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Membrane permeable, bioreversibly modified Prodrugs of Nucleoside Diphosphate-γ-phosphonates

*Xiao Jia,[1] Stefan Weber,[1] Dominique Schols,[2] Chris Meier[1]**

[1]Organic Chemistry, Department of Chemistry, Faculty of Mathematics, Informatics and Natural Sciences, Universität Hamburg, Martin-Luther-King-Platz 6, D-20146 Hamburg, Germany.

[2]Laboratory of Virology and Chemotherapy, Department of Microbiology and Immunology and Transplantation, Rega Institute for Medical Research, KU Leuven, Herestraat 49, B-3000 Leuven, Belgium.

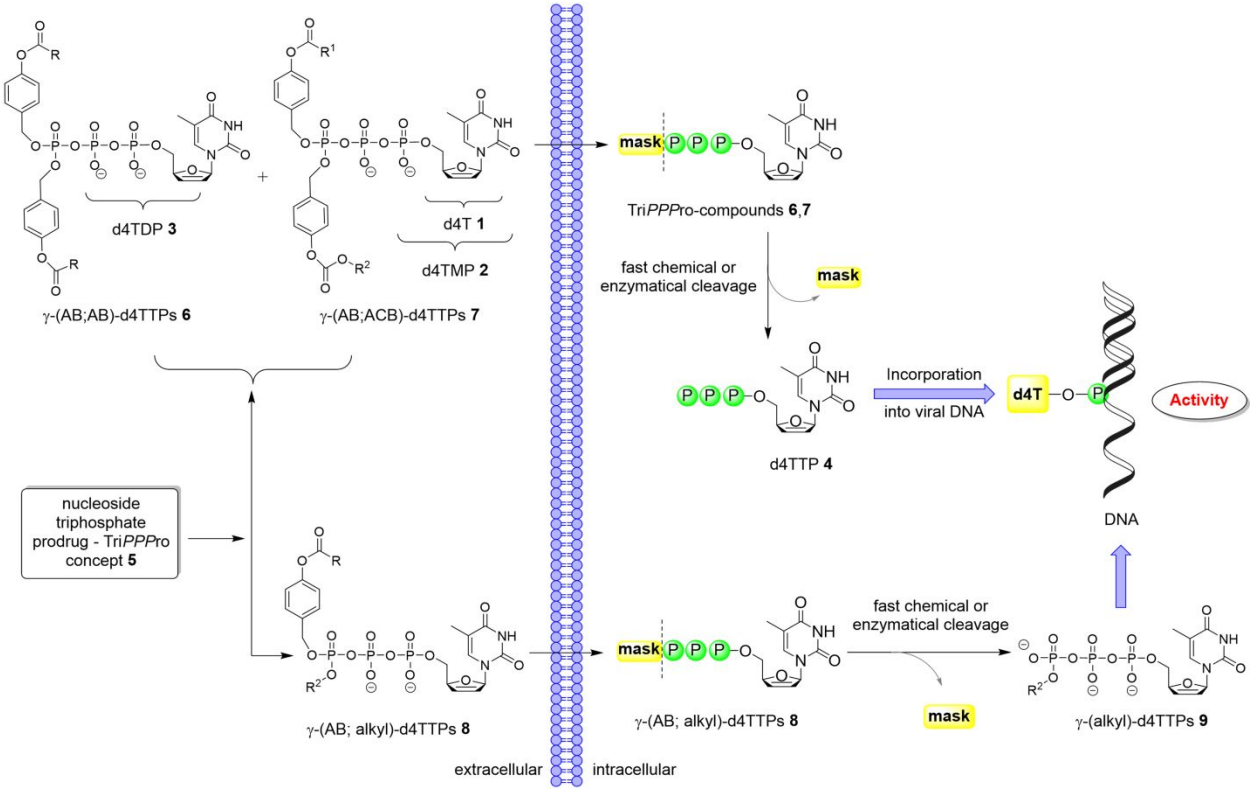
ABSTRACT Nucleoside reverse transcriptase inhibitors (NRTIs) are widely used as antiviral and anticancer agents although they require intracellular phosphorylation into their antivirally active form, the triphosphorylated nucleoside analogue metabolites. We report on the synthesis and characterisation of a new class of nucleoside triphosphate analogues comprising a C-alkyl-phosphonate moiety replacing the γ-phosphate. These compounds were converted into bioreversibly modified lipophilic prodrugs at the γ-phosphonate by the attachment of an acyloxybenzyl- (AB; ester) or an alkoxycarbonyloxybenzyl- (ACB; carbonate) group. Such compounds formed γ-C-(alkyl)-nucleoside triphosphate analogues with high selectivity due to an enzyme-triggered delivery mechanism. The later compounds were very stable in CEM cell extracts and they were substrates for HIV-RT without being substrates for DNA-polymerases α, β and γ. In antiviral assays, excellent antiviral activity of the prodrugs was found in CEM/0 cells was completely kept in CEM/TK$^-$ cells. The activity was improved by 3 logs as compared to the parent nucleoside d4T.

Introduction

Nucleoside analogues play an important role in combatting virus infections as can be seen in their impressive potency against HIV, herpes virus, influenza, hepatitis B and hepatitis C and since very recently SARS-CoV-2.[1-5] Generally, they target the viral RNA- or DNA-polymerase needed for virus replication. Today, several nucleoside analogues are approved as HIV reverse transcriptase (RT)[2,3] inhibitors (NRTIs)[6] blocking RT's enzymatic function in the early phase of the infection and prevent completion of the synthesis of the viral DNA, act as chain terminators of DNA synthesis, thus preventing HIV from replicating. The efficacy of antiviral or antitumor active nucleoside analogues is highly dependent on the efficient intracellular conversion by cellular kinases to yield ultimately the bioactive nucleoside analogue triphosphate derivatives (NTP)[7,8] which block the function of the viral DNA polymerase. Former studies have shown that cellular kinases often perform these transformations ineffectively, resulting in low or no antiviral activity of the corresponding nucleoside analogue,[8-10] and insufficient phosphorylation may led to adverse effects.[11,12] Within the stepwise biotransformation process, often the initial phosphorylation step yielding the nucleoside monophosphate (NMP) performed by the salvage pathway enzyme thymidine kinase (TK) was identified as limiting step, for example, for the NRTI 3'-deoxy-2',3'-dehydrothymidine (d4T **1**; Scheme 1) and the formation of its monophosphate metabolite (d4TMP **2**). Moreover, the clinical efficacy of such drugs is adversed by further limitations such as poor biological half-lives, mutations of nucleoside transporters, unsuitable bioavailability after oral administration, or drug resistant virus strain development.[13-15] Prodrug strategies to overcome these problems resulted in orally administrable forms of a few antiviral nucleoside monophosphates. Others are currently under ongoing development.[10,16-25]

Two recent examples of a very successful nucleoside monophosphate prodrug are the anti-HCV active drug sofosbuvir[26-30] and Remdesivir.[30-32]

Recently, we reported on an technology to deliver nucleoside diphosphates (NDPs) to cells (Di*PP*ro-approach).[33-37] However, although both approaches deliver the mono- or the diphosphorylated derivatives of the nucleosides, respectively, the delivered nucleotides need further phosphorylation into the triphosphate forms in order to block the viral replication process. As a consequence, the development of nucleoside triphosphate (NTP) prodrugs is still highly desirable (Tri*PPP*ro-concept **5**; Scheme 1) because *all* phosphorylation steps from the nucleoside analogue to the nucleoside triphosphate are bypassed.[38] Thus, this approach should principle optimise the intracellular amount of the antivirally active NTP.



Scheme 1: Cell uptake and metabolism of Tri*PPP*ro-d4TTP prodrugs.

Despite these obvious advantages, almost no reports on attempts to design triphosphate prodrugs have been disclosed.[39,40] However, the significant challenges should not be overlooked : (a) a triphosphate is highly negatively charged and thus requires extensive masking to produce a lipophilic compound, (b) the intrinsic lability of the triphosphate moiety comprising reactive phosphate anhydride linkages, (c) the requirement for the NTP to act selectively as a substrate for viral polymerases and not for cellular polymerases, and (d) the risk of low stability of the delivered NTP toward phosphatases/kinases leading to dephosphorylation and thus clearance of the bioactive metabolite. Therefore, it was in the past common sense that the design of nucleoside triphosphate prodrugs would be almost impossible.[41]

We disclosed recently a unique pronucleotide approach based on partially masking the negative charges and improving the lipophilicity of nucleoside triphosphates, namely, the Tri*PPP*ro-approach **5**.[42-47] At first, Tri*PPP*ro-compounds **6** comprising two lipophilic and bioreversible acyloxybenzyl- (AB) masking groups at the γ-phosphate and d4T **1** as a nucleoside analogue were disclosed.[42] The successful formation of a nucleoside triphosphate inside cells was proven in a celluptake study involving fluorescent nucleoside analogues.[43] Recently, we also reported on non-symmetric Tri*PPP*ro-d4TTPs **7**.[45] In the case of Tri*PPP*ro-compounds **7**, an enzymatic break-down of the Tri*PPP*ro-derivatives to yield d4TTP was finally achieved in CEM cell extracts. Tri*PPP*ro-d4TTPs **6**,**7** retained their anti-HIV activity in CEM/TK$^-$ cell assays whereas the parent d4T **1** was inactive due to the lack of cytosolic monophosphorylating enzyme TK. The Tri*PPP*ro-strategy was also used to convert so-far inactive nucleoside analogues into biologically active metabolites.[43,46] Both compounds **6**,**7** were rapidly hydrolysed in cell extracts. However, it was very difficult to detect d4TTP **4** because of its relatively fast enzymatic

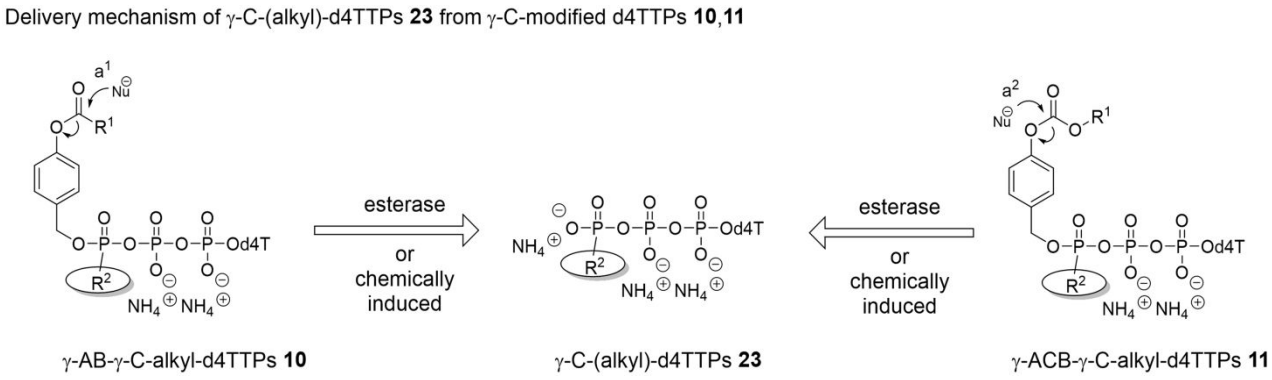
dephosphorylation ($t_{1/2}$ = 38 min) by phosphorylases/kinases to form d4TDP **3** first and ultimately d4TMP **2**.[42,45]

It should be added that we proved that the γ-double bioreversibly masked Tri*PPP*ro-compounds **6** did not act as substrates for DNA polymerases, e.g HIV-RT or DNA pol β.[42] In contrast, recently we have shown that NTP analogues such as γ-alkylketobenzyl-d4TTP and γ-(alkyl-C18)-d4TTPs **9** that comprised one non-hydrolyzable lipophilic alkyl group were substrates for HIV-RT and d4TMP **2** was incorporated in a primer extension assay into the primer strand while these compounds were non-substrates for cellular DNA polymerases α, β and γ.[47] More importantly, prodrug versions of compounds **9** like γ-(AB;alkyl)-d4TTPs **8** showed even higher antiviral activity against HIV-2 in thymidine kinase-deficient cell assays (CEM/TK$^-$ cells). Further studies concerning the antiviral activity showed that also γ-(alkyl-C18)-d4TTP **9** was a potent compound with EC_{50} values 3logs lower (EC_{50}: 0.05 μM) as compared to d4T **1** and in contrast to d4TTP **4**, γ-(alkyl-C18)-d4TTP **9** proved to be very highly stable in cell extracts towards dephosphorylation.[47]

Earlier studies showed that the carbonate Tri*PPP*ro-compounds **7** and intermediates were more stable as compared to Tri*PPP*ro-compounds **6** and intermediates, respectively.[42,45] The above summarized results motivated us to develop a new series of γ-C-alkylphosphonate-d4TDP prodrugs **10**,**11**, bearing γ-C-*n*-alkyl chains of different length combined with an acyloxybenzyl-(AB; ester) or an alkoxycarbonyloxybenzyl- (ACB; carbonate) moiety (Scheme 2). We expected that such a design would lead to a rapid conversion of the Tri*PPP*ro-compounds **10**,**11** (Scheme 2) into γ-C-(alkyl)-d4TTPs **23** and thereby should avoid a side reaction which we have observed from prodrugs **6**-**8** that led to the formation of (although small amounts) of d4TMP **2** or d4TDP **3**. Moreover, the introduction of a P-C-bond in **10**,**11** instead of a P-O-bond in **6**,**7** will lead to a

chemically and enzymatically stable compound because P-C-bond are not hydrolysed. This will then also lead to metabolically very resistant nucleoside triphosphate analogues.

We report here on the synthesis of these γ-C-modified d4TTP prodrugs **10**,**11** as well as their hydrolysis products γ-C-(alkyl)-d4TTPs **23**. The hydrolysis characteristics in phosphate buffer (PB, pH 7.3), pig liver esterase (PLE), human $CD4^+$ T-lymphocyte CEM cell extracts and the anti-HIV activity against HIV-1- and HIV-2 in infected wild-type CEM/0 cell cultures and in HIV-2-infected mutant thymidine kinase-deficient CEM/TK^- cell cultures will be described. Primer extension assays were conducted studying the substrate properties of such γ-C-(alkyl)-d4TTPs **23** for four different DNA-polymerases.



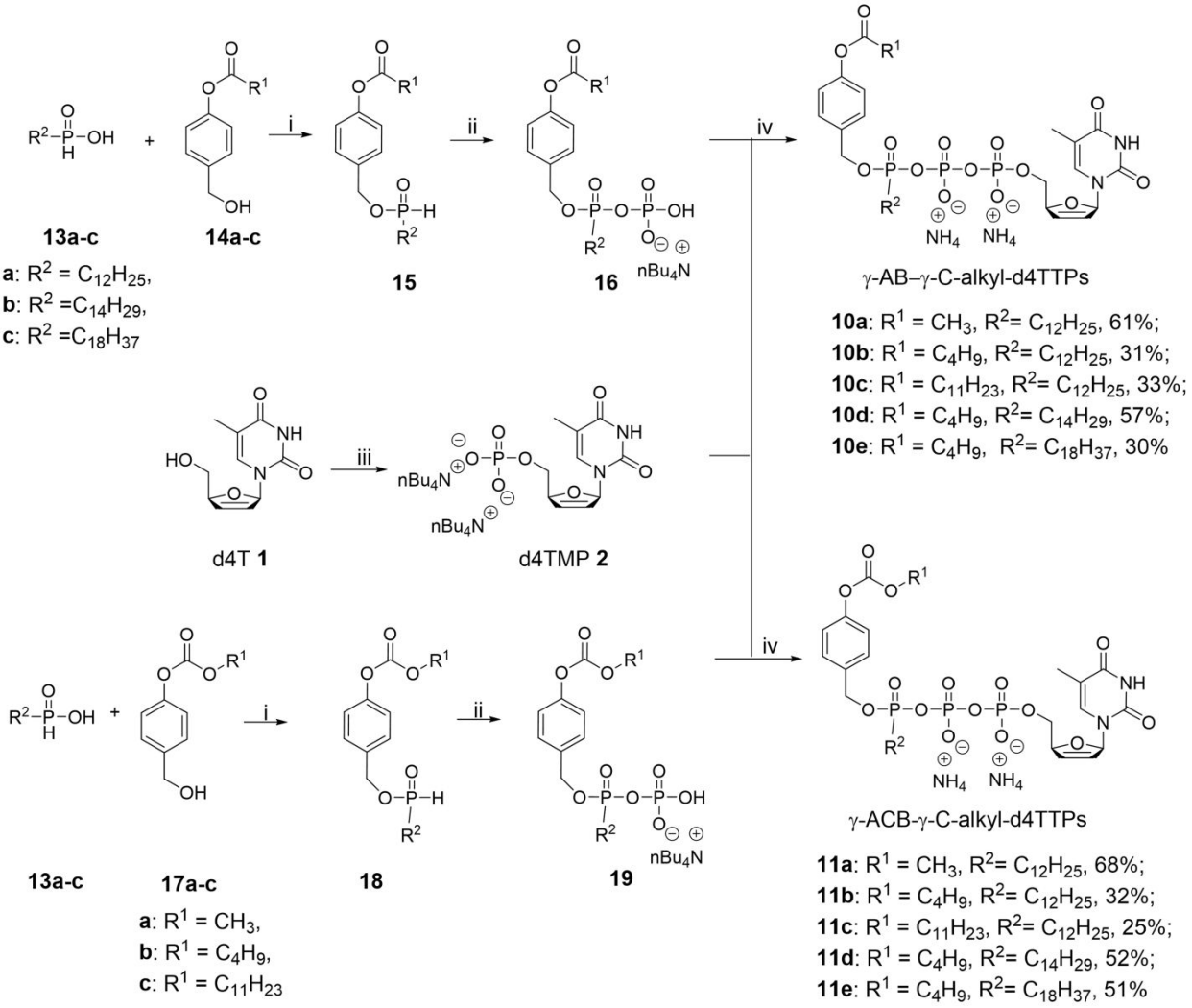
Scheme 2: γ-AB-γ-C-alkyl-d4TTPs **10** and γ-ACB-γ-C-alkyl-d4TTPs **11** and the cleavage pathways leading to γ-C-alkyl-d4TTPs **23**.

Results and Discussion

Synthesis of γ-acyloxybenzyl (AB)-γ-C-alkyl-d4TTPs 10 and γ-alkyloxycarbonyloxybenzyl (ACB)-γ-C-alkyl-d4TTPs 11.

γ-AB-γ-C-alkyl-d4TTPs **10** were synthesized using *H*-phosphinate chemistry. D4TMP **2** was prepared from d4T **1** as described before.[48] *H*-Phosphinates **15** were synthesized from *H*-phosphinic acids **13**, 4-dimethylaminopyridine (DMAP), 1-(3-dimethylaminopropyl)-3-

ethylcarbodiimide (EDC) and 4-acyloxybenzyl alcohols **14** in yields between 48% and 85%. Next, *H*-phosphinates **15** were reacted to give phosphonochloridates by an oxidative chlorination with *N*-chlorosuccinimide (NCS).[49] Subsequent phosphorylation with tetra-*n*-butylammonium phosphate yielded phosphonate-phosphates **16** in almost quantitative yields. Due to chemical instability, compounds **16** were rapidly purified by extraction and immediately used in the next step. The final coupling reaction was achieved by an activation of compounds **16** with trifluoroacetic acid anhydride (TFAA) and *N*-methylimidazole,[50,51] followed by the reaction with d4TMP **2** to give γ-AB-γ-C-alkyl-d4TTPs **10** (*n*-Bu_4N^+ form). γ-AB-γ-C-alkyl-d4TTPs **10** (NH_4^+ counterion) were obtained as colorless solids after reversed-phase (rp) column chromatography (18-68% yield), a Dowex 50WX8 (NH_4^+) ion exchange and freeze-drying. A series of γ-ACB-γ-C-alkyl-d4TTPs **11** was also prepared using the identical approach (Scheme 3). Using this *H*-phosphinate pathway, the bond between the α- and the β-phosphate was formed without the need of a subsequent oxidation after forming the P-O-P-linkage, which was in agreement with the *H*-phosphonate synthesis sequence reported before.[43] Thus, also oxidation sensitive nucleoside analogues or masking units might be used in the future. Target compounds **10** and **11** were isolated as diastereomeric mixtures. These diastereomeric mixtures were inseparable by chromatography and generally indistinguishable in the ^{31}P-NMR spectra.

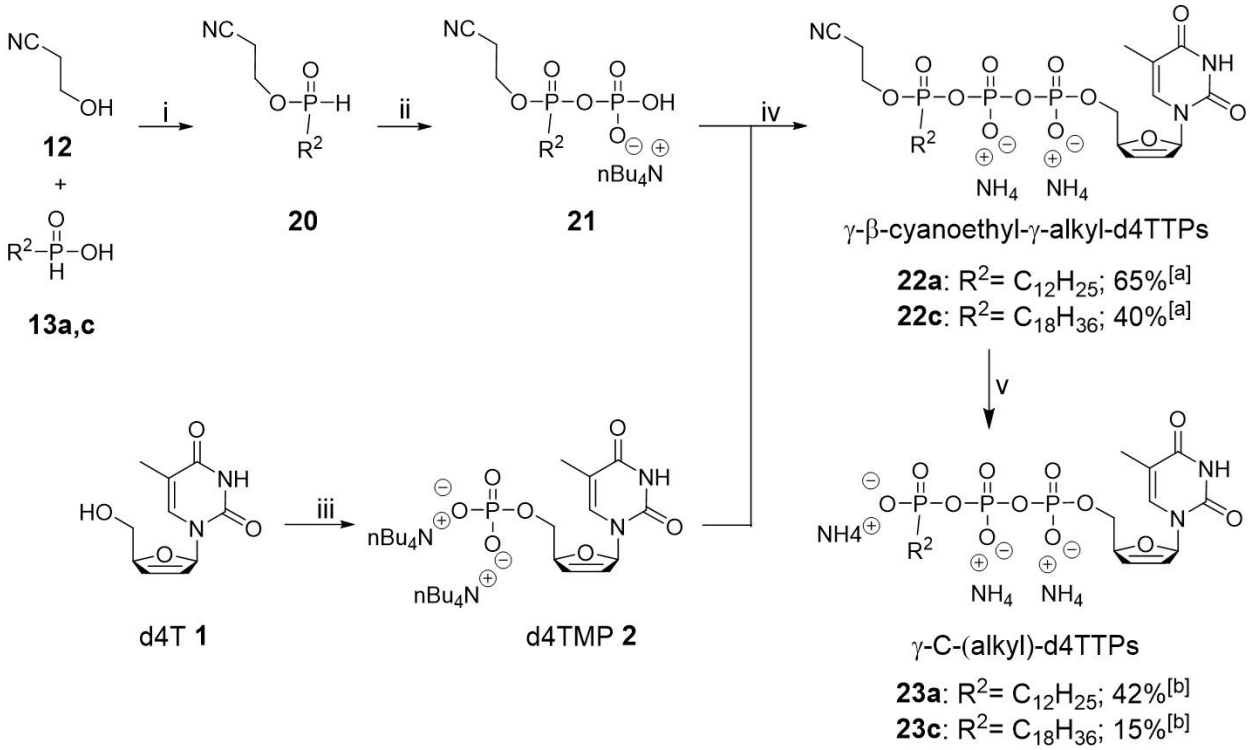


Scheme 3. Reagents and conditions: i) EDC, DMAP, CH_2Cl_2, rt, 12 h; ii) a. NCS, CH_3CN, rt, 2 h, b) $N(Bu)_4(H_2PO_4)$, CH_3CN, rt, 1 h; iii) d4T **1**, $POCl_3$, pyridine, H_2O, CH_3CN, 0 °C-rt, 5 h; iv) a. TFAA, Et_3N, CH_3CN, 0 °C, 10 min, b. 1-methylimidazole, Et_3N, CH_3CN, 0 °C-rt, 10 min, c. d4TMP **2**, rt, 2 h.

