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Synthesis of potential bisubstrate inhibitors of *Leishmania* elongating α-D-mannosyl phosphate transferase

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Abstract—Synthesis of a potential mechanism-based bisubstrate inhibitor **1** of the elongating α-D-mannosyl phosphate transferase in *Leishmania*, comprising a guanosine subunit bound to the synthetic acceptor substrate through the methylenebisphosphonate linker, as well as its analogues **2** and **3** has been successfully accomplished.

Sandfly-transmitted protozoan parasites of the *Leishmania* genus cause a variety of debilitating or fatal diseases throughout the tropical and subtropical regions of the globe. It has been shown that survival and infectivity of the parasite in the mammalian host and in the insect vector are both dependent on the lipophosphoglycan (LPG) molecules, which are ubiquitous at the parasite's cell surface.[1] The biosynthesis of the polymeric backbone portion of this glycoconjugate [-6)-β-D-Gal*p*-(1→4)-α-D-Man*p*-(1-PO_3^--]$_n$ proceeds through the consecutive action of the elongating α-mannosyl phosphate transferase (eMPT) and β-galactosyltransferase enzyme activities with the former only being found in the *Leishmania* parasite,[2] thus endorsing the search for selective eMPT inhibitors as prospective anti-leishmaniasis drugs. As a part of our ongoing programme towards elucidation of the enzyme's mode of action by means of synthetic probes,[3] we wish to disclose here preliminary results aimed at the preparation of potential mechanism-based eMPT bisubstrate inhibitors.[4]

Little is known so far about the structure of the eMPT active site. However, a putative transition state model (**TS**) for α-mannosyl phosphate transfer could be devised (Fig. 1) taking into account that α-mannosyl phosphate is transferred to the growing end of the LPG chain (i.e., an acceptor substrate, **AS**, X = O, R = LPG) from the donor substrate GDP–mannose en block with retention of anomeric configuration.[2,3] Whereas the synthetic phosphonodisaccharide, dec-9-enyl β-D-galactopyranosyl-(1→4)-α-D-mannopyranosyl methanephosphonate was shown to be an effective exogenous acceptor substrate [**AS**, X = CH_2, R = $(CH_2)_8CH{=}CH_2$] for the eMPT,[5] we assume the enzyme simultaneously hosts the acceptor and the donor substrates, while the entire bond breaking–bond forming process occurs in one step in the ternary complex formed.

Thus, the covalently bound transition state analogue **1** with a nonhydrolysable methylenebisphosphonate (MBP) bridge mimicking the pyrophosphate moiety of GDP–mannose while linking together the acceptor substrate and the guanosine residue was recognised as a potential eMPT bisubstrate-type inhibitor. Moreover, the simpler analogues **2** and **3**, either lacking the nucleoside subunit (**2**) or bearing a 2-dimethylaminoethylphosphonate residue instead of MBP (**3**), both being, however, able to take part in the chelation phenomena with divalent metal ion(s) presumably present in the active site of the enzyme, were also considered as synthetic targets.

Our first goal was the preparation of the simplified analogue **3** (Scheme 1). Phosphonylation of the 6′-OH in

