis-((pivaloyloxy) methyl)-2'-deoxy-5-fluorouridine-5'-monophosphate (FdUMP): a strategy to introduce nucleotides into cells. *J. Med. Chem.* **37**, 3902-3909 (1994).

113 Kearney, B. P., Flaherty, J. F. & Shah, J. Tenofovir disoproxil fumarate. *Clin. Pharm.* **43**, 595-612 (2004).

114 Yuan, L.-C., Dahl, T. C. & Oliyai, R. Degradation kinetics of oxycarbonyloxymethyl prodrugs of phosphonates in solution. *Pharm. Res.* **18**, 234-237 (2001).

115 Yuan, L.-C., Dahl, T. C. & Oliyai, R. Effect of carbonate salts on the kinetics of acid-catalyzed dimerization of adefovir dipivoxil. *Pharm. Res.* **17**, 1098-1103 (2000).

116 Shaw, J.-P. *et al.* Metabolism and pharmacokinetics of novel oral prodrugs of 9-[(R)-2-(phosphonomethoxy) propyl] adenine (PMPA) in dogs. *Pharm. Res.* **14**, 1824-1829 (1997).

117 Van Gelder, J. *et al.* Intestinal absorption enhancement of the ester prodrug tenofovir disoproxil fumarate through modulation of the biochemical barrier by defined ester mixtures. *Drug Metab. Dispos.* **30**, 924-930 (2002).

118 Robbins, B. L., Srinivas, R. V., Kim, C., Bischofberger, N. & Fridland, A. Anti-human immunodeficiency virus activity and cellular metabolism of a potential prodrug of the acyclic nucleoside phosphonate 9-R-(2-phosphonomethoxypropyl) adenine (PMPA), bis (isopropyloxymethylcarbonyl) PMPA. *Antimicrob. Agents Chemother.* **42**, 612-617 (1998).

119 Abrahamsson, K. *et al.* Impaired ketogenesis in carnitine depletion caused by short-term administration of pivalic acid prodrug. *Biochem. Med. Metab. Biol.* **52**, 18-21 (1994).

120 Mehellou, Y., Balzarini, J. & McGuigan, C. Aryloxy phosphoramidate triesters: a technology for delivering monophosphorylated nucleosides and sugars into cells. *ChemMedChem* **4**, 1779-1791 (2009).

121 Mehellou, Y., Balzarini, J. & McGuigan, C. An investigation into the anti-HIV activity of 2 ',3 '-didehydro-2 ',3 '-dideoxyuridine (d4U) and 2 ',3 '-dideoxyuridine (ddU) phosphoramidate 'ProTide' derivatives. *Org. Biomol. Chem.* **7**, 2548-2553 (2009).

122 Mehellou, Y., McGuigan, C., Brancale, A. & Balzarini, J. Design, synthesis, and anti-HIV activity of 2',3'-didehydro-2',3'-dideoxyuridine (d4U), 2',3'-dideoxyuridine (ddU) phosphoramidate "ProTide' derivatives. *Bioorg. Med. Chem. Lett.* **17**, 3666-3669 (2007).

123 Benzaria, S. *et al.* Synthesis, in vitro antiviral evaluation, and stability studies of bis (S-acyl-2-thioethyl) ester derivatives of 9-[2-(phosphonomethoxy) ethyl]

adenine (PMEA) as potential PMEA prodrugs with improved oral bioavailability. *J. Med. Chem.* **39**, 4958-4965 (1996).

124 Girardet, J.-L. *et al.* Increase of the anti-HIV activity of d4T in human T-cell culture by the use of the SATE pronucleotide approach. *Bioorg. Med. Chem. Lett.* **5**, 2981-2984 (1995).

125 Shafiee, M. *et al.* New bis (SATE) prodrug of AZT 5′ ‐monophosphate: In vitro anti ‐HIV activity, stability, and potential oral absorption. *J. Pharm. Sci.* **90**, 448-463 (2001).

126 Erion, M., Bullough, D. A., Lin, C.-C. & Hong, Z. HepDirect prodrugs for targeting nucleotide-based antiviral drugs to the liver. *Curr. Opin. Investig. Drugs* **7**, 109-117 (2006).

127 Tillmann, H. L. The treatment of chronic hepatitis B: Focus on adefovir-like antivirals. *Ther. Clin. Risk Manag.***4**, 797 (2008).

128 Meier, C. 2-Nucleos-5′-O-yl-4H-1,3,2-benzodioxaphos-phinin-2-oxides------A New Concept for Lipophilic, Potential Prodrugs of Biologically Active Nucleoside Monophosphates. *Angew. Chem. Int. Ed. in Eng.* **35**, 70-72 (1996).

129 Meier, C., Lorey, M., De Clercq, E. & Balzarini, J. cyclo Sal-2 ‘, 3 ‘-dideoxy-2 ‘, 3 ‘-didehydrothymidine Monophosphate (cyclo Sal-d4TMP): Synthesis and Antiviral Evaluation of a New d4TMP Delivery System. *J. Med. Chem.* **41**, 1417-1427 (1998).

130 Vukadinović, D., Boege, N., Balzarini, J. & Meier, C. “Lock-in” modified cyclosal nucleotides—the second generation of cyclosal prodrugs. *Nucleosides Nucleotides Nucleic Acids* **24**, 939-942 (2005).