Synthesis of γ-(β-cyanoethyl)-γ-C-alkyl-d4TTPs 22 and γ-C-(alkyl)-d4TTPs 23.

As concluded from previous studies,[45,47] a suitable access to γ-C-(alkyl)-d4TTPs **23** was achieved starting from γ-(β-cyanoethyl)-γ-C-alkyl-d4TTPs **22** and treatment with tetra-*n*-butylammonium phosphate. Thus, γ-(β-cyanoethyl)-γ-C-alkyl-d4TTPs **22** were synthesized by the *H*-phosphinate route. The β-cyanoethyl group was included here as protective group at the γ-

phosphonate moiety. After final coupling of compounds **21** and d4TMP **2**, leading to γ-(β-cyanoethyl)-γ-C-alkyl-d4TTPs **22** (*n*-Bu_4N^+ form), the crude product was stirred in a mixture of 40% $nBu_4N^+OH^-$ (water solution) and CH_3CN at rt for 8h to obtain products **23** (*n*-Bu_4N^+ form). After ion exchange (Dowex 50WX8 (NH_4^+)), followed by a rp-column chromatography and freeze-drying, the γ-C-(alkyl)-d4TTPs **23** (NH_4^+ form) were isolated as colorless solids. The obtained yields for the conversions of d4TMP **2** to compounds **22** varied between 40% to 65% and γ-C-(alkyl)-d4TTPs **23** were isolated in 15% to 42% yield.



Scheme 4. Reagents and conditions: i) EDC, DMAP, CH_2Cl_2, rt, 12 h; ii) a. NCS, CH_3CN, rt, 2 h, b) $N(Bu)_4(H_2PO_4)$, CH_3CN, rt, 1 h; iii) d4T **1**, $POCl_3$, pyridine, H_2O, CH_3CN, 0 °C-rt, 5 h; iv) a. TFAA, Et_3N, CH_3CN, 0 °C, 10 min, b. 1-methylimidazole, Et_3N, CH_3CN, 0 °C-rt, 10 min, c. d4TMP **2**, rt, 2 h. v) *n*-$Bu_4N^+OH^-$, 8h, Dowex 50WX8 (NH_4^+ form) ion exchange. [a] yields are

calculated for the conversion from **20** to **22**. [b] yields are calculated for the conversion from **20** to **23**.

Stability studies

Previously we reported how nucleotides were released from different Tri*PPP*ro-compounds.[42,45] In the case of compounds **6**,**7**, the enzymatically-catalyzed cleavage of both bioreversible masks led to d4TTP **4** formation which is subsequently dephosphorylated to yield d4TDP. However, in the metabolism of compounds **8**, a predominate formation of γ-(alkyl)-d4TTPs **9** companied by a very small amount of d4TDP **3** was detected.[47] As expected, no d4TTP formation was observed in these studies using compounds **8**. Prior to the antiviral evaluation of compounds **10**,**11**,**22**,**23**, we verified their chemical or enzyme-triggered nucleotide release. The determined half-lives of prodrugs **10**,**11** (Table 1, $t_{1/2}$) reflect the removal of the bioreversible AB-group or the ACB-group, respectively, to give γ-C-(alkyl)-d4TTPs **23**. All mixtures were analyzed by means of analytical RP18-HPLC.

a. Chemical stability in aqueous phosphate buffer (PB, pH 7.3)

Tri*PPP*ro-compounds **10**,**11**,**22**,**23** were incubated in aqueous phosphate buffer (PB, 25 mM, pH 7.3) to study their stability and the product distribution. The stability of Tri*PPP*ro-d4TTP prodrugs **10a-c** (AB:C1-C11;alkyl-C12), **11a-c** (ACB:C1-C11;alkyl-C12) increased with increasing alkyl chain lengths (Table 1). As expected from previous work, the half-lives for the carbonate compounds γ-ACB-γ-C-alkyl-d4TTPs **11** were higher than the corresponding ester derivatives γ-AB-γ-C-alkyl-d4TTPs **10**. Indeed, these compounds were more stable as compared to the studies of Tri*PPP*ro-d4TTPs **6**,**7**.[42,45] Compared to previously studied γ-(AB-C4;alkyl-C18)-d4TTP **8** ($t_{1/2}$ = 237 h),[47] the half-life for γ-(AB-C4)-γ-C-(alkyl-C18)-d4TTP **10e** was also significantly higher by almost a factor of 3 ($t_{1/2}$ = 646 h, Table 1). Unexpectedly, the chemical

stability of compounds **23a**,**c** (alkyl:C12-C18) decreased with increasing alkyl chain lengths and the half-life of γ-C-(alkyl-C18)-d4TTP **23c** ($t_{1/2}$ = 535 h) was found to be lower than those of the prodrugs **10e** ($t_{1/2}$ = 646 h) or **11e** ($t_{1/2}$ = 1240 h), which was in contrast to the results as γ-(ACB)-d4TTPs reported before.[45]

As an example, the hydrolysis of γ-(AB-C4)-γ-C-(alkyl-C12)-d4TTP **10b** is shown in Figure 1. Clearly, as hydrolysis products starting from compound **10b** a predominant formation of γ-C-(alkyl-C12)-d4TTP **23a** (85%) and also a small amount of d4TDP **3** (12%) was detected, indicating an almost selective cleavage of the biodegradable AB group of compounds **10**. In PB, the cleavage of the AB group in compounds **10** or the ACB group in compounds **11** is initiated by an ester or carbonate hydrolysis and thus proceed similar to the previously published cleavage pathway for Tri*PPP*ro-d4TTPs **7**.[45] Due to the very long hydrolysis periods, side-reaction was observed which has been reported previously by us.[37,42,47] The nucleobase thymine was detected which was the result of the cleavage of the glycosidic bond in d4T. Therefore, prior to complete consumption of the starting material, an increase of thymine concentration was detected. In all cases, γ-C-(alkyl-C12)-d4TTP **23a** ($t_{1/2}$ > 1000 h) was mainly formed (between 84% (**11b**) and 96% (**11a**) and a very small amount of d4TMP **2** was detected (<4%) in chemical hydrolysis studies (Figure S1-S3; Supporting Information). Additionally, after complete conversion of the starting γ-(AB-C4)-γ-C-(alkyl-C12)-d4TTP **10b**, no increase of d4TDP **3** concentrations were detected which leading to the conclusion that γ-(AB-C4)-γ-C-(alkyl-C12)-d4TTP **10b** were prone to a breakage of the γ-phosphonate -β-phosphate bond. If an additional charge appears at the γ-phosphonate group due to the cleavage of the biodegradable masking moiety, the hydrolysis of the γ-phosphonate-β-phosphate anhydride bond is prevented. In the case of γ-(β-cyanoethyl)-γ-C-alkyl-d4TTPs **22** in PB, a marked selective cleavage of β-cyanoethyl moiety led to the

formation of γ-(alkyl)-d4TTPs **23** by β-elimination as can be seen in Figure S4-S5 (Supporting Information). It should also be noted that d4TTP **4** was not detected using **10**,**11**,**22**,**23**.

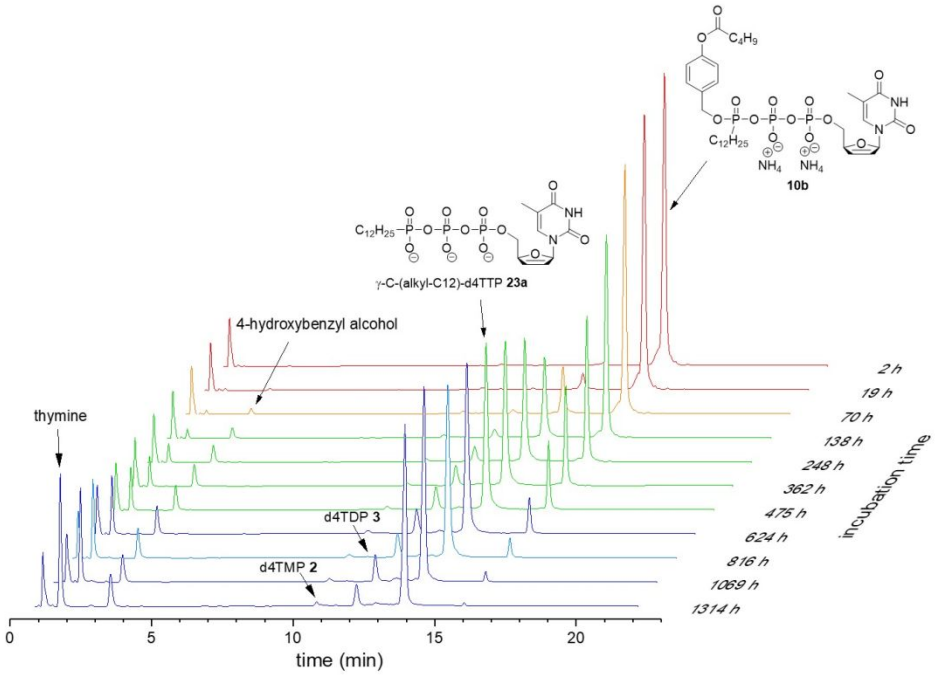


Figure 1: HPLC profile for γ-(AB-C4)-γ-C-(alkyl-C12)-d4TTP **10b** after incubation in PB (pH 7.3).

b. Hydrolysis study using pig liver esterase

Next, the enzymatic stability of Tri*PPP*ro-compounds **10**,**11** was studied by incubation with pig liver esterase (PLE) in phosphate buffer (pH 7.3) to confirm the γ-C-(alkyl)-d4TTPs (**23**) formation. Compared to the chemical hydrolyses, Tri*PPP*ro-compounds **10**,**11** were quickly hydrolyzed and delivered γ-C-alkyl-d4TTP derivatives **23** much faster than in PB, proving a significant contribution of the enzymatic cleavage (Table 1). In PLE, the stability of Tri*PPP*ro-compounds **10**,**11** was found to be between 0.009 h and 10.6 h. The enzymatic stability of

Tri*PPP*ro-d4TTP prodrugs **10b**,**10d**,**10e** (AB-C4;alkyl:C12,C14,C18), **11b**,**11d**,**11e** (ACB-C4;alkyl:C12,C14,C18), raised with increasing alkyl chain lengths (R^2).

The AB masking group cleavage in **10b** (AB-C4;alkyl-C12) occurred readily ($t_{1/2}$ = 0.79 min) leading selectively to γ-C-(alkyl-C12)-d4TTP **23a** (Figure 2). For γ-(AB-C4)-γ-C-(alkyl-C18)-d4TTP **10e** comprising a long, lipophilic alkyl chain, the half-life was found to be 2h, which was in agreement with the results from the studies of Tri*PPP*ro-compounds **7** described before.[45] However, the half-lives for γ-(ACB-C4)-γ-C-(alkyl-C12)-d4TTP **11b** ($t_{1/2}$ = 0.53 min), γ-(ACB-C4)-γ-C-(alkyl-C18)-d4TTP **11e** ($t_{1/2}$ = 1.04 h) was found to be lower than the corresponding compounds **10b** (AB-C4;alkyl-C12) or **10e** (AB-C4;alkyl-C18), respectively. Interestingly, the stability of γ-(AB-C4)-γ-C-(alkyl-C18)-d4TTP **10e** ($t_{1/2}$ = 2 h) was 15-fold higher as compared to the corresponding prodrug γ-(AB-C4;alkyl-C18)-d4TTP **8** ($t_{1/2}$ = 8.1 min).[47] Always, the exclusive formation of γ-C-(alkyl)-d4TTP derivatives **23** and no d4TTP **4** (Figure S6-S10; Supporting Information) was detected in contrast to the studies using the previously described Tri*PPP*ro-compounds **6**,**7**[42,45] and this is in agreement with the results reported before.[47] More importantly, the half-lives of γ-(β-cyanoethyl)-γ-C-(alkyl)-d4TTPs **22** ($t_{1/2}$ > 80 h, Table 1) and γ-C-(alkyl)-d4TTP derivatives **23** ($t_{1/2}$ > 80 h) showed that the delivery of d4TDP (Figure S11; Supporting Information) was most probably due to a pure chemical cleavage of the γ-phosphonate moiety, which proved our initial design of attaching an enzymaticallystable group to the γ-phosphonate unit.

Table 1: Half-lives of Tri*PPP*ro-d4TTP prodrugs **10**,**11**,**22** and γ-C-(alkyl)-d4TTPs **23** in different media.

Comp.	R^1	R^2	PB pH=7.3 $t_{1/2}$ [h]	CEM/0 cell extracts $t_{1/2}$ [h]	PLE $t_{1/2}$ [h]	Comp.	R^1	R^2	PB pH=7.3 $t_{1/2}$ [h]	CEM/0 cell extracts $t_{1/2}$ [h]	PLE $t_{1/2}$ [h]
10a	CH_3	$C_{12}H_{25}$	103	0.043	0.46	**11c**	$C_{11}H_{23}$	$C_{12}H_{25}$	1150	>10	10.6
10b	C_4H_9	$C_{12}H_{25}$	215	0.81	0.013	**11d**	C_4H_9	$C_{14}H_{29}$	518	6.4	0.032
10c	$C_{11}H_{23}$	$C_{12}H_{25}$	919	>8	0.917	**11e**	C_4H_9	$C_{18}H_{37}$	1240	>10	1.04
10d	C_4H_9	$C_{14}H_{29}$	509	4.5	0.081	**22a**	C_2H_4CN	$C_{12}H_{25}$	148	>15	>80
10e	C_4H_9	$C_{18}H_{37}$	646	6.7	2.0	**22c**	C_2H_4CN	$C_{18}H_{37}$	479	>10	>150
11a	CH_3	$C_{12}H_{25}$	123	0.65	0.7	**23a**		$C_{12}H_{25}$	>3000	>30	>150
11b	C_4H_9	$C_{12}H_{25}$	345	4.3	0.009	**23c**		$C_{18}H_{37}$	535	>30	>80

The hydrolysis experiments of γ-modified-d4TTPs **10**,**11**,**22** and **23** were conducted in aqueous 25 mM phosphate buffer (PB, pH=7.3), pig liver esterase (PLE) and human $CD4^+$ T-lymphocyte cell extracts. The hydrolysis products were detected by analytical rp18 HPLC.

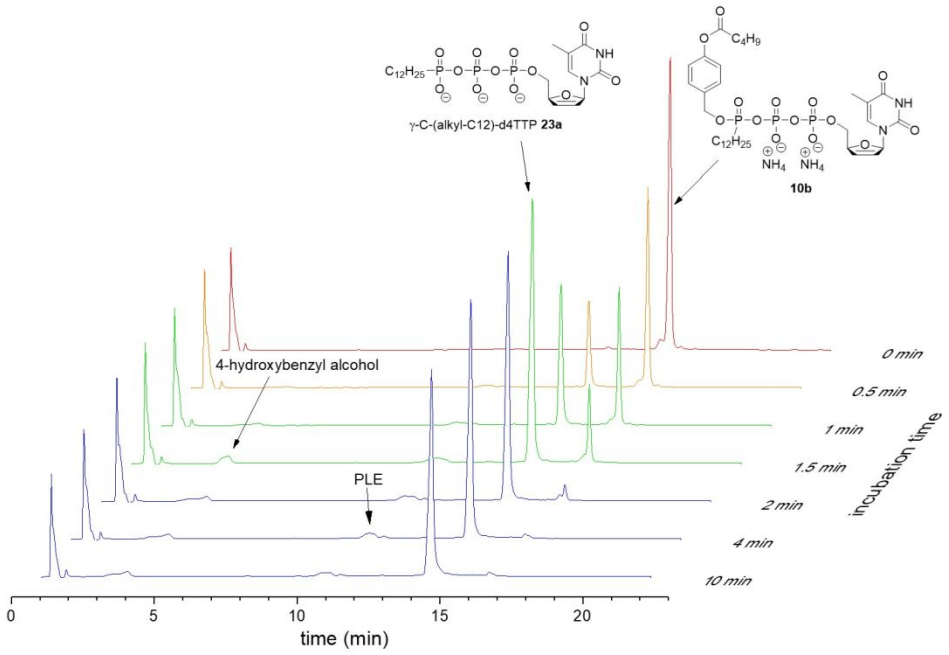


Figure 2: HPLC profile for PLE hydrolysis of γ-(AB-C4)-γ-C-(alkyl-C12)-d4TTP **10b**.

c. Hydrolysis in cell extracts.

The incubation of Tri*PPP*ro-d4TTP prodrugs **10**,**11**,**22** with human $CD4^+$ T-lymphocyte CEM cell extracts led to a significant acceleration of the formation of γ-C-(alkyl)-d4TTPs **23**. The half-lives of Tri*PPP*ro-d4TTPs **10**,**11** correlated with the alkyl chain length and were also significantly lower than the half-lives in PB (up to 2300-fold; Table 1), which clearly indicates the significant contribution of enzymatic cleavage as in the PLE studies.

The cleavage of the AB- or the ACB group is driven initially by an ester or a carbonate hydrolysis and thus proceed similar as previously reported,[45] showing a clear trend (Table 1). The stability of Tri*PPP*ro-d4TTP prodrugs **10a-c** (AB:C1,C4,C11;alkyl-C12), **11a-c** (ACB:C1,C4,C11;alkyl-C12) increased with the raising lipophilicity of the alkyl chain (R^1).

While the half-lives determined for compounds **10b**,**10d**,**10e** (AB-C4;alkyl:C12,C14,C18) as well as **11b**,**11d**,**11e** (ACB-C4;alkyl:C12,C14,C18) increased with the lipophilicity of the second alkyl chain (R^2). Moreover, the half-lives of compounds γ-ACB-γ-C-alkyl-d4TTPs **11** were also higher than the corresponding prodrugs γ-AB-γ-C-alkyl-d4TTPs **10** (Table 1). As compared to γ-(AB-C1)-γ-C-(alkyl-C12)-d4TTP **10a** ($t_{1/2}$ = 0.043 h, Table 1), the half-life for γ-(ACB-C1)-γ-C-(alkyl-C18)-d4TTP **11a** ($t_{1/2}$ = 0.65 h, Table 1) was found to be remarkably higher by almost a factor of 15. This is in accordance to our previously published results of Tri*PPP*ro-compounds **6**.[42] In all cases, before complete consumption of the starting compounds, an increase of γ-C-(alkyl)-d4TTPs **23** concentrations and a very low concentration of d4TDP **3** (<3%) was observed, but no d4TTP **4** was detected in these studies (Figure 3 and Figure S12-15; supporting information). Additionally, in the case of γ-C-(alkyl)-d4TTPs **23** ($t_{1/2}$ >30 h, Table 1), a very small amount of d4TDP **3** was detected (Figure S16; supporting information), which supports the hypothesis that γ-C-(alkyl)-d4TTPs **23** were subject to a bond breakage between the γ-phosphonate and β-phosphate in very small extend. Interestingly, the half-lives for γ-(AB-C4)-γ-C-(alkyl-C18)-d4TTP **10e** ($t_{1/2}$ = 6.7 h) and γ-(ACB-C4)-γ-C-(alkyl-C18)-d4TTP **11e** ($t_{1/2}$ >10 h) were higher than the corresponding prodrug γ-(AB-C4; alkyl-C18)-d4TTP **8** ($t_{1/2}$ = 4.8 h).[47]

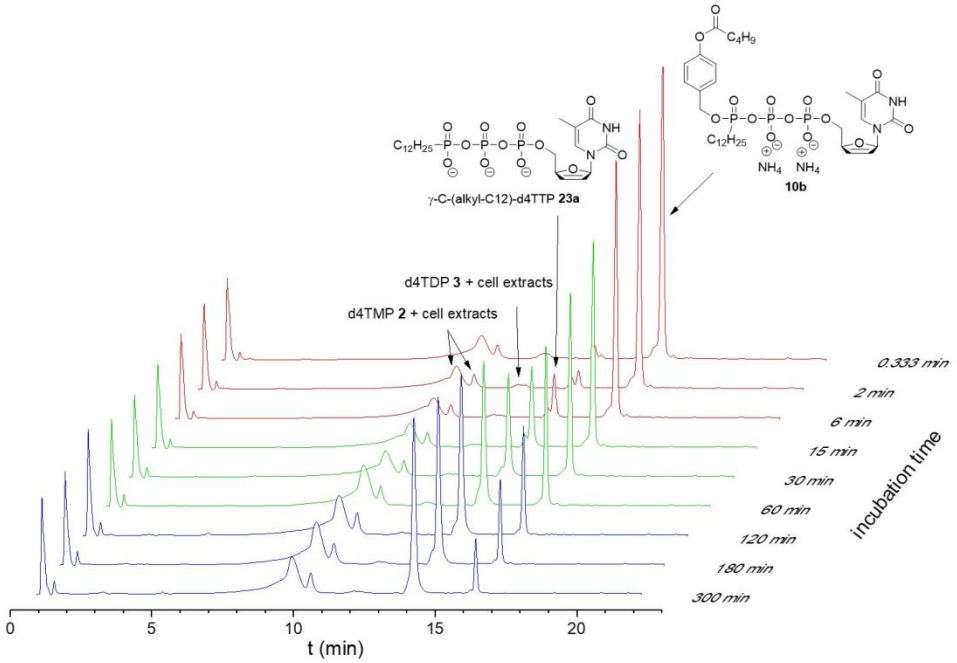


Figure 3: HPLC profiles of γ-(AB-C4)-γ-C-(alkyl-C12)-d4TTP **10b;** incubation in CEM/0 cell extracts.

d. Antiviral evaluation

Tri*PPP*ro-d4TTP prodrugs **10**,**11**,**22** and γ-C-(alkyl)-d4TTPs **23** were evaluated for their ability to inhibit HIV replication. Therefore, HIV-1- and HIV-2-infected wild-type CEM/0 as well as HIV-2-infected mutant thymidine kinase-deficient CEM cell cultures (CEM/TK$^-$) were treated with these compounds. Table 2 summarizes the antiviral and cytostatic data of the Tri*PPP*ro-d4TTP prodrugs **10**,**11**,**22**, γ-C-(alkyl)-d4TTPs **23** and the parent nucleoside d4T **1** as reference compound. As can be seen, the inhibition of the replication of HIV-1 and HIV-2 by prodrugs **10**,**11**,**22**,**23** was much higher (**11e**: EC_{50}: 0.0018 μM/HIV-1; EC_{50}: 0.026 μM/HIV-2), or at least similar (**11d**: EC_{50}: 0.12 μM/HIV-1; EC_{50}: 0.22 μM/HIV-2), compared to their parent nucleoside **1** (EC_{50}: 0.43 μM/HIV-1; EC_{50}: 0.31 μM/HIV-2) in wild-type CEM/0 cells.