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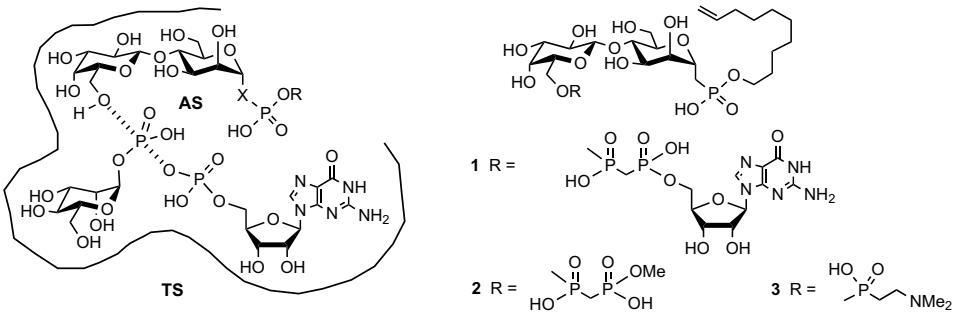
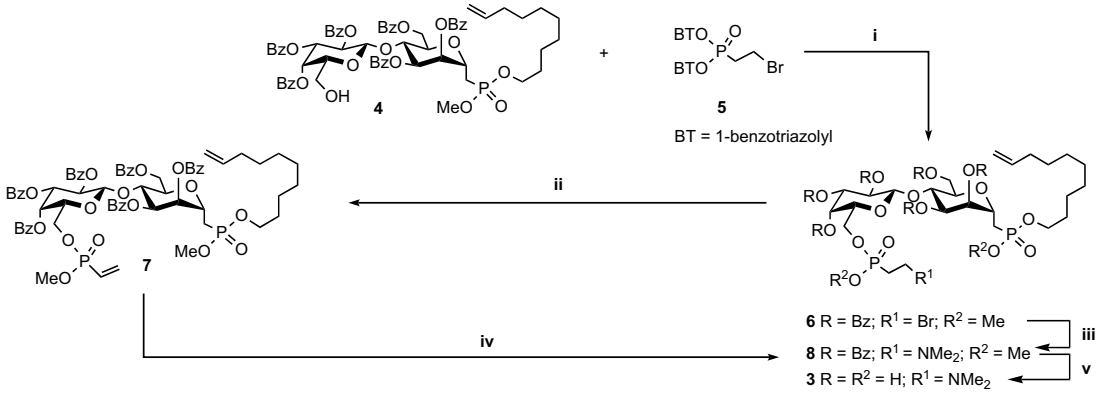


Figure 1. Putative transition state model for the α-D-mannosyl phosphate transfer and structures of the potential bisubstrate-type inhibitors.



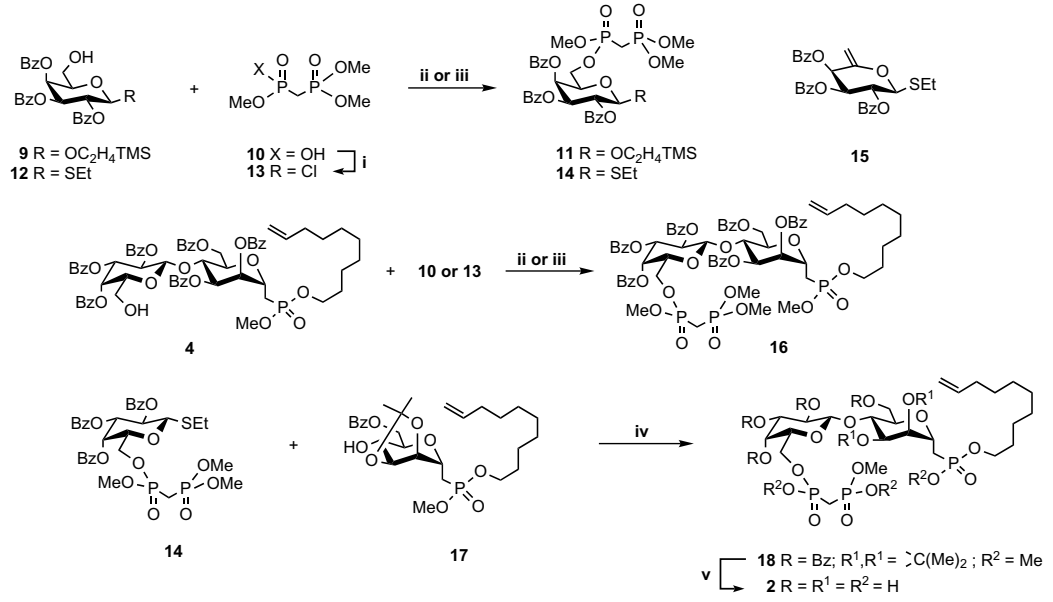
Scheme 1. Reagents and conditions: (i) DCM, 1,4-dioxane, rt, 30 min, then MeOH excess, 16 h, 85%; (ii) Et_3N, DCM, rt, 30 min, 81%; (iii) NaI, Me_2CO, 60 °C, 4 h, then Me_2NH, THF, rt, 16 h, 54%; (iv) Me_2NH, THF, rt, 16 h, 86%; (v) PhSH, Et_3N, DMF, rt, 16 h, then MeONa, MeOH, rt, 5 h, 90%.

