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Synthesis of 2′,3′-dihydrosolanesyl analogues of β-D-arabinofuranosyl-1-monophosphoryldecaprenol with promising antimycobacterial activity

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Abstract—Two new hydrolytically stable analogues of β-D-arabinofuranosyl-1-monophosphoryldecaprenol, the donor substrate for mycobacterial arabinosyltransferase, have been prepared. Biological evaluation of these compounds in vitro against *Mycobacterium tuberculosis* $H_{37}Rv$ strain revealed a promising activity.

The cell wall of *Mycobacterium tuberculosis* features two complex lipopolysaccharides (lipoarabinomannan (LAM) and lipoarabinogalactan (LAG)), which act together as a barrier, largely responsible for the strong resistance of this microorganism to antibiotics. The arabinane moieties of LAM and LAG are made of repeating D-arabinofuranosyl units[1] that are assembled by specific arabinosyltransferases (AraT's). Inhibition of AraT's has been suggested as a new target for antimycobacterial agents, an hypothesis supported by the fact that ethambutol (**2**), one of the first line antituberculars still in use, is believed to work through inhibition of these enzymes.[2] Although AraT's have not been isolated, their main substrate, β-D-arabinofuranosyl-1-monophosphory-ldecaprenol **1** (DPA), whose role is to 'donate' arabinofuranosyl units to the growing arabinan chain has been identified.[2] This knowledge has been used for the rational design of hydrolytically stable DPA analogues as AraT's inhibitors, some of which (**3**,[3] **4**,[4] **5**[5]) are shown in Figure 1. Only phosphonate **3** (when $n = 15$) showed significant biological activity, inhibiting the growth of *M. tuberculosis* as efficiently as ethambutol.[3] The importance of the lipophilic chain length for recognition of DPA-like alternative arabinose donors by AraT's had been reported earlier by Lee et al.[6]

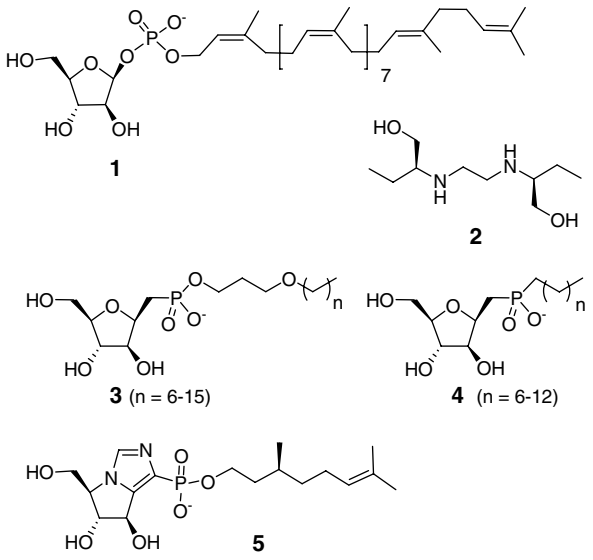

Figure 1. DPA (**1**), ethambutol (**2**) and DPA analogues.

In this work, we describe the synthesis of two new arabinose- and aza-arabinose-derived phosphonates **8** and **9**

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and their in vitro activity against *M. tuberculosis* strain $H_{37}Rv$ in comparison to ethambutol **2** and the two phosphonates **6** and **7** previously synthesized by us (Fig. 2).[7]

Our first target on the way to the aza-arabinose derivative **8**, was the key tri-*O*-benzyl-*N*-PMB-aza-arabinosylphosphonate intermediate **14**. This was synthesized in a few steps from L-xylose by adapting a methodology previously used for the preparation of (difluoromethyl)phosphonyl azasugars (Scheme 1).[8]

Thus, 2,3,5-tri-*O*-benzyl-xylofuranose **11**[9] was readily prepared from L-xylose and quantitatively converted to the furanosylamine **12**, of low hydrolytic stability, obtained as an anomeric ($\alpha/\beta = 1/3$) mixture by treatment with *p*-methoxybenzylamine in the presence of molecular sieves. Reaction of **12**, freshly prepared, with excess