For example, with compound **11e** (EC_{50}: 0.0018 μM) the antiviral activity in this infected cell line was improved by a 240-fold. In general, the antiviral activity determined for Tri*PPP*ro-prodrugs **10a-c** (AB:C1,C4,C11;alkyl-C12), **11a-c** (ACB:C1,C4,C11;alkyl-C12) as well as **10b**,**10d**,**10e** (AB-C4;alkyl:C12,C14,C18), **11b**,**11d**,**11e** (ACB-C4;alkyl:C12,C14,C18) was dependent on the increasing lipophilicity due to the increasing alkyl chain lengths (R^1 and R^2). However, prodrugs **10a**,**b** (AB:C1,C4;alkyl-C12), **11a**,**b** (ACB:C1,C4;alkyl-C12) showed a marked loss of activity in thymidine-kinase-(TK)-deficient cell cultures, respectively. This points to an insufficient lipophilicity of these compounds in addition to a fast cleavage of the bioreversible AB- or ACB-moiety. This led to the fast formation of the more polar γ-C-(alkyl-C12)-d4TTP **23a**. γ-C-(alkyl-C12)-compound **23a** lost its activity in the TK-deficient cell line. In contrast, the antiviral activity observed in the wild-type CEM/0 cell cultures was almost fully retained in the case of γ-(AB-C4)-γ-C-(alkyl-C18)-d4TTP **10e** (EC_{50}: 0.042 μM) and γ-(ACB-C4)-γ-C-(alkyl-C18)-d4TTP **11e** (EC_{50}: 0.032 μM) in mutant TK-deficient CEM cells (TK^-). γ-(ACB-C4)-γ-C-(alkyl-C18)-d4TTP **11e** is the most active compounds of all the listed derivatives (1000-fold more active as d4T **1**, which showed a 100-fold loss in activity in the TK-deficient cell cultures; EC_{50}: 31 μM/HIV-2). The retention of the antiviral activity in TK-deficient cells strongly supports a cell uptake of the Tri*PPP*ro-compounds **10**,**11**. Although markedly less active than the prodrugs **10e**,**11e** (about a 38-fold and 49-fold, respectively), γ-C-(alkyl-C18)-d4TTP **23c** was also active against HIV-2 in CEM/TK^- cells (EC_{50}: 1.58 μM) indicating a cell membrane passage of this compound**.** Compounds **22c** (β-cyanoethyl;alkyl-C18) and **23c** (alkyl-C18) bearing the C18 chain proved to be more active as compared to the compounds γ-C-(β-cyanoethyl;alkyl-C12)-d4TTP **22a**, γ-C-(alkyl-C12)-d4TTP **23a**, respectively. In contrast to γ-C-(alkyl-C12)-d4TTP **23a** (EC_{50}: 41.1 μM), the antiviral activity of compound **10c** (EC_{50}: 0.066

μM) in cultures of infected wild-type CEM/0 cells was improved by a 623-fold, indicating the advantage to combine the prodrug strategy with the stable γ-alkylphosphonate modification. Ester prodrugs γ-AB-γ-C-alkyl-d4TTPs **10** reported here had similar or even better activities against HIV-1 and HIV-2 in cultures of infected wild-type CEM/0 cells and more importantly in TK-deficient $CD4^+$ T-cells as compared to the corresponding carbonate compounds γ-ACB-γ-C-alkyl-d4TTPs **11**. For instance, γ-(AB-C11)-γ-C-(alkyl-C12)-d4TTP **10c** (EC_{50}: 0.0074 μM/HIV-1; EC_{50}: 0.091 μM/HIV-2) proved to be more active in cultures of infected wild-type CEM/0 cells than γ-(ACB-C11)-γ-C-(alkyl-C12)-d4TTP **11c** (EC_{50}: 0.04 μM/HIV-1; EC_{50}: 0.11 μM/HIV-2). Moreover, the antiviral activity of γ-(AB-C11)-γ-C-(alkyl-C12)-d4TTP **10c** was found to be higher by almost a factor of 5 as compared to γ-(ACB-C11)-γ-C-(alkyl-C12)-d4TTP **11c** in CEM/TK^- cell cultures, probably due to the different stability of the prodrugs **10c** ($t_{1/2}$ > 8 h),**11c** ($t_{1/2}$ > 10 h) in CEM cell extracts.

As compared to our previously reported γ-alkylphosphate compounds **8** (γ-(AB-C4;alkyl-C18)-d4TTP; EC_{50}: 0.17 μM/HIV-2),[47] the inhibitory effect in CEM/TK^- cells of γ-alkylphosphonates such as γ-(AB-C4)-γ-C-(alkyl-C18)-d4TTP **10e** (EC_{50}: 0.042 μM/HIV-2) and γ-(ACB-C4)-γ-C-(alkyl-C18)-d4TTP **11e** (EC_{50}: 0.032 μM/HIV-2) disclosed here was improved by a 4-fold and 5-fold, respectively. It should also be noticed that the cleavage of the AB masking unit or the ACB masking unit in **10** and **11**, respectively, occurred readily in the biological medium leading to the formation of 4-hydroxybenzyl alcohol.[52]

Table 2: Antiviral activity and cytotoxicity of Tri*PPP*ro-d4TTPs **10**,**11**,**22** and γ-C-alkyl-d4TTPs **23** and d4T **1**.

Comp.	HIV-1 (HE)	HIV-2 (ROD)	CEM/TK-	cellular toxicity

	HIV-2 (ROD)			
	EC_{50}[a] [μM]	EC_{50}[a] [μM]	EC_{50}[a] [μM]	CC_{50}[b] [μM]
10a	0.023 ±0.025	0.021±0.07	26.3±0.85	>100±0
10b	0.065±0.064	0.025±0.021	3.43±1.88	86±33
10c	0.0074±0.008	0.091±0.008	0.066±0.063	37±9
10d	0.031±0.039	0.16±0.021	0.53±0.11	56±29
10e	0.0074±0.0014	0.021±0.006	0.042±0.002	19±8
11a	0.095±0.002	0.35±0.01	36.8±4	>100±0
11b	0.097±0.004	0.20±0.2	9.9±4	38±2.5
11c	0.04±0.03	0.11±0.1	0.32±0.7	39±6
11d	0.12±0.2	0.22±0.3	1.76±2	32±6
11e	0.0018±0.002	0.026±0.04	0.032±0.02	16±2
22a	0.21±0.12	0.087±0.014	20.9±4.1	>100±0
22c	0.095±0.032	0.046±0.056	0.14±0.02	38±2
23a	0.15±0.039	0.17±0.035	41.1±0.71	>100±0
23c	0.046±0.021	0.11±0.064	1.58±0.75	21±9
d4T	0.43±0.23	0.31±0.13	31.05±5.25	>50

[a] Antiviral activity determined 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.

Primer extension assays using γ-C-(alkyl)-d4TTPs 23 and cellular DNA polymerases and HIV's reverse transcriptase (RT).

In antiviral assays, all compounds were highly active against HIV-1 and HIV-2 in cultures of infected wild-type CEM/0 cells and also in thymidine kinase-deficient $CD4^+$ T-cells (CEM/TK^-). We have proven that no d4TTP was formed in all hydrolysis studies. From the results summarized above, it appeared that the delivered γ-C-(alkyl)-d4TTPs **23** was responsible for the inhibitory effect of these compounds, which is a marked difference to the formerly reported

Tri*PPP*ro-compounds **6**. Next, primer extension assays were done investigating γ-C-(alkyl)-d4TTPs (**23**) substrate properties towards four DNA-polymerases: reverse transcriptase (RT) which is an RNA-dependent-DNA-polymerase, DNA polymerase α, DNA polymerase β, and DNA polymerase γ, which is a mitochondrial polymerase and often made responsible for marked toxicity effects caused by nucleoside analogue triphosphates.[53,54]

Figure 4 shows the results of a primer extension assay in which HIV-RT was used. In the case of a missing RT no elongation happened (- lane) while full extension to the 30mer occurred (+ lane) when all four canonical triphosphates were present (controls). γ-C-(alkyl-C18)-d4TTP **23c** (lane 3), γ-C-(alkyl-C12)-d4TTP **23a** (lane 4), and d4TTP (lane 6), only show the n+1 band due to d4TMP incorporation. Thus, γ-C-(alkyl)-d4TTPs **23** were accepted by HIV-RT as a substrate although the elongation with γ-C-(alkyl-C18)-d4TTP **23c** (lane 3) was not complete. As control TTP (lane 5) was also used.

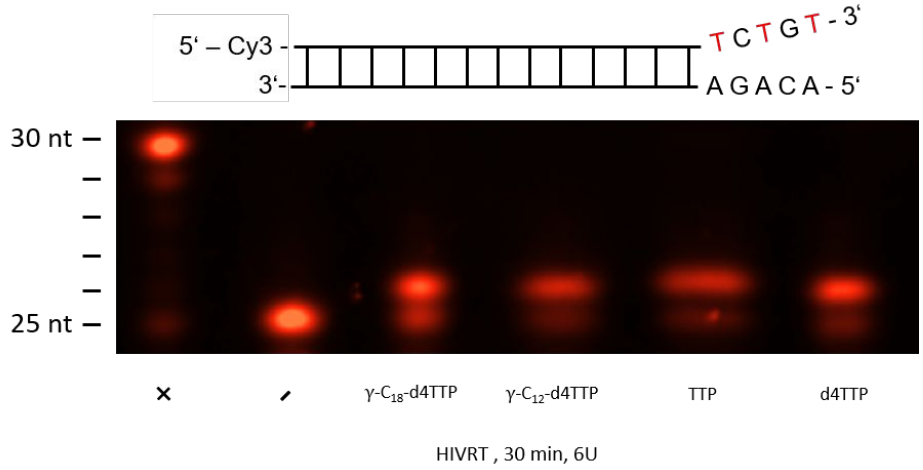
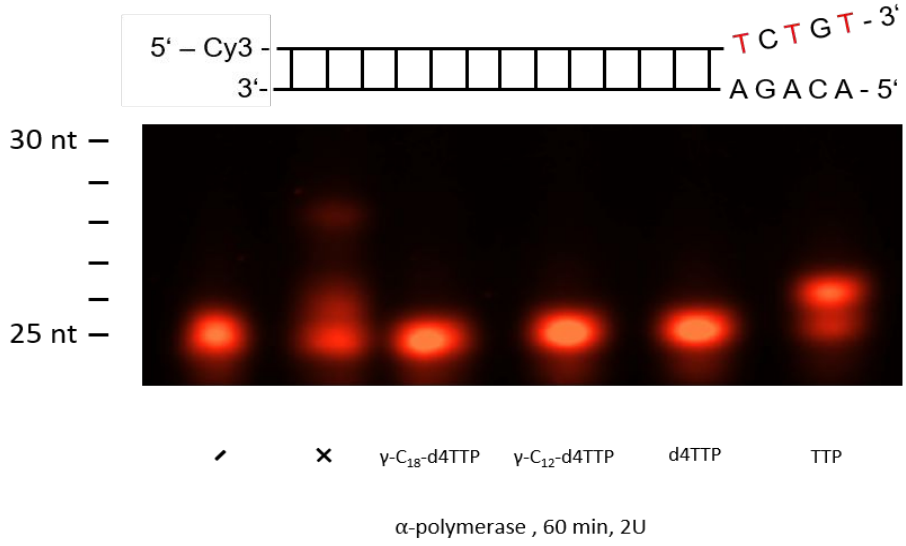


Figure 4. Primer extension assay using HIV's RT. lane 1 (+): dATP, dGTP, dCTP and TTP with HIV-RT; lane 2 (-): dATP, dGTP, dCTP and TTP without HIV-RT; lane 3 (γ-C_{18}-d4TTP): γ-C-(alkyl-C18)-d4TTP **23c**; lane 4 (γ-C_{12}-d4TTP): γ-C-(alkyl-C12)-d4TTP **23a**; lane 5: TTP; lane 6: d4TTP.

Next, γ-C-(alkyl)-d4TTPs **23** were studied using human DNA polymerases α, β and γ (Figure 5, A-C). Figure 5 A shows the result using human DNA polymerase α and γ-C-(alkyl)-d4TTPs **23** compared to TTP. Obviously, with TTP the expected the canonical incorporation of TMP was observed which led to the presence of the n + 1 band (26 nt, lane 6). However, when γ-C-(alkyl)-d4TTPs **23** and d4TTP were used, no incorporation was detectable (Figure A; lanes 3-5). Thus, γ-C-(alkyl)-d4TTPs **23** were not substrates for DNA polymerase α.

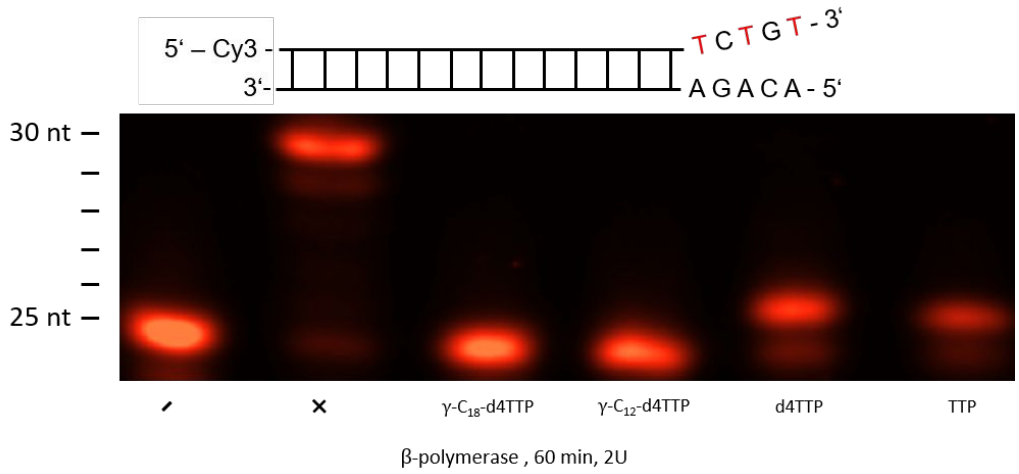
As is shown in Figure 5B and 5C, also *no* incorporation was detected in primer extension assays using γ-C-(alkyl)-d4TTPs **23** in combination with DNA pol β (B) and DNA pol γ (C). Interestingly, in contrast to DNA polymerases α and γ, DNA polymerase β used d4TTP as a good substrate which led to the n + 1 band in Figure 5B. As a conclusion DNA polymerase α, β and γ did not recognize the γ-C-(alkyl)-d4TTPs **23** as a substrate. In contrast, these compounds acted as substrates for HIV-RT.

Figure 5. Primer extension assay with human DNA polymerases α (A), β (B) and γ (C).

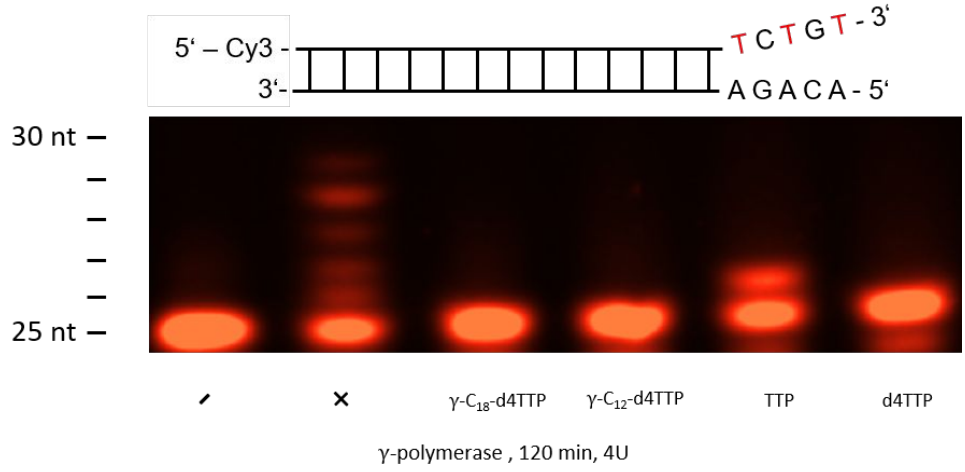


A. For human DNA pol α assay: lane 1 (-): dATP, dGTP, dCTP and TTP without human Pol α; lane 2 (+): dATP, dGTP, dCTP and TTP with human Pol α; lane 3 (γ-C18-d4TTP): γ-C-(alkyl-

C18)-d4TTP **23c**; lane 4 (γ-C12-d4TTP): γ-C-(alkyl-C12)-d4TTP **23a**; lane 5 (d4TTP): d4TTP; lane 6 (TTP): TTP.



B. For human DNA pol β assay: lane 1 (-): dATP, dGTP, dCTP and TTP without human DNA pol β; lane 2 (+): dATP, dGTP, dCTP and TTP with human DNA pol β; lane 3 (γ-C18-d4TTP): γ-C-(alkyl-C18)-d4TTP **23c**; lane 4 (γ-C12-d4TTP): γ-C-(alkyl-C12)-d4TTP **23a**; lane 5 (d4TTP): d4TTP; lane 6 (TTP): TTP.



C. For human DNA pol γ assay: lane 1 (-): dATP, dGTP, dCTP and TTP without human DNA pol γ; lane 2 (+): dATP, dGTP, dCTP and TTP with human DNA pol γ; lane 3 (γ-C18-d4TTP): γ-C-(alkyl-C18)-d4TTP **23c**; lane 4 (γ-C12-d4TTP): γ-C-(alkyl-C12)-d4TTP **23a**; lane 5 (TTP): TTP; lane 6 (d4TTP): d4TTP.

Summary and Conclusion

In summary, we disclosed the synthesis of a new class of nucleoside triphosphate prodrugs, γ-AB-γ-C-alkyl-d4TTPs **10** and γ-ACB-γ-C-alkyl-d4TTPs **11**, which differ from earlier reported Tri*PPP*ro-derivatives **6**,**7** by bearing a non-cleavable γ-alkyl-phosphonate moiety instead of the normal γ-phosphate group in addition to a biodegradable prodrug moiety at the γ-phosphonate unit. As γ-C-alkyl-group three *n*-alkyl moieties were used in combination with the previously used acyloxybenzyl- or alkoxycarbonyloxybenzyl-prodrug groups. Tri*PPP*ro-d4TTPs **10**,**11**,**22**, as well as γ-C-(alkyl)-d4TTPs **23**, were synthesized by using the *H*-phosphinate route with satisfying yields (up to 68%). We have proven that the hydrolysis, stability, and antiviral activity were significantly influenced by two different alkyl chain lengths of the prodrug moieties (R^1 and R^2). The prodrug group was cleaved to give γ-C-(alkyl)-d4TTPs **23** by chemical hydrolysis or by enzymes present in cell extracts, no d4TTP **4** was detected in all hydrolysis studies using prodrugs **10**,**11**,**22**. Interestingly, γ-C-(alkyl)-d4TTPs **23** proved to be stable in PLE and cell extracts. All these compounds showed at least similar, often even much better antiviral activity against HIV-1 and HIV-2 than the parent nucleoside d4T **1** in wild-type CEM/0 cells. The strong antiviral activities of Tri*PPP*ro-d4TTP prodrugs **10**,**11** (e,g. γ-(AB-C4)-γ-C-(alkyl-C18)-d4TTP **10e** and γ-(ACB-C4)-γ-C-(alkyl-C18)-d4TTP **11e**) in the wild-type CEM/0 cell cultures were completely retained in mutant thymidine-deficient CEM cells (TK^-), proving the successful cellular uptake of these derivatives and an intracellular delivery of the nucleoside diphosphate-γ-alkylphosphonate. The half-lives of prodrugs γ-ACB-γ-C-alkyl-d4TTPs **11** ($t_{1/2}$ = 0.65-10 h, Table 1) in cell extracts were found to be higher than those for γ-AB-γ-C-alkyl-d4TTPs **10** ($t_{1/2}$ = 0.044-8 h, Table 1). However, the inhibition of the replication of HIV-1- and HIV-2 in infected

wild-type CEM/0 as well as HIV-2-infected mutant thymidine kinase-deficient CEM cell cultures (CEM/TK$^-$) by prodrugs γ-ACB-γ-C-alkyl-d4TTPs **11** was found to be lower or at its best similar to the corresponding γ-AB-γ-C-alkyl-d4TTPs **10**. It was concluded that the antiviral activity determined for Tri*PPP*ro-prodrugs **10**,**11** correlated well with the biological stability of the prodrugs **10**,**11** in CEM cell extracts.

We have proven that γ-C-(alkyl-C18)-d4TTP **23c** was also, although weakly potent against HIV-2 in thymidine kinase-deficient cell cultures (CEM/TK$^-$, EC_{50}: 1.58 μM). However, this compound was less active than its prodrug forms **10e**,**11e**,**22c** (about a 38-fold, 49-fold, and 11-fold, respectively). γ-C-(alkyl)-d4TTPs **23** were substrates for HIV-RT as shown in primer extension assays while they proved to be non-substrates for the cellular DNA-polymerases α, β and γ. Thus, a high degree of differentiation between the viral polymerase and the cellular one was discovered. We are currently working on the question if this increased selectivity towards the viral enzyme is a general property of these γ-modified phosphonate compounds by using different nucleoside analogues and further viral polymerases.

As a conclusion, the described prodrugs are able to efficiently enter cells and deliver γ-C-alkyl-NTPs highly selectively by an enzyme-triggered reaction. This Tri*PPP*ro-approach offers high potential to be used in antiviral and antitumoral chemotherapies: i) would bypass *all* phosphorylation steps normally needed for the activation of a nucleoside analogue ii) the delivered γ-C-alkyl-d4TTPs **23** proved to be highly stable in cell extracts against dephosphorylation in contrast to natural NTPs and iii) γ-C-alkyl-d4TTPs **23** were substrates for a viral polymerase (RT) but did not act as substrates for three cellular DNA-polymerases with DNA pol α being the most important one. Moreover, we are convinced that this Tri*PPP*ro-

strategy is not limited to HIV inhibition but may also be used for other viral targets. Using these insights, γ-C-modified NTP-prodrugs of other nucleoside analogues will be explored next.