the phosphonodisaccharide **4**[6] with bis(benzotriazolyl) 2-bromoethylphosphonate **5**[7] provided the mixed phosphonodiester **6**[8] in 85% yield. However, further transformation into the required *N*,*N*-dimethylamino derivative **8** either by direct treatment of **6** with Me_2NH/THF or through the intermediate iodide was not as effective as expected. Repeatedly, the yield of the required product did not exceed 54%. This product was obtained more effectively through the conjugate addition of the same reagent to the vinyl phosphonate **7** easily prepared from **6** by short treatment with Et_3N.[7] Finally, deprotection provided the target compound **3** as the bis-ammonium salt after isolation by ion-exchange chromatography.[9]

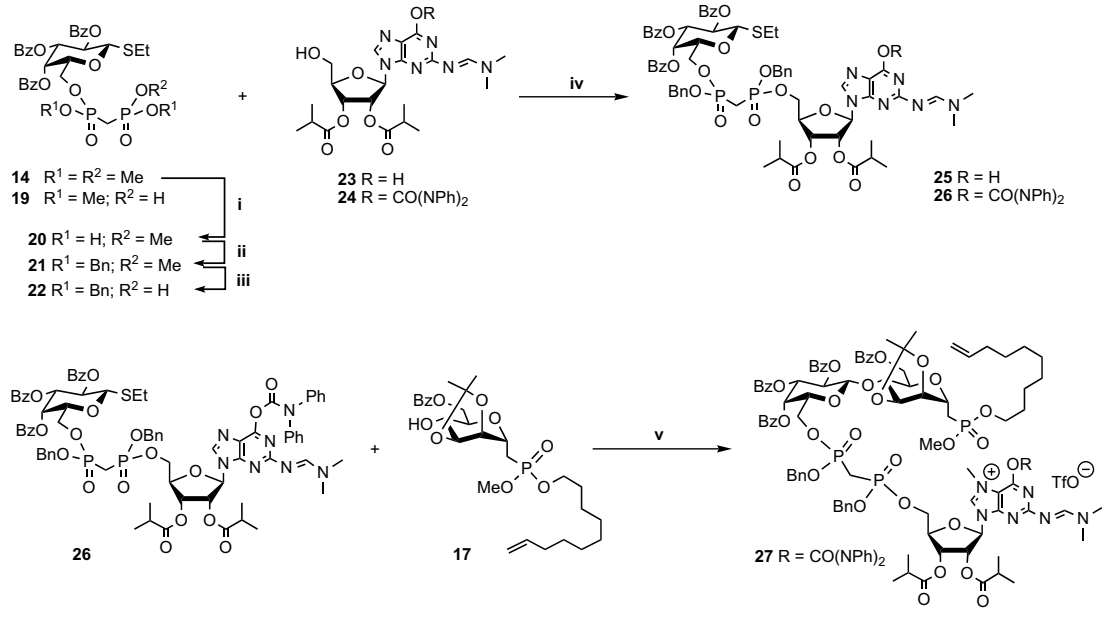
Encouraged by the straightforward implementation of the first objective we turned our efforts to the preparation of the methylenebisphosphonate derivative **2** (Scheme 2). Direct introduction of the MBP group via the monochloridate reagent **13** (derived from methylenebisphosphonic acid trimethyl ester **10**[10]) in the presence of Et_3N and DMAP,[11] while being successful when applied to the simple galactose derivative **9** (65% isolated yield of MBP-carrying glycoside **11**), completely failed in the case of the phosphonodisaccharide **4**. These results were replicated in a parallel Mitsunobu esterification[12] of **9** and **4** with the triester **10**. In this case, a high yield of the phosphonylated monosaccharide derivative **11** (83%) again contrasted with the formation of only traces of the desired disaccharide product **16**. In agreement with our previous observations,[6] the apparently diminished reactivity of the 6′-OH in compound **4** could be accounted for by the steric hindrance imposed by the benzoate protection at 3-OH of the mannose residue closely flanking the reactive site, as revealed by inspection of molecular models.

