Synthesis of Stable Dolichylphosphomannose Analogues

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Four oleyl or dolichyl thiophosphate esters **16**, **17**, **21**, and **22**, analogues of Dol-P-Man possessing C(1)–S and/or P–S bonds, were synthesized as potential inhibitors of mannosyl transferases operating in the endoplasmic reticulum (ER). The β-mannosyl derivatives were prepared by a *Mitsunobu* reaction of 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranose (**1**) with the thiophosphate **2** that provided *O*- and *S*-glycosides with good-to-excellent diastereoselectivity. A second route to β-mannosyl derivatives is based on the phosphitylation of the β-D-mannopyranosyl thiol **3** with the phosphoramidites **4a** and **4b**. Oxidation of the intermediate oleyl thiophosphite with *t*-BuOOH led to mono- and dithiophosphates. The thiophospholipids **16**, **21**, and **22** were inactive as inhibitors of the $Man_6(GlcNAc)_2$-PP-Dol glycolipid elongation.

Introduction. – Most secretory proteins in eukaryotic organisms are glycosylated by enzymatic transfer of the branched tetradecasaccharide $Glc_3Man_9(GlcNAc)_2$. Multiple glycosyltransferases catalyse the construction of $Glc_3Man_9(GlcNAc)_2$ attached to dolichyl pyrophosphate (Dol-PP), the lipid carrier embedded in the endoplasmic reticulum (ER) membrane. The synthesis starts on the outer (cytoplasmic) side of the ER membrane and ends on the inner side (*Fig. 1*) [1] [2]. Soluble sugar nucleotides serve as activated monosaccharide donors for the cytoplasmic glycosyl transfer, while dolichylphosphate-mannose and -glucose (Dol-P-Man and Dol-P-Glc) are the monosaccharide donors used in the lumen. Dol-P-Man is also a biosynthetic precursor of the GPI-anchor [3] and involved in *O*- and *C*-mannosylation of proteins [4]. The biosynthesis of Dol-PP-linked $Glc_3Man_9(GlcNAc)_2$ is highly conserved in eukaryotic organisms, and the results of studies of yeast genomics of that process were applied to analyse human glycosylation disorders [5].

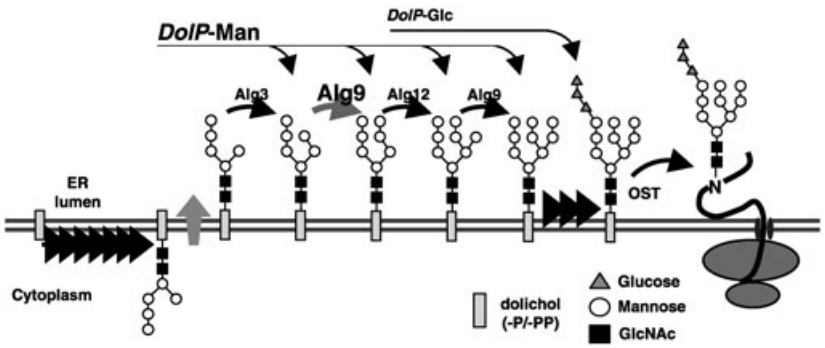


Fig. 1. *Biosynthesis of lipid-linked oligosaccharide in the endoplasmic reticulum membrane*

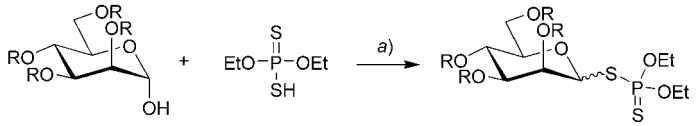
Although there are no known inhibitors of the mannosyl and glucosyl transferases that take part in the synthesis of the tetradecasaccharide $Glc_3Man_9(GlcNAc)_2$, many aspects of the oligosaccharide assembly at the ER membrane have been elucidated [2]. We have now designed the Dol-P-β-Man analogues **16**, **17**, **21**, and **22**, thiophosphates possessing a C(1)–S and/or P–S bond, as potential inhibitors of mannosyl transferases. These thiophosphates are intended to serve as tools for a more-detailed analysis of the mechanism of mannosyl transfer to the growing oligosaccharide core in the ER. The thiophosphates should be significantly less reactive towards enzymatic cleavage of the glycosidic bond, and function as competitive inhibitors [6].

