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Note

Synthesis of lipid II phosphonate analogues

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Dedicated to Professor András Lipták on the occasion of his 75th birthday

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ABSTRACT

Simple analogues of lipid II were synthesized from 3,4,6-tri-*O*-acetyl-2-acetamido-2-deoxy-1-thio-β-D-glucopyranose using conjugate addition onto ethylidene bisphosphonate and subsequent Wadsworth–Horner–Emmons reaction with long chain aliphatic aldehydes.

The bacterial cell wall is a resistant exoskeleton based on a polymeric network of a peptidoglycan consisting of *N*-acetyl-D-glucosaminyl-*N*-acetylmuramyl (*N*AcGlu-*N*AcMur) peptide as the repeating unit.[1] This building block is synthesized within the bacterial cell in activated form as an undecaprenyl diphosphate derivative, which is known as lipid II (Fig. 1). It is anchored through the lipophilic side chain in the membrane. Lipid II, once synthesized within the cell, is transported to the external surface of the cell membrane where it reacts with the reducing end of the growing peptidoglycan chain. This reaction is catalysed by transglycosylase enzymes. Since these molecules are building up outside of the bacterial membrane, possible inhibitors, antibiotics, targeting this process do not have to cross the membrane to reach their target, therefore, such inhibitors can be of therapeutic importance.[2–6] Inhibitors of bacterial transglycosylases can be classified into two types: the substrate binders and the enzyme binders.[4] Glycopeptide-type antibiotics, such as vancomycin and teicoplanin belong to the first class,[6] and the only known natural product that binds to transglycosylases is moenomycin A. The latter antibiotic contains a characteristic side chain attached to an oligosaccharide through a phosphate ester, mimicking the substrate of a transglycosylase. In a systematic, pioneering work of Welzel[7] many structural fragments of this antibiotic have been prepared and several simple analogues have been synthesized by Wong[8] and also by Vederas.[9]

In this paper we report the synthesis of simple, glycosylthio analogues of lipid II carrying a lipophilic chain and a phosphonate moiety.

In the first step, the conjugate addition of the 1-thio-*N*-acetyl-D-glucosamine derivative **1**[10] to the activated double bond of diphosphonate **2**[11] afforded the glycosylthio-diphosphonate **3**. Wadsworth–Horner–Emmons reaction of the latter with *n*-octanal or *n*-decanal resulted in the formation of mixtures of the *Z* and *E* isomers **4a**, **4b** and **5a**, **5b**, respectively. The concomitant formation of **1** was also observed, due to a concurrent elimination reaction of the intermediate carbanion. The two diastereoisomers were formed in a ~1:1 ratio in both cases. Column chromatographic separation of these mixtures afforded the pure **4a**, **4b**, **5a** and **5b**, and their structures could be determined on the basis of three-bond proton-phosphorous couplings along the carbon–carbon double bond (Table 1). Coupling constants of 22 Hz in the proton spectra indicated *syn* relationship between the coupled nuclei (*E*-isomers, **4b** and **5b**),[12] while *anti* relationship of P and H resulted in couplings of 47 Hz (*Z*-isomers, **4a** and **5a**). The two synthetic steps could also be performed in one flask starting from **1** by means of subsequent addition of sodium hydride, compound **2** and the appropriate aldehyde (Scheme 1, condition c).

Deprotection[13] of the diethylphosphonate moieties of intermediates **4** and **5** using the classical Rabinowitz-procedure[14] followed by deacetylation afforded the *Z* isomers **6** and **7** and the *E* isomers **8** and **9**, respectively (Scheme 2).

We also attempted to alkylate the diphosphonate intermediate **3** with 1-bromodecane but, owing to a concurrent elimination reaction of the 1-thio sugar, its S-alkylated product, the thio glycoside **10** could only be isolated (Scheme 3). This case is similar to the concurrent formation of **1** from **3** during the Wadsworth–Horner–Emmons reaction (Scheme 1). In this reaction the reactivity of the intermediate carbanion towards the alkyl halide was lower than