Experimental Section

General: All experiments were carried out under anhydrous conditions and nitrogen atmosphere. All anhydrous solvents were purchased from Acros Organics (Extra Dry over molecular sieves) and obtained by the MBraun solvent purification system (MB SPS-800). CH_3CN for HPLC was purchased from VWR (HPLC grade). Ultrapure water was produced by a Sartorius Aurium® pro (Sartopore 0.2 µm, UV). All other organic solvents were purchased technical grade and distilled prior to use. Commercially available solvents and reagents were used as received without further purification. General flash column chromatography was performed with silica gel 60 M (0.04-0.063 mm, Macherey-Nagel). For reversed phase automated flash chromatography, an Interchim Puriflash 430 was used in combination with Chromabond® Flash RS40 C_{18} ec. All High Performance Liquid Chromatography (HPLC) measurements were carried out using a VWR-Hitachi LaChromElite HPLC system (L-2130, L-2200, L-2455) and EzChromElite software. TBAA buffer: 2 mM tetra-*n*-Butylammonium acetate solution (pH 6.3). Method: Nucleodur 100-5 C18ec; 0-20 min: TBAA buffer/CH_3CN gradient (5-80%); 20-30 min: buffer/CH_3CN (80%); 30-33 min: buffer/CH_3CN (80-5%); 33-38 min: buffer/CH_3CN (5%); flow: 1 mL/min. Compound purity: All final compounds were isolated analytically pure, ≥95% purity by HPLC and NMR spectroscopy.

All NMR spectra were carried out using Bruker spectrometers: Bruker AMX 400, Bruker DRX 500 or Bruker AVIII 600. All chemical shifts (δ) were given in ppm and calibrated on solvent signals. HRMS (ESI) mass spectra were performed with a VG Analytical Finnigan ThermoQuest

MAT 95 XL or an Agilent 6224 EIS-TOF spectrometer. MALDI mass spectra were recorded on an ultrafleXtreme MALDI-TOF-TOF mass spectrometer by Bruker Daltonik with 9-AA as matrix.

General procedures

General Procedure 1: Preparation of *H*-phosphinates 15.

The reactions carried out under nitrogen (N_2) atmosphere under dry conditions. 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide (EDC; 1.2 equiv.) was added to a solution of 4-acyloxybenzyl alcohols **14** (2.0 equiv.), *H*-phosphinic acid **13** (1.0 equiv.) and 4-dimethylaminopyridine (DMAP; 0.2 equiv.) in dry CH_2Cl_2. The mixture was stirred for 12 h at room temperature (rt). The solvent was removed in vaccum and the residue was purified using column chromatography to give compounds *H*-phosphinates **15**.

General Procedure 2: Preparation of *H*-phosphinates 18.

The reactions carried out under nitrogen (N_2) atmosphere under dry conditions. EDC (1.2 equiv.) was added to a solution of and 4-alkoxycarbonyloxybenzyl alcohols **17** (2.0 equiv.), *H*-phosphinic acid **13** (1.0 equiv.) and DMAP (0.2 equiv.) in dry CH_2Cl_2. The mixture was stirred for 12 h at room temperature (rt). The solvent was removed in vaccum and the residue was purified using column chromatography to obtain compounds *H*-phosphinates **18**.

General Procedure 3: Preparation of γ-AB-γ-C-alkyl-d4TTPs 10 and γ-ACB-γ-C-alkyl-d4TTPs 11.

The reactions were performed in a nitrogen (N_2) atmosphere and dry conditions. Firstly, *H*-phosphinates **15**,**18** (0.3 mmol, 1.0 equiv.) were dissolved in 6 mL CH_3CN. *N*-chlorosuccinimide (NCS, 0.6 mmol, 2.0 equiv.) was added and the mixture was stirred for 2 h at room temperature. Subsequently, tetrabutylammonium phosphate solution (0.4 M in CH_3CN, 9 mmol, 3.0 equiv.) was added quickly and was stirred for 1 h at room temperature and the solvent was removed in vacuum under reduced pressure. The residue was extracted with CH_2Cl_2/H_2O (three times), The organic phase was dried over sodium sulfate and the solvent was removed to afford pyrophosphate. Then, pyrophosphate was dissolved in 4 mL CH_3CN at 0 °C. Then, a mixture of Et_3N (2.4 mmol, 8.0 equiv.) and trifluoroacetic anhydride (TFAA, 1.5 mmol, 5.0 equiv.) in 4 mL CH_3CN was cooled to 0 °C and added to the mixture of pyrophosphate. All volatile components were removed in vacuum under reduced pressure after 10 min. The residue was dissolved in 6 mL CH_3CN and cooled to 0 °C. 1-Methylimidazole (0.9 mmol, 3.0 equiv.) and Et_3N (TEA, 1.5 mmol, 5.0 equiv.) were added at 0 °C. The mixture was warmed to room temperature and stirred for 10 min. Finally, d4TMP (0.5-0.7 equiv.) dissolved in 6 mL CH_3CN was added to the mixture and stirred at room temperature for 2-5 h. All volatile components were removed in vacuum. The crude product was purified by automatic RP18 flash chromatography, and 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 freeze-dried and the products **10**,**11** were obtained as white solids.

Dodecyl-*H*-phosphinic acid 13a.

Under dry conditions, a solution of diethyl chlorophosphite (2.9 mL, 20 mmol) in dry Et_20 (40 mL) was cooled to 0 °C. A 1.0 M solution of dodecylmagnesium bromide in Et_2O (20 mL, 20

mmol) was added dropwise over 30 min and stirred at 0 °C. Following, the white suspension was heated at 50 °C for 4 h, cooled, and filtered. The Et_20 was removed by distillation at atmospheric pressure and the residue distilled under reduced pressure to give the corresponding phosphonous diester. 2) 2 mL concentrated HC1 was adeed to a solution of phosphonous diester and 20 mL H_2O. After being stirred at room temperature for 1 h, the mixture was extracted with CH_2Cl_2. The organic phase was washed with saturated NaCl, dried over $MgSO_4$, and evaporated. The crude phosphonous monoester was taken up in 4.0 N NaOH (40 mL) and stirred at room temperature for 1 h. The aqueous mixture was washed with Et_20, acidified with concentrated HCl (pH 1), and extracted with CH_2Cl_2. The CH_2Cl_2 was washed with saturated NaCl, dried over $MgSO_4$, and evaporated. Recrystallization of the crude product from CH_2Cl_2/Et_2O give pure phosphonous acid **13a** (3.28 g, 70%) as a while solid. ^{1}H-NMR (400 MHz, $CDCl_3$): δ [ppm] = 12.22 (m, 1H, OH), 7.05 (d, $^1J_{HH}$= 540.1 Hz, 1H, P-H), 1.78-1.68 (m, 2H, H-a), 1.62-1.52 (m, 2H, H-b), 1.41-1.34 (m, 2H, H-c), 1.31-1.24 (m, 16H, H-d, H-e, H-f, H-g, H-h, H-i, H-j, H-k), 0.86 (t, $^3J_{HH}$= 6.9 Hz, 3H, H-l). ^{13}C NMR (101 MHz, $CDCl_3$): δ [ppm] = 31.9, 30.4, 30.3, 29.57, 29.53, 29.3, 29.1, 28.8, 22.6 (C-a, C-c, C-d, C-e, C-f, C-g, C-h, C-i, C-j, C-k), 20.6 (d, $^3J_{CP}$= 2.7 H_Z, C-b), 14.0 (C-l). ^{31}P NMR (162 MHz, $CDCl_3$): δ [ppm] = 38.7. HRMS (ESI^+, m/z): calculated for $C_{12}H_{27}O_2P$, $[M+H]^+$ 235.1822; found, 235.2083.

Tetradecyl-*H*-phosphinic acid 13b.

Under dry conditions, a solution of diethyl chlorophosphite (4.3 mL, 30 mmol) in dry THF (60 mL) was cooled to 0 °C. A 1.0 M solution of tetradecylmagnesium chloride solution in THF (30 mL, 30 mmol) was added dropwise over 30 min and stirred at 0 °C. Following, the mixture was heated at 50 °C for 6 h, cooled, and the THF was removed under reduced pressure. 150 mL

CH_2Cl_2 was added to the residue, and filtered. The CH_2Cl_2 was removed under reduced pressure to give the corresponding phosphonous diester. 2) 4 mL concentrated HC1 was adeed to a solution of phosphonous diester and 60 mL H_2O. After being stirred at room temperature for 2 h, the mixture was extracted with CH_2Cl_2. The organic phase was washed with saturated NaCl, dried over $MgSO_4$, and evaporated. The crude phosphonous monoester was taken up in 4.0 N NaOH (60 mL) and stirred at room temperature for 2 h. The mixture was washed with Et_20, acidified with concentrated HCl (pH 1), and extracted with CH_2Cl_2. The CH_2Cl_2 was washed with saturated NaCl, dried over $MgSO_4$, and evaporated. Recrystallization of the crude product from CH_2Cl_2/Et_2O give pure phosphonous acid **13b** (5.11 g, 65%) as a while solid. ^{1}H-NMR (400 MHz, $CDCl_3$): δ [ppm] = 12.3 (m, 1H, OH), 7.09 (d, $^1J_{HH}$= 541.5 Hz, 1H, P-H), 1.84-1.66 (m, 2H, H-a), 1.65-1.50 (m, 2H, H-b), 1.44-1.34 (m, 2H, H-c), 1.33-1.22 (m, 20H, H-d, H-e, H-f, H-g, H-h, H-i, H-j, H-k, H-l, H-m), 0.87 (t, $^3J_{HH}$= 6.7 Hz, 3H, H-n). ^{13}C NMR (101 MHz, $CDCl_3$): δ [ppm] = 31.9, 30.5, 30.3, 29.65, 29.63, 29.61, 29.54, 29.3, 29.1, 28.6, 22.6 (C-a, C-c, C-d, C-e, C-f, C-g, C-h, C-i, C-j, C-k, C-l, C-m), 20.6 (C-b), 14.1 (C-n). ^{31}P NMR (162 MHz, $CDCl_3$): δ [ppm] = 39.1. HRMS (ESI^+, m/z): calculated for $C_{14}H_{31}O_2P$, $[M+Na]^+$ 285.1954; found, 285.2142.

Octadecyl-*H*-phosphinic acid 13c.

Under dry conditions, a solution of diethyl chlorophosphite (2.9 mL, 20 mmol) in dry THF (40 mL) was cooled to 0 °C. A 0.5 M solution of octadecylmagnesium chloride solution in THF (40 mL, 20 mmol) was added dropwise over 30 min and stirred at 0 °C. Following, the mixture was heated at 50 °C for 12 h, cooled, and the THF was removed under reduced pressure. 200 mL CH_2Cl_2 was added to the residue, and filtered. The CH_2Cl_2 was removed under reduced pressure

to give the corresponding phosphonous diester. 2) 4 mL concentrated HC1 was adeed to a solution of phosphonous diester and 60 mL H_2O. After being stirred at room temperature for 2 h, the mixture was extracted with CH_2Cl_2. The organic phase was washed with saturated NaCl, dried over $MgSO_4$, and evaporated. The crude phosphonous monoester was taken up in 4.0 N NaOH (80 mL) and stirred at room temperature for 3 h. The mixture was filtered and washed with Et_20, the solid was acidified with concentrated HCl (pH 1), and extracted with CH_2Cl_2. The CH_2Cl_2 was washed with saturated NaCl, dried over $MgSO_4$, and evaporated. Recrystallization of the crude product from CH_2Cl_2/Et_2O give pure phosphonous acid **13c** (3.50 g, 55%) as a while solid. ^{1}H-NMR (400 MHz, $CDCl_3$): δ [ppm] = 10.97 (m, 1H, OH), 7.09 (d, $^1J_{HH}$= 541.8 Hz, 1H, P-H), 1.84-1.68 (m, 2H, H-a), 1.64-1.54 (m, 2H, H-b), 1.45-1.36 (m, 2H, H-c), 1.34-1.16 (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-p, H-q), 0.88 (t, $^3J_{HH}$= 6.8 Hz, 3H, H-r). ^{13}C NMR (101 MHz, $CDCl_3$): δ [ppm] = 31.9, 30.5, 30.3, 29.69, 29.66, 29.65, 29.62, 29.57, 29.35, 29.34, 29.1, 28.6, 22.7 (C-a, C-c, 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), 20.5 (d, $^3J_{CP}$= 2.4 H_Z, C-b), 14.1 (C-r). ^{31}P NMR (162 MHz, $CDCl_3$): δ [ppm] = 39.2. HRMS (ESI^+, m/z): calculated for $C_{18}H_{39}O_2P$, $[M+NH]^+$ 319.2761; found, 319.2844.

Synthesis of *H*-phosphinates 15 and *H*-phosphinates 18.

(AB-C1;alkyl-C12)-*H*-phosphinate 15a

According to general procedure 1, with 0.23 g dodecyl-*H*-phosphinic acid **13a** (1.0 mmol, 1.0 equiv.), 0.33 g 4-(hydroxymethyl)phenyl acetate **14a** (2.0 mmol, 2.0 equiv.), 0.19 g EDC (1.2 mmol, 1.2 equiv.), 24 mg DMAP (0.2 mmol, 0.2 equiv.) dissolved in 5 mL CH_2Cl_2. Column chromatography (SiO_2, petrol ether/ethyl acetate/CH_3COOH 6:4:0.005 v/v/v). Yield: 0.24 g

(0.62 mmol, 62%) white solid. ^{1}H-NMR (400 MHz, CD_3OD): δ [ppm] = 7.43-7.38 (m, 2H, H-c^{1}), 7.14-7.10 (m, 2H, H-d^{1}), 7.12 (dt, $^{1}J_{HH}$= 530.7 Hz, $^{4}J_{HH}$= 1.9 Hz, 1H, P-H), 5.16-4.95 (m, 2H, H-a^{1}), 2.31 (s, 3H, H-g^{1}), 1.83-1.75 (m, 2H, H-a^{2}), 1.64-1.55 (m, 2H, H-b^{2}), 1.42-1.36 (m, 2H, H-c^{2}), 1.32-1.24 (m, 16H, H-d^{2}, H-e^{2}, H-f^{2}, H-g^{2}, H-h, H-i, H-j, H-k), 0.89 (t, $^{3}J_{HH}$= 7.0 Hz, 3H, H-l). ^{13}C NMR (101 MHz, CD_3OD): δ [ppm] = 169.3 (C-f^{1}), 150.8 (C-e^{1}), 133.4 (d, $^{3}J_{CP}$= 6.0 H_Z, C-b^{1}), 129.5 (C-c^{1}), 121.9 (C-d^{1}), 67.8 (d, $^{3}J_{CP}$= 6.6 H_Z, C-a^{1}), 31.9, 31.5, 30.3, 29.57, 29.51, 29.34, 29.30, 29.28, 22.6 (C-c^{2}, C-d^{2}, C-e^{2}, C-f^{2}, C-g^{2}, C-h, C-i, C-j, C-k), 29.0, 28.1 (C-a^{2}), 21.1 (C-g^{1}), 20.6 (d, $^{3}J_{CP}$= 3.0 H_Z, C-b^{2}), 14.1 (C-l). ^{31}P NMR (162 MHz, CD_3OD): δ [ppm] = 39.6. HRMS (ESI^+, m/z): calculated for $C_{21}H_{35}O_4P$, $[M+Na]^+$ 405.2165; found, 405.2455.

(AB-C4;alkyl-C12)-H-phosphinate 15b

According to general procedure 1, with 0.23 g dodecyl-*H*-phosphinic acid **13a** (1.0 mmol, 1.0 equiv.), 0.42 g 4-(hydroxymethyl)phenyl pentanoate **14b** (2.0 mmol, 2.0 equiv.), 0.19 g EDC (1.2 mmol, 1.2 equiv.), 24 mg DMAP (0.2 mmol, 0.2 equiv.) dissolved in 5 mL CH_2Cl_2. Column chromatography (SiO_2, petrol ether/ethyl acetate/CH_3COOH 6:4:0.005 v/v/v). Yield: 0.22 g (0.53 mmol, 53%) white solid. ^{1}H-NMR (400 MHz, CD_3OD): δ [ppm] = 7.42-7.37 (m, 2H, H-c^{1}), 7.12-7.06 (m, 2H, H-d^{1}), 7.13 (dt, $^{1}J_{HH}$= 529.9 Hz, $^{4}J_{HH}$= 1.9 Hz, 1H, P-H), 5.15-4.96 (m, 2H, H-a^{1}), 2.55 (t, $^{3}J_{HH}$= 7.4 Hz, 2H, H-g^{1}), 1.83-1.68 (m, 4H, H-a^{2}, H-h^{1}), 1.64-1.50 (m, 2H, H-b^{2}), 1.44 (sext, $^{3}J_{HH}$= 7.4 Hz, 2H, H-i^{1}), 1.40-1.32 (m, 2H, H-c^{2}), 1.30-1.24 (m, 16H, H-d^{2}, H-e^{2}, H-f^{2}, H-g^{2}, H-h^{2}, H-i^{2}, H-j^{2}, H-k), 0.95 (t, $^{3}J_{HH}$= 7.3 Hz, 3H, H-j^{1}), 0.87 (t, $^{3}J_{HH}$= 6.8 Hz, 3H, H-l). ^{13}C NMR (101 MHz, CD_3OD): δ [ppm] = 172.1 (C-f^{1}), 150.9 (C-e^{1}), 133.2 (d, $^{3}J_{CP}$= 6.0 H_Z, C-b^{1}), 129.2 (C-c^{1}), 121.9 (C-d^{1}), 66.7 (d, $^{3}J_{CP}$= 6.6 H_Z, C-a^{1}), 34.0 (C-g^{1}), 31.8, 30.4, 30.3, 29.54, 29.48, 29.27, 29.25, 29.22, 29.0, 28.3, 22.6 (C-a^{2}, C-c^{2}, C-d^{2}, C-e^{2}, C-f^{2}, C-g^{2}, C-h^{2}, C-i^{2},

C-j^2, C-k), 26.9 (C-h^1), 22.2 (C-i^1), 20.6 (d, $^3J_{CP}$= 3.0 H_Z, C-b^2), 14.1 (C-l), 13.4 (C-j^1). ^{31}P NMR (162 MHz, CD_3OD): δ [ppm] = 39.4. HRMS (ESI$^+$, m/z): calculated for $C_{24}H_{41}O_4P$, $[M+Na]^+$ 447.2634; found, 447.2975.

(AB-C11;alkyl-C12)-*H*-phosphinate 15c

According to general procedure 1, with 0.23 g dodecyl-*H*-phosphinic acid **13a** (1.0 mmol, 1.0 equiv.), 0.61 g 4-(hydroxymethyl)phenyl dodecanoate **14c** (2.0 mmol, 2.0 equiv.), 0.19 g EDC (1.2 mmol, 1.2 equiv.), 24 mg DMAP (0.2 mmol, 0.2 equiv.) dissolved in 5 mL CH_2Cl_2. Column chromatography (SiO_2, petrol ether/ethyl acetate/CH_3COOH 7:3:0.005 v/v/v). Yield: 0.35 g (0.59 mmol, 59%) white solid. ^{1}H-NMR (500 MHz, CD_3OD): δ [ppm] =7.42-7.37 (m, 2H, H-c^1), 7.12-7.07 (m, 2H, H-d^1), 7.11 (dt, $^1J_{HH}$= 530.1 Hz, $^4J_{HH}$= 1.9 Hz, 1H, P-H), 5.15-4.98 (m, 2H, H-a^1), 2.55 (t, $^3J_{HH}$= 7.5 Hz, 2H, H-g^1), 1.82-1.72 (m, 4H, H-a^2, H-h^1), 1.63-1.53 (m, 2H, H-b^2), 1.43-1.23 (m, 34H, H-c^2, H-d^2, H-e^2, H-f^2, H-g^2, H-h^2, H-i^1, H-i^2, H-j^1, H-j^2, H-k^1, H-k^2, H-l^1, H-m, H-n, H-o, H-p), 0.92-0.85 (m, 6H, H-l^2, H-q). ^{13}C NMR (126 MHz, CD_3OD): δ [ppm] = 172.2 (C-f^1), 150.9 (C-e^1), 133.2 (d, $^3J_{CP}$= 5.9 H_Z, C-b^1), 129.3 (C-c^1), 121.9 (C-d^1), 66.8 (d, $^3J_{CP}$= 6.7 H_Z, C-a^1), 34.4 (C-g^1), 31.9, 30.5, 30.3, 29.58, 29.52, 29.43, 29.37, 29.31, 29.2, 29.1, 28.1, 22.7 (C-a^2, C-c^2, C-d^2, C-e^2, C-f^2, C-g^2, C-h^2, C-i^1, C-i^2, C-j^1, C-j^2, C-k^1, C-k^2, C-l^1, C-m, C-n, C-o, C-p), 24.9 (C-h^1), 20.6 (d, $^3J_{CP}$= 3.0 H_Z, C-b^2), 14.1 (C-l^2, C-q). ^{31}P NMR (202 MHz, CD_3OD): δ [ppm] = 40.7. HRMS (ESI$^+$, m/z): calculated for $C_{31}H_{55}O_4P$, $[M+Na]^+$ 545.3730; found, 545.3571.

(AB-C4;alkyl-C14)-*H*-phosphinate 15d

According to general procedure 1, with 0.26 g tetradecyl-*H*-phosphinic acid **13b** (1.0 mmol, 1.0 equiv.), 0.42 g 4-(hydroxymethyl)phenyl pentanoate **14b** (2.0 mmol, 2.0 equiv.), 0.19 g EDC (1.2 mmol, 1.2 equiv.), 24 mg DMAP (0.2 mmol, 0.2 equiv.) dissolved in 5 mL CH_2Cl_2. Column chromatography (SiO_2, petrol ether/ethyl acetate/CH_3COOH 6:4:0.005 v/v/v). Yield: 0.23 g (0.51 mmol, 51%) white solid. ^{1}H-NMR (600 MHz, CD_3OD): δ [ppm] = 7.39-7.35 (m, 2H, H-c^1), 7.10-7.05 (m, 2H, H-d^1), 7.14 (dt, $^1J_{HH}$= 530.1 Hz, $^4J_{HH}$= 1.9 Hz, 1H, P-H), 5.15-4.96 (m, 2H, H-a^1), 2.53 (t, $^3J_{HH}$= 7.5 Hz, 2H, H-g^1), 1.83-1.68 (m, 4H, H-a^2, H-h^1), 1.60-1.54 (m, 2H, H-b^2), 1.43 (sext, $^3J_{HH}$= 7.4 Hz, 2H, H-i^1), 1.40-1.32 (m, 2H, H-c^2), 1.30-1.24 (m, 20H, H-d^2, H-e^2, H-f^2, H-g^2, H-h^2, H-i^2, H-j^2, H-k, H-l, H-m), 0.94 (t, $^3J_{HH}$= 7.4 Hz, 3H, H-j^1), 0.85 (t, $^3J_{HH}$= 6.9 Hz, 3H, H-n). ^{13}C NMR (151 MHz, CD_3OD): δ [ppm] = 172.2 (C-f^1), 150.9 (C-e^1), 133.2 (d, $^3J_{CP}$= 6.0 H_Z, C-b^1), 129.3 (C-c^1), 121.9 (C-d^1), 66.8 (d, $^3J_{CP}$= 6.6 H_Z, C-a^1), 34.1 (C-g^1), 31.9, 30.5, 30.3, 29.65, 29.62, 29.58, 29.52, 29.32, 29.28, 22.7 (C-c^2, C-d^2, C-e^2, C-f^2, C-g^2, C-h^2, C-i^2, C-j^2, C-k, C-l, C-m), 29.0, 28.1 (C-a^2), 26.9 (C-h^1), 22.2 (C-i^1), 20.6 (d, $^3J_{CP}$= 3.0 H_Z, C-b^2), 14.1 (C-n), 13.7 (C-j^1). ^{31}P NMR (243 MHz, CD_3OD): δ [ppm] = 39.4. HRMS (ESI$^+$, m/z): calculated for $C_{26}H_{45}O_4P$, $[M+Na]^+$ 475.2947; found, 475.3245.