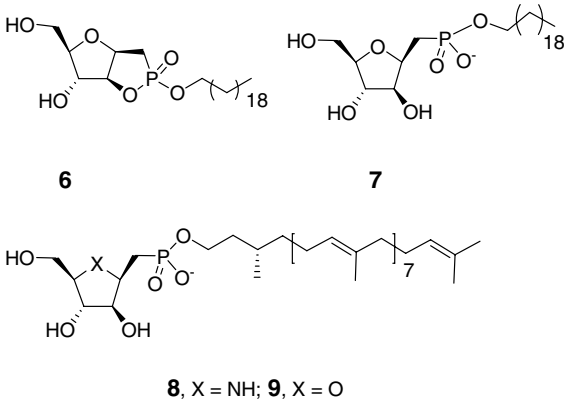
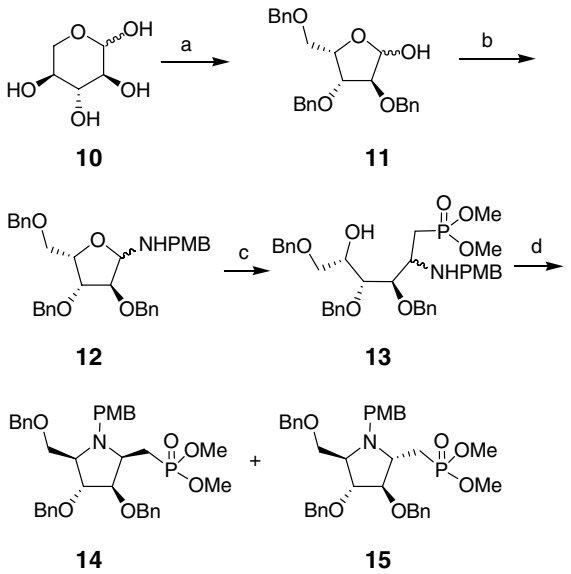


Figure 2. Synthetic DPA analogues.



Scheme 1. Reagents and conditions: (a) Ref. 10, 68%; (b) *p*-methoxybenzylamine, CH_2Cl_2, 50 °C, molecular sieves 4 Å, 15 h, quant.; (c) (i) $BF_3{\cdot}Et_2O$ (5 equiv), THF, −78 °C; (ii) $LiCH_2P(O)(OMe)_2$ (5 equiv), THF, −78 °C, 3 h, 56%; (d) MsCl, pyridine, 14 h then SiO_2 chromatography, **14** (63%), **15** (31%).

dimethyl(lithiomethyl)phosphonate (prepared by butyllithium treatment of dimethyl(methyl)phosphonate at −78 °C), as described by Guillerm et al.,[8] was very sluggish, yielding a mixture of stereoisomers **13** in only 10% yield. Much better results were obtained by pretreating **12** with $BF_3{\cdot}Et_2O$ (5 equiv) at 0 °C for 15 min. The mixture of isomers **13** was then converted in excellent yield into the two aza-arabinosylphosphonates **14** and **15** which, unlike **13**, could be easily separated by chromatography. The configuration of the newly created stereocentres was firmly assigned by NOE experiments and by chemical correlation with the corresponding *N*-benzyloxycarbonyl derivatives previously prepared by us.[10] Although moderate, the diastereoselection appears to favour the formation of the desired phosphonate **14**, in line with earlier observations.[8a]