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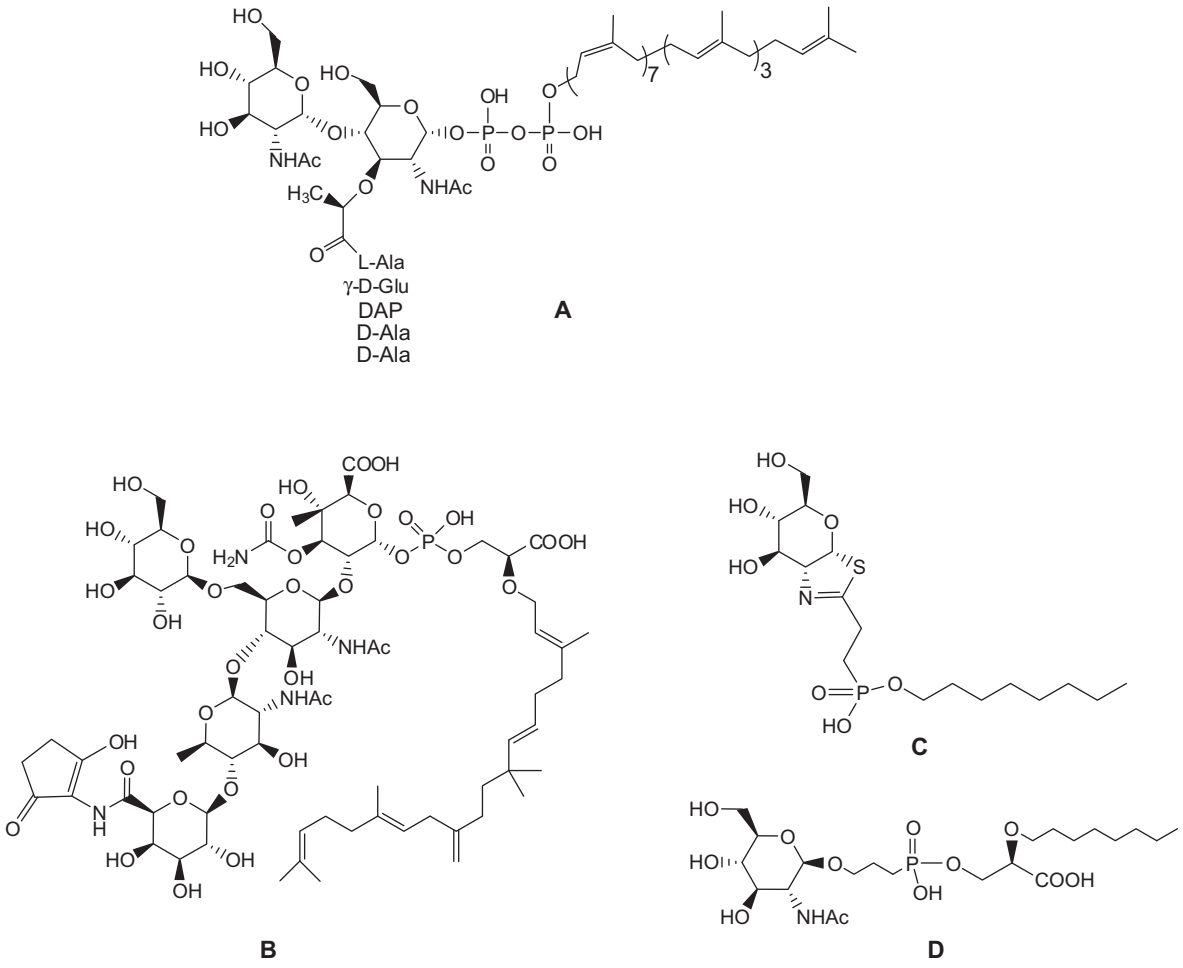
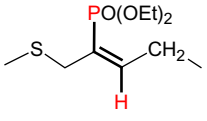
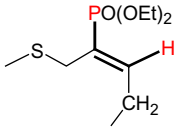


Figure 1. Structures of lipid II and its natural and synthetic analogues. (A) lipid II; (B) moenomycin A; (C) synthetic analogue[8] and (D) synthetic analogue.[9]

Table 1
Characteristic $^{3}J_{P,H}$ couplings, and chemical shifts of the vinylic protons of the (*Z*)-isomers (**4a**, **5a**) and (*E*)-isomers (**4b**, **5b**)

		
	4a or **5a**	**4b** or **5b**
$^{3}J_{P,H}$ (Hz) (^{1}H NMR: δ)	47 (6.20 ppm)	22.4 (6.54 ppm)

that of the aldehydes in the WHE reaction, therefore, the elimination of the thiol took place exclusively. A detailed literature search for similar alkylation of alkylthiomethyl-substituted bisphosphonates, phosphonoacetates and malonates gave no results, a mechanistic study of such reactions is worth to be done.

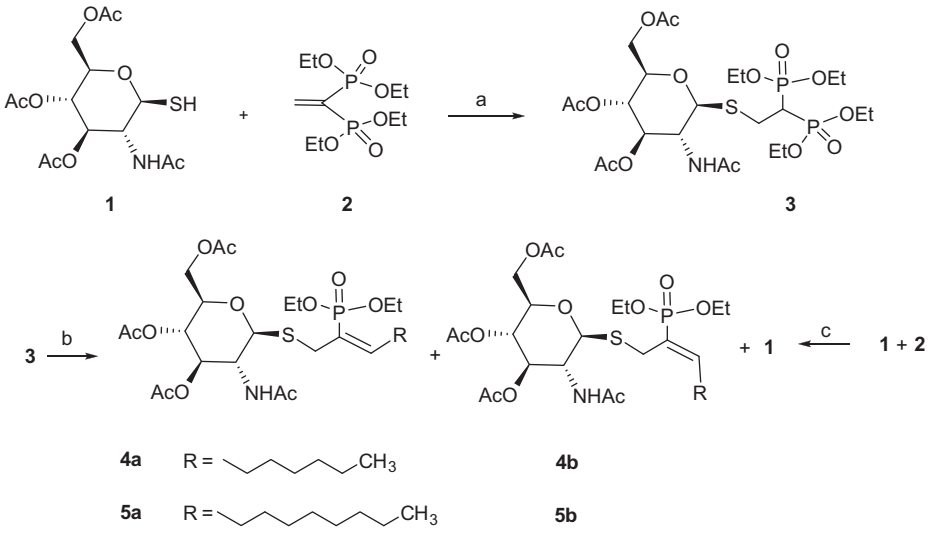
We postulated that analogues of lipid II would inhibit the biosynthesis of the bacterial cell wall. Unfortunately, compounds **6**–**9** did not show any antibacterial activity in a test against a panel of various Gram positive bacteria including methicillin resistant and sensitive *Staphylococcus aureus*, *Streptococcus epidermidis*, vancomycin and teicoplanin resistant *Enterococcus faecalis* and *Bacillus subtilis*.