At this juncture we decided that the synthetic approach had to be diverted to the consecutive esterification–glycosylation concept developed by us previously.[6] Thus, a suitable glycosyl donor already bearing the MBP moiety had to be prepared from **11** and reacted with the known mannosyl methanephosphonate acceptor **17**[6] to give access to a suitable precursor of **2**.

All attempts to elaborate the 2-(trimethylsilyl)ethyl galactoside **11** (via the hemiacetal derivative) into the corresponding glycosyl trichloroacetimidate or bromide proved to be unsuccessful due to the instability of the MBP substructure towards either basic (DBU or Cs_2CO_3/DCM) or mild brominating agents [HBr/DCM or $(COBr)_2$/DMF/DCM]. The solution was eventually found when we tested the MBP-carrying thioglycoside **14** as a stable glycosyl donor. Mitsunobu esterification of the parent thiogalactoside **12** with the triester **10** provided **14** in 67% optimised yield along with ca. 20% of the *exo*-methylene by-product **15**. Rewardingly, the MeOTf-promoted glycosylation of **17** with **14** proceeded



Scheme 2. Reagents and conditions: (i) $(COCl)_2$, DMF cat, DCM, 0 °C, 1 h; (ii) Et_3N, DMAP, DCM, 0 °C to rt, 6 h, 65% for **9**+**13**, no reaction for **4**+**13**; (iii) PPh_3, DIAD, THF, 60 °C, 1 h, 83% for **9**+**10**, 67% for **12**+**10**, traces for **4**+**10**; (iv) MeOTf, MS 4 Å, DCM, rt, 2.5 h, 72%; (v) TFA, DCM–H_2O, rt, 15 min, then PhSH, Et_3N, DMF, rt, 16 h, then MeONa, MeOH, rt, 5 h, 80%.



Scheme 3. Reagents and conditions: (i) PhSH, Et_3N, DMF, rt, 16 h, quant; (ii) $PhCHN_2$, *t*-BuOMe, HBF_4 cat, rt, 67%; (iii) KCN, DMF, 70 °C, 6 h, 88%; (iv) PPh_3, DIAD, THF, 60 °C, 4 h, 32% for **22**+**23**, 65% for **22**+**24**; (v) MeOTf, MS 4 Å, DCM, rt, 4 h, 50%.

with complete stereoselectivity to give the β-linked phosphonodisaccharide **18** ($J_{1',2'} = 8.0$ Hz; $\delta_{C1'} = 101.7$–101.9) as a mixture of four diastereomers at phosphorus in 72% yield. Finally, the stepwise removal of the protecting groups from **18** afforded the required compound **2** as the tris-ammonium salt after purification by ion-exchange chromatography.[13]

Going ahead with the construction of the guanosine-containing compound **1** we first intended this to be accomplished through the stereoselective glycosylation of the mannosyl methanephosphonate acceptor **17** with the complex guanosine–MBP–thioglycoside donor **25** (Scheme 3) that could be prepared, in turn, via the Mitsunobu esterification of the monodeprotected MBP-carrying thioglycoside **19** with a suitable guanosine derivative **23**.[14a] It is worthy to note, that the protective group pattern in the derivative **23** meant that the final deprotection could be performed in a nonhydrogenolytic way[14b] thus keeping the dec-9-enyl moiety,