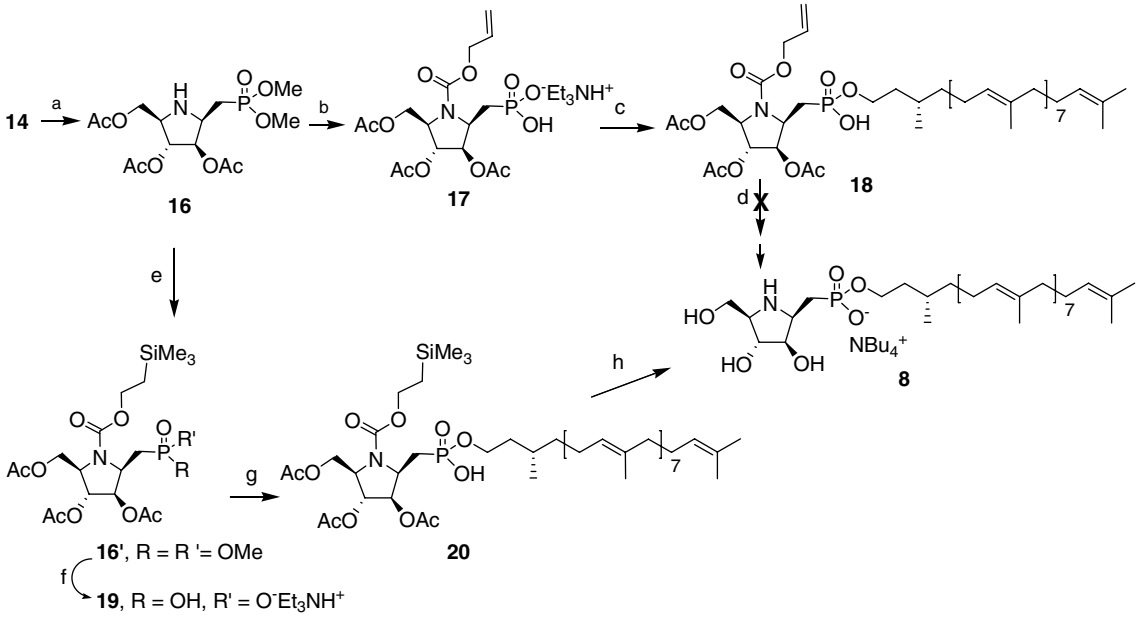
Completion of the synthesis is shown in Scheme 2. First, **14** was converted to the corresponding tri-*O*-acetyl derivative **16**,[11] by sequential treatment with BCl_3 (10 equiv), acetylation and removal of the *N*-PMB group by hydrogenolysis under pressure (50 bar) in isopropanol.

The *N*-allyloxycarbonyl (*N*-Alloc) derivative **17** was obtained in excellent yield from pyrrolidine **16** by action of allyl chloroformate in pyridine followed by TMSBr treatment but, despite several attempts varying the temperature and the reaction time, trichloroacetonitrile-assisted coupling with 2,3-dihydrosolanesol[12] led to the prenyl derivative **18** in (at best) 11% yield. Furthermore, removal of the Alloc group in **18** proved unsuccessful. We then switched to the *N*-trimethylsilylethoxycarbonyl (*N*-TEOC) **19** as coupling partner. The protection of **16** as a TEOC derivative worked reasonably well but required conditions more drastic than those described elsewhere.[13] After brief TMSBr treatment, coupling of the C_{45} prenol could be accomplished in good (61%) yield. The final steps of the synthesis involve deacetylation (sodium methanolate) and tetrabutylammonium fluoride treatment to provide **8** that was obtained in >90% purity (as judged by ^1H NMR) by preparative TLC (eluent AcOEt/*i*PrOH/H_2O (3/2/1, v/v)).[14]

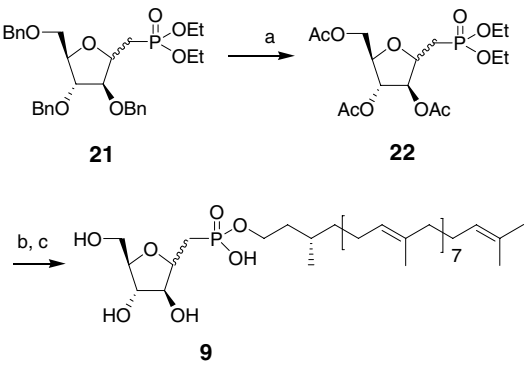
Our second target molecule, the tetrahydrofuranyl derivative **9** was easily prepared starting from an anomeric mixture (α/β = ca. 2/1) of diethylphosphonates **21**[7] and obtained as a mixture of anomers (α/β = ca. 2/1) in >90% purity (as judged by ^1H NMR) by preparative TLC (eluent AcOEt/*i*PrOH/H_2O (6/4/1, v/v)) (Scheme 3).

The activities of polyprenyl derivatives **8** and **9**[15] against *M. tuberculosis* strain $H_{37}Rv$ were then measured in vitro and compared with the activities of the two eicosanylphosphonates **6** and **7**[7] and ethambutol (**2**). Apart from the bicyclic phostone **6**, which appeared to be inactive, all compounds showed interesting MICs, comparable to that of ethambutol (MIC = 5 μM): **7** (18 μM), **8** (35 μM), **9** (50 μM).