1. Experimental

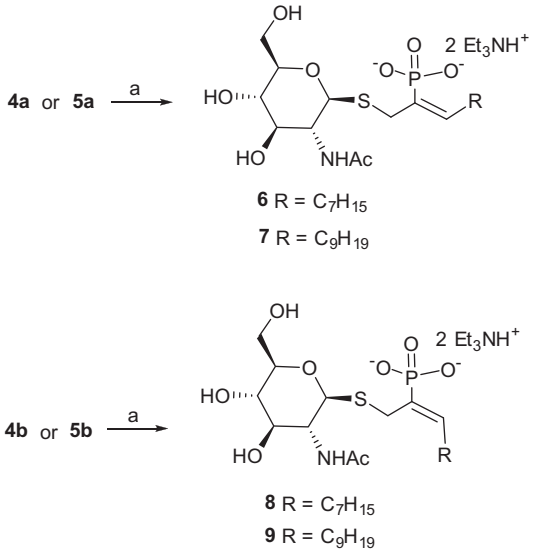
Optical rotations were measured at room temperature with a Perkin–Elmer 241 automatic polarimeter. Melting points were determined on a Cole-Palmer hot-stage apparatus and are uncorrected. TLC was performed on Kieselgel 60 F_{254} (Merck) with detection by immersing into molybdenum blue (6 g ammonium molybdate dissolved in 100 mL of 5% aq sulfuric acid soln) followed by heating. Column chromatography was performed on Silica Gel 60 (E. Merck, 0.063–0.200 mm) and reversed phase silica gel C_{18} (Kieselgel 60 RP-18, 40–63 μm, Merck). The organic solutions were dried over $MgSO_4$ and concentrated in vacuo. The ^{1}H (200, 400 and 500 MHz) and ^{13}C NMR (50, 100 and 125 MHz) spectra were recorded with Bruker WP-200 SY, DRX-400 and DRX-500 Avance spectrometers. Chemical shifts are referenced to Me_4Si (0.00 ppm for ^{1}H) or to the residual solvent signals (77.00 ppm ($CDCl_3$) or 49.05 ppm (MeOD) for ^{13}C). MALDI-TOF MS analyses of the compounds were carried out in the positive reflectron mode using a BIFLEX III mass spectrometer (Bruker, Germany) equipped with delayed-ion extraction. The 2,4,6-trihydroxy-acetophenone (THAP) matrix solution was saturated THAP solution in MeCN.

1.1. Tetraethyl 2-(3,4,6-tri-*O*-acetyl-2-acetamido-2-deoxy-β-D-glucopyranosyl-1-thio)ethane-1,1-diyldiphosphonate (3)

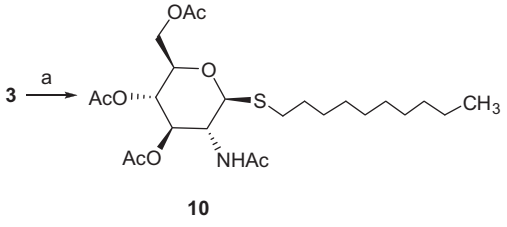
A solution of 3,4,6-tri-*O*-acetyl-2-acetamido-2-deoxy-1-thio-β-D-glucopyranose[10] (**1**, 2.18 g, 6 mmol), tetraethyl ethene-1,1-diyldiphosphonate[11] (**2**, 1.80 g, 6 mmol) and triethylamine (840 μL, 6 mmol) was stirred overnight at rt. Then the mixture was concentrated and the residue was purified by silica column chromatography (CH_2Cl_2/MeOH 95:5) to yield **3** (3.6 g, 90%) as a colourless syrup. $[\alpha]_D$ −37.5 (*c* 0.10, $CHCl_3$); ^{1}H NMR (500 MHz,



Scheme 1. Reagents and conditions: (a) abs CH_2Cl_2, Et_3N, rt, 24 h (90%); (b) dioxane, 60% NaH, 30 min, then *n*-octanal or *n*-decanal, 1 h (with *n*-octanal **1**: 31%, **4a**: 18%, **4b**: 15%; with *n*-decanal **1**: 31%, **5a**: 19%, **5b**: 14%); (c) dioxane, 60% NaH, *n*-octanal or *n*-decanal, 2 h (with *n*-octanal **4a**: 20%, **4b**: 17%, recovered **1**: 21%; with *n*-decanal **5a**: 20%, **5b**: 15%, recovered **1**: 24%).