(AB-C4;alkyl-C18)-*H*-phosphinate 15e

According to general procedure 1, with 0.32 g octadecyl-*H*-phosphinic acid **13c** (1.0 mmol, 1.0 equiv.), 0.42 g 4-(hydroxymethyl)phenyl pentanoate **14b** (2.0 mmol, 2.0 equiv.), 0.19 g EDC (1.2 mmol, 1.2 equiv.), 24 mg DMAP (0.2 mmol, 0.2 equiv.) dissolved in 5 mL CH_2Cl_2. Column chromatography (SiO_2, petrol ether/ethyl acetate/CH_3COOH 7:3:0.005 v/v/v). Yield: 0.25 g (0.48 mmol, 48%) white solid. ^{1}H-NMR (400 MHz, CD_3OD): δ [ppm] = 7.42-7.36 (m, 2H, H-c^1), 7.12-7.06 (m, 2H, H-d^1), 7.11 (dt, $^1J_{HH}$= 530.0 Hz, $^4J_{HH}$= 1.8 Hz, 1H, P-H), 5.15-4.96 (m,

2H, H-a[1]), 2.56 (t, $^3J_{HH}$= 7.5 Hz, 2H, H-g[1]), 1.85-1.68 (m, 4H, H-a[2], H-h[1]), 1.65-1.54 (m, 2H, H-b[2]), 1.52-1.41 (m, 2H, H-i[1]), 1.40-1.32 (m, 2H, H-c[2]), 1.37-1.24 (m, 30H, H-c[2], H-d[2], H-e[2], H-f[2], H-g[2], H-h[2], H-i[2], H-j[2], H-k, H-l, H-m, H-n, H-o, H-p, H-q), 0.96 (t, $^3J_{HH}$= 7.3 Hz, 3H, H-j[1]), 0.87 (t, $^3J_{HH}$= 6.6 Hz, 3H, H-r). ^{13}C NMR (101 MHz, CD_3OD): δ [ppm] = 172.1 (C-f[1]), 150.9 (C-e[1]), 133.2 (d, $^3J_{CP}$= 6.0 H_Z, C-b[1]), 129.3 (C-c[1]), 121.9 (C-d[1]), 66.8 (d, $^3J_{CP}$= 6.6 H_Z, C-a[1]), 34.1 (C-g[1]), 31.9, 30.5, 30.3, 29.66, 29.63, 29.58, 29.52, 29.36, 29.33, 29.29, 22.7 (C-c[2], C-d[2], C-e[2], C-f[2], C-g[2], C-h[2], C-i[2], C-j[2], C-k, C-l, C-m, C-n, C-o, C-p, C-q), 29.0, 28.1 (C-a[2]), 26.9 (C-h[1]), 22.2 (C-i[1]), 20.6 (d, $^3J_{CP}$= 3.0 H_Z, C-b[2]), 14.1 (C-n), 13.7 (C-j[1]). ^{31}P NMR (162 MHz, CD_3OD): δ [ppm] = 39.4. HRMS (ESI^+, m/z): calculated for $C_{30}H_{53}O_4P$, $[M+Na]^+$ 531.3573; found, 531.3517.

(ACB-C1;alkyl-C12)-*H*-phosphinate 18a

According to general procedure 2, with 0.23 g dodecyl-*H*-phosphinic acid **13a** (1.0 mmol, 1.0 equiv.), 0.36 g 4-(hydroxymethyl)phenyl methyl carbonate **17a** (2.0 mmol, 2.0 equiv.), 0.19 g EDC (1.2 mmol, 1.2 equiv.), 24 mg DMAP (0.2 mmol, 0.2 equiv.) dissolved in 5 mL CH_2Cl_2. Column chromatography (SiO_2, petrol ether/ethyl acetate/CH_3COOH 6:4:0.005 v/v/v). Yield: 0.22 g (0.55 mmol, 55%) white solid. ^{1}H-NMR (400 MHz, CD_3OD): δ [ppm] =7.42-7.36 (m, 2H, H-c[1]), 7.18-7.13 (m, 2H, H-d[1]), 7.13 (dt, $^1J_{HH}$= 530.3 Hz, $^4J_{HH}$= 1.9 Hz, 1H, P-H), 5.16-4.94 (m, 2H, H-a[1]), 3.86 (s, 3H, H-g[1]), 1.80-1.70 (m, 2H, H-a[2]), 1.62-1.48 (m, 2H, H-b[2]), 1.38-1.30 (m, 2H, H-c[2]), 1.26-1.18 (m, 16H, H-d[2], H-e[2], H-f[2], H-g[2], H-h, H-i, H-j, H-k), 0.84 (t, $^3J_{HH}$= 6.8 Hz, 3H, H-l). ^{13}C NMR (101 MHz, CD_3OD): δ [ppm] = 154.0 (C-f[1]), 151.1 (C-e[1]), 133.6 (d, $^3J_{CP}$= 6.0 H_Z, C-b[1]), 129.2 (C-c[1]), 121.3 (C-d[1]), 66.6 (d, $^3J_{CP}$= 6.6 H_Z, C-a[1]), 55.4 (C-g[1]), 31.8, 30.4, 30.3, 29.54, 29.47, 29.33, 29.26, 29.24, 22.6 (C-c[2], C-d[2], C-e[2], C-f[2], C-g[2], C-h, C-i, C-j, C-k), 29.0, 28.1 (C-a[2]), 20.6 (d, $^3J_{CP}$= 3.0 H_Z, C-b[2]), 14.0 (C-l). ^{31}P NMR (162 MHz, CD_3OD): δ

[ppm] = 39.6. HRMS (ESI^+, m/z): calculated for $C_{21}H_{35}O_5P$, $[M+Na]^+$ 421.2114; found, 421.2207.

(ACB-C4;alkyl-C12)-*H*-phosphinate 18b

According to general procedure 2, with 0.23 g dodecyl-*H*-phosphinic acid **13a** (1.0 mmol, 1.0 equiv.), 0.45 g butyl (4-(hydroxymethyl)phenyl) carbonate **17b** (2.0 mmol, 2.0 equiv.), 0.19 g EDC (1.2 mmol, 1.2 equiv.), 24 mg DMAP (0.2 mmol, 0.2 equiv.) dissolved in 5 mL CH_2Cl_2. Column chromatography (SiO_2, petrol ether/ethyl acetate/CH_3COOH 6:4:0.005 v/v/v). Yield: 0.21 g (0.48 mmol, 48%) white solid. ^{1}H-NMR (400 MHz, CD_3OD): δ [ppm] = 7.42-7.36 (m, 2H, H-c^1), 7.21-7.14 (m, 2H, H-d^1), 7.10 (dt, $^1J_{HH}$= 530.5 Hz, $^4J_{HH}$= 1.9 Hz, 1H, P-H), 5.16-4.94 (m, 2H, H-a^1), 4.24 (t, $^3J_{HH}$= 6.7 Hz, 2H, H-g^1), 1.84-1.66 (m, 4H, H-a^2, H-h^1) 1.64-1.50 (m, 2H, H-b^2), 1.48-1.28 (m, 2H, H-i^1), 1.38-1.20 (m, 18H, H-c^2, H-d^2, H-e^2, H-f^2, H-g^2, H-h^2, H-i^2, H-j^2, H-k), 0.95 (t, $^3J_{HH}$= 7.3 Hz, 3H, H-j^1), 0.86 (t, $^3J_{HH}$= 6.8 Hz, 3H, H-l). ^{13}C NMR (101 MHz, CD_3OD): δ [ppm] = 153.5 (C-f^1), 151.1 (C-e^1), 133.4 (d, $^3J_{CP}$= 6.0 H_Z, C-b^1), 129.2 (C-c^1), 121.3 (C-d^1), 68.7 (C-g^1), 66.7 (d, $^3J_{CP}$= 6.6 H_Z, C-a^1), 31.8, 30.5, 30.4, 30.2, 29.51, 29.45, 29.28, 29.24, 29.22, 29.0, 22.6 (C-a^2, C-c^2, C-d^2, C-e^2, C-f^2, C-g^2, C-h^1, C-h^2, C-i^2, C-j^2, C-k), 20.6 (d, $^3J_{CP}$= 3.0 H_Z, C-b^2), 18.8(C-i^1), 14.0 (C-l), 13.6 (C-j^1). ^{31}P NMR (162 MHz, CD_3OD): δ [ppm] = 39.4. HRMS (ESI^+, m/z): calculated for $C_{24}H_{41}O_5P$, $[M+Na]^+$ 463.2584; found, 463.2699.

(ACB-C11;alkyl-C12)-*H*-phosphinate 18c

According to general procedure 2, with 0.23 g dodecyl-*H*-phosphinic acid **13a** (1.0 mmol, 1.0 equiv.), 0.64 g 4-(hydroxymethyl)phenyl undecyl carbonate **17c** (2.0 mmol, 2.0 equiv.), 0.19 g EDC (1.2 mmol, 1.2 equiv.), 24 mg DMAP (0.2 mmol, 0.2 equiv.) dissolved in 5 mL CH_2Cl_2.

Column chromatography (SiO_2, petrol ether/ethyl acetate/CH_3COOH 6:4:0.005 v/v/v). Yield: 0.29 g (0.54 mmol, 54%) white solid. ^{1}H-NMR (400 MHz, CD_3OD): δ [ppm] = 7.44-7.38 (m, 2H, H-c^1), 7.22-7.18 (m, 2H, H-d^1), 7.11 (dt, $^1J_{HH}$= 529.8 Hz, $^4J_{HH}$= 1.9 Hz, 1H, P-H), 5.16-4.96 (m, 2H, H-a^1), 4.24 (t, $^3J_{HH}$= 6.7 Hz, 2H, H-g^1), 1.84-1.70 (m, 4H, H-a^2, H-h^1) 1.64-1.56 (m, 2H, H-b^2), 1.44-1.22 (m, 34H, H-c^2, H-d^2, H-e^2, H-f^2, H-g^2, H-h^2, H-i^1, H-i^2, H-j^1, H-j^2, H-k^1, H-k^2, H-l^1, H-m, H-n, H-o, H-p), 0.90-0.84 (m, 6H, H-l^2, H-q). ^{13}C NMR (101 MHz, CD_3OD): δ [ppm] = 153.6 (C-f^1), 151.2 (C-e^1), 133.5 (d, $^3J_{CP}$= 6.6 H_Z, C-b^1), 129.3 (C-c^1), 121.4 (C-d^1), 69.1 (C-g^1), 66.7 (d, $^3J_{CP}$= 6.6 H_Z, C-a^1), 31.9, 30.5, 30.3, 29.8, 29.58, 29.56, 29.52, 29.45, 29.29, 29.23, 29.17, 29.0, 28.5, 28.3, 25.6, 22.6 (C-a^2, C-c^2, C-d^2, C-e^2, C-f^2, C-g^2, C-h^1, C-h^2, H-i^1, H-i^2, H-j^1, H-j^2, H-k^1, H-k^2, H-l^1, H-m, H-n, H-o, H-p), 20.6 (d, $^3J_{CP}$= 3.0 H_Z, C-b^2), 14.1 (C-l^2, C-q). ^{31}P NMR (162 MHz, CD_3OD): δ [ppm] = 39.5. HRMS (ESI^+, m/z): calculated for $C_{31}H_{55}O_5P$, $[M+Na]^+$ 561.3679; found, 561.3762.

(ACB-C4;alkyl-C14)-*H*-phosphinate 18d

According to general procedure 2, with 0.26 g tetradecyl-*H*-phosphinic acid **13b** (1.0 mmol, 1.0 equiv.), 0.45 g butyl (4-(hydroxymethyl)phenyl) carbonate **17b** (2.0 mmol, 2.0 equiv.), 0.19 g EDC (1.2 mmol, 1.2 equiv.), 24 mg DMAP (0.2 mmol, 0.2 equiv.) dissolved in 5 mL CH_2Cl_2. Column chromatography (SiO_2, petrol ether/ethyl acetate/CH_3COOH 6:4:0.005 v/v/v). Yield: 0.25 g (0.53 mmol, 53%) white solid. ^{1}H-NMR (500 MHz, CD_3OD): δ [ppm] = 7.44-7.36 (m, 2H, H-c^1), 7.23-7.16 (m, 2H, H-d^1), 7.11 (dt, $^1J_{HH}$= 529.7 Hz, $^4J_{HH}$= 1.8 Hz, 1H, P-H), 5.17-4.95 (m, 2H, H-a^1), 4.26 (t, $^3J_{HH}$= 6.6 Hz, 2H, H-g^1), 1.84-1.68 (m, 4H, H-a^2, H-h^1) 1.64-1.53 (m, 2H, H-b^2), 1.52-1.42 (m, 2H, H-i^1), 1.40-1.20 (m, 22H, H-c^2, H-d^2, H-e^2, H-f^2, H-g^2, H-h^2, H-i^2, H-j^2, H-k, H-l, H-m), 0.97 (t, $^3J_{HH}$= 7.3 Hz, 3H, H-j^1), 0.87 (t, $^3J_{HH}$= 6.7 Hz, 3H, H-n). ^{13}C NMR (126

MHz, CD_3OD): δ [ppm] = 153.6 (C-f^1), 151.2 (C-e^1), 133.5 (d, $^3J_{CP}$= 6.0 H_Z, C-b^1), 129.3 (C-c^1), 121.4 (C-d^1), 68.8 (C-g^1), 66.7 (d, $^3J_{CP}$= 6.6 H_Z, C-a^1), 31.8, 30.55, 30.49, 30.3, 29.65, 29.62, 29.58, 29.52, 29.33, 29.29, 29.1, 28.1, 22.7 (C-a^2, C-c^2, C-d^2, C-e^2, C-f^2, C-g^2, C-h^1, C-h^2, C-i^2, C-j^2, C-k, C-l, C-m), 20.6 (d, $^3J_{CP}$= 3.0 H_Z, C-b^2), 18.9 (C-i^1), 14.1 (C-n), 13.6 (C-j^1). ^{31}P NMR (202 MHz, CD_3OD): δ [ppm] = 41.0. HRMS (ESI^+, m/z): calculated for $C_{26}H_{45}O_5P$, $[M+Na]^+$ 491.2897; found, 491.2910.

(ACB-C4;alkyl-C18)-*H*-phosphinate 18e

According to general procedure 2, with 0.32 g octadecyl-*H*-phosphinic acid **13c** (1.0 mmol, 1.0 equiv.), 0.45 g butyl (4-(hydroxymethyl)phenyl) carbonate **17b** (2.0 mmol, 2.0 equiv.), 0.19 g EDC (1.2 mmol, 1.2 equiv.), 24 mg DMAP (0.2 mmol, 0.2 equiv.) dissolved in 5 mL CH_2Cl_2. Column chromatography (SiO_2, petrol ether/ethyl acetate/CH_3COOH 6:4:0.005 v/v/v). Yield: 0.45 g (0.85 mmol, 85%) white solid. ^{1}H-NMR (600 MHz, CD_3OD): δ [ppm] = 7.39-7.35 (m, 2H, H-c^1), 7.18-7.14 (m, 2H, H-d^1), 7.13 (dt, $^1J_{HH}$= 530.0 Hz, $^4J_{HH}$= 1.8 Hz, 1H, P-H), 5.12-4.95 (m, 2H, H-a^1), 4.22 (t, $^3J_{HH}$= 6.7 Hz, 2H, H-g^1), 1.78-1.66 (m, 4H, H-a^2, H-h^1) 1.64-1.51 (m, 2H, H-b^2), 1.42 (sext, $^3J_{HH}$= 7.5 Hz, 2H, H-i^1), 1.36-1.31 (m, 2H, H-c^2), 1.40-1.20 (m, 28H, H-d^2, H-e^2, H-f^2, H-g^2, H-h^2, H-i^2, H-j^2, H-k, H-l, H-m, H-n, H-o, H-p, H-q), 0.93 (t, $^3J_{HH}$= 7.4 Hz, 3H, H-j^1), 0.84 (t, $^3J_{HH}$= 7.0 Hz, 3H, H-r). ^{13}C NMR (151 MHz, CD_3OD): δ [ppm] = 153.6 (C-f^1), 151.2 (C-e^1), 133.5 (d, $^3J_{CP}$= 6.0 H_Z, C-b^1), 129.3 (C-c^1), 121.4 (C-d^1), 68.8 (C-g^1), 66.7 (d, $^3J_{CP}$= 6.6 H_Z, C-a^1), 31.8, 30.55, 30.49, 30.3, 29.67, 29.63, 29.59, 29.53, 29.33, 29.30, 29.1, 28.1, 22.7 (C-a^2, C-c^2, C-d^2, C-e^2, C-f^2, C-g^2, C-h^1, C-h^2, C-i^2, C-j^2, C-k, C-l, C-m, C-n, C-o, C-p, C-q), 20.6 (d, $^3J_{CP}$= 3.0 H_Z, C-b^2), 18.9 (C-i^1), 14.1 (C-r), 13.6 (C-j^1). ^{31}P NMR (243 MHz, CD_3OD): δ

[ppm] = 39.5. HRMS (ESI$^+$, m/z): calculated for $C_{30}H_{53}O_5P$, $[M+Na]^+$ 547.3523; found, 547.3518.

(β-cyanoethyl;alkyl-C12)-*H*-phosphinate 20a

According to general procedure 2, with 0.23 g dodecyl-*H*-phosphinic acid **13a** (1.0 mmol, 1.0 equiv.), 0.14 g 3-hydroxypropionitrile **12** (2.0 mmol, 1.0 equiv.), 0.19 g EDC (1.2 mmol, 1.2 equiv.), 24 mg DMAP (0.2 mmol, 0.2 equiv.) dissolved in 5 mL CH_2Cl_2. Column chromatography (SiO_2, petrol ether/ethyl acetate/CH_3COOH 4:6:0.005 v/v/v). Yield: 0.12 g (0.42 mmol, 42%) white solid. ^{1}H NMR (400 MHz, CD_3OD): δ [ppm] = 7.11 (dt, $^1J_{HH}$= 535.2 Hz, $^4J_{HH}$= 1.9 Hz, 1H, P-H), 4.32-4.10 (m, 2H, H-a^1), 2.82-2.62 (m, 2H, H-b^1), 1.84-1.70 (m, 2H, H-a^2), 1.62-1.48 (m, 2H, H-b^2), 1.38-1.30 (m, 2H, H-c^2), 1.27-1.16 (m, 16H, H-d, H-e, H-f, H-g, H-h, H-i, H-j, H-k), 0.81 (t, $^3J_{HH}$= 6.7 Hz, 3H, H-l). ^{13}C NMR (101 MHz, CD_3OD): δ [ppm] = 118.8 (C-c^1), 60.2 (d, $^3J_{CP}$= 6.4 H_Z, C-a^1), 31.8, 31.4, 30.2, 29.54, 29.48, 29.31, 29.27, 29.25, 22.6 (C-c^2, C-d, C-e, C-f, C-g, C-h, C-i, C-j, C-k), 29.0, 28.1 (C-a^2), 20.4 (d, $^3J_{CP}$= 3.0 H_Z, C-b^2), 20.0 (d, $^3J_{CP}$= 6.3 H_Z, C-b^1), 14.1 (C-l). ^{31}P NMR (162 MHz, CD_3OD): δ [ppm] = 41.9. HRMS (ESI$^+$, m/z): calculated for $C_{15}H_{30}NO_2P$, $[M+Na]^+$ 310.1906; found, 310.2115.

(β-cyanoethyl;alkyl-C18)-*H*-phosphinate 20c

According to general procedure 2, with 0.32 g octadecyl-*H*-phosphinic acid **13c** (1.0 mmol, 1.0 equiv.), 0.14 g 3-hydroxypropionitrile **12** (2.0 mmol, 1.0 equiv.), 0.19 g EDC (1.2 mmol, 1.2 equiv.), 24 mg DMAP (0.2 mmol, 0.2 equiv.) dissolved in 5 mL CH_2Cl_2. Column chromatography (SiO_2, petrol ether/ethyl acetate/CH_3COOH 4:6:0.005 v/v/v). Yield: 0.19 g (0.51 mmol, 51%) white solid. ^{1}H NMR (400 MHz, CD_3OD): δ [ppm] = 7.16 (dt, $^1J_{HH}$= 534.7

Hz, $^4J_{HH}$= 1.9 Hz, 1H, P-H), 4.38-4.12 (m, 2H, H-a^1), 2.86-2.66 (m, 2H, H-b^1), 1.88-1.75 (m, 2H, H-a^2), 1.67-1.52 (m, 2H, H-b^2), 1.43-1.34 (m, 2H, H-c^2), 1.33-1.20 (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-p, H-q), 0.86 (t, $^3J_{HH}$= 6.7 Hz, 3H, H-l). ^{13}C NMR (101 MHz, CD_3OD): δ [ppm] = 116.5 (C-c^1), 60.3 (d, $^3J_{CP}$= 6.6 H_Z, C-a^1), 31.9, 31.4, 30.3, 29.63, 29.60, 29.55, 29.49, 29.30, 29.25, 28.4, 22.6 (C-c^2, 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), 29.0, 28.1 (C-a^2), 20.4 (d, $^3J_{CP}$= 3.3 H_Z, C-b^2), 20.0 (d, $^3J_{CP}$= 6.6 H_Z, C-b^1), 14.1 (C-r). ^{31}P NMR (162 MHz, CD_3OD): δ [ppm] = 42.0. HRMS (ESI^+, m/z): calculated for $C_{21}H_{42}NO_2P$, $[M+Na]^+$ 394.2845; found, 394.3081.