131 Gisch, N., Balzarini, J. & Meier, C. Enzymatically Activated cyclo Sal-d4T-monophosphates: The Third Generation of cyclo Sal-Pronucleotides. *J. Med. Chem.* **50**, 1658-1667 (2007).

132 Painter, G. R. *et al.* Evaluation of hexadecyloxypropyl-9-R-[2-(phosphonomethoxy) propyl]-adenine, CMX157, as a potential treatment for human immunodeficiency virus type 1 and hepatitis B virus infections. *Antimicrob. Agents Chemother.* **51**, 3505-3509 (2007).

133 Heussner, P. *et al.* in *Acute Leukemias VI* 882-885 (Springer, 1997).

134 Girard, P.-M. *et al.* Phase II placebo-controlled trial of fozivudine tidoxil for HIV infection: pharmacokinetics, tolerability, and efficacy. *J. Acquir. Immune Defic. Syndr.* **23**, 227-235 (2000).

135 Thomson, W. *et al.* Synthesis, bioactivation and anti-HIV activity of the bis (4-acyloxybenzyl) and mono (4-acyloxybenzyl) esters of the 5′ -monophosphate of AZT. *J. Chem. Soc. Perkin Trans. 1*, 1239-1245 (1993).

136 Hostetler, K. Y., Stuhmiller, L., Lenting, H., Van den Bosch, H. & Richman, D. Synthesis and antiretroviral activity of phospholipid analogs of azidothymidine and other antiviral nucleosides. *J. Biol. Chem.* **265**, 6112-6117 (1990).

137 van Wijk, G. M., Hostetler, K. Y. & van den Bosch, H. Lipid conjugates of antiretroviral agents: release of antiretroviral nucleoside monophosphates by a nucleoside diphosphate diglyceride hydrolase activity from rat liver

mitochondria. *Biochim. Biophys. Acta (BBA)-Lipids Lipid Metab.* **1084**, 307-310 (1991).

138 Bonnaffé, D., Dupraz, B., Ughetto-Monfrin, J., Namane, A. & Dinh, T. H. Synthesis of acyl pyrophosphates. Application to the synthesis of nucleotide lipophilic prodrugs. *Tetrahedron lett.* **36**, 531-534 (1995).

139 Bonnaffe, D., Dupraz, B., Ughetto-Monfrin, J., Namane, A. & Dinh, T. H. Synthesis of nucleotide lipophilic prodrugs containing two inhibitors targeted against different phases of the HIV replication cycle. *Nucleosides Nucleotides Nucleic Acids* **14**, 783-787 (1995).

140 Bonnaffé, D. *et al.* Potential lipophilic nucleotide prodrugs: synthesis, hydrolysis, and antiretroviral activity of AZT and d4T acyl nucleotides. *J. Org. Chem.* **61**, 895-902 (1996).

141 Schulz, T., Balzarini, J. & Meier, C. The DiPPro approach: synthesis, hydrolysis, and antiviral activity of lipophilic d4T diphosphate prodrugs. *ChemMedChem* **9**, 762-775 (2014).

142 Weinschenk, L., Gollnest, T., Schols, D., Balzarini, J. & Meier, C. Bis (benzoyloxybenzyl) ‐ DiPPro Nucleoside Diphosphates of Anti ‐ HIV Active Nucleoside Analogues. *ChemMedChem* **10**, 891-900 (2015).

143 Van Wijk, G. *et al.* Synthesis and antiviral activity of 3′ -azido-3′ -deoxythymidine triphosphate distearoylglycerol: a novel phospholipid conjugate of the anti-HIV agent AZT. *Chem. Physics Lipids* **70**, 213-222 (1994).

144 Kreimeyer, A., Andrɛ, F., Gouyette, C. & Huynh-Dinh, T. Transmembrane Transport of Adenosine 5´-Triphosphate Using a Lipophilic Cholesteryl Derivative. *Angew. Chem. Int. Ed.* **37**, 2853-2855 (1998).

145 Kreimeyer, A., Andre, F., Bluzat, A., Gouyette, C. & Huynh-Dinh, T. Synthesis and transmembrane transport studies of lipophilic adenosine 5 '-triphosphate derivatives. *Nucleosides Nucleotides* **18**, 995-999, (1999).

146 Yarbrough, L. R. Synthesis and properties of a new fluorescent analog of ATP - adenosine-5'-triphosphoro-gamma-1-(5-sulfonic acid) napthylamidate. *Biochem. Biophys. Res. Comm.* **81**, 35-41 (1978).

147 Reimer, I. S. *3'-S-Phosphorthiolatverbrückte Oligonucleotide und Fluoreszierende Nucleotid-Prodrugs für Zellaufnahmestudien*. (Staats- und Universitätsbibliothek Hamburg, 2017).

148 Montanez, M. I. *et al.* Bifunctional Dendronized Cellulose Surfaces as Biosensors. *Biomacromolecules* **12**, 2114-2125 (2011).