Scheme 2. Reagents and conditions: (a) CH_3CN, pyr, Me_3SiBr, 0 °C, 2 h; $MeOH/H_2O$/Et_3N (7:3:1), rt, 24 h, (**6**: 46%, **7**: 42%, **8**: 43%, **9**: 45%).



Scheme 3. Reagents and conditions: (a) dioxane, 60% NaH, *n*-bromodecane, 1 h, then *N*,*N*-DMF, 30 min (85%).

$CDCl_3$): δ 6.77 (d, 1H, *J* 9.0 Hz, N*H*), 5.29 (t, 1H, $J_{2,3} = J_{3,4}$ 9.5 Hz, H-3), 5.09 (t, 1H, $J_{4,5}$ 9.5 Hz, H-4), 4.85 (d, 1H, $J_{1,2}$ 10.5 Hz, H-1), 4.26–4.12 (m, 11H, 4 OCH_2CH_3, H-2, H-6a,b), 3.77–3.74 (m, 1H, H-5), 3.29–3.08 (m, 2H, CH_2CHP_2), 3.00–2.84 (m, 1H, CH_2CHP_2), 2.10, 2.01, 1.94 (3s, 12H, 4 $COCH_3$), 1.39–1.34 (m, 12H, 4 OCH_2CH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.5, 170.5, 170.1, 169.1 (4 $COCH_3$), 84.7 (C-1), 75.6, 74.0 (C-3, C-5), 68.3 (C-4), 62.9 62.8, 62.8, 62.7, 62.6 (4d, $^3J_{C,P}$ 6.6 Hz, 4 OCH_2CH_3), 62.0 (C-6), 52.6 (C-2), 38.9 (t, 1C, $^1J_{C,P}$ 132 Hz, CHP_2), 25.91 (SCH_2), 22.9, 20.5, 20.0 (4 $COCH_3$), 16.2 (d, 4C, $^4J_{C,P}$ 6.3 Hz, 4 OCH_2CH_3). MALDI-TOF: *m*/*z* 686.24 $[M+Na]^+$ (Calcd [M] 663.19). Anal. Calcd for $C_{24}H_{43}NO_{14}P_2S$: C, 43.44; H, 6.53; N, 2.11. Found: C, 43.12; H, 6.69; N, 2.01.

1.2. (*Z*)-Diethyl 1-(3,4,6-tri-*O*-acetyl-2-acetamido-2-deoxy-β-D-glucopyranosyl-1-thio)dec-2-en-2-ylphosphonate (4a), (*E*)-Diethyl 1-(3,4,6-tri-*O*-acetyl-2-acetamido-2-deoxy-β-D-glucopyranosyl-1-thio)dec-2-en-2-ylphosphonate (4b)

To a stirred solution of **3** (350 mg, 0.52 mmol) in abs dioxane were successively added 1.2 equiv of NaH (25 mg, 0.62 mmol) and 1 equiv of *n*-octanal (83 μL, 0.52 mmol) at rt. TLC showed complete conversion of the starting material after 1 h, then 50% aq acetic acid was added until neutral and the mixture was evaporated. The residue was dissolved in CH_2Cl_2, washed with water, dried and concentrated. The crude product was purified on silica by two subsequent chromatographic steps (first eluent CH_2Cl_2/acetone 9:1–7:3; second eluent *n*-hexane: CH_2Cl_2/MeOH 5:5:0.4) to give **1** (60 mg, 31%), **4a** (60 mg 18%) and **4b** (50 mg, 15%).

Compound **1**: mp 168–170 °C, lit.[10] 173 °C; $[\alpha]_D$ −14.4 (*c* 0.86, $CHCl_3$), lit.[10] $[\alpha]_D$ −16; MALDI-TOF: *m*/*z* 386.28 $[M+Na]^+$ (Calcd [M] 363.10).