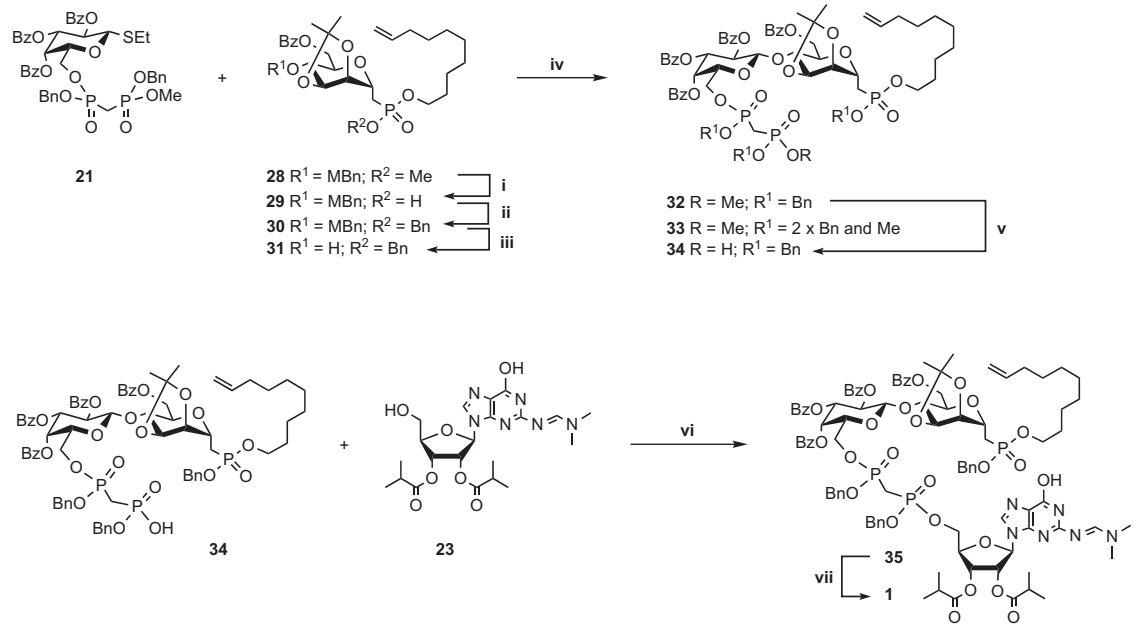
important for the preparation of neoglycoconjugates,[15] intact in the targeted product.

In the event, an attempted *regioselective* monodemethylation of the phosphonic dimethyl ester in **14** with either neat *t*-$BuNH_2$ or DABCO in THF only resulted in the formation of an inseparable 1:1 mixture of **19** and its regioisomer with the phosphonic monoacid proximal to the galactose residue.[16] Obviously, the protecting groups at the MBP moiety had to be remodeled to make a *chemoselective* deprotection feasible. To fulfil this objective the starting compound **14** was treated with PhSH–Et_3N–DMF[17] to produce the phosphonic diacid **20** almost quantitatively. This was exhaustively esterified[18] with phenyl diazomethane in the presence of HBF_4 to provide the compound **21** with the selectively protected MBP group in 67% yield, which was smoothly converted into the required monoacid derivative **22** on treatment with KCN in DMF.[19]

The Mitsunobu esterification of **22** with the guanosine synthone **23** provided the targeted thiogalactoside–MBP–guanosine building block **25** albeit in a meagre 32% yield. Predictably, when 6-*O*-protected guanosine derivative **24**[20] was used as the hydroxylic component in the same reaction, the yield of the corresponding ester **26** was almost twice as high. The standard MeOTf promoted glycosylation of the mannosyl methanephosphonate acceptor **17** with the thioglycoside **26** did furnish some unstable material with a newly formed β-glycosidic bond ($\delta_{C1'} = 101.2$–101.4). On the basis of the MALDI-TOF spectrum however, the structure of this product was tentatively assigned as the corresponding *N*-methylimidazolium salt **27** in accordance with the presence of a molecular ion with m/z 2037.72, exactly one methyl group heavier than required.

Despite seemingly being unproductive, the above approach already encompassed all the synthetic essentials needed for the successful preparation of the target compound **1** (vide infra). Specifically, with the established protocol of chemoselective monodeprotection of the MBP moiety in hand the introduction of the guanosine block could be advantageously switched further to the end of the synthetic sequence thus avoiding the presence of the incompatible heterocyclic base at the glycosylation step. This required, however, that the methyl protecting group for the phosphonodiester function in the mannosyl methanephosphonate acceptor **17** had to be changed to a benzyl one in order to be stable during the MBP monodeprotection.