149 McGuigan, C., Cahard, D., Sheeka, H. M., DeClercq, E. & Balzarini, J. Aryl phosphoramidate derivatives of d4T have improved anti-HIV efficacy in tissue culture and may act by the generation of a novel intracellular metabolite. *J. Med. Chem.* **39**, 1748-1753 (1996).

150 Sowa, T. & Ouchi, S. The facile synthesis of 5'-nucleotides by the selective phosphorylation of a primary hydroxyl group of nucleosides with phosphoryl chloride. *Bulletin Chem. Soc. Japan* **48**, 2084-2090 (1975).

151 Gollnest, T. *Das TriPPPro-Konzept : Entwicklung und Charakterisierung von antiviralen Nukleosidtriphosphat-Prodrugs*. (2015).

152 Pertenbreiter, F., Balzarini, J. & Meier, C. Nucleoside Mono- and Diphosphate Prodrugs of 2 ', 3 '-Dideoxyuridine and 2 ', 3 '-Dideoxy-2 ', 3 '-didehydrouridine. *ChemMedchem* **10**, 94-106 (2015).

153 Merkel, M. *et al.* Scope and Limitations of Typical Copper-Free Bioorthogonal Reactions with DNA: Reactive 2 '-Deoxyuridine Triphosphates for Postsynthetic Labeling. *J. Org. Chem.* **81**, 7527-7538 (2016).

154 Wenge, U., Ehrenschwender, T. & Wagenknecht, H.-A. Synthesis of 2 '-O-Propargyl Nucleoside Triphosphates for Enzymatic Oligonucleotide Preparation and "Click" Modification of DNA with Nile Red as Fluorescent Probe. *Bioconjug. Chem.* **24**, 301-304 (2013).

155 Martinez, S.E., Bauman, J. D., Das, K., Arnold, E. Structure of HIV-1 reverse transcriptase/d4TTP complex: Novel DNA cross-linking site and pH-dependent conformational changes. *Protein Science*. pp 1-11 (2018).

156 VK, B. *et al.* Magnesium-induced assembly of a complete DNA polymerasecatalytic complex. *Structure* **14**, 757-766 (2006).

8 Attachment

8.1 Chemicals an Hazards

8.1.1 Hazardous Substances Directory with HP-statements

The Globally Harmonized System of Classification and Labelling of Chemicals (GHS) is an internationally agreed-upon standard managed by the United Nations that was set up to replace the assortment of hazardous material classification and labelling schemes previously used around the world. The following is a list of compounds and solvents that were used during the research. The list contains GHS pictograms, GHS hazard statements and GHS precautionary statements for the corresponding hazardous substances. Substances for which unknown classification exists are to be classified as dangerous. Therefore, the contamination of the own or other person as well as the exposure into the environment must be avoided in every condition.

Substance	Pictograms	Hazard statements	Precautionary statements
Acetic acid glacial		226-314	280-305+351+338-310
Acetone		225-319-336-373	210-235-260-305+351+338
Acetonitrile		225-302-312-319-332	210-280-305+351+338
Acetyl chloride		225-302-314-318-335-402-412	210-240-280-310-301+312-301+330+331-305+351+338-402+404
Acrylamide		301-312-332-315-317-319-340-350-361f-372	201-280-302+352-304+340-305+351+338-308+310
Ammonia 25%		221-280-314-331-400	210-261-273-280-305+351+338-310

Attachment

1-Butanol		226-302-318-315-335-336	210-280-302+352-304+340-305+351+338-313
Ammonium acetate		303-316-320-333	281-335
Chloroform-d_1		302-315-319-331-336-351-361d-372	261-281-305+351+338-311
N-Chlorosuccinimide		302-314	280-305+351+338-310
Deuterium oxide		315-319-335	261-264-271-280-302+352-304+340-305+351+338-312-321-332+313-337+313-362-403+233-405-501
Dichloromethane		315-319-335-336-351-373	261-281-305-+351+338
Diethylene glycol monoethyl ether (Ethoxy diglycol)		302-319-331	261-264-270-271-280-301+312-304+340-305+351+338-311-321-330-337+313-403+233-405-501
Diethylether		224-302-336 EUH: 019-066	210-240-403+235
4-Dimethylaminopyridine		310-301-315-319	302+352-305+351+338
Diphenyl phosphonate		302-314-340-360FD-370	260-280-301-330-331-310-303-361-353-310-363-304-340-310-305-351-338-310
Dowex-H^+ (50WX8)		315-319-335	261-305-351+338

Ethanol		225-319	210-240-305+351+338-403+233
Ethyl acetate		225-319-336 EUH: 066	210-233-240-305+351+338-403+235
9-Fluorenemethanol			
Glutaric anhydride		302-312-315-318-335	261-264-270-271-280-301+312-302+352-304+340-305+351+338-310-312-321-322-330-332+313-362-363-403+233-405-501
Heptanoyl chloride		314-226 EUH: 014	210-260-303+361+353-305+351+338-405-501
n-Hexane		225-304-361f-373-315-336-411	210-240-273-301+310-331-302+352-403+235
Hydrochloric acid		290-314-335	260-280-303+361+353-304+340+310-305+351+338
4-Hydroxybenzyl alcohol		315-319-335	261-280-304+340-305+351+388-405-501
3-hydroxypropionitrile		315-319-335	261-264-271-280-302+352-304+340-305+351+338-312-321-332+313-337+313-362-403+233-405-501
Isovaleryl chloride		314-226	210-260-303+361+353-305+351+338-405-501
Methanol		225-331-311-301-370	280-301+312+330-303+361+353-304+340+310-305+351+338