Compound **4a**: $[\alpha]_D$ −26.6 (*c* 0.10, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$): δ 6.87 (d, 1H, *J* 8.8 Hz, N*H*), 6.20 (dt, $^3J_{P,H}$ 47 Hz, $^3J_{H,CH2}$ 7.5 Hz, PC=C*H*), 5.19 (t, 1H, $J_{2,3} = J_{3,4}$ 9.5 Hz, H-3), 5.07 (t, 1H, $J_{4,5}$ 9.5 Hz, H-4), 4.64 (d, 1H, $J_{1,2}$ 10.5 Hz, H-1), 4.21–4.04 (m, 7H), 3.61–3.58 (m, 1H, H-5), 3.53–3.41 (m, 2H, SCH_2), 2.43–2.40 (m, 2H, CH_2), 2.08, 2.01, 1.93 (3s, 12H, 4 $COCH_3$), 1.42–1.19 (m, 16H, 5 CH_2, 2 OCH_2CH_3), 0.89 (t, 3H, $^3J_{H,CH3}$ 6.7 Hz, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.6, 170.5, 170.4, 169.3 (4 $COCH_3$), 150.5 (d, 1C, $^2J_{C,P}$ 11.3 Hz, PC=CH), 124.7 (d, 1C, $^1J_{C,P}$ 181 Hz, PC=CH) 82.2 (C-1), 75.7, 74.3 (C-3, C-5), 68.6 (C-4), 62.8–61.9 (m, 2C, OCH_2CH_3, C-6), 61.6 (d, 1C, $^3J_{C,P}$ 6.2 Hz, OCH_2CH_3), 52.4 (C-2), 35.2 (d, 1C, $^3J_{C,P}$ 13.5 Hz, SCH_2), 32.5 (CH_2), 30.6 (d, 1C, $^3J_{C,P}$ 6.3 Hz, =$CHCH_2$), 29.2, 29.0, 28.7 (3 CH_2), 22.9 ($COCH_3$), 22.5 (CH_2) 20.7, 20.6, 20.5 (3 $COCH_3$), 16.3 (m, 2C, 2 OCH_2CH_3) 14.0 (CH_3). MALDI-TOF: *m*/*z* 660.26 $[M+Na]^+$ (Calcd [M] 637.27). Anal. Calcd for $C_{28}H_{48}NO_{11}PS$: C, 52.73; H, 7.59; N, 2.20. Found: C, 53.12; H, 6.69.

Compound **4b**: $[\alpha]_D$ −23.6 (*c* 0.20, $CHCl_3$); ^1H NMR (200 MHz, $CDCl_3$): δ 6.87 (d, 1H, *J* 9.0 Hz, N*H*), 6.20 (dt, $^3J_{P,H}$ 22.4 Hz, $^3J_{H,CH2}$ 7.3 Hz, PC=C*H*), 5.14–5.07 (m, 2H, H-3, H-4), 4.64 (d, 1H, $J_{1,2}$ 10.6 Hz, H-1), 4.21–4.09 (m, 7H, 2 OCH_2CH$_3$, H-2, H-6a,b), 3.74–3.56 (m, 2H, H-5, SCH_2), 3.45 (t, 1H, *J* 14 Hz, SCH_2), 2.44–2.41 (m, 2H, CH_2), 2.09, 2.01, 1.92 (3s, 12H, 4 COCH_3), 1.36–1.29 (m, 16H, 5 CH_2, 2 OCH$_2$CH_3), 0.95–0.87 (m, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.6, 170.5, 170.3, 169.2 (4 *C*OCH$_3$), 149.7 (d, 1C, $^2J_{C,P}$ 8 Hz, PC=*C*H), 124.7 (d, 1C, $^1J_{C,P}$ 183.2 Hz, PC=CH) 83.4 (C-1), 75.7, 74.4 (C-3, C-5), 68.3 (C-4), 62.5–62.2 (m, 2C, 2 OCH$_2$CH$_3$, C-6), 52.6 (C-2), 31.7 (SCH$_2$), 29.2, 29.0, 28.7 (4 *C*H$_2$), 26.3 (d, 1C, $^3J_{C,P}$ 13.1 Hz, =CH*C*H$_2$), 23.0 (CO*C*H$_3$), 22.6 (*C*H$_2$), 20.7, 20.6 (3 CO*C*H$_3$), 16.3 (2 OCH$_2$*C*H$_3$) 14.0 (*C*H$_3$). MALDI-TOF: *m/z* 660.34 $[M+Na]^+$ MALDI-TOF: *m/z* 660.34 $[M+Na]^+$ (Calcd [M] 637.27). Anal. Calcd for $C_{28}H_{48}NO_{11}PS$: C, 52.73; H, 7.59; N, 2.20. Found: C, 52.47; H, 7.70; N, 2.02.

1.3. (*Z*)-Diethyl 1-(3,4,6-tri-*O*-acetyl-2-acetamido-2-deoxy-β-D-glucopyranosyl-1-thio)dodec-2-en-2-ylphosphonate (5a) and (*E*)-Diethyl 1-(3,4,6-tri-*O*-acetyl-2-acetamido-2-deoxy-β-D-glucopyranosyl-1-thio)dodec-2-en-2-ylphosphonate (5b)

Compound **3** (350 mg, 0.52 mmol) was treated with 1.2 equiv of NaH (25 mg, 0.62 mmol) and 1.2 equiv of *n*-decanal (120 μL, 0.52 mmol) as described for the synthesis of **4** to give **1** (60 mg, 31%), **5a** (66 mg, 19%) and **5b** (50 mg, 14%).