γ-(AB-C1)-γ-C-(alkyl-C12)-d4TTP 10a. According to general procedure 3 with 115 mg *H*-phosphinate **15a** (0.3 mmol, 1.0 equiv.), 80 mg NCS (0.6 mmol, 2.0 equiv.), 2.3 mL tetrabutylammonium phosphate (0.9 mmol, 3.0 equiv.) and 165 mg d4TMP 2×nBu_4N^+ salt (0.21 mmol, 0.7 equiv.). Reaction time was 3 h at room temperature. Yield: 103 mg (0.13 mmol, 61%) white solid. HPLC-UV analysis confirmed purity: > 96%, t_R = 14.7 min. ^{1}H-NMR (400 MHz, CD_3OD): δ [ppm] = 7.69 (dd, $^3J_{HH}$= 3.1 Hz, $^4J_{HH}$=1.3 Hz, 1H, H-6), 7.52-7.44 (m, 2H, H-c^1), 7.12-7.06 (m, 2H, H-d^1), 6.94 (dt, $^3J_{HH}$= 3.5 Hz, $^4J_{HH}$=1.6 Hz, 1H, H-1′), 6.49 (dt, $^3J_{HH}$= 5.9 Hz, $^4J_{HH}$=1.5 Hz, 1H, H-3′), 5.84 (ddd, $^3J_{HH}$= 6.1 Hz, $^3J_{HH}$= 3.4 Hz, $^4J_{HH}$= 1.8 Hz, 1H, H-2′), 5.30-5.10 (m, 2H, H-a^1), 5.00-4.94 (m, 1H, H-4′), 4.31–4.15 (m, 2H, H-5′), 3.28-3.17 (m, 0.12H, H-A), 2.27 (s, 3H, H-g^1), 2.08-1.96 (m, 2H, H-a^2), 1.90 (dd, $^3J_{HH}$= 4.9 Hz, $^4J_{HH}$= 1.1 Hz, 3H, H-7), 1.70-1.55 (m, 4.24H, H-B, H-C, H-b^2, H-c^2), 1.42-1.20 (m, 16H, H-d^2, H-e^2, H-f^2, H-g^2, H-h, H-i, H-j, H-k), 1.01 (t, $^3J_{HH}$= 7.3 Hz, 0.18H, H-D), 0.89 (t, $^3J_{HH}$= 7.0 Hz, 3H, H-l). ^{13}C NMR (101 MHz, CD_3OD): δ [ppm] = 171.1 (C-f^1), 166.5 (d, $^3J_{CP}$= 2.8 H_Z, C-4), 152.8 (C-2), 152.2 (d, $^4J_{CP}$= 1.8 H_Z, C-e^1), 138.6 (C-6), 135.7 (C-3′), 135.56 (dd, $^3J_{CP}$= 7.4 H_Z, $^3J_{CP}$= 3.8 Hz, C-b^1),

130.30 (d, $^{3}J_{CP}$= 8.3 Hz, C-c^{1}), 127.2 (C-2′), 122.9 (C-d^{1}), 112.1 (C-5), 90.8 (C-1′), 87.1 (d, $^{3}J_{CP}$= 8.8 H_Z, C-4′), 67.96, 67.88, 67.81 (C-a^{1}, C-5′), 59.5 (C-A), 39.6, 33.0, 31.6, 31.4, 30.74, 30.72, 30.68, 30.5, 30.4, 30.2, 23.7 (C-c^{2}, C-d^{2}, C-e^{2}, C-f^{2}, C-g^{2}, C-h, C-i, C-j, C-k), 27.9, 26.1 (C-a^{2}), 24.7 (C-B), 23.2 (d, $^{3}J_{CP}$= 5.4 H_Z, C-b^{2}), 20.7 (C-C), 20.9 (C-g^{1}), 14.4 (C-l), 13.9 (C-D), 12.5 (C-7). ^{31}P NMR (162 MHz, CD_3OD): δ [ppm] = 24.42 (dd, $^{2}J_{pp}$= 23.6, $^{2}J_{pp}$= 11.8 Hz, P-γ), -11.68 (d, $^{2}J_{pp}$= 19.2 Hz, P-α), -23.53 (t, $^{2}J_{pp}$= 26.5 Hz, P-β). MALDI-MS (m/z): calculated for $C_{31}H_{47}N_2O_{14}P_3$ $[M-H]^-$ 763.217; found, 763.207.

γ-(AB-C4)-γ-C-(alkyl-C12)-d4TTP 10b. According to general procedure 3 with 127 mg *H*-phosphinate **15b** (0.3 mmol, 1.0 equiv.), 80 mg NCS (0.6 mmol, 2.0 equiv.), 2.3 mL tetrabutylammonium phosphate (0.9 mmol, 3.0 equiv.) and 165 mg d4TMP 2×nBu_4N^+ salt (0.21 mmol, 0.7 equiv.). Reaction time was 2 h at room temperature. Yield: 52 mg (0.065 mmol, 31%) white solid. HPLC-UV analysis confirmed purity: > 96%, t_R = 16.1 min. ^{1}H-NMR (400 MHz, CD_3OD-d_4): δ [ppm] = 7.68 (dd, $^{3}J_{HH}$= 2.9 Hz, $^{4}J_{HH}$=1.3 Hz, 1H, H-6), 7.50-7.45 (m, 2H, H-c^{1}), 7.10-7.05 (m, 2H, H-d^{1}), 6.94 (dt, $^{3}J_{HH}$= 3.3 Hz, $^{4}J_{HH}$=1.5 Hz, 1H, H-1′), 6.49 (dt, $^{3}J_{HH}$= 5.9 Hz, $^{4}J_{HH}$=1.8 Hz, 1H, H-3′), 5.84 (ddd, $^{3}J_{HH}$= 6.1 Hz, $^{3}J_{HH}$= 3.4 Hz, $^{4}J_{HH}$= 1.8 Hz, 1H, H-2′), 5.26-5.13 (m, 2H, H-a^{1}), 5.00-4.94 (m, 1H, H-4′), 4.30-4.15 (m, 2H, H-5′), 2.58 (t, $^{3}J_{HH}$= 7.4 Hz, 2H, H-g^{1}), 2.06-1.96 (m, 2H, H-a^{2}), 1.90 (dd, $^{3}J_{HH}$= 4.6 Hz, $^{4}J_{HH}$= 1.1 Hz, 3H, H-7), 1.71 (quint, $^{3}J_{HH}$= 7.3 Hz, 2H, H-h^{1}), 1.64-1.54 (m, 2H, H-b^{2}), 1.44 (sext, $^{3}J_{HH}$= 7.7 Hz, 2H, H-i^{1}), 1.38-1.24 (m, 18H, H-c^{2}, H-d^{2}, H-e^{2}, H-f^{2}, H-g^{2}, H-h^{2}, H-i^{2}, H-j^{2}, H-k), 0.99 (t, $^{3}J_{HH}$= 7.3 Hz, 3H, H-j^{1}), 0.89 (t, $^{3}J_{HH}$= 6.7 Hz, 3H, H-l). ^{13}C NMR (101 MHz, CD_3OD): δ [ppm] = 173.8 (C-f^{1}), 166.5 (d, $^{3}J_{CP}$= 2.7 H_Z, C-4), 152.8 (C-2), 152.2 (d, $^{4}J_{CP}$= 1.7 H_Z, C-e^{1}), 138.6 (C-6), 135.7 (C-3′), 135.50 (dd, $^{3}J_{CP}$= 7.2 H_Z, $^{3}J_{CP}$= 3.9 Hz, C-b^{1}), 130.34 (d, $^{3}J_{CP}$= 7.7 Hz, C-c^{1}), 127.2 (C-2′), 122.8 (d,

$^{4}J_{CP}$= 1.1 H_Z, C-d^1), 112.0 (C-5), 90.9 (C-1´), 87.1 (d, $^{3}J_{CP}$= 9.1 H_Z, C-4´), 67.99, 67.89, 67.80 (C-a^1, C-5´), 34.7 (C-g^1), 27.97, 26.1 (C-a^2), 28.03 (C-h^1), 33.0, 31.6, 31.4, 30.76, 30.73, 30.69, 30.51, 30.45, 30.2, 23.7 (C-c^2, C-d^2, C-e^2, C-f^2, C-g^2, C-h^2, C-i^1, C-i^2, C-j^2, C-k), 23.2 (d, $^{3}J_{CP}$= 5.2 H_Z, C-b^2), 14.5 (C-l), 14.1 (C-j^1), 12.5 (C-7). ^{31}P NMR (162 MHz, CD_3OD): δ [ppm] = 24.44 (dd, $^{2}J_{pp}$= 23.6, $^{2}J_{pp}$= 11.8 Hz, P-γ), -11.68 (d, $^{2}J_{pp}$= 17.8 Hz, P-α), -23.49 (t, $^{2}J_{pp}$= 26.5 Hz, P-β). MALDI-MS (m/z): calculated for $C_{34}H_{53}N_2O_{14}P_3$ $[M-H]^-$ 805.264; found, 805.227.

γ-(AB-C11)-γ-C-(alkyl-C12)-d4TTP 10c. According to general procedure 3 with 157 mg *H*-phosphinate **15c** (0.3 mmol, 1.0 equiv.), 80 mg NCS (0.6 mmol, 2.0 equiv.), 2.3 mL tetrabutylammonium phosphate (0.9 mmol, 3.0 equiv.) and 165 mg d4TMP 2×nBu_4N^+ salt (0.21 mmol, 0.7 equiv.). Reaction time was 4 h at room temperature. Yield: 65 mg (0.069 mmol, 33%) white solid. HPLC-UV analysis confirmed purity: > 98%, t_R = 18.2 min. ^{1}H-NMR (400 MHz, CD_3OD): δ [ppm] = 7.67 (dd, $^{3}J_{HH}$= 2.3 Hz, $^{4}J_{HH}$=1.1 Hz, 1H, H-6), 7.52-7.45 (m, 2H, H-c^1), 7.15-7.05 (m, 2H, H-d^1), 6.94 (dt, $^{3}J_{HH}$= 3.3 Hz, $^{4}J_{HH}$=1.5 Hz, 1H, H-1´), 6.49 (dt, $^{3}J_{HH}$= 5.9 Hz, $^{4}J_{HH}$=1.5 Hz, 1H, H-3´), 5.84 (ddd, $^{3}J_{HH}$= 6.0 Hz, $^{3}J_{HH}$= 3.3 Hz, $^{4}J_{HH}$= 1.5 Hz, 1H, H-2´), 5.25-5.15 (m, 2H, H-a^1), 5.05-4.97 (m, 1H, H-4´), 4.30-4.15 (m, 2H, H-5´), 3.27-3.17 (m, 2H, H-A), 2.57 (t, $^{3}J_{HH}$= 7.4 Hz, 2H, H-g^1), 2.05-1.95 (m, 2H, H-a^2), 1.90 (dd, $^{3}J_{HH}$= 3.7 Hz, $^{4}J_{HH}$= 1.5 Hz, 3H, H-7), 1.80-1.55 (m, 8H, H-B, H-C, H-b^2, H-h^1), 1.45-1.24 (m, 34H, H-c^2, H-d^2, H-e^2, H-f^2, H-g^2, H-h^2, H-i^1, H-i^2, H-j^1, H-j^2, H-k^1, H-k^2, H-l^1, H-m, H-n, H-o, H-p), 1.01 (t, $^{3}J_{HH}$= 7.3 Hz, 3H, H-D), 0.89 (t, $^{3}J_{HH}$= 6.6 Hz, 6H, H-l^2, H-q). ^{13}C NMR (101 MHz, CD_3OD): δ [ppm] = 173.7 (C-f^1), 166.5 (d, $^{3}J_{CP}$= 2.7 H_Z, C-4), 152.8 (C-2), 152.2 (d, $^{4}J_{CP}$= 1.6 H_Z, C-e^1), 138.6 (C-6), 135.7 (C-3´), 135.50 (dd, $^{3}J_{CP}$= 7.2 H_Z, $^{3}J_{CP}$= 3.9 Hz, C-b^1), 130.35 (d, $^{3}J_{CP}$= 7.7 Hz, C-c^1), 127.2 (C-2´), 122.8 (C-d^1), 112.0 (C-5), 90.8 (C-1´), 87.1 (d, $^{3}J_{CP}$= 9.1 H_Z, C-4´), 67.98, 67.88, 67.78

(C-a[1], C-5′), 59.5 (C-A), 35.0 (C-g[1]), 39.6, 33.06, 33.04, 31.6, 31.4, 30.76, 30.74, 30.70, 30.57, 30.51, 30.47, 30.44, 30.37, 30.2, 30.1, 23.72, 23.71 (C-c[2], C-d[2], C-e[2], C-f[2], C-g[2], C-h[2], C-i[1], C-i[2], C-j[1], C-j[2], C-k[1], C-k[2], C-l[1], C-m, C-n, C-o, C-p), 27.9, 26.1 (C-a[2]), 25.9 (C-h[1]), 24.8 (C-B), 23.2 (d, $^3J_{CP}$= 4.8 H_Z, C-b[2]), 20.7 (C-C), 14.5 (C-l[2], C-q), 13.9 (C-D), 12.5 (C-7). ^{31}P NMR (162 MHz, CD_3OD): δ [ppm] = 25.75 (dd, $^2J_{pp}$= 23.6, $^3J_{pp}$= 9.5 Hz, P-γ), -10.48 (d, $^2J_{pp}$= 16.4 Hz, P-α), -22.26 (t, $^2J_{pp}$= 18.5 Hz, P-β). MALDI-MS (m/z): calculated for $C_{41}H_{67}N_2O_{14}P_3$ $[M-H]^-$ 903.373; found, 903.340.

γ-(AB-C4)-γ-C-(alkyl-C14)-d4TTP 10d. According to general procedure 3 with 136 mg *H*-phosphinate **15d** (0.3 mmol, 1.0 equiv.), 80 mg NCS (0.6 mmol, 2.0 equiv.), 2.3 mL tetrabutylammonium phosphate (0.9 mmol, 3.0 equiv.) and 165 mg d4TMP 2×nBu_4N^+ salt (0.21 mmol, 0.7 equiv.). Reaction time was 4 h at room temperature. Yield: 104 mg (0.12 mmol, 57%) white solid. HPLC-UV analysis confirmed purity: > 97%, t_R = 16.7 min. ^{1}H-NMR (400 MHz, CD_3OD): δ [ppm] = 7.68 (dd, $^3J_{HH}$= 2.2 Hz, $^4J_{HH}$=1.2 Hz, 1H, H-6), 7.50-7.45 (m, 2H, H-c[1]), 7.11-7.06 (m, 2H, H-d[1]), 6.94 (dt, $^3J_{HH}$= 3.4 Hz, $^4J_{HH}$=1.6 Hz, 1H, H-1′), 6.49 (dt, $^3J_{HH}$= 5.9 Hz, $^4J_{HH}$=1.8 Hz, 1H, H-3′), 5.84 (ddd, $^3J_{HH}$= 6.0 Hz, $^3J_{HH}$= 3.7 Hz, $^4J_{HH}$= 1.6 Hz, 1H, H-2′), 5.26-5.14 (m, 2H, H-a[1]), 5.00-4.95 (m, 1H, H-4′), 4.30-4.16 (m, 2H, H-5′), 3.28-3.20 (m, 0.12H, H-A), 2.58 (t, $^3J_{HH}$= 7.5 Hz, 2H, H-g[1]), 2.06-1.96 (m, 2H, H-a[2]), 1.90 (dd, $^3J_{HH}$= 3.8 Hz, $^4J_{HH}$= 1.0 Hz, 3H, H-7), 1.71 (quint, $^3J_{HH}$= 7.5 Hz, 2H, H-h[1]), 1.66-1.54 (m, 2.12H, H-B, H-b[2]), 1.46 (sext, $^3J_{HH}$= 7.5 Hz, 2H, H-i[1]), 1.40-1.24 (m, 22.12H, H-C, H-c[2], H-d[2], H-e[2], H-f[2], H-g[2], H-h[2], H-i[2], H-j[2], H-k, H-l, H-m), 1.02 (t, $^3J_{HH}$= 7.2 Hz, 0.18H, H-D), 0.98 (t, $^3J_{HH}$= 7.3 Hz, 3H, H-j[1]), 0.89 (t, $^3J_{HH}$= 7.0 Hz, 3H, H-n). ^{13}C NMR (101 MHz, CD_3OD): δ [ppm] = 173.8 (C-f[1]), 166.6 (d, $^3J_{CP}$= 4.5 H_Z, C-4), 152.8 (C-2), 152.2 (d, $^4J_{CP}$= 2.7 H_Z, C-e[1]), 138.7 (C-6), 135.8 (C-3′), 135.6, 135.1

(C-b[1]), 130.3 (d, $^{2}J_{CP}$= 13.8 Hz, C-c[1]), 127.2 (C-2´), 122.8 (d, $^{4}J_{CP}$= 1.1 H_Z, C-d[1]), 112.1 (C-5), 90.9 (d, $^{4}J_{CP}$= 1.8 Hz, C-1´), 87.2 (d, $^{3}J_{CP}$= 9.2 H_Z, C-4´), 67.9, 67.8 (2 x d, $^{3}J_{CP}$= 6.2 H_Z, $^{3}J_{CP}$= 5.6 H_Z, C-a[1], C-5´), 59.5 (t, $^{3}J_{CP}$= 2.8 H_Z, C-A), 34.8 (C-g[1]), 28.1 (C-h[1]), 27.6, 26.5 (C-a[2]), 33.1, 31.6, 31.4, 30.79, 30.77, 30.75, 30.69, 30.52, 30.46, 30.2, 23.7 (C-c[2], C-d[2], C-e[2], C-f[2], C-g[2], C-h[2], C-i[1], C-i[2], C-j[2], C-k, C-l, C-m), 24.8 (C-B), 23.2 (d, $^{3}J_{CP}$= 5.4 H_Z, C-b[2]), 20.7 (C-C), 14.3 (C-n), 14.1 (C-j[1]), 13.9 (C-D), 12.5 (C-7). ^{31}P NMR (162 MHz, CD_3OD): δ [ppm] = 25.74 (dd, $^{2}J_{pp}$= 25.2, $^{3}J_{pp}$= 9.2 Hz, P-γ), -10.47 (d, $^{2}J_{pp}$= 18.3 Hz, P-α), -22.24 (t, $^{2}J_{pp}$= 16.5 Hz, P-β). MALDI-MS (m/z): calculated for $C_{36}H_{57}N_2O_{14}P_3$ $[M-H]^-$ 833.295; found, 833.260.

γ-(AB-C4)-γ-C-(alkyl-C18)-d4TTP 10e. According to general procedure 3 with 153 mg *H*-phosphinate **15e** (0.3 mmol, 1.0 equiv.), 80 mg NCS (0.6 mmol, 2.0 equiv.), 2.3 mL tetrabutylammonium phosphate (0.9 mmol, 3.0 equiv.) and 165 mg d4TMP 2×nBu_4N^+ salt (0.21 mmol, 0.7 equiv.). Reaction time was 5 h at room temperature. Yield: 58 mg (0.063 mmol, 30%) white solid. HPLC-UV analysis confirmed purity: > 98%, t_R = 18.2 min. ^{1}H NMR (400 MHz, CD_3OD): δ [ppm] = 7.68 (dd, $^{3}J_{HH}$= 2.8 Hz, $^{4}J_{HH}$= 1.3 Hz, 1H, H-6), 7.50-7.45 (m, 2H, H-c[1]), 7.11–7.05 (m, 2H, H-d[1]), 6.94 (dt, $^{3}J_{HH}$= 3.4 Hz, $^{4}J_{HH}$=1.6 Hz, 1H, H-1´), 6.49 (dt, $^{3}J_{HH}$= 6.0 Hz, $^{4}J_{HH}$=1.4 Hz, 1H, H-3´), 5.84 (ddd, $^{3}J_{HH}$= 6.0 Hz, $^{3}J_{HH}$= 2.2 Hz, $^{4}J_{HH}$= 1.4 Hz, 1H, H-2´), 5.28-5.12 (m, 2H, H-a[1]), 5.02-4.96 (m, 1H, H-4´), 4.30-4.22 (m, 1H, H-5´$_a$), 4.20-4.15 (m, 1H, H-5´$_b$), 2.58 (t, $^{3}J_{HH}$= 7.4 Hz, 2H, H-g[1]), 2.07-1.95 (m, 2H, H-a[2]), 1.90 (dd, $^{3}J_{HH}$=2.8 Hz, $^{4}J_{HH}$=1.0 Hz, 3H, H-7), 1.71 (quint, $^{3}J_{HH}$= 7.4 Hz, 2H, H-h[1]), 1.66-1.54 (m, 2H, H-b[2]), 1.46 (sext, $^{3}J_{HH}$= 7.5 Hz, 2H, H-i[1]), 1.37-1.25 (m, 30H, H-c[2], H-d[2], H-e[2], H-f[2], H-g[2], H-h[2], H-i[2], H-j[2], H-k, H-l, H-m, H-n, H-o, H-p, H-q), 0.98 (t, $^{3}J_{HH}$= 7.3 Hz, 3H, H-j[1]), 0.89 (t, $^{3}J_{HH}$= 6.8 Hz, 3H, H-r). ^{13}C NMR (101 MHz, CD_3OD): δ [ppm] = 173.7 (d, $^{3}J_{CP}$= 2.1 H_Z, C-f[1]), 166.5 (d, $^{3}J_{CP}$= 3.5 H_Z, C-4), 152.8

(C-2), 152.2 (d, $^{3}J_{CP}$= 2.2 H_Z, C-e^{1}), 138.6 (C-6), 135.7 (C-3´), 135.5 (dd, $^{3}J_{CP}$= 6.6 H_Z, $^{3}J_{CP}$= 5.1 Hz, C-b^{1}), 130.3 (d, $^{2}J_{CP}$= 10.3 H_Z, C-c^{1}), 127.2 (C-2´), 122.8 (d, $^{4}J_{CP}$= 1.4 H_Z, C-d^{1}), 112.0 (C-5), 90.8 (d, $^{3}J_{CP}$= 2.2 H_Z, C-1´), 87.2 (d, $^{3}J_{CP}$= 9.0 H_Z, C-4´), 67.96, 67.88, 67.80 (C-a^{1}, C-5´), 34.7 (C-g^{1}), 43.1, 33.1, 31.6, 31.4, 30.80, 30.75, 30.72, 30.54, 30.47, 30.2, 23.7, 11.6 (C-c^{2}, C-d^{2}, C-e^{2}, C-f^{2}, C-g^{2}, C-h^{2}, C-i^{2}, C-j^{2}, C-k, C-l, C-m, C-n, C-o, C-p, C-q), 28.0 (C-h^{1}), 27.7, 26.3 (C-a^{2}), 23.28, 23.24 (C-b^{2}, C-i^{1}), 14.4 (C-r), 14.1 (C-j^{1}), 12.5 (C-7). ^{31}P NMR (162 MHz, CD_3OD): δ [ppm] = 24.55 (dd, $^{2}J_{pp}$=23.8 Hz, $^{3}J_{pp}$=8.0 Hz, P-γ), -11.56 (d, $^{2}J_{pp}$=17.5 Hz, P-α), -23.39 (t, $^{2}J_{pp}$= 20.5 Hz, P-β). MALDI-MS (m/z): calculated for $C_{40}H_{65}N_2O_{14}P_3$ $[M-H]^-$ 889.358; found, 889.334.