In conclusion, we have prepared two new hydrolytically stable analogues of DPA, the immediate arabinose do-



Scheme 2. Reagents and conditions: (a) (i) BCl_3, CH_2Cl_2, −78 °C then CH_3OH; (ii) Ac_2O (20 equiv), pyridine, 24 h, 69% (two steps); (iii) H_2 (50 bar), *i*PrOH, Pd/C (10%), 15 h, 70%; (b) (i) AllylOC(O)Cl, pyridine, CH_2Cl_2, 1 h, 0 °C, 94%; (ii) TMSBr, CH_2Cl_2, 12 h, rt then CH_3OH/Et_3N (3/1, v/v), quant.; (c) CCl_3CN, 2,3-dihydrosolanesol, pyridine, 11%; (d) $Pd(PPh_3)_4$, pyrrolidine, EtOAc/CH_2Cl_2 (1/1, v/v), 12 h, rt, 0%; (e) 2-(trimethylsilyl)ethyl-*p*-nitrophenyl carbonate (2 equiv), DMAP (2 equiv), toluene, 50 °C, 2 h, 53%; (f) TMSBr, CH_2Cl_2, 0 °C, 90 min then CH_3OH/Et_3N (3/1, v/v), quant.; (g) CCl_3CN, pyridine, 2,3-dihydrosolanesol, 60 °C, 16 h, 61%; (h) (i) NaOMe, CH_3OH, 1 h then DOWEX 50W (H^+ form), 95%; (ii) $NBu_4^+F^-$, THF, 16 h, 49%.



Scheme 3. Reagents and conditions: (a) (i) H_2, $CH_3OH/CHCl_3$ (1/1, v/v), $Pd(OH)_2/C$, quant.; (ii) Ac_2O, pyridine, 86%; (b) (i) TMSBr, CH_2Cl_2, 15 h, rt then Et_3N; (ii) CCl_3CN, 2,3-dihydrosolanesol, pyridine, 65 °C, 17 h, 31% (two steps); (iii) CH_3ONa, CH_3OH, 84%.

nor for mycobacterial arabinosyltransferases. The two compounds feature a C_{45} prenyl moiety that closely matches the decaprenyl DPA chain. In contrast to most arabinosylphosphonates (phosphinates) prepared so far, preliminary biological results against *M. tuberculosis* are encouraging, as both analogues exhibit an activity in the range of ethambutol.

Acknowledgements

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*i*PrOH) followed by benzyloxycarbonylation of the resulting free amine (ZCl, CH_2Cl_2, Na_2CO_3, 2 h).