In a first approach to these analogues, we planned to introduce the thiophosphate substituent at the anomeric center of 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranose (**1**) by a *Mitsunobu* reaction [7]. The substitution should proceed by inversion of configuration, considering that the AcO groups disfavour a S_N1-type process [8] [9] and should not participate in the substitution by the highly nucleophilic thiophosphates. Indeed, the synthesis of mannopyranosyl benzoates by *Mitsunobu* substitution of **1** afforded predominantly the β-D-anomer (β-D/α-D 4:1) [10].

In a second approach, aiming at reducing the number of steps involving dolichylated intermediates, we planned to couple the known β-D-mannopyranosyl thiol **3** [11] with the phosphoramidites **4a** and **4b** derived from oleyl alcohol and dolichol, respectively, and to oxidize the deprotected products to the desired thiophosphates. A similar route was described by *Crich* and *Dudkin* [12] for the synthesis of β-D-mannopyranosyl phosphoisoprenoids.

Results and Discussion. – To establish the extent of the S_N2 or S_N1 displacement at the anomeric centre in the presence of *O*-Ac groups, we investigated the *Mitsunobu* reaction of **1** with commercially available *O*,*O*′-diethyl dithiophosphate (**6**) in solvents differing by their polarity and their ability to favour an S_N1- or S_N2-type substitution (*Scheme 1*) [13]. Although the choice of solvents was restricted by the requirements of the *Mitsunobu* reaction, the results are in keeping with the expected preferred inversion of configuration. The α/β anomeric composition of **1** in THF and MeCN, as established by ^{1}H-NMR spectroscopy was 95:5 (THF) and 91:9 (MeCN). Substitution in THF led to β-D-**7**/α-D-**7** 93:7, the ratio of anomers decreasing to 91:9 upon addition of 2 equiv. of the polar and strongly solvating hexamethylphosphoric triamide (HMPA). Further increasing the ratio HMPA/THF to 3:1 resulted in a 80:20 ratio of anomers (*Table*). The substitution in MeCN (β-D/α-D 45:55) may reflect solvent [14] or neighbouring group participation.

Scheme 1



1 R = Ac
5 R = Bn

6

7 R = Ac
8 R = Bn

a) Diethyl azodicarboxylate (DEAD), Ph_3P, THF; 94% of β-D-**7**; 76% of β-D-**8**/α-D-**8** 3:2.

Hemiacetal

	Solvent	Product (yield)	β/α
1	THF	**7** (97%)	93 : 7
1	THF/2 mol.-equiv. of HMPA	**7** (95%)	91 : 9
1	THF/HMPA 3 : 1	**7** (96%)	80 : 20
1	MeCN	**7** (89%)	45 : 55
5	THF	**8** (76%)	60 : 40
5	MeCN	**8** (68%)	83 : 17

The crystal structure of the dithiophosphate **7** was established by X-ray analysis (*Fig. 2*)[1][2]). It evidences the β-D-configuration of the mannopyranosyl unit, a $^4C_1$ conformation of the pyranose ring, the *gt*-conformation of the $AcOCH_2$ group, and an antiperiplanar arrangement of the C(1)–S and P=S bonds (torsion angle C(1)–S–P=S 175.12°). This antiperiplanar arrangement of C(1)–S and P=S bonds is preferred for *S*,*O*,*O*-trialkyl dithiophosphates (6 out of 7 in *Cambridge Database*). The C(1)–S and P=S bond lengths (1.836 and 1.913 Å, resp.) are in the range of published bond lengths of such dithiophosphates.

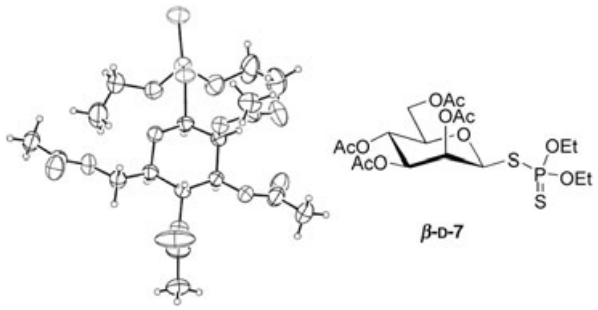


Fig. 2. *Crystal structure of di*