Compound **5a** $[\alpha]_D$ −23.9 (*c* 0.14, $CHCl_3$); ^1H NMR (200 MHz, $CDCl_3$): δ 6.88 (d, 1H, *J* 8.8 Hz, N*H*), 6.20 (dt, $^3J_{P,H}$ 47 Hz, $^3J_{H,CH2}$ 7.5 Hz, PC=C*H*), 5.18 (t, 1H, $J_{2,3}$ = $J_{3,4}$ 9.5 Hz, H-3), 5.05 (t, 1H, $J_{4,5}$ 9.5 Hz, H-4), 4.61 (d, 1H, $J_{1,2}$ 10.5 Hz, H-1), 4.31–4.00 (m, 7H), 3.66–3.41 (m, 3H, H-5, SCH_2), 2.42–2.38 (m, 2H, CH_2), 2.07, 2.00, 1.91 (3s, 12H, 4 COCH_3), 1.37–1.25 (m, 20H, 7 CH_2, 2 OCH$_2$CH_3), 0.86 (t, 3H, $^3J_{H,CH3}$ 6.6 Hz, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.5, 170.4, 170.3, 169.2 (4 *C*OCH$_3$), 150.5 (d, 1C, $^2J_{C,P}$ 11 Hz, PC=CH), 124.7 (d, 1C, $^1J_{C,P}$ 179.2 Hz, PC=CH) 82.1 (C-1), 75.7, 74.3 (C-3, C-5), 68.5 (C-4), 62.2–62.4 (m, 2C, OCH$_2$CH$_3$, C-6), 61.6 (d, 1C, $^3J_{C,P}$ 5.3 Hz, OCH$_2$CH$_3$), 52.4 (C-2), 35.3 (d, 1C, $^3J_{C,P}$ 12 Hz, SCH$_2$), 31.8 (*C*H$_2$), 30.7 (d, 1C, $^3J_{C,P}$ 4.9 Hz, =CH*C*H$_2$), 29.7, 29.5, 29.4, 29.3 (5 *C*H$_2$), 23.0 (CO*C*H$_3$), 22.7 (*C*H$_2$) 20.8, 20.7, 20.6 (3 CO*C*H$_3$), 16.3 (2 OCH$_2$*C*H$_3$) 14.0 (*C*H$_3$). MALDI-TOF: *m/z* 688.45 $[M+Na]^+$ (Calcd [M] 665.30). Anal. Calcd for $C_{30}H_{52}NO_{11}PS$: C, 54.12; H, 7.87; N, 2.10. Found: C, 53.62; H, 7.10; N, 2.01.

Compound **5b** $[\alpha]_D$ −23.8 (*c* 0.11, $CHCl_3$); ^1H NMR (200 MHz, $CDCl_3$): δ 6.85 (d, 1H, *J* 9.0 Hz, N*H*), 6.56 (dt, $^3J_{P,H}$ 22.4 Hz, $^3J_{H,CH2}$ 7.3 Hz, PC=C*H*), 5.17–5.04 (m, 2H, H-3, H-4), 4.67 (d, 1H, $J_{1,2}$ 10.6 Hz, H-1), 4.27–4.01 (m, 7H, 2 OCH_2CH$_3$, H-2, H-6a,b), 3.79–3.48 (m, 2H, H-5, SCH$_2$), 3.40 (t, 1H, *J* 13.8 Hz, SCH_2), 2.30–2.19 (m, 2H, CH_2), 2.09, 2.01, 1.92 (3s, 12H, 4 COCH_3), 1.36–1.26 (m, 20H, 7 CH_2, 2 OCH$_2$CH_3), 0.88 (t, 3H, $^3J_{H,CH3}$ 6.7 Hz, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.6, 170.5, 170.3, 169.2 (4 *C*OCH$_3$), 149.7 (d, 1C, $^2J_{C,P}$ 8.3 Hz, PC=CH), 125.7 (d, 1C, $^1J_{C,P}$ 183.5 Hz, PC=CH) 83.4 (C-1), 75.8, 74.5 (C-3, C-5), 68.3 (C-4), 62.5–62.3 (m, 2C, 2 OCH$_2$CH$_3$, C-6), 52.6 (C-2), 31.8 (SCH$_2$), 29.4–28.7 (6 *C*H$_2$), 26.4 (d, 1C, $^3J_{C,P}$ 12.5 Hz, =CH*C*H$_2$), 23.1 (CO*C*H$_3$), 22.7 (*C*H$_2$), 20.7, 20.6 (3 CO*C*H$_3$), 16.4 (2 OCH$_2$*C*H$_3$) 14.1 (*C*H$_3$). MALDI-TOF: *m/z* 688.43 $[M+Na]^+$ (Calcd [M] 665.30). Anal. Calcd for $C_{30}H_{52}NO_{11}PS$: 54.12; H, 7.87; N, 2.10. Found: C, 53.80; H, 7.11, N, 2.11.

1.4. (*Z*)-Bis(triethylammonium) 1-(2-acetamido-2-deoxy-β-D-glucopyranosyl-1-thio)dec-2-en-2-ylphosphonate (6)