11. Initial experiments had shown that the benzyl groups could not be removed at the end of the synthesis while preserving the integrity of the prenyl chain.
12. In order to guarantee a better hydrolytic stability of the final sugar phosphonates, experiments were run with (*S*)-2,3-dihydrosolanesol, provided by Hoffmann–La Roche (Basel), instead of commercial solanesol.
13. The use of 1 equiv each of DMAP and 2-(trimethylsilyl)ethyl-*para*-nitrophenyl carbonate in dichloromethane at room temperature has been recommended: Jewel, C. F., Jr.; Brinkmann, J.; Petter, R. C. *Tetrahedron* **1994**, *50*, 3849–3856, We used 2 equiv of these reagents in toluene at 50 °C.
14. *Selected analytical data*: Compound **12** (anomeric mixture): ^{1}H NMR (250 MHz, $CDCl_3$, 300 K) δ 7.40–7.23 (15H, m), 4.99 (0.3H, d, $J = 3.8$ Hz), 4.71 (0.7H, d, $J = 2.3$ Hz), 4.65–4.42 (6H, m), 4.38 (0.3H, dd, $J = 1.9$, 6 Hz), 4.38 (0.7H, q, $J = 5.1$ Hz), 4.1–3.96 (1.7H, m), 3.91 (0.7H, t, $J = 2.3$ Hz), 3.90 (0.3H, dd, $J = 1.5$, 3.8 Hz), 3.84–3.67 (3H, m), 3.80 (3H, s). Compound **13** (major isomer): ^{1}H NMR (400 MHz, $CDCl_3$, 300 K) δ 7.32–7.24 (17H, m), 7.22 (2H, d, $J = 8.4$ Hz), 6.82 (2H, d, $J = 8.4$ Hz), 4.72 (1H, ABq, $J = 11.5$ Hz), 4.65 (1H, ABq, $J = 11.3$ Hz), 4.50 (1H, ABq, $J = 11.5$ Hz), 4.49 (1H, ABq, $J = 11.3$ Hz), 4.47 (1H, ABq, $J = 12$ Hz), 4.40 (1H, ABq, $J = 12$ Hz), 4.22 (1H, d, $J = 6.6$ Hz), 4.05 (1H, dd, $J = 6$, 8 Hz), 3.89 (1H, d, $J = 6.6$ Hz), 3.76 (3H, s), 3.74 (1H, ABq, $J = 12$ Hz), 3.69 (3H, d, $J = 10.8$ Hz), 3.64 (3H, d, $J = 10.8$ Hz), 3.62 (1H, ABq, $J = 12$ Hz), 3.59 (1H, dd, $J = 6$, 8.8 Hz), 3.52 (1H, dd, $J = 8$, 8.8 Hz), 3.47 (1H, dd, $J = 3.3$, 9.2 Hz), 2.23 (1H, ddd, $J = 3.3$, 15.3, 21 Hz), 2.06 (1H, ddd, $J = 9.2$, 15.3, 17.1 Hz). ^{31}P NMR (161.9 MHz, $CDCl_3$, 300 K) δ 30.5. ^{13}C NMR (100.69 MHz, $CDCl_3$, 300 K) δ 159.03, 138.52, 138.26, 137.91, 129.92 (2C), 128.44–127.44 (15C), 114.06 (2C), 78.80 (d, $J = 3$ Hz), 77.23, 75.48, 73.44, 73.12, 70.79, 66.62, 55.24, 52.42 (d, $J = 6.9$ Hz), 52.35 (d, $J = 6.8$ Hz), 50.61, 49.99, 25.35 (d, $J = 135.9$ Hz). Compound **14**: ^{1}H NMR (400 MHz, $CDCl_3$, 300 K) δ 7.35–7.16 (17H, m), 6.82 (2H, d, $J = 8.4$ Hz), 4.53 (1H, ABq, $J = 12$ Hz), 4.48 (1H, ABq, $J = 12$ Hz), 4.46 (1H, ABq, $J = 12$ Hz), 4.42 (1H, ABq, $J = 12$ Hz), 4.37 (1H, ABq, $J = 12$ Hz), 4.25 (1H, ABq, $J = 12$ Hz), 3.98 (1H, d, $J = 4.4$ Hz), 3.90 (1H, s), 3.88 (1H, ABq, $J = 10.4$ Hz), 3.79 (3H, s), 3.66 (6H, d, $J = 11.2$), 3.64 (1H, ABq, $J = 10.4$ Hz), 3.40 (1H, ddd, $J = 3$, 4.4, 11 Hz), 3.27 (1H, dd, $J = 5.2$, 12 Hz), 3.11–3.05 (2H, m), 2.41 (1H, ddd, $J = 11$, 15.6, 16 Hz), 1.96 (1H, ddd, $J = 3$, 15.6,18.4 Hz). ^{31}P NMR (161.9 MHz, $CDCl_3$, 300 K) δ 31.8. ^{13}C NMR (100.69 MHz, $CDCl_3$, 300 K) δ 158.79, 138.52, 138.40, 138.18, 130.76, 130.45 (2C), 128.31–127.39 (15C), 113.60 (2C), 82.74 (d, $J = 1.4$ Hz), 82.21, 72.88, 71.97, 71.83, 70.73, 68.29, 61.28, 57.39, 55.22, 52.16 (d, $J = 6.4$ Hz), 52.10 (d, $J = 6.6$ Hz), 23.76 (d, $J = 136$ Hz). HRMS (electrospray) calcd for $C_{37}H_{45}NO_7P$ ($M+H^+$) 646.2934, found 646.2902. Compound **15**: ^{1}H NMR (400 MHz, $CDCl_3$, 300 K) δ 7.36–7.26 (17H, m), 6.83 (2H, d, $J = 8.8$ Hz), 4.55 (1H, ABq, $J = 12.4$ Hz), 4.48 (2H, s), 4.47 (1H, ABq, $J = 12.4$ Hz), 4.46 (2H, s), 4.13 (1H, s), 4.03 (1H, ABq, $J = 14.2$ Hz), 3.89 (1H, d, $J = 2.8$ Hz), 3.80 (3H, s), 3.64 (1H, ABq, $J = 14.2$ Hz), 3.60 (1H, dd, $J = 5.2$, 9.6 Hz), 3.55–3.47 (2H, m), 3.53 (3H, d, $J = 10.8$ Hz), 3.51 (3H, d, $J = 10.8$ Hz), 3.16–3.12 (1H, m), 2.14 (1H, ddd, $J = 3.6$, 15.6, 18.8 Hz), 2.05 (1H, td, $J = 10$, 15.6 Hz). ^{31}P NMR (161.9 MHz, $CDCl_3$, 300 K) δ 32.0. ^{13}C NMR (100.69 MHz, $CDCl_3$, 300 K) δ 158.60, 138.74, 138.37, 138.25, 131.01, 129.30 (2C), 128.29–127.39 (15C), 113.71 (2C), 86.52, 85.11, 73.21, 71.35, 71.24, 71.12, 66.27, 59.36, 55.27, 52.10 (d, $J = 6.4$ Hz), 51.93 (d, $J = 6.2$ Hz), 51.13, 21.03 (d, $J = 134.2$ Hz). HRMS (electrospray) calcd for $C_{37}H_{45}NO_7P$ ($M+H^+$) 646.2934, found 646.2902. Compound **16**: ^{1}H NMR (250 MHz, $CDCl_3$, 300 K) δ 5.09 (1H, d, $J = 4.2$ Hz), 4.80 (1H, d, $J = 3.8$ Hz), 4.24 (1H, dd, $J = 5.3$, 11.1 Hz), 4.04 (1H, dd, $J = 7.1$, 11.1 Hz), 3.75 (6H, d, $J = 10.9$ Hz), 3.65 (1H, ddd, $J = 4.2$, 9, 13.6 Hz), 3.29 (1H, ddd, $J = 3.8$, 5.3, 7.1 Hz), 2.13–1.82 (2H, m), 2.07 (3H, s), 2.04 (3H, s), 2.03 (3H, s). ^{31}P NMR (101.2 MHz, $CDCl_3$, 300 K) δ 28.34. ^{13}C NMR (62.9 MHz, $CDCl_3$, 300 K) δ 170.78, 169.82, 169.66, 78.79, 77.89 (d, $J = 13$ Hz), 65.35, 61.78, 54.80 (d, $J = 6.4$ Hz), 52.44 (d, $J = 6.6$ Hz), 25.34 (d, $J = 142$ Hz), 20.81, 20.80, 20.75. Compound **16′**: ^{1}H NMR(250 MHz, CD_3OD, 300 K) δ 5.33 (1H, d, $J = 5.9$ Hz), 5.02 (1H, s), 4.35 (1H, ddd, $J = 3.2$, 5.9, 11.3 Hz), 4.22–4.08 (4H, m), 3.74 (3H, d, $J = 10.6$ Hz), 3.70 (3H, d, $J = 10.9$ Hz), 4.13–4.05 (1H, m), 3.05–2.71 (1H, m), 2.23–1.98 (1H, m), 2.13 (3H, s), 2.08 (3H, s), 2.05 (3H, s), 1.06–0.99 (92H, m), 0.01 (9H,s). ^{31}P NMR (101.2 MHz, $CDCl_3$, 300 K) δ 28.34. ^{13}C NMR (62.9 MHz, $CDCl_3$, 300 K) δ 170.73, 169.31, 169.12, 155.84, 74.92, 74.71, 64.42, 61.97, 52.55 (d, $J = 6.3$ Hz), 52.36 (d, $J = 6.8$ Hz), 23.90, 20.77, 17.72, −1.55. HRMS (electrospray) calcd for $C_{20}H_{36}NO_{11}NaSiP$ ($M+Na^+$) 548.1699, found 548.1693. Compound **20**: ^{1}H NMR (400 MHz, CD_3OD, 300 K) δ 5.35 (1H, broad s), 5.10 (8H, t, $J = 6.8$ Hz), 5.04 (1H, s), 4.36 (2H, broad t, $J = 9$ Hz), 4.25–4.17 (2H, m), 4.15 (1H, dd, $J = 5$, 10.8 Hz), 