Methanol-d_4		225-301-311-331-370	210-260-280-301+310-311
1-Methylimidazole		302-311-314	280-305+351+338-310
1-Octadecanol		315	264-280-302+352-321-332+313-362
Octadecanoyl chloride		314 EUH: 014	260-264-280-301+330+331-303+361+353-304+340-305+351+338-310-321-405-501
Oxalic chloride		260-301-314-318-331-335	223-231+232-260-261-264-270-271-280-301+310-301+330+331-303+361+353-304+340-305+351+338-310-311-312-321-330-335+334-363-370+378-402+404-403+233-405-501
Palmitoyl chloride		314	260-301+330+331-303+361+353-305+351+338-405-501
Pentadecanoic acid		315-319-335-413	261-305+351+338
1-Pentadecanol		315-319-400-410	264-273-280-302+352-305+351+338-321-332+313-337-313-362-391-501
Petroleum ether 50-70		225-304-315-336-411	210-243-273-301+310-303+361+353-301+330+331-403+235
Phosphomolybdic acid		272-314	220-280-305+351+338-310

Phosphorus oxychloride		300+330-314-372 EUH: 014-029	280-301+330+331-304+340-305+351+338-308+310
Potassium permanganate		272-302-314-410	221-273-280-301+330+331-305+351+338-308+310
2-Propanol		225-319-336	210-233-240-305+351+338-403+235
Propionyl chloride		225-302-331-314 EUH: 014	210-240-280-301+330+331-305+351+338-310-402+404
Pyridine		225-332-302-312-319-315	210-280-305+351+338
Sodium bicarbonate			
Sodium carbonate		319	305+351+338
Sodium chloride			
Sodium hydroxide		290-314	280-305+351+338-310
Sodium sulfate			
Succinic anhydride		302-314-317-334-335	280-301+330+331-302+352-304+340-305+351+338-308+310
Tetrabutylammonium hydroxide (40% in H2O)		314	280-305+351+338-309
Triethylamine		225-302-311+331-314-335	210-280-303+361+353-304+340-310-305+351+338-403+233
Triethylene glycol monomethyl ether (Methoxytriglycol)		315-319	264-280-302+352-305+351+338-321-332+313-337+313-362

Tetrahydrofuran		225-302-319-335-351 EUH:019	210-280-301+312+330-305+351+338-370+378
Thionyl chloride		302-314-332	260-261-264-270-271-280-301+312-301+330+331-303+361+353-304+312-304+340-305+351+338-310-312-321-330-363-405-501
Toluene		225-304-315-336-361D-373	210-240-301+310-302+352-308+313-314-403+233
1-Undecanol		315-319-411	273-280-305+351+338
Urea		315-319-335-351-371	201-202-260-261-264-270-271-280-281-302+352-304+340-305+351+338-308+313-309+311-312-321-332+313-337+313-362-403+233-405-501
Valeryl chloride		226-314-331	261-280-305+351+338-310

8.1.2 GHS Hazards Pictograms

Physical hazards pictograms:

GHS01: Explosive

GHS02: Flammable

GHS03: Oxidizing

GHS04: Compressed Gas

GHS05: Corrosive

Health hazards pictograms

GHS06: Toxic

GHS07: Harmful

GHS08: Health hazard

Environmental hazards pictograms:

GHS09: Environmental hazard

8.1.3 Hazard Statements

H2xx: Physical hazards;

H3xx: Health hazards;

H4xx: Environmental hazards

H201: Explosive; mass explosion hazard

H202: Explosive; severe projection hazard

H220: Extremely flammable gas

H221: Flammable gas

H223: Flammable aerosol

H224: Extremely flammable liquid and vapour

H225: Highly flammable liquid and vapour

H226: Flammable liquid and vapour

H231: May react explosively even in the absence of air at elevated pressure and/or temperature

H232: May ignite spontaneously if exposed to air

H240: Heating may cause an explosion

H260: In contact with water releases flammable gases which may ignite spontaneously

H261: In contact with water releases flammable gas

H270: May cause or intensify fire; oxidizer

H271: May cause fire or explosion; strong oxidizer
H272: May intensify fire; oxidizer
H280: Contains gas under pressure; may explode if heated
H281: Contains refrigerated gas; may cause cryogenic burns or injury
H290: May be corrosive to metals
H300: Fatal if swallowed
H301: Toxic if swallowed
H302: Harmful if swallowed
H303: May be harmful if swallowed
H304: May be fatal if swallowed and enters airways
H305: May be harmful if swallowed and enters airways
H310: Fatal in contact with skin
H311: Toxic in contact with skin
H312: Harmful in contact with skin
H313: May be harmful in contact with skin
H314: Causes severe skin burns and eye damage
H315: Causes skin irritation
H316: Causes mild skin irritation
H317: May cause an allergic skin reaction
H318: Causes serious eye damage
H319: Causes serious eye irritation
H320: Causes eye irritation
H330: Fatal if inhaled
H331: Toxic if inhaled
H332: Harmful if inhaled
H333: May be harmful if inhaled
H334: May cause allergy or asthma symptoms or breathing difficulties if inhaled
H335: May cause respiratory irritation
H336: May cause drowsiness or dizziness
H340: May cause genetic defects
H350: May cause cancer
H351: Suspected of causing cancer
H360: May damage fertility or the unborn child
H361: Suspected of damaging fertility or the unborn child
H361d: Suspected of damaging the unborn child
H362: May cause harm to breast-fed children
H370: Causes damage to organs
H371: May cause damage to organs
H372: Causes damage to organs through prolonged or repeated exposure
H373: May cause damage to organs through prolonged or repeated exposure
H400: Very toxic to aquatic life