To a solution of **4a** (110 mg, 0.17 mmol) in anhydrous CH_3CN (5 mL) pyridine (1.7 mmol, 140 μL) was added at 0 °C, followed by dropwise addition of Me_3SiBr (1.7 mmol, 227 μL). After stirring for 2 h at 0 °C, a mixture of water and pyridine (9:1, 200 μL) was added, then the reaction mixture was evaporated. The residue was purified by chromatography on reversed phase silica (RP-18, H_2O/MeOH 1:1) to obtain the acetylated phosphonic acid intermediate. The product was stirred in H_2O/MeOH/Et_3N (7:3:1, 3 mL) overnight then evaporated and the residue was purified (RP-18, H_2O/MeOH 1:1) to give **6** (70 mg, 62%). $[\alpha]_D$ −5.41 (*c* 0.11, H_2O); ^1H NMR (500 MHz, D_2O + CD_3OD): δ 6.05 (dt, $^3J_{P,H}$ 42 Hz, $^3J_{H,CH2}$ 6 Hz, PC=C*H*), 4.54 (d, 1H, $J_{1,2}$ 10 Hz, H-1), 3.89–3.32 (m, 8H), 2.42–2.38 (m, 2H, CH_2), 2.00, (s, 3H, COCH_3), 1.42–1.38 (m, 2H, CH_2), 1.32–1.24 (m, 8H, 4 CH_2), 0.86 (br t, 3H, *J* 6 Hz, CH_3); ^{13}C NMR (125 MHz, D_2O + CD_3OD): δ 175.4 (*C*OCH$_3$), 147.6 (PC=*C*H), 130.2 (d, 1C, $^1J_{C,P}$ 170.9 Hz, PC=CH) 83.1 (C-1), 81.0 (C-5), 76.6 (C-3), 71.0 (C-4), 62.0 (C-6), 55.5 (C-2), 36.0 (d, 1C, $^2J_{C,P}$ 15 Hz, SCH$_2$), 32.3, 31.4, 29.9, 29.8, 29.5 (5 *C*H$_2$), 23.3 (CO*C*H$_3$), 23.2 (*C*H$_2$), 14.8 (*C*H$_3$). MALDI-TOF: *m/z* 478.38 $[M^{2-}+2H^++Na^+]$, 578.2 $[M^-+H^++Na^+]$, (Calcd $[M^{2-}]$ 453.16). Anal. Calcd for $C_{30}H_{64}N_3O_8PS$: C, 54.77; H, 9.81; N, 6.39. Found: C, 55.12; H, 9.69, N, 6.15.

1.5. (*E*)-[Bis(triethylammonium) 1-(2-acetamido-2-deoxy-β-D-glucopyranosyl-1-thio)dec-2-en-2-ylphosphonate] (7)

Compound **4b** (110 mg, 0.17 mmol) was treated analogously as described for **4a** to give **7** (76 mg, 67%); $[\alpha]_D$ −4.12 (*c* 0.09, H_2O); ^1H NMR (500 MHz, D_2O + CD_3OD): δ 6.43 (br d, $^3J_{P,H}$ 21 Hz, PC=C*H*), 4.62 (d, 1H, $J_{1,2}$ 10.0 Hz, H-1), 3.91–3.41 (m, 8H), 2.24–2.12 (m, 2H, CH_2), 2.02, (s, 3H, COCH_3),1.42–1.29 (m, 14H, 7 CH_2), 0.86 (br s, 3H, CH_3); ^{13}C NMR (100 MHz, D_2O + CD_3OD): δ 175.1 (*C*OCH$_3$), 147.1 (PC=*C*H), 129.7 (d, 1C, $^1J_{C,P}$ 183 Hz, PC=CH), 84.7 (C-1), 80.9, 76.4 (C-3, C-5), 70.8 (C-4), 62.0 (C-6), 55.5 (C-2), 31.0, 28.8, 28.0, 27.8 (5 *C*H$_2$), 26.0 (d, 1C, $^3J_{C,P}$ 15 Hz, =CH*C*H$_2$), 22.0 (CO*C*H$_3$), 21.6 (*C*H$_2$), 13.0 (*C*H$_3$). MALDI-TOF: *m/z* 478.40 $[M^{2-}+2H^++Na^+]^+$, 500.40 $[M^{2-}+H^++2Na^+]$, (Calcd $[M^{2-}]$ 453.16). Anal. Calcd for $C_{30}H_{64}N_3O_8PS$: C, 54.77; H, 9.81; N, 6.39. Found: C, 55.12; H, 9.69, N, 6.22.

1.6. (*Z*)-[Bis(triethylammonium) 1-(2-acetamido-2-deoxy-β-D-glucopyranosyl-1-thio)dodec-2-en-2-ylphosphonate] (8)

Compound **5a** (160 mg, 0.24 mmol) was treated analogously as described for **4a** to give **8** (85 mg, 52%); $[\alpha]_D$ −8.96 (*c* 0.22, H_2O); ^1H NMR (500 MHz, D_2O + CD_3OD): δ 6.1 (br d, $^3J_{P,H}$ 42 Hz, PC=C*H*), 4.56 (d, 1H, $J_{1,2}$ 10 Hz, H-1), 3.87–3.36 (m, 8H), 2.48–2.38 (m, 2H, CH_2), 2.00, (s, 3H, COCH_3), 1.42–1.24 (m, 14H, 7 CH_2), 0.86 (br s, 3H, CH_3); ^{13}C NMR (125 MHz, D_2O + CD_3OD): δ 175.4 (*C*OCH$_3$), 147.6 (PC=*C*H), 129.7 (d, 1C, $^1J_{C,P}$ 171 Hz, PC=CH) 83.1 (C-1), 81.0 (C-5), 76.6 (C-3), 70.8 (C-4), 61.9 (C-6), 55.6 (C-2), 36.1 (SCH$_2$), 32.7, 31.6, 30.4, 30.3, 30.2, 30.1 (7 *C*H$_2$), 23.5 (CO*C*H$_3$), 23.4 (*C*H$_2$), 14.8 (*C*H$_3$). Anal. Calcd for $C_{32}H_{68}N_3O_8PS$: C, 56.03; H, 9.99; N, 6.13. Found: C, 55.12; H, 9.69, N, 6.04.