3.98 (1H, dd, $J = 5$, 9.2 Hz), 3.96–3.83 (2H, m), 2.80–2.52 (1H, m), 2.28–1.88 (31H, m), 2.15 (3H, s), 2.07 (3H, s), 2.06 (3H, s), 1.74–1.52 (2H, m), 1.66 (3H, s), 1.59 (24H, s), 1.50–1.25 (2H, m), 1.22–1.13 (1H, m), 1.07 (2H, broad t, $J = 9$ Hz), 0.92 (3H, d, $J = 6.4$ Hz), 0.05 (9H, s). ^{31}P NMR (161.9 MHz, CD_3OD, 300 K) δ 21.3. ^{13}C NMR (100.69 MHz, CD_3OD, 300 K) δ 172.59, 171.38, 171.14, 135.82–135.75 (7C), 132.08, 126.07, 125.61–125.52 (7C), 77.18, 76.11, 65.23, 64.35, 63.33, 63.13, 58.80, 40.96–40.86 (7C), 39.24, 38.51, 30.71, 27.88–27.62 (7C), 26.50, 25.96, 21.14, 20.78, 20.75, 20.08, 18.81, 17.84, 16.21 (7C), −1.39 (3C). HRMS (electrospray) recorded on the deacetylated POMe derivative obtained after NaOMe and CH_2N_2 treatments, calcd for $C_{58}H_{102}NO_8PNaSi$ ($M+H+Na^+$) 1022.7010, found 1022.7092. Compound **8**: ^{1}H NMR (400 MHz, CD_3OD, 300 K) δ 5.10 (8H, t, $J = 6.8$ Hz), 4.09 (2H, q, $J = 7$ Hz), 3.95–3.90 (2H, m), 3.88 (1H, broad s), 3.81 (1H, dd, $J = 6.8$, 11.6 Hz), 3.75–3.68 (1H, m), 3.71 (1H, dd, $J = 8$, 11.6 Hz), 2.42–2.15 (2H, m), 2.11–1.85 (30H, m), 1.81–1.14 (5H, m), 1.66 (3H, s), 1.59 (24H, s), 0.93 (3H, d, $J = 6.5$ Hz). ^{31}P NMR (161.9 MHz, CD_3OD, 300 K) δ 18.3. Compound **22** (mixture of anomers): ^{1}H NMR (400 MHz, $CDCl_3$, 300 K) δ 5.17 (0.33H, d, $J = 3.2$ Hz), 5.14 (0.66H, dd, $J = 2.5$, 3 Hz), 5.04 (0.66H, t, $J = 2.5$ Hz), 4.88 (0.33H, d, $J = 2.8$ Hz), 4.39 (0.66H, dq, $J = 3$, 7 Hz), 4.36–4.31 (0.33H, m), 4.27–4.16 (2H, m), 4.14–4.11 (0.66H, m), 4.08 (4H, quint, $J = 6.8$ Hz), 3.96–3.93 (0.33H, m), 2.30–2.20 (0.33H, m), 2.17 (1.32H, dd, $J = 7$, 18 Hz), 2.11–2.03 (9.33H, m), 1.28 (4H, t, $J = 6.8$ Hz), 1.27 (2H, t, $J = 6.8$ Hz). ^{31}P NMR (161.9 MHz, $CDCl_3$, 300 K) δ 28.06 (0.33P), 27.31 (0.66P). Compound **9** (mixture of epimers): ^{1}H NMR (400 MHz, $CDCl_3$, 300 K) δ 5.11 (8H, t, $J = 6.3$ Hz), 4.28 (0.33H, qd, $J = 3.3$, 7 Hz), 4.11–4.01 (2.66H, m), 3.97–3.94 (1H, m), 3.91 (0.33H, d, $J = 3.3$ Hz), 3.88–3.82 (1.32H, m), 3.78–3.73 (0.33H, m), 3.68 (1H, dd, $J = 3.5$, 12 Hz), 3.63 (1H, dd, $J = 7$, 12 Hz), 2.22–1.95 (32H, m), 1.75–1.69 (1H, m), 1.67 (1H, s), 1.66–1.59 (1H, m), 1.60 (24H, s), 1.51–1.44 (1H, m), 1.43–1.32 (1H, m), 1.29–1.21 (1H, m), 0.93 (3H,

d, $J = 6.5$ Hz). ^{31}P NMR (161.9 MHz, $CDCl_3$, 300 K) δ 26.38 (0.33P), 25.55 (0.66P). HRMS (electrospray) recorded on the POMe derivative obtained by CH_2N_2 treatment, calcd for $C_{52}H_{89}O_7NaP$ ($M+Na^+$) 879.6244, found 879.6268.

15. Bacterial growth was followed using a fast colorimetric method based on the use of a diphenyltetrazolium bromide derivative: Abate, G.; Aseffa, A.; Selassie, S.; Goshu, B.; Fekade, D.; WoldeMeskal, D.; Miorner, H. *J. Clin. Microbiol.* **2004**, *42*, 871–873.