γ-(ACB-C1)-γ-C-(alkyl-C12)-d4TTP 11a. According to general procedure 3 with 119 mg *H*-phosphinate **18a** (0.3 mmol, 1.0 equiv.), 80 mg NCS (0.6 mmol, 2.0 equiv.), 2.3 mL tetrabutylammonium phosphate (0.9 mmol, 3.0 equiv.) and 165 mg d4TMP 2×nBu_4N^+ salt (0.21 mmol, 0.7 equiv.). Reaction time was 3 h at room temperature. Yield: 119 mg (0.14 mmol, 68%) white solid. HPLC-UV analysis confirmed purity: > 97%, t_R = 14.0 min. ^{1}H-NMR (600 MHz, CD_3OD): δ [ppm] = 7.67 (dd, $^{3}J_{HH}$= 2.2 Hz, $^{4}J_{HH}$=1.2 Hz, 1H, H-6), 7.52-7.46 (m, 2H, H-c^{1}), 7.20-7.15 (m, 2H, H-d^{1}), 6.94 (dt, $^{3}J_{HH}$= 3.2 Hz, $^{4}J_{HH}$=1.3 Hz, 1H, H-1´), 6.49 (dt, $^{3}J_{HH}$= 6.2 Hz, $^{4}J_{HH}$=1.8 Hz, 1H, H-3´), 5.84 (ddd, $^{3}J_{HH}$= 6.0 Hz, $^{3}J_{HH}$= 3.7 Hz, $^{4}J_{HH}$= 1.6 Hz, 1H, H-2´), 5.27-5.14 (m, 2H, H-a^{1}), 5.00-4.95 (m, 1H, H-4´), 4.30-4.22 (m, 1H, H-5´$_a$), 4.20-4.15 (m, 1H, H-5´$_b$), 3.87 (s, 3H, H-g^{1}), 2.06-1.96 (m, 2H, H-a^{2}), 1.90 (dd, $^{3}J_{HH}$= 3.8 Hz, $^{4}J_{HH}$= 1.2 Hz, 3H, H-7), 1.70-1.55 (m, 2H, H-b^{2}), 1.40-1.34 (m, 2H, H-c^{2}), 1.33-1.24 (m, 16H, H-d^{2}, H-e^{2}, H-f^{2}, H-g^{2}, H-h, H-i, H-j, H-k), 0.89 (t, $^{3}J_{HH}$= 7.5 Hz, 3H, H-l). ^{13}C NMR (151 MHz, CD_3OD): δ [ppm] = 166.5 (d, $^{3}J_{CP}$= 2.7 H_Z, C-4), 155.6 (C-f^{1}), 152.8 (C-2), 152.5 (d, $^{4}J_{CP}$= 1.4 H_Z, C-e^{1}), 138.5 (C-

6), 135.80 (dd, $^{3}J_{CP}$= 7.2 H_Z, $^{3}J_{CP}$= 3.8 Hz, C-b^{1}), 135.6 (C-3´), 130.42 (d, $^{3}J_{CP}$= 8.0 Hz, C-c^{1}), 127.2 (C-2´), 122.2 (d, $^{4}J_{CP}$= 1.1 H_Z, C-d^{1}), 112.0 (C-5), 90.9 (C-1´), 87.1 (d, $^{3}J_{CP}$= 8.9 H_Z, C-4´), 67.88, 67.78 (C-a^{1}, C-5´), 56.0 (C-g^{1}), 27.9, 26.1 (C-a^{2}), 33.0, 31.6, 31.3, 30.72, 30.70, 30.66, 30.5, 30.4, 30.2, 23.7 (C-c^{2}, C-d^{2}, C-e^{2}, C-f^{2}, C-g^{2}, C-h, C-i, C-j, C-k), 23.2 (d, $^{3}J_{CP}$= 5.5 H_Z, C-b^{2}), 14.5 (C-l), 12.5 (C-7). ^{31}P NMR (243 MHz, CD_3OD): δ [ppm] = 25.74 (dd, $^{2}J_{pp}$= 23.5, $^{2}J_{pp}$= 11.5 Hz, P-γ), -10.46 (d, $^{2}J_{pp}$= 18.6 Hz, P-α), -22.20 (dt, $^{2}J_{pp}$= 27.5 Hz, $^{3}J_{pp}$= 8.9 Hz, P-β). MALDI-MS (m/z): calculated for $C_{31}H_{47}N_2O_{15}P_3$ $[M-H]^-$ 779.212; found, 779.215.

γ-(ACB-C4)-γ-C-(alkyl-C12)-d4TTP 11b. According to general procedure 3 with 132 mg *H*-phosphinate **18b** (0.3 mmol, 1.0 equiv.), 80 mg NCS (0.6 mmol, 2.0 equiv.), 2.3 mL tetrabutylammonium phosphate (0.9 mmol, 3.0 equiv.) and 165 mg d4TMP 2×nBu_4N^+ salt (0.21 mmol, 0.7 equiv.). Reaction time was 2 h at room temperature. Yield: 57 mg (0.067 mmol, 32%) white solid. HPLC-UV analysis confirmed purity: > 98%, t_R = 15.8 min. ^{1}H-NMR (600 MHz, CD_3OD): δ [ppm] = 7.69 (dd, $^{3}J_{HH}$= 3.1 Hz, $^{4}J_{HH}$=1.3 Hz, 1H, H-6), 7.52-7.46 (m, 2H, H-c^{1}), 7.20-7.15 (m, 2H, H-d^{1}), 6.94 (dt, $^{3}J_{HH}$= 3.1 Hz, $^{4}J_{HH}$=1.5 Hz, 1H, H-1´), 6.49 (dt, $^{3}J_{HH}$= 5.9 Hz, $^{4}J_{HH}$=1.8 Hz, 1H, H-3´), 5.84 (ddd, $^{3}J_{HH}$= 6.0 Hz, $^{3}J_{HH}$= 3.4 Hz, $^{4}J_{HH}$= 1.8 Hz, 1H, H-2´), 5.26-5.14 (m, 2H, H-a^{1}), 5.00-4.95 (m, 1H, H-4´), 4.30-4.15 (m, 2H, H-5´), 4.24 (t, $^{3}J_{HH}$= 6.5 Hz, 2H, H-g^{1}), 2.06-1.98 (m, 2H, H-a^{2}), 1.91 (dd, $^{3}J_{HH}$= 4.9 Hz, $^{4}J_{HH}$= 1.1 Hz, 3H, H-7), 1.71 (quint, $^{3}J_{HH}$= 6.8 Hz, 2H, H-h^{1}), 1.65-1.56 (m, 2H, H-b^{2}), 1.45 (sext, $^{3}J_{HH}$= 7.6 Hz, 2H, H-i^{1}), 1.37-1.25 (m, 18H, H-c^{2}, H-d^{2}, H-e^{2}, H-f^{2}, H-g^{2}, H-h^{2}, H-i^{2}, H-j^{2}, H-k), 0.98 (t, $^{3}J_{HH}$= 7.4 Hz, 3H, H-j^{1}), 0.89 (t, $^{3}J_{HH}$= 6.9 Hz, 3H, H-l). ^{13}C NMR (151 MHz, CD_3OD): δ [ppm] = 166.5 (d, $^{3}J_{CP}$= 2.8 H_Z, C-4), 155.1 (C-f^{1}), 152.8 (C-2), 152.6 (d, $^{4}J_{CP}$= 1.6 H_Z, C-e^{1}), 138.6 (C-6), 135.8 (dd, $^{3}J_{CP}$= 7.2 H_Z, $^{3}J_{CP}$= 3.9 Hz, C-b^{1}), 135.7 (C-3´), 130.47 (d, $^{3}J_{CP}$= 8.2 Hz, C-c^{1}), 127.2 (C-2´), 122.3 (d,

$^{4}J_{CP}$= 1.1 H_Z, C-d^1), 112.1 (C-5), 90.9 (d, $^{4}J_{CP}$= 1.4 Hz, C-1′), 87.1 (d, $^{3}J_{CP}$= 9.1 H_Z, C-4′), 69.7 (C-g^1), 67.88, 67.80 (C-a^1, C-5′), 33.1, 32.2, 31.8, 31.6, 31.4, 30.77, 30.74, 30.70, 30.52, 30.49, 30.46, 30.2, 23.7 (C-c^2, C-d^2, C-e^2, C-f^2, C-g^2, C-h^1, C-h^2, C-i^2, C-j^2, C-k), 28.0, 26.1 (C-a^2), 23.2 (d, $^{3}J_{CP}$= 5.5 H_Z, C-b^2), 20.0 (C-i^1), 14.4 (C-l), 14.0 (C-j^1), 12.5 (C-7). ^{31}P NMR (243 MHz, CD_3OD): δ [ppm] = 24.35 (dd, $^{2}J_{pp}$= 23.6, $^{2}J_{pp}$= 11.7 Hz, P-γ), -11.87 (d, $^{2}J_{pp}$= 20.2 Hz, P-α), -23.60 (dt, $^{2}J_{pp}$= 26.5 Hz, $^{2}J_{pp}$= 11.5 Hz, P-β). MALDI-MS (m/z): calculated for $C_{34}H_{53}N_2O_{15}P_3$ $[M-H]^-$ 821.259; found, 821.230.

γ-(ACB-C11)-γ-C-(alkyl-C12)-d4TTP 11c. According to general procedure 3 with 161 mg *H*-phosphinate **18c** (0.3 mmol, 1.0 equiv.), 80 mg NCS (0.6 mmol, 2.0 equiv.), 2.3 mL tetrabutylammonium phosphate (0.9 mmol, 3.0 equiv.) and 118 mg d4TMP 2×nBu_4N^+ salt (0.15 mmol, 0.5 equiv.). Reaction time was 5 h at room temperature. Yield: 36 mg (0.038 mmol, 25%) white solid. HPLC-UV analysis confirmed purity: > 96%, t_R = 18.7 min. ^{1}H-NMR (400 MHz, CD_3OD): δ [ppm] = 7.68 (dd, $^{3}J_{HH}$= 2.0 Hz, $^{4}J_{HH}$=1.2 Hz, 1H, H-6), 7.52-7.46 (m, 2H, H-c^1), 7.20-7.15 (m, 2H, H-d^1), 6.94 (dt, $^{3}J_{HH}$= 3.3 Hz, $^{4}J_{HH}$=1.5 Hz, 1H, H-1′), 6.49 (dt, $^{3}J_{HH}$= 5.9 Hz, $^{4}J_{HH}$=1.5 Hz, 1H, H-3′), 5.84 (ddd, $^{3}J_{HH}$= 6.0 Hz, $^{3}J_{HH}$= 3.7 Hz, $^{4}J_{HH}$= 1.5 Hz, 1H, H-2′), 5.27-5.13 (m, 2H, H-a^1), 5.02-4.95 (m, 1H, H-4′), 4.30-4.15 (m, 2H, H-5′), 4.23 (t, $^{3}J_{HH}$= 6.5 Hz, 2H, H-g^1), 2.07-1.95 (m, 2H, H-a^2), 1.90 (dd, $^{3}J_{HH}$= 3.2 Hz, $^{4}J_{HH}$= 1.1 Hz, 3H, H-7), 1.72 (quint, $^{3}J_{HH}$= 6.8 Hz, 2H, H-h^1), 1.64-1.55 (m, 2H, H-b^2), 1.45-1.20 (m, 34H, H-c^2, H-d^2, H-e^2, H-f^2, H-g^2, H-h^2, H-i^1, H-i^2, H-j^1, H-j^2, H-k^1, H-k^2, H-l^1, H-m, H-n, H-o, H-p), 0.94-0.87 (m, 6H, H-l^2, H-q). ^{13}C NMR (101 MHz, CD_3OD): δ [ppm] = 166.5 (d, $^{3}J_{CP}$= 2.7 H_Z, C-4), 155.1 (C-f^1), 152.8 (C-2), 152.6 (d, $^{4}J_{CP}$= 1.4 H_Z, C-e^1), 138.6 (C-6), 135.8 (dd, $^{3}J_{CP}$= 7.2 H_Z, $^{3}J_{CP}$= 3.9 Hz, C-b^1), 135.7 (C-3′), 130.4 (d, $^{3}J_{CP}$= 7.7 Hz, C-c^1), 127.2 (C-2′), 122.3 (d, $^{4}J_{CP}$= 1.1 H_Z, C-d^1), 112.0 (C-

5), 90.9 (d, $^4J_{CP}$= 1.4 Hz, C-1´), 87.1 (d, $^3J_{CP}$= 9.1 H_Z, C-4´), 70.0 (C-g^1), 67.88, 67.80 (C-a^1, C-5´), 33.06, 33.04, 31.6, 31.4, 30.77, 30.75, 30.70, 30.67, 30.61, 30.52, 30.47, 30.44, 30.3, 30.2, 26.8, 23.7 (C-c^2, C-d^2, C-e^2, C-f^2, C-g^2, C-h^2, C-i^1, C-i^2, C-j^1, C-j^2, C-k^1, C-k^2, C-l^1, C-m, C-n, C-o, C-p), 29.7 (C-h^1), 28.0, 26.1 (C-a^2), 23.2 (d, $^3J_{CP}$= 5.9 H_Z, C-b^2), 14.5 (C-l^2, C-q), 12.5 (C-7). ^{31}P NMR (162 MHz, CD_3OD): δ [ppm] = 24.48 (dd, $^2J_{pp}$= 25.2, $^3J_{pp}$= 7.8 Hz, P-γ), -11.77 (d, $^2J_{pp}$= 19.4 Hz, P-α), -23.58 (t, $^2J_{pp}$= 22.5 Hz, P-β). MALDI-MS (m/z): calculated for $C_{41}H_{67}N_2O_{15}P_3$ $[M-H]^-$ 919.368; found, 919.342.

γ-(ACB-C4)-γ-C-(alkyl-C14)-d4TTP 11d. According to general procedure 3 with 140 mg *H*-phosphinate **18d** (0.3 mmol, 1.0 equiv.), 80 mg NCS (0.6 mmol, 2.0 equiv.), 2.3 mL tetrabutylammonium phosphate (0.9 mmol, 3.0 equiv.) and 165 mg d4TMP 2×nBu_4N^+ salt (0.21 mmol, 0.7 equiv.). Reaction time was 2 h at room temperature. Yield: 97 mg (0.11 mmol, 52%) white solid. HPLC-UV analysis confirmed purity: > 96%, t_R = 16.8 min. ^{1}H-NMR (400 MHz, CD_3OD): δ [ppm] = 7.69 (dd, $^3J_{HH}$= 2.2 Hz, $^4J_{HH}$=1.2 Hz, 1H, H-6), 7.53-7.46 (m, 2H, H-c^1), 7.21-7.15 (m, 2H, H-d^1), 6.94 (dt, $^3J_{HH}$= 3.4 Hz, $^4J_{HH}$=1.6 Hz, 1H, H-1´), 6.49 (dt, $^3J_{HH}$= 5.9 Hz, $^4J_{HH}$=1.4 Hz, 1H, H-3´), 5.84 (ddd, $^3J_{HH}$= 6.0 Hz, $^3J_{HH}$= 3.4 Hz, $^4J_{HH}$= 1.5 Hz, 1H, H-2´), 5.26-5.14 (m, 2H, H-a^1), 5.00-4.94 (m, 1H, H-4´), 4.32-4.15 (m, 2H, H-5´), 4.24 (t, $^3J_{HH}$= 6.6 Hz, 2H, H-g^1), 2.06-1.96 (m, 2H, H-a^2), 1.91 (d, $^3J_{HH}$= 2.2 Hz, 3H, H-7), 1.72 (quint, $^3J_{HH}$= 6.7 Hz, 2H, H-h^1), 1.66-1.55 (m, 2H, H-b^2), 1.44 (sext, $^3J_{HH}$= 7.6 Hz, 2H, H-i^1), 1.38-1.24 (m, 22H, H-c^2, H-d^2, H-e^2, H-f^2, H-g^2, H-h^2, H-i^2, H-j^2, H-k, H-l, H-m), 0.98 (t, $^3J_{HH}$= 7.3 Hz, 3H, H-j^1), 0.89 (t, $^3J_{HH}$= 6.7 Hz, 3H, H-n). ^{13}C NMR (101 MHz, CD_3OD): δ [ppm] = 166.5 (d, $^3J_{CP}$= 2.8 H_Z, C-4), 155.1 (C-f^1), 152.8 (C-2), 152.6 (d, $^4J_{CP}$= 1.3 H_Z, C-e^1), 138.6 (C-6), 135.83 (dd, $^3J_{CP}$= 7.2 H_Z, $^3J_{CP}$= 3.8 Hz, C-b^1), 135.68 (C-3´), 130.4 (d, $^3J_{CP}$= 7.8 Hz, C-c^1), 127.2 (C-2´), 122.3 (d, $^4J_{CP}$=

1.1 H_Z, C-d^1), 112.0 (C-5), 90.9 (d, $^4J_{CP}$= 1.3 Hz, C-1′), 87.2 (d, $^3J_{CP}$= 8.9 H_Z, C-4′), 69.7 (C-g^1), 67.88, 67.80 (C-a^1, C-5′), 33.0, 31.7, 31.6, 31.4, 30.77, 30.75, 30.69, 30.61, 30.51, 30.45, 30.2, 23.7 (C-c^2, C-d^2, C-e^2, C-f^2, C-g^2, C-h^1, C-h^2, C-i^2, C-j^2, C-k, C-l, C-m), 28.0, 26.1 (C-a^2), 23.2 (d, $^3J_{CP}$= 5.6 H_Z, C-b^2), 20.0 (C-i^1), 14.5 (C-n), 14.0 (C-j^1), 12.5 (C-7). ^{31}P NMR (162 MHz, CD_3OD): δ [ppm] = 24.58 (dd, $^2J_{pp}$= 25.2, $^3J_{pp}$= 7.8 Hz, P-γ), -11.71 (d, $^2J_{pp}$= 19.7 Hz, P-α), -22.24 (dt, $^2J_{pp}$= 25.1 Hz, $^3J_{pp}$= 5.9 Hz, P-β). MALDI-MS (m/z): calculated for $C_{36}H_{57}N_2O_{15}P_3$ $[M-H]^-$ 849.290; found, 849.247.

γ-(ACB-C4)-γ-C-(alkyl-C18)-d4TTP 11e. According to general procedure 3 with 157 mg *H*-phosphinate **18e** (0.3 mmol, 1.0 equiv.), 80 mg NCS (0.6 mmol, 2.0 equiv.), 2.3 mL tetrabutylammonium phosphate (0.9 mmol, 3.0 equiv.) and 165 mg d4TMP 2×nBu_4N^+ salt (0.21 mmol, 0.7 equiv.). Reaction time was 3 h at room temperature. Yield: 101 mg (0.11 mmol, 51%) white solid. HPLC-UV analysis confirmed purity: > 98%, t_R = 18.4 min. ^{1}H NMR (400 MHz, CD_3OD): δ [ppm] = 7.67 (dd, $^3J_{HH}$= 2.5 Hz, $^4J_{HH}$= 1.2 Hz, 1H, H-6), 7.50-7.45 (m, 2H, H-c^1), 7.20–7.15 (m, 2H, H-d^1), 6.94 (dt, $^3J_{HH}$= 3.4 Hz, $^4J_{HH}$=1.6 Hz, 1H, H-1′), 6.49 (dt, $^3J_{HH}$= 6.0 Hz, $^4J_{HH}$=1.4 Hz, 1H, H-3′), 5.84 (ddd, $^3J_{HH}$= 6.0 Hz, $^3J_{HH}$= 2.9 Hz, $^4J_{HH}$= 1.4 Hz, 1H, H-2′), 5.28-5.14 (m, 2H, H-a^1), 5.00-4.96 (m, 1H, H-4′), 4.30-4.16 (m, 2H, H-5′), 4.24 (t, $^3J_{HH}$= 6.5 Hz, 2H, H-g^1), 3.26-3.20 (m, 0.24H, H-A), 2.05-1.95 (m, 2H, H-a^2), 1.91 (dd, $^3J_{HH}$=4.5 Hz, $^4J_{HH}$=1.1 Hz, 3H, H-7), 1.71 (quint, $^3J_{HH}$= 6.7 Hz, 2H, H-h^1), 1.66-1.54 (m, 2.24H, H-b^2, H-B), 1.46 (sext, $^3J_{HH}$= 7.5 Hz, 2H, H-i^1), 1.37-1.25 (m, 30.24H, H-c^2, H-C, H-d^2, H-e^2, H-f^2, H-g^2, H-h^2, H-i^2, H-j^2, H-k, H-l, H-m, H-n, H-o, H-p, H-q), 1.01 (t, $^3J_{HH}$= 7.4 Hz, 0.36H, H-D), 0.98 (t, $^3J_{HH}$= 7.4 Hz, 3H, H-j^1), 0.89 (t, $^3J_{HH}$= 6.9 Hz, 3H, H-r). ^{13}C NMR (101 MHz, CD_3OD): δ [ppm] = 166.5 (d, $^3J_{CP}$= 5.5 H_Z, C-4), 155.1 (C-f^1), 152.8 (d, $^3J_{CP}$= 2.1 H_Z, C-2), 152.6 (d, $^3J_{CP}$= 2.4 H_Z, C-e^1),

138.6 (C-6), 135.8 (dd, $^{3}J_{CP}$= 7.5 H_Z, $^{3}J_{CP}$= 3.8 Hz, C-b^{1}), 135.7 (d, $^{3}J_{CP}$= 2.1 H_Z, C-3′), 130.3 (d, $^{2}J_{CP}$= 16.4 H_Z, C-c^{1}), 127.2 (C-2′), 122.3 (d, $^{3}J_{CP}$= 3.2 H_Z, C-d^{1}), 112.0 (C-5), 90.8 (d, $^{3}J_{CP}$= 3.2 H_Z, C-1′), 87.2 (d, $^{3}J_{CP}$= 8.8 H_Z, C-4′), 69.7 (C-g^{1}), 67.85, 67.81 (C-a^{1}, C-5′), 59.5 (C-A), 33.1, 31,7, 31.5, 31.4, 30.79, 30.75, 30.71, 30.53, 30.46, 30.2, 23.7 (C-c^{2}, C-d^{2}, C-e^{2}, C-f^{2}, C-g^{2}, C-h^{1}, C-h^{2}, C-i^{2}, C-j^{2}, C-k, C-l, C-m, C-n, C-o, C-p, C-q), 27.5, 26.6 (C-a^{2}), 24.7 (C-B), 23.2 (d, $^{3}J_{CP}$= 5.4 H_Z, C-b^{2}), 20.7 (C-C), 20.0 (C-i^{1}), 14.5 (C-r), 14.03 (C-j^{1}), 13.96 (C-D), 12.5 (C-7). ^{31}P NMR (162 MHz, CD_3OD): δ [ppm] = 24.54 (dd, $^{2}J_{pp}$=23.5 Hz, $^{3}J_{pp}$=11.8 Hz, P-γ), -11.60 (d, $^{2}J_{pp}$=17.8 Hz, P-α), -23.40 (t, $^{2}J_{pp}$= 20.5 Hz, P-β). MALDI-MS (m/z): calculated for $C_{40}H_{65}N_2O_{15}P_3$ [M-H]$^{-}$ 905.353; found, 905.308.