1.7. (*E*)-[Bis(triethylammonium) 1-(2-acetamido-2-deoxy-β-D-glucopyranosyl-1-thio)dodec-2-en-2-ylphosphonate] (9)

Compound **5b** (90 mg, 0.13 mmol) was treated analogously as described for **4a** to give **9** (60 mg, 67%). $[\alpha]_D$ −6.92 (*c* 0.35, $CHCl_3$); ^1H NMR (500 MHz, D_2O + CD_3OD): δ 6.38 (br t, $^3J_{P,H}$ 21 Hz, PC=C*H*), 4.62 (d, 1H, $J_{1,2}$ 10 Hz, H-1), 3.87–3.46 (m, 8H), 2.22–2.09 (m, 2H, CH_2), 2.00, (s, 3H, COCH_3), 1.42–1.37 (m, 2H, CH_2), 1.32–1.24 (m, 12H, 6 CH_2), 0.86 (br s, 3H, CH_3); ^{13}C NMR (125 MHz, D_2O+CD_3OD): δ 175.2 (*C*OCH$_3$), 146.4 (PC=*C*H), 130.5 (d, 1C, $^1J_{C,P}$ 170 Hz, PC=CH) 84.9 (C-1), 81.3 (C-5), 76.7 (C-3), 71.1 (C-4), 62.3 (C-6), 55.9 (C-2), 33.1, 30.8, 30.7, 30.5, 30.2, 30.1, 29.7, 27.7 (7 *C*H$_2$, SCH$_2$), 23.9 (CO*C*H$_3$), 23.8 (*C*H$_2$), 15.2 (*C*H$_3$). Anal. Calcd for $C_{32}H_{68}N_3O_8PS$: C, 56.03; H, 9.99; N, 6.13. Found: C, 55.12; H, 9.69, N, 6.30.

1.8. *n*-Decyl 3,4,6-tri-*O*-acetyl-2-acetamido-2-deoxy-1-thio-β-D-glucopyranoside (10)

To a stirred solution of **3** (98 mg, 0.15 mmol) in abs dioxane (5 mL) 1.2 equiv of NaH (7 mg, 0.17 mmol) and 1.5 equiv of *n*-decyl bromide (46 μL, 0.22 mmol) were added at 10 °C. There was no reaction after 2 h, then 1 mL of DMF was added. TLC showed complete conversion of the starting compound after 20 min. MeOH (0.5 mL) was added, the reaction mixture was stirred for 15 min, then the solvents were evaporated. The residue was dissolved in CH_2Cl_2, washed with water, dried and concentrated. The crude product was purified on silica (CH_2Cl_2/acetone 9:1) to give **10** as white needles (63 mg, 85%), mp 148–150 °C, $[\alpha]_D$ –42.6 (*c* 0.10, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.75 (br d, 1H, *J* 10 Hz, N*H*), 5.19 (t, 1H, $J_{2,3} = J_{3,4}$ 10 Hz, H-3), 5.09 (t, 1H, $J_{4,5}$ 9.5 Hz, H-4), 4.61 (d, 1H, $J_{1,2}$ 10 Hz, H-1), 4.24 (d, 1H, J_{gem} 12.5 Hz, $J_{5,6a}$ 5.5 Hz, H-6a), 4.13 (dd, 1H, $J_{5,6b}$ 2 Hz, H-6b), 4. 09 (dd, 1H, H-2), 3.72–3.69 (m, 1H, H-5), 2.73–2.63 (m, 2H, SCH_2), 2.08, 2.03, 2.02, 1.96 (4s, 12H, 4 COCH_3), 1.61–1.57 (m, 2H, CH_2), 1.36–1.32 (m, 2H, CH_2), 1.32–1.25 (m, 12H, 6 CH_2), 0.88 (t, 3H, $J_{H,CH3}$ 6.5 Hz, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.9, 170.6, 170.0, 169.2 (4 *C*OCH$_3$), 84.4 (C-1), 75.8 (C-5), 73.8 (C-3), 68.4 (C-4), 62.3 (C-6), 53.2 (C-2), 31.8 (SCH_2), 29.9–28.8 (7 CH_2), 23.2 (COCH_3), 22.6 (CH_2), 20.7, 20.6, 20.5 (3 COCH_3), 14.0 (CH_3). Anal. Calcd for $C_{24}H_{43}NO_8S$: C, 57.01; H, 8.57; N, 2.77. Found: C, 57.12; H, 8.69, N, 2.53.

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