H402: Harmful to aquatic life

H410: Very toxic to aquatic life with long-lasting effects

H411: Toxic to aquatic life with long-lasting effects

H412: Harmful to aquatic life with long-lasting effects

H413: May cause long-lasting harmful effects to aquatic life

EU Supplementary Hazard Statements List:

EUH014: Reacts violently with water.

EUH019: May form explosive peroxides.

EUH029: Contact with water liberates toxic gas.

EUH066: Repeated exposure may cause skin dryness or cracking.

8.1.4 Precautionary Statements

P1xx: general precautionary statement;

P2xx: prevention precautionary statement;

P3xx: response precautionary statement;

P4xx: storage precautionary statement;

P5xx: disposal precautionary statement;

P201: Obtain special instructions before use.

P202: Do not handle until all safety precautions have been read and understood.

P210: Keep away from heat/sparks/open flames/hot surfaces. - No smoking.

P220: Keep/Store away from clothing/.../combustible materials.

P221: Take any precaution to avoid mixing with combustibles...

P223: Keep away from any possible contact with water, because of violent reaction and possible flash fire.

P231: Handle under inert gas.

P232: Protect from moisture.

P233: Keep container tightly closed.

P235: Keep cool.

P240: Ground/bond container and receiving equipment.

P243: Take precautionary measures against static discharge.

P260: Do not breathe dust/fume/gas/mist/vapours/spray.

P261: Avoid breathing dust/fume/gas/mist/vapours/spray.

P264: Wash … thoroughly after handling.

P270: Do not eat, drink or smoke when using this product.

P271: Use only outdoors or in a well-ventilated area.

P272: Contaminated work clothing should not be allowed out of the workplace.

P273: Avoid release to the environment.

P280: Wear protective gloves/protective clothing/eye protection/face protection.

P281: Use personal protective equipment as required.

P231 + P232: Handle under inert gas. Protect from moisture.

P301: IF SWALLOWED:

Attachment

P302: IF ON SKIN:

P303: IF ON SKIN (or hair):

P304: IF INHALED:

P305: IF IN EYES:

P308: IF exposed or concerned:

P309: IF exposed or if you feel unwell:

P310: Immediately call a POISON CENTER or doctor/physician.

P311: Call a POISON CENTER or doctor/physician.

P312: Call a POISON CENTER or doctor/physician if you feel unwell.

P313: Get medical advice/attention.

P314: Get medical advice/attention if you feel unwell.

P315: Get immediate medical advice/attention.

P320: Specific treatment is urgent (see ... on this label).

P321: Specific treatment (see ... on this label).

P322: Specific measures (see ... on this label).

P330: Rinse mouth.

P331: Do NOT induce vomiting.

P332: If skin irritation occurs:

P333: If skin irritation or rash occurs:

P334: Immerse in cool water/wrap in wet bandages.

P335: Brush off loose particles from skin.

P336: Thaw frosted parts with lukewarm water. Do no rub affected area.

P337: If eye irritation persists:

P338: Remove contact lenses, if present and easy to do. Continue rinsing.

P340: Remove victim to fresh air and keep at rest in a position comfortable for breathing.

P350: Gently wash with plenty of soap and water.

P351: Rinse cautiously with water for several minutes.

P352: Wash with plenty of soap and water.

P353: Rinse skin with water/shower.

P360: Rinse immediately contaminated clothing and skin with plenty of water before removing clothes.

P361: Remove/Take off immediately all contaminated clothing.

P362: Take off contaminated clothing and wash before reuse.

P363: Wash contaminated clothing before reuse.

P370: In case of fire:

P371: In case of major fire and large quantities:

P372: Explosion risk in case of fire.

P373: DO NOT fight fire when fire reaches explosives.

P378: Use ... for extinction.

P391: Collect spillage.

P301 + P310: IF SWALLOWED: Immediately call a POISON CENTER or doctor/physician.

P301 + P312: IF SWALLOWED: Call a POISON CENTER or doctor/physician if you feel unwell.

Attachment

P301 +P330 + P331: IF SWALLOWED: rinse mouth. Do NOT induce vomiting.

P302 + P334: IF ON SKIN: Immerse in cool water/wrap in wet bandages.

P302 + P350: IF ON SKIN: Gently wash with plenty of soap and water.

P302 + P352: IF ON SKIN: Wash with plenty of soap and water.

P303 + P361 + P353: IF ON SKIN (or hair): Remove/Take off immediately all contaminated clothing. Rinse skin with water/shower.