Fortunately, preparation of the required mannosyl methanephosphonate acceptor **31** proved to be quite straightforward (Scheme 4). Selective monodeprotection of the starting mixed phosphonodiester **28**[6] with PhSH–Et_3N (nearly quantitative yield) followed by the Mitsunobu-type esterification with benzyl alcohol (92% yield) and removal of the *p*-methoxybenzyl (MBn) protecting group (90%) provided the desired product seamlessly. Next, the standard MeOTf promoted glycosylation of the acceptor **31** with the MBP-carrying thioglycoside **21** was again completely β-selective giving the phosphonodisaccharide MBP derivative **32** ($\delta_{C1'} = 101.6$–101.8) in an acceptable yield of 47% along with some 20% of compound **33** presumably arising from transesterification of one of the phosphonodiester groups present in the molecule with MeOTf. Selective saponification of the single methyl phosphonic ester in **32** with KCN in DMF proceeded without incident (75% yield), as did the crucial Mitsunobu esterification of **34** thus prepared with the guanosine derivative **23**, which produced the required compound **35** in 50% yield.



Scheme 4. Reagents and conditions: (i) PhSH, Et_3N, DMF, rt, 16 h, quant; (ii) BnOH, PPh_3, DIAD, THF, rt, 1 h, 92%; (iii) DDQ, DCM–H_2O, rt, 2 h, 90%; (iv) MeOTf, MS, 4 Å, DCM, rt, 4 h, 47%; (v) KCN, DMF, 70 °C, 6 h, 75%; (vi) PPh_3, DIAD, THF, rt, 6 h, 50%; (vii) TFA, DCM–H_2O, rt, 15 min, then PhSH, Et_3N, DMF, rt, 16 h, then *t*-BuOK, *t*-BuOH–THF–H_2O, rt, 16 h, 80%.

Finally, sequential deprotection[21] of **35** performed without isolation of any intermediates followed by ion-exchange chromatographic purification delivered the phosphonodisaccharide–MBP–guanosine hybrid **1**[22] as the tris-ammonium salt in 80% yield.

In conclusion, we report here the synthesis of a potential mechanism-based bisubstrate inhibitor of the elongating α-mannosyl phosphate transferase in *Leishmania* designed with the emphasis on the incorporation of a guanosine moiety linked to the acceptor substrate through the methylenebisphosphonate bridge mimicking the important guanosine-pyrophosphate motif present in the natural substrate donor GDP–mannose, as well as its simplified analogues. The results of a biological evaluation of these compounds will be disclosed elsewhere in due course.