γ-(β-cyanoethyl)-γ-(alkyl-C12)-d4TTP 22a. According to general procedure 3 with 86 mg *H*-phosphinate **20a** (0.3 mmol, 1.0 equiv.), 80 mg NCS (0.6 mmol, 2.0 equiv.), 2.3 mL tetrabutylammonium phosphate (0.9 mmol, 3.0 equiv.) and 165 mg d4TMP 2×nBu_4N^+ salt (0.21 mmol, 0.7 equiv.). Reaction time was 2 h at room temperature. Yield: 96 mg (0.14 mmol, 65%) white solid. HPLC-UV analysis confirmed purity: > 96%, t_R = 13.6 min. ^{1}H NMR (400 MHz, CD_3OD): δ [ppm] = 7.69 (dd, $^{4}J_{HH}$= 1.2 Hz, $^{4}J_{HH}$= 0.7 Hz, 1H, H-6), 6.96 (dt, $^{3}J_{HH}$= 3.4 Hz, $^{4}J_{HH}$=1.6 Hz, 1H, H-1′), 6.52 (dt, $^{3}J_{HH}$= 6.0 Hz, $^{4}J_{HH}$=1.6 Hz, 1H, H-3′), 5.88 (ddd, $^{3}J_{HH}$= 6.0 Hz, $^{3}J_{HH}$= 2.2 Hz, $^{4}J_{HH}$= 1.4 Hz, 1H, H-2′), 5.02-4.96 (m, 1H, H-4′), 4.42-4.32 (m, 2H, H-a^{1}), 4.30-4.21 (m, 1H, H-5′$_a$), 4.21-4.15 (m, 1H, H-5′$_b$), 2.95-2.85 (m, 2H, H-b^{1}), 2.10-1.97 (m, 2H, H-a^{2}), 1.92 (d, $^{4}J_{HH}$=1.1 Hz, 3H, H-7), 1.74-1.60 (m, 2H, H-b^{2}), 1.46-1.38 (m, 2H, H-c^{2}), 1.37-1.25 (m, 16H, H-d, H-e, H-f, H-g, H-h, H-i, H-j, H-k), 0.89 (t, $^{3}J_{HH}$= 6.8 Hz, 3H, H-l). ^{13}C NMR (101 MHz, CD_3OD): δ [ppm] = 166.7 (C-4), 152.9 (C-2), 138.7 (C-6), 135.8 (C-3′), 127.2 (C-2′), 118.8 (C-c^{1}), 112.1 (C-5), 90.9 (C-1′), 87.2 (d, $^{3}J_{CP}$= 9.0 H_Z, C-4′), 67.8 (d, $^{3}J_{CP}$= 5.7 H_Z, C-5′),

62.0 (d, $^{3}J_{CP}$= 7.0 H_Z, C-a[1]), 33.1, 31.7, 31.5, 30.8, 30.6, 30.5, 30.4, 30.3, 23.7 (C-c[2], C-d, C-e, C-f, C-g, C-h, C-i, C-j, C-k), 27.8, 26.0 (C-a[2]), 23.2 (d, $^{3}J_{CP}$= 5.3 H_Z, C-b[2]), 20.2 (d, $^{3}J_{CP}$= 7.1 H_Z, C-b[1]), 14.4 (C-r), 12.5 (C-7). ^{31}P NMR (162 MHz, CD_3OD): δ [ppm] = 24.39 (dd, $^{2}J_{pp}$= 25.5 Hz, $^{3}J_{pp}$= 4.0 Hz, P-γ), -11.64 (d, $^{2}J_{pp}$=19.5 Hz, P-α), -23.65 (dd, $^{2}J_{pp}$= 25.3 Hz, $^{2}J_{pp}$= 19.5 Hz, P-β). MALDI-MS (m/z): calculated for $C_{25}H_{42}N_3O_{12}P_3$ $[M-H]^-$ 668.191; found, 668.200.

γ-C-(alkyl-C12)-d4TTP 23a. According to general procedure 3 with 86 mg *H*-phosphinate **20a** (0.3 mmol, 1.0 equiv.), 80 mg NCS (0.6 mmol, 2.0 equiv.), 2.3 mL tetrabutylammonium phosphate (0.9 mmol, 3.0 equiv.), 165 mg d4TMP 2×nBu_4N^+ salt (0.21 mmol, 0.7 equiv.) and 0.97 mL 40% $nBu_4N^+OH^-$ in H_2O (1.50 mmol, 5 equiv.) and then stirred for 8 h at room temperature. 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: 60 mg (0.088 mmol, 42%) white solid. HPLC-UV analysis confirmed purity: > 96%, t_R = 12.9 min. ^{1}H NMR (400 MHz, CD_3OD): δ [ppm] = 7.66 (d, $^{4}J_{HH}$= 1.2 Hz, 1H, H-6), 6.95 (dt, $^{3}J_{HH}$= 3.4 Hz, $^{4}J_{HH}$=1.6 Hz, 1H, H-1´), 6.53 (dt, $^{3}J_{HH}$= 6.0 Hz, $^{4}J_{HH}$=1.6 Hz, 1H, H-3´), 5.89 (ddd, $^{3}J_{HH}$= 6.0 Hz, $^{3}J_{HH}$= 2.3 Hz, $^{4}J_{HH}$= 1.3 Hz, 1H, H-2´), 5.04-4.98 (m, 1H, H-4´), 4.28-4.10 (m, 2H, H-5´), 1.91 (d, $^{4}J_{HH}$=1.0 Hz, 3H, H-7), 1.80-1.68 (m, 2H, H-a), 1.68-1.56 (m, 2H, H-b), 1.46-1.25 (m, 18H, H-c, H-d, H-e, H-f, H-g, H-h, H-i, H-j, H-k), 0.89 (t, $^{3}J_{HH}$= 6.6 Hz, 3H, H-l). ^{13}C NMR (101 MHz, CD_3OD): δ [ppm] = 166.6 (C-4), 152.9 (C-2), 138.6 (C-6), 135.8 (C-3´), 127.2 (C-2´), 112.0 (C-5), 90.9 (C-1´), 87.2 (d, $^{3}J_{CP}$= 9.0 H_Z, C-4´), 67.8 (d, $^{3}J_{CP}$= 5.6 H_Z, C-5´), 33.1, 30.81, 30.79, 30.77, 30.73, 30.48, 23.7 (C-a, C-b, C-c, 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), 32.4, 32.2 (C-c), 30.53, 28.9

(C-a), 24.6 (d, $^{3}J_{CP}$= 4.7 H_Z, C-b), 14.5 (C-l), 12.5 (C-7). ^{31}P NMR (162 MHz, CD_3OD): δ [ppm] = 19.47 (d, $^{2}J_{pp}$=25.4 Hz, P-γ), -11.43 (d, $^{2}J_{pp}$=19.5 Hz, P-α), -22.42 (t, $^{2}J_{pp}$= 21.8 Hz, P-β). MALDI-MS (m/z): calculated for $C_{22}H_{39}N_2O_{12}P_3$ $[M-H]^-$ 615.164; found, 615.198.

γ-(β-cyanoethyl)-γ-(alkyl-C18)-d4TTP 22c. According to general procedure 3 with 111 mg *H*-phosphinate **20c** (0.3 mmol, 1.0 equiv.), 80 mg NCS (0.6 mmol, 2.0 equiv.), 2.3 mL tetrabutylammonium phosphate (0.9 mmol, 3.0 equiv.) and 165 mg d4TMP 2×nBu_4N^+ salt (0.21 mmol, 0.7 equiv.). Reaction time was 2 h at room temperature. Yield: 66 mg (0.084 mmol, 40%) white solid. HPLC-UV analysis confirmed purity: > 98%, t_R = 16.6 min. ^{1}H NMR (400 MHz, CD_3OD): δ [ppm] = 7.69 (dd, $^{4}J_{HH}$= 1.2 Hz, $^{4}J_{HH}$= 0.6 Hz, 1H, H-6), 6.96 (dt, $^{3}J_{HH}$= 3.4 Hz, $^{4}J_{HH}$=1.6 Hz, 1H, H-1′), 6.52 (dt, $^{3}J_{HH}$= 6.0 Hz, $^{4}J_{HH}$=1.6 Hz, 1H, H-3′), 5.88 (ddd, $^{3}J_{HH}$= 6.0 Hz, $^{3}J_{HH}$= 2.2 Hz, $^{4}J_{HH}$= 1.4 Hz, 1H, H-2′), 5.02-4.96 (m, 1H, H-4′), 4.40-4.32 (m, 2H, H-a^1), 4.30-4.21 (m, 1H, H-5′$_a$), 4.21-4.15 (m, 1H, H-5′$_b$), 2.95-2.85 (m, 2H, H-b^1), 2.10-1.97 (m, 2H, H-a^2), 1.92 (d, $^{4}J_{HH}$=1.1 Hz, 3H, H-7), 1.74-1.64 (m, 2H, H-b^2), 1.46-1.39 (m, 2H, H-c^2), 1.37-1.25 (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-p, H-q), 0.89 (t, $^{3}J_{HH}$= 6.8 Hz, 3H, H-r). ^{13}C NMR (101 MHz, CD_3OD): δ [ppm] = 166.6 (C-4), 152.8 (C-2), 138.5 (C-6), 135.7 (C-3′), 127.2 (C-2′), 118.7 (C-c^1), 112.0 (C-5), 90.9 (C-1′), 87.2 (d, $^{3}J_{CP}$= 9.0 H_Z, C-4′), 67.8 (d, $^{3}J_{CP}$= 5.5 H_Z, C-5′), 62.0 (d, $^{3}J_{CP}$= 7.0 H_Z, C-a^1), 39.7, 33.0, 31.7, 31.4, 30.77, 30.73, 30.6, 30.5, 30.4, 30.2, 27.5, 23.7 (C-c^2, 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), 27.8, 25.9 (C-a^2), 23.2 (d, $^{3}J_{CP}$= 5.4 H_Z, C-b^2), 20.2 (d, $^{3}J_{CP}$= 7.1 H_Z, C-b^1), 14.5 (C-r), 12.5 (C-7). ^{31}P NMR (162 MHz, CD_3OD): δ [ppm] = 24.43 (dd, $^{2}J_{pp}$=25.4 Hz, $^{3}J_{pp}$=4.0 Hz, P-γ), -11.64 (d, $^{2}J_{pp}$=19.5 Hz, P-α), -23.39 (dd, $^{2}J_{pp}$= 25.3 Hz, $^{2}J_{pp}$= 19.6 Hz, P-β). MALDI-MS (m/z): calculated for $C_{31}H_{54}N_3O_{12}P_3$ $[M-H]^-$ 752.285; found, 752.285.

γ-C-(alkyl-C18)-d4TTP 23c. According to general procedure 3 with 111 mg *H*-phosphinate **20c** (0.3 mmol, 1.0 equiv.), 80 mg NCS (0.6 mmol, 2.0 equiv.), 2.3 mL tetrabutylammonium phosphate (0.9 mmol, 3.0 equiv.) and 165 mg d4TMP 2×nBu_4N^+ salt (0.21 mmol, 0.7 equiv.) and 0.97 mL 40% $nBu_4N^+OH^-$ in H_2O (1.50 mmol, 5 equiv.) and then stirred for 8 h at room temperature. 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: 24 mg (0.031 mmol, 15%) white solid. HPLC-UV analysis confirmed purity: > 96%, t_R = 15.2 min. ^{1}H NMR (400 MHz, CD_3OD): δ [ppm] = 7.69 (d, $^4J_{HH}$= 1.2 Hz, 1H, H-6), 6.95 (dt, $^3J_{HH}$= 3.4 Hz, $^4J_{HH}$=1.6 Hz, 1H, H-1´), 6.54 (dt, $^3J_{HH}$= 6.0 Hz, $^4J_{HH}$=1.6 Hz, 1H, H-3´), 5.88 (ddd, $^3J_{HH}$= 6.0 Hz, $^3J_{HH}$= 2.3 Hz, $^4J_{HH}$= 1.3 Hz, 1H, H-2´), 5.05-4.96 (m, 1H, H-4´), 4.30-4.21 (m, 1H, H-5´$_a$), 4.21-4.15 (m, 1H, H-5´$_b$), 1.92 (d, $^4J_{HH}$=1.0 Hz, 3H, H-7), 1.84-1.75 (m, 2H, H-a), 1.74-1.56 (m, 4H, H-b, H-c), 1.37-1.25 (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-p, H-q), 0.89 (t, $^3J_{HH}$= 6.8 Hz, 3H, H-r). ^{13}C NMR (101 MHz, CD_3OD): δ [ppm] = 166.7 (C-4), 152.9 (C-2), 138.7 (C-6), 135.9 (C-3´), 127.1 (C-2´), 112.0 (C-5), 90.9 (C-1´), 87.2 (d, $^3J_{CP}$= 9.0 Hz, C-4´), 67.8 (C-5´), 39.7, 33.1, 32.5, 30.78, 30.74, 30.53, 30.45, 24.6, 23.7 (C-c, 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), 29.0, 27.6 (C-a^2), 23.2 (C-b), 14.4 (C-r), 12.5 (C-7). ^{31}P NMR (162 MHz, CD_3OD): δ [ppm] = 19.60 (d, $^2J_{pp}$=25.4 Hz, P-γ), -11.23 (d, $^2J_{pp}$=19.5 Hz, P-α), -22.30 (dd, $^2J_{pp}$= 25.3 Hz, $^2J_{pp}$= 19.6 Hz, P-β). MALDI-MS (m/z): calculated for $C_{28}H_{51}N_2O_{12}P_3$ $[M-H]^-$ 699.258; found, 699.256.

Preparation of phosphate buffer (PB, pH 7.3)

1.55 g Potassium dihydrogen phosphate and 5.47 g disodium hydrogen phosphate were dissolved in 1L ultrapure water. Next, diluted phosphoric acid was added to adjust solution pH 7.3. All prodrugs **10**,**11**,**22** as well as γ-C-(alkyl)-d4TTPs **23** were incubated in this phosphate buffer to study their chemical stability.

Chemical hydrolysis of the Tri*PPP*ro-compounds 10,**11**,**22 as well as the γ-C-(alkyl)-d4TTPs 23:**

Stock solutions (50mM in DMSO) of compounds **10**,**11**,**22**,**23** were prepared. 1.9 mM hydrolysis solutions were prepared from 22 µL 50mM solutions, 378 µL milliQ water, 200 µL DMSO, and 600 µL phosphate buffer (PB, 50mM, pH 7.3). The solution was incubated at 37 °C in a thermomixer. 25 µL solutions (aliquots) were taken directly and analyzed by analytical HPLC. The exponential decay curves were calculated with OriginPro 9.0G and yielded the half-lives ($t_{1/2}$) of the prodrugs via one determination.

Hydrolysis of compounds 10,**11**,**22**,**23 with *pig liver esterase* (PLE):**

6.0 mM hydrolysis solutions of compounds **10**,**11**,**22**,**23** were prepared from 10 µL 50 mM DMSO stock solution, 31.7 µL DMSO and 41.7 µL ultrapure water. Next, 833 µL 50 mM phosphate buffer (PB, pH 7.3) and 125 µL DMSO were added to the 6.0 mM hydrolysis solutions. The reaction was started by addition of 62.5 µL of PLE in phosphate buffer (100 units/mL) and the mixture was incubated with 800 rpm at 37 °C in a thermomixer. At different times, 100 µL solutions (aliquots) were taken and stopped by addition of 106 µL MeOH. The mixture was kept for 5 min on ice followed by centrifugation at 13 000 rpm (Heraeus, Biofuge Pico) for 5 min, the mixture was filtered (Chromafil RC-20/15 MS, 0.2 mm) and stored in liquid nitrogen until an amount of 80 µL was injected into the analytical RP-18-HPLC instrument. The calculation of $t_{1/2}$ was performed analogously to that for the chemical hydrolysis studies.

CEM cell extracts hydrolysis of the Tri*PPP*ro-compounds 10,11,22 and γ-C-(alkyl)-d4TTPs 23:

6.0 mM hydrolysis solutions of compounds **10**,**11**,**22**,**23** were prepared from 21 µL 50 mM DMSO stock solutions and 154 µL DMSO. Nine-twelve different samples including 10 µL water and 10 µL 6.0 mM hydrolysis solution were prepared in 2 mL Eppendorf® vials. Then, 50 µL human CEM cell extracts was added in the mixture, the reaction was started and the mixture was incubated at 37 °C for different time periods. For work-up the reactions were stopped by addition of 150 µL MeOH. After 5 min on ice, 5 min in an ultrasonic bath, and centrifugation at 13,000 rpm for 5 min, the supernatants were filtered (Chromafil® RC-20/15 MS, 0.2 µm) and stored in liquid nitrogen. Finally, samples were warmed up to room temperature and 80 µL liquid was directly injected HPLC analysis. The calculation of $t_{1/2}$ was performed analogously to that for the chemical hydrolysis studies.

Preparation of cell extracts: Human $CD4^+$ T-lymphocyte CEM cells were grown in RPMI-1640-based cell culture medium to a final density of about $3 \cdot 10^6$ cells/mL. After centrifugation for 10 min at 1,250 rpm at 4 °C, washing twice with cold PB, and the pellet was re-suspended at 10^8 cells/mL and sonicated (Hielscher Ultrasound Techn., 100% amplitude, 3·times for 10 sec) to destroy cell integrity. After a second centrifugation of cell suspension at 10,000 rpm, the cell debris was removed, and the supernatant divided in aliquots before being frozen at -80 °C and used.

Anti-HIV activity assay: Inhibition of HIV-1(III_B)- and HIV-2(ROD)-induced cytopathogenicity in wild-type CEM/0 $CD4^+$ T-cells and thymidine kinase-deficient CEM/TK^- T-cells was measured in microtiter 96-well plates containing ~$3 \cdot 10^5$ 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 cellular effects and syncytia 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%.

Primer-extension assays:

HIV-RT and the human polymerases β and γ were obtained from Roboklon, human polymerase α was obtained from Chimerx. The flurescent labled primer and template were obtained from Metabion. Gel size was adjusted to the electrophoresis chamber (450 mm×200 mm×1.0 mm)

Primer sequence for the Cy3-fluorescent labled primer extension experiment:

5′-Cy3-CGTTG GTCCT GAAGG AGGAT AGGTT-3′

Template-sequence:

3′-GCAAC CAGGA CTTCC TCCTA TCCAA AGACA-5′

Following conditions were used in the primer extension experiments:

1.) Hybridisation: Primer and template (10 μM) were mixed in 1:1.5 reatio. The Mixture was incubated for 5 min. at 95 °C and then cooled to -20 °C over 3 hours.

2.) HIV-RT assay reaction conditions: 50 mM Tris-HCl (pH 8.6), 10 mM $MgCl_2$, 40 mM KCl, 250 μM dNTPs, HIV-RT 6 U, 0.20 μM DNA hybrid, incubated at 37 °C for 5 min without dNTPs, then incubated at 37 °C for 30 min, 80 °C for 7 min.

3.) Human DNA polymerase β assay conditions: 60 mM Tris-HCl (pH 8.7), 5 mM MgOAc, 1.0 mM dithiothreitol, 0.1 mM spermine, 0.01% (w/v) bovine serum albumin, 15% glycerol, 250 μM dNTPs, 2 U human DNA polymerase β, 0.20 μM DNA hybrid, incubated at 37 °C for 5 min without dNTPs, then incubated at 37 °C for 60 min, 80 °C for 7 min.

4.) Human DNA polymeras α assay conditions: 60 mM Tris-HCl (pH 8), 5 mM MgOAc, 1.0 mM dithiothreiol, 0.1 mM spermine, 0.01% (w/v) bovine serum albumin, 250 μM dNTPs, 2 U human polymerase α, 0.20 μM DNA hybrid, incubated at 37 °C for 5 min without dNTPs, then incubated at 37 °C for 60 min, 80 °C for 7 min.

5.) Human polymerase γ assay conditions: 60 mM Tris-HCl (pH 8), 5 mM MgOAc, 1.0 mM dithiothreiol, 0.1 mM spermine, 0.01% (w/v) bovine serum albumin, 250 μM dNTPs, 2 U human polymerase α, 0.20 μM DNA hybrid, incubated at 37 °C for 5 min without dNTPs, then incubated at 37 °C for 60 min, 80 °C for 7 min. For all assays: 50 mA, 45w for 3 h.

AUTHOR INFORMATION

Corresponding Authors

chris.meier@chemie.uni-hamburg.de.

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Author contributions

CM headed the project; XJ performed the chemical synthesis and did the biochemical assays, SW performed the primer extension assays, DS carried out the antiviral testing of the synthesized compounds. All authors were involved in the preparation of the manuscript.

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ABBREVIATIONS

AB, acyloxybenzyl; ACB, alkoxycarbonyloxybenzyl; CC_{50}, 50% cytotoxic concentration; CEM cells, $CD4^+$ T-lymphocytes; Di*PP*ro, nucleoside diphosphate prodrugs; d4T, 3'-deoxy-2',3'-didehydrothymidine; EC_{50}, 50% effective concentration; NTP, nucleoside triphosphate; NRTI, nucleoside reverse transcriptase inhibitor; PB, phosphate buffer; PLE, pig liver esterase; Tri*PPP*ro, nucleoside triphosphate prodrugs; TK, thymidine kinase.

SUPPORTING INFORMATION: Supplementary figures for chemical hydrolysis and biological hydrolysis studies of compounds **10a**,**10e**,**11a**,**11b**,**11e**,**22a**,**22b**,**23a** as well as NMR-spectra of all presented compounds are available. Molecular Formular Strings are available as well. This material is available free of charge via the Internet at http://pubs.acs.org.

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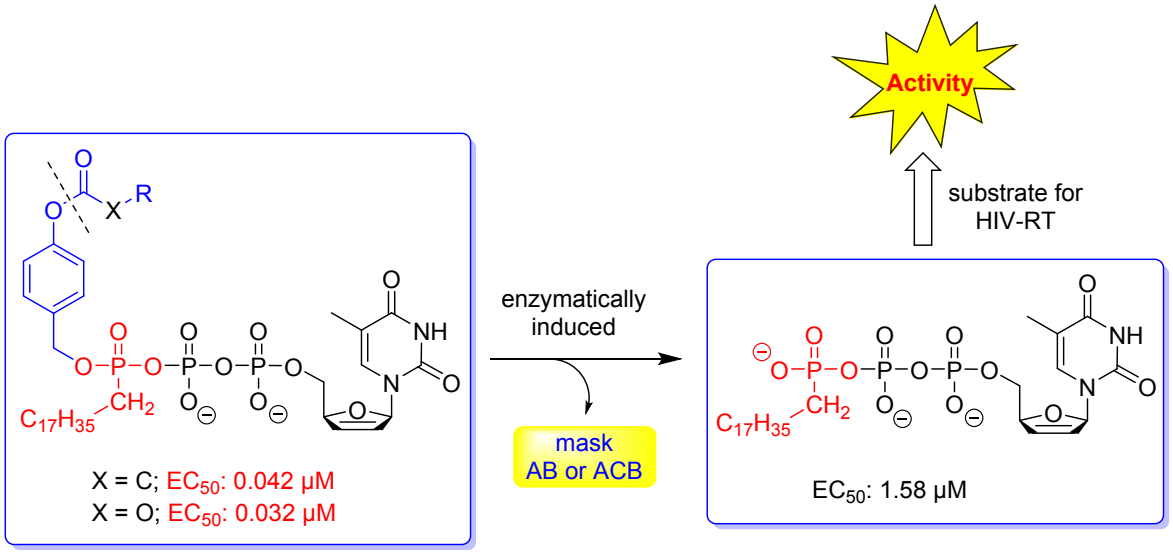
Activity
substrate for HIV-RT
enzymatically induced
mask AB or ACB
X = C; EC50: 0.042 μM
X = O; EC50: 0.032 μM
EC50: 1.58 μM