P304 + P340: IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing.

P304 + P341: IF INHALED: If breathing is difficult, remove victim to fresh air and keep at rest in a position comfortable for breathing.

P305 + P351 + P338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

P308 + P313: IF exposed or concerned: Get medical advice/attention.

P309 + P311: IF exposed or if you feel unwell: Call a POISON CENTER or doctor/physician.

P332 + P313: If skin irritation occurs: Get medical advice/attention.

P333 + P313: If skin irritation or rash occurs: Get medical advice/attention.

P335 + P334: Brush off loose particles from skin. Immerse in cool water/wrap in wet bandages.

P337 + P313: If eye irritation persists: Get medical advice/attention.

P370 + P376: In case of fire: Stop leak if safe to do so.

P370 + P378: In case of fire: Use ... for extinction.

P370 + P380: In case of fire: Evacuate area.

P370 + P380 + P375: In case of fire: Evacuate area. Fight fire remotely due to the risk of explosion.

P371 + P380 + P375: In case of major fire and large quantities: Evacuate area. Fight fire remotely due to the risk of explosion.

P402: Store in a dry place.

P403: Store in a well-ventilated place.

P404: Store in a closed container.

P405: Store locked up.

P410: Protect from sunlight.

P411: Store at temperatures not exceeding ... oC/...oF.

P412: Do not expose to temperatures exceeding 50 oC/122oF.

P413: Store bulk masses greater than ... kg/... lbs at temperatures not exceeding ... oC/...oF.

P420: Store away from other materials.

P403 + P233: Store in a well-ventilated place. Keep container tightly closed.

P403 + P235: Store in a well-ventilated place. Keep cool.

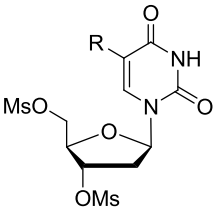
P410 + P403: Protect from sunlight. Store in a well-ventilated place.

P410 + P412: Protect from sunlight. Do no expose to temperatures exceeding 50 celcius degress.

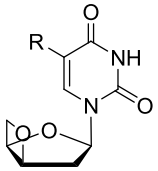
P411 + P235: Store at temperatures not exceeding ...Keep cool.

P501: Dispose of contents/container to...

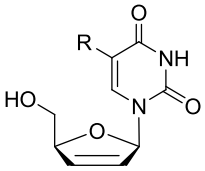
8.2 Overview of the Compound Structures



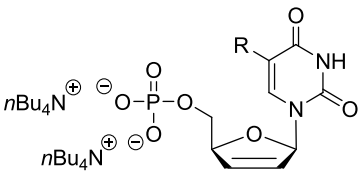
52a: R = CH_3
52b: R = H



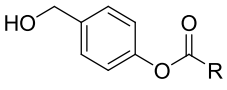
53a: R = CH_3
53b: R = H



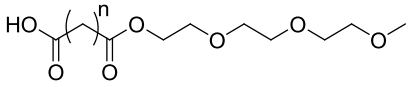
4: R = CH_3
54: R = H



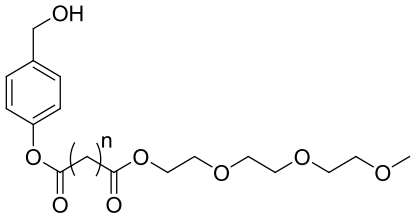
4m: R = CH_3
54m: R = H



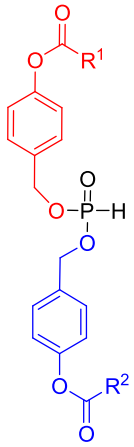
44b: R = CH_3
44c: R = C_2H_5
44e: R = *n*-C_4H_9
44ei: R = *iso*-C_4H_9
44g: R = *n*-C_6H_{13}
44o: R = *n*-$C_{14}H_{29}$
44u: R = *n*-$C_{15}H_{31}$
44w: R = *n*-$C_{17}H_{35}$



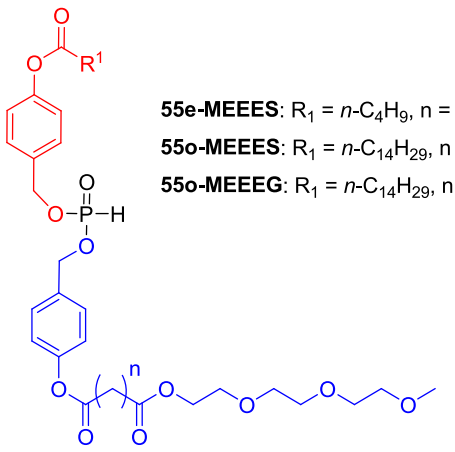
48b: n = 2
48c: n = 3



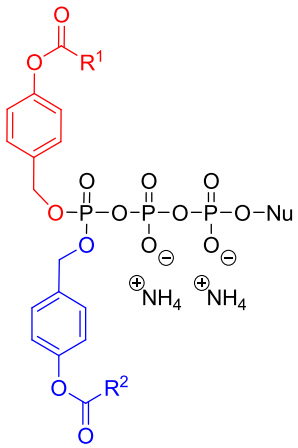
50b: n = 2
50c: n = 3



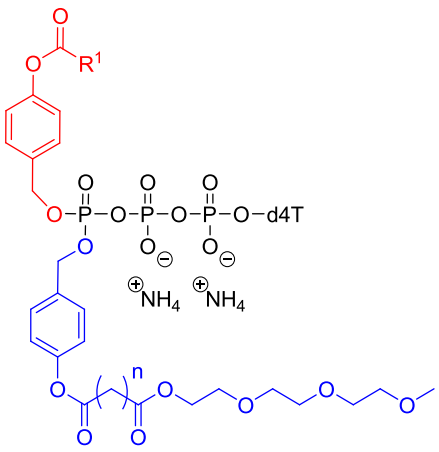
55co: R_1 = C_2H_5, R_2 = *n*-$C_{14}H_{29}$
55eo: R_1 = *n*-C_4H_9, R_2 = *n*-$C_{14}H_{29}$
55go: R_1 = *n*-C_6H_{13}, R_2 = *n*-$C_{14}H_{29}$
55eio: R_1 = *iso*-C_4H_9, R_2 = *n*-$C_{14}H_{29}$
55eu: R_1 = *n*-C_4H_9, R_2 = *n*-$C_{15}H_{31}$
55ew: R_1 = *n*-C_4H_9, R_2 = *n*-$C_{17}H_{35}$
55uu: R_1 = *n*-$C_{15}H_{31}$, R_2 = *n*-$C_{15}H_{31}$



55e-MEEES: R_1 = *n*-C_4H_9, n = 2
55o-MEEES: R_1 = *n*-$C_{14}H_{29}$, n = 2
55o-MEEEG: R_1 = *n*-$C_{14}H_{29}$, n = 3

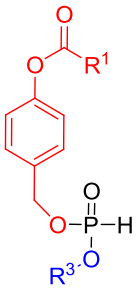


56co: Nu = d4T, R_1 = C_2H_5, R_2 = *n*-$C_{14}H_{29}$
56eo: Nu = d4T, R_1 = *n*-C_4H_9, R_2 = *n*-$C_{14}H_{29}$
56go: Nu = d4T, R_1 = *n*-C_6H_{13}, R_2 = *n*-$C_{14}H_{29}$
56eio: Nu = d4T, R_1 = *iso*-C_4H_9, R_2 = *n*-$C_{14}H_{29}$
56eu: Nu = d4T, R_1 = *n*-C_4H_9, R_2 = *n*-$C_{15}H_{31}$
56ew: Nu = d4T, R_1 = *n*-C_4H_9, R_2 = *n*-$C_{17}H_{35}$
56uu: Nu = d4U, R_1 = *n*-$C_{15}H_{31}$, R_2 = *n*-$C_{15}H_{31}$

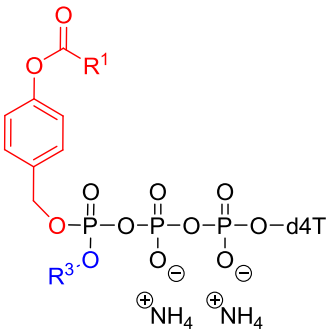


56e-MEEES: R_1 = *n*-C_4H_9, n = 2
56o-MEEES: R_1 = *n*-$C_{14}H_{29}$, n = 2
56o-MEEEG: R_1 = *n*-$C_{14}H_{29}$, n = 3

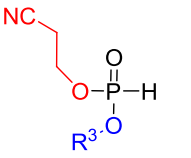
Attachment



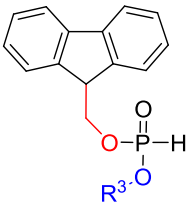
57ed: R_1 = *n*-C_4H_9, R_3 = *n*-C_4H_9
57ud: R_1 = *n*-$C_{15}H_{31}$, R_3 = *n*-C_4H_9
57bo: R_1 = CH_3, R_3 = *n*-$C_{15}H_{31}$
57eo: R_1 = *n*-C_4H_9, R_3 = *n*-$C_{15}H_{31}$
57uo: R_1 = *n*-$C_{15}H_{31}$, R_3 = *n*-$C_{15}H_{31}$
57br: R_1 = CH_3, R_3 = *n*-$C_{18}H_{37}$
57cr: R_1 = C_2H_5, R_3 = *n*-$C_{18}H_{37}$
57er: R_1 = *n*-C_4H_9, R_3 = *n*-$C_{18}H_{37}$
57gr: R_1 = *n*-C_6H_{13}, R_3 = *n*-$C_{18}H_{37}$



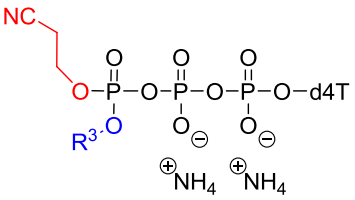
58ed: R_1 = *n*-C_4H_9, R_3 = *n*-C_4H_9
58ud: R_1 = *n*-$C_{15}H_{31}$, R_3 = *n*-C_4H_9
58bo: R_1 = CH_3, R_3 = *n*-$C_{15}H_{31}$
58eo: R_1 = *n*-C_4H_9, R_3 = *n*-$C_{15}H_{31}$
58uo: R_1 = *n*-$C_{15}H_{31}$, R_3 = *n*-$C_{15}H_{31}$
58br: R_1 = CH_3, R_3 = *n*-$C_{18}H_{37}$
58cr: R_1 = C_2H_5, R_3 = *n*-$C_{18}H_{37}$
58er: R_1 = *n*-C_4H_9, R_3 = *n*-$C_{18}H_{37}$
58gr: R_1 = *n*-C_6H_{13}, R_3 = *n*-$C_{18}H_{37}$



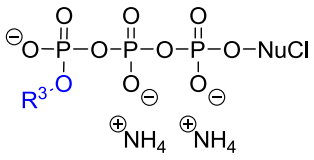
59d: R^3 = -C_4H_9
59k: R^3 = -$C_{11}H_{23}$
59r: R^3 = -$C_{18}H_{37}$



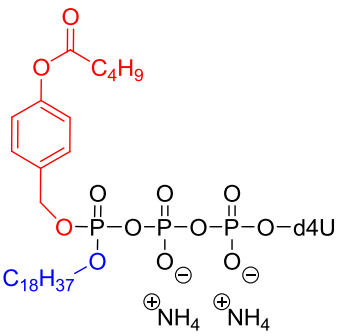
63d: R^3 = -C_4H_9



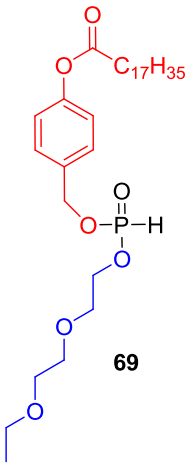
61r: R^3 = -$C_{18}H_{37}$



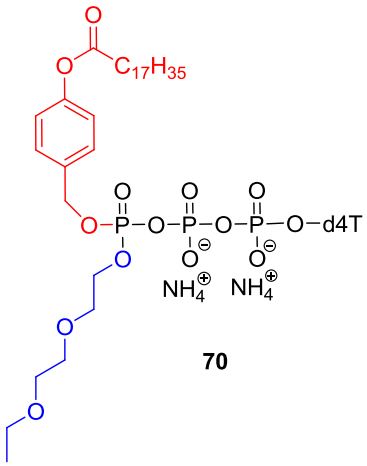
60d: R^3 = -C_4H_9, NuCl = d4T
60k: R^3 = -$C_{11}H_{23}$, NuCl = d4T
60r: R^3 = -$C_{18}H_{37}$, NuCl = d4T
62d: R^3 = -C_4H_9, NuCl = dT
62r: R^3 = -$C_{18}H_{37}$, NuCl = dT



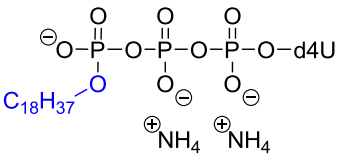
64er



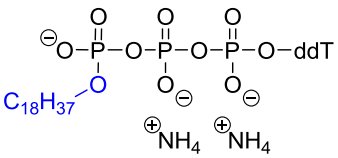
69



70



65r



67r

8.3 Curriculum Vitae

Personal data:	M. Sc. Chenglong Zhao Born on 21.10.1988 in Xi´an, China
Education:	**Ph.D. study (2014-2018)**
	Institute of Organic Chemistry, MIN Department University of Hamburg. Supervisor: Prof. Dr. Chris Meier.
	Master study (2011-2014)
	Institute of Organic Chemistry, Science Department University of Shanghai. Supervisor: Prof. Dr. Hegui Gong.
	Bachelor study (2006-2010)
	Institute of Organic Chemistry, College of Science (now College of Chemistry & Pharmacy) Northwest Agriculture & Forestry University. Supervisor: Prof. Dr. Wenming Zhou.
Patent:	Nucleoside Triphosphate and Nucleoside Triphosphate analogue prodrugs. Bibliographic data: WO2018100137(A1)-2018-06-07; LU2016-93331 2016-12-02.
Conference:	31st International Conference on Antiviral Research (ICAR). 2018, Porto Poster and presentation: Gamma-Non-Symmetrically-Modified d4T Triphosphate as Anti-HIV Prodrugs.
Awards:	2nd Poster awards in the graduate student category in 2018 ICAR.

8.4 Publication List

Patent: Nucleoside Triphosphate and Nucleoside Triphosphate analogue prodrugs. Bibliographic data: WO2018100137(A1)-2018-06-07; LU2016-93331 2016-12-02.

8.5 Eidesstattliche Versicherung

Hiermit erkläre ich an Eides statt, dass ich die vorliegende Dissertationsschrift ``Non-Symmetrically-Masked Tri*PPP*ro Prodrugs and γ-Modified Nucleoside Triphosphate Compounds as Potential Antivirals against HIV´´ selbst verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel benutzt habe.

I hereby declare on oath, that I have written the present dissertation ``Non-Symmetrically-Masked Tri*PPP*ro Prodrugs and γ-Modified Nucleoside Triphosphate Compounds as Potential Antivirals against HIV´´ by my own and have not used other than the acknowledged resources and aids.

_____ _____

Ort, Datum M.Sc. Chenglong Zhao