Acknowledgements

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8. All new compounds showed satisfactory analytical and spectral data.
9. Compound **3**: ^{1}H NMR (300 MHz, D_2O): δ 1.16–1.31 (10H, m, 5×CH_2), 1.51 (2H, quintet, J 6.5, –OCH$_2$CH_2–), 1.85–2.07 (6H, m, H-1*a, -1*b, –CH_2CH$_2$NMe$_2$ and –CH_2CH=CH$_2$), 2.76 and 2.77 (6H, 2×s, –NMe$_2$), 3.23 (2H, m, –CH_2NMe$_2$), 3.44 (1H, dd, $J_{2',3'}$ 9.8, H-2′), 3.58 (1H, dd, $J_{3',4'}$ 3.5, H-3′), 3.60 (1H, m, H-5), 3.70–4.00 (11H, m, H-2, -3, -4, -4′, -5′, -6a, -6b, -6′a, -6′b and –OCH_2CH$_2$–), 4.13 (1H, m, H-1), 4.35 (1H, d, $J_{1',2'}$ 7.8, H-1′), 4.86 and 4.94 (2H, 2×br d, J 10.4, J 17.2, –CH$_2$CH=CH_2), 5.81 (1H, ddt, $J_{H,CH2}$ 6.5, –CH$_2$CH=CH$_2$); ^{13}C NMR (75 MHz, D_2O): δ 22.0 (d, $J_{C,P}$ 133.7, –CH$_2$CH$_2$NMe$_2$), 26.6 (d, $J_{C,P}$ 133.7, C-1*), 25.0, 28.1, 28.3, 28.4, 28.5 (5×CH_2), 30.1 (d, $J_{C,P}$ 5.8, –OCH$_2$$CH_2$–), 33.1 (–$CH_2$CH=CH$_2$), 42.3 (–NMe$_2$), 53.1 (–$CH_2NMe_2$), 60.3 (C-6), 63.1 (d, $J_{C,P}$ 4.5, C-6′), 64.7 (d, $J_{C,P}$ 4.5, –OCH$_2$CH$_2$–), 68.1 (C-4′), 69.1 (C-3), 70.7 (C-2′), 70.8 (d, $J_{C,P}$ 9.0, C-2), 72.3 (C-3′), 72.5 (C-5), 73.1 (C-1), 74.1 (d, $J_{C,P}$ 8.5, C-5′), 77.7 (C-4), 103.2 (C-1′), 114.0 (–CH=CH$_2$), 140.2 (–CH=CH$_2$); ^{31}P NMR (121 MHz, D_2O): δ 20.6 (P′), 22.3 (P); ES-MS (−) data: m/z 692.28 (100%, [M−H]$^-$) (expected m/z 692.29; $C_{27}H_{53}NO_{15}P_2$ requires M, 693.29); $[\alpha]_D^{22}$ +15.2° (c 0.5, MeOH).
10. Compound **10** was prepared from tetramethyl methylenebisphosphonate by saponification with neat t-BuNH$_2$ at rt for 28 h, followed by evaporation of the amine, treatment of the residue with Dowex-50 (H^+) in MeOH and concentration. The triester **10** (which contained up to 10% of the symmetric dimethyl ester) was used without additional purification.
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13. Compound **2**: ^{1}H NMR (300 MHz, D_2O): δ 1.20–1.35 (10H, m, 5×CH_2), 1.54 (2H, quintet, J 6.6, –OCH$_2$CH_2–), 1.89–2.02 (4H, m, H-1*a, -1*b and –CH_2CH=CH$_2$), 2.10 (2H, br t, $J_{H,P}$ 19.5, –PCH$_2$P–), 3.46 (1H, dd, $J_{2',3'}$ 10.0, H-2′), 3.50 (3H, d, $J_{H,P}$ 10.3, OMe), 3.62 (1H, dd, $J_{3',4'}$ 3.5, H-3′), 3.65 (1H, m, H-5), 3.74–3.98 (11H, m, H-2, -3, -4, -4′, -5′, -6a, -6b, -6′a, -6′b and –OCH_2CH$_2$–), 4.16 (1H, m, H-1), 4.38 (1H, d, $J_{1',2'}$ 7.8, H-1′), 4.89 and 4.97 (2H, 2×br d, J 10.2, J 17.3, –CH$_2$CH=CH_2), 5.85 (1H, ddt, $J_{H,CH2}$ 6.7, –CH$_2$CH=CH$_2$); ^{13}C NMR (75 MHz, D_2O): δ 25.2(t, $J_{C,P}$ 125.9, –PCH$_2$P–), 26.8 (d, $J_{C,P}$ 133.2, C-1*), 25.0, 28.2, 28.3, 28.4, 28.6 (5×CH_2), 30.2 (d, $J_{C,P}$ 6.0, –OCH$_2$$CH_2$–), 33.2 (–$CH_2$CH=CH$_2$), 51.6 (d, $J_{C,P}$ 3.5, –OMe), 60.4 (C-6), 62.6 (br, C-6′), 64.9 (d, $J_{C,P}$ 5.6, –OCH$_2$CH$_2$–), 68.0 (C-4′), 69.3 (C-3), 70.9 (d, $J_{C,P}$ 8.0, C-2), 71.0 (C-2′), 72.4 (C-3′), 72.9 (C-5), 73.5 (C-1), 73.8 (br, C-5′), 77.6 (C-4), 103.3 (C-1′), 114.0 (–CH=CH$_2$), 140.6 (–CH=CH$_2$); ^{31}P NMR (121 MHz, D_2O): δ 18.8 (br, P′ + P″), 22.5 (P); ES-MS (−) data: m/z 364.33 (100%, [M−2H]$^{2-}$) (expected m/z 364.10), 729.05 (40%, [M−H]$^-$) (expected m/z 729.21; $C_{25}H_{49}O_{18}P_3$ requires M, 730.21); $[\alpha]_D^{22}$ +11.2° (c 0.52, MeOH–H_2O, 1:1).
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20. Compound **24** was synthesised following the preparation of **23** (Ref. 14a), by treatment of the common 6′-*O*-TBDPS precursor with Ph$_2$NCOCl–DIPEA in pyridine (rt, 16 h, 50%) prior to desilylation with TBAF–AcOH in THF (rt, 1 h, 90%).
21. Simultaneous de-*O*-acylation and cleavage of the *N*,*N*-dimethylformamidine *N*-protecting group was performed with t-BuOK in t-BuOH–THF–H_2O as recommended in Ref. 14b.
22. Compound **1**: ^{1}H NMR (300 MHz, D_2O): δ 1.15–1.31 (10H, m, 5×CH_2), 1.51 (2H, quintet, J 6.8, –OCH$_2$CH_2–), 1.87–1.98 (4H, m, H-1*a, -1*b and –CH_2CH=CH$_2$),

2.11 (2H, br t, $J_{H,P}$ 19.9, –PCH_2P–), 3.42 (2H, m, H-2′ and -3′), 3.65 (1H, dt, $J_{4,5}$ 8.7, $J_{5,6a} = J_{5,6b} = 4.1$, H-5), 3.65–3.95 [11H, includes d (at δ 3.84), $J_{3',4'}$ 2.6, H-4′ and m, H-2, -3, -4, -5′, -6a, -6b, -6′a, -6′b and –OCH_2CH_2–], 4.07 (2H, m, H-5″a and -5″b), 4.15 (1H, m, H-1), 4.24 (1H, m, H-4″), 4.27 (1H, d, $J_{1',2'}$ 7.7, H-1′), 4.42 (1H, dd, $J_{2'',3''}$ 5.1, $J_{3'',4''}$ 3.6, H-3″), 4.70 (1H, dd, H-2″), 4.86 and 4.94 (2H, 2×br d, J 10.2, J 17.3, –$CH_2CH{=}CH_2$), 5.81 (1H, ddt, $J_{H,CH2}$ 6.7, –$CH_2CH{=}CH_2$), 5.84 (1H, d, $J_{1'',2''}$ 5.9, H-1″), 8.09 (1H, br, H-8‴); ^{13}C NMR (75 MHz, D_2O): δ 26.9 (d, $J_{C,P}$ 133.1, C-1*), 25.0, 28.2, 28.3, 28.5, 28.6 (5×CH_2), 30.2 (d, $J_{C,P}$ 5.8, –OCH_2CH_2–), 33.2 (–$CH_2CH{=}CH_2$), 60.5 (C-6), 62.8 (br, C-6′), 63.9 (br, C-5″), 64.9 (d, $J_{C,P}$ 5.6, –OCH_2CH_2–), 68.0 (C-4′), 69.3 (C-3), 70.5 (C-3″), 70.9 (2C, C-2′ and d, $J_{C,P}$ 8.5, C-2), 72.4 (C-3′), 72.9 (C-5), 73.6 (br, C-1), 73.8 (C-2″), 73.9 (br, C-5′), 77.8 (C-4), 84.0 (d, $J_{C,P}$ 6.1, C-4″), 87.0 (C-1″), 103.3 (C-1′), 114.0 (–$CH{=}CH_2$), 116.3 (br, C-5‴), 137.8 (br, C-8‴), 140.5 (–$CH{=}CH_2$), 151.9 (br, C-4‴), 154.0 (C-2‴), 158.9 (C-6‴); ^{31}P NMR (121 MHz, D_2O): δ 17.2, 17.5 (2×br, P′ and P″), 22.3 (P); ES-MS (−) data: m/z 489.67 (50%, $[M-2H]^{2-}$) (expected m/z 489.63), 500.69 (50%, $[M-3H+Na]^{2-}$) (expected m/z 500.62), 508.65 (100%, $[M-3H+K]^{2-}$) (expected m/z 508.61; $C_{34}H_{58}N_5O_{22}P_3$ requires M, 981.28); $[\alpha]_D^{22}$ −2.5° (c 0.5, MeOH–H_2O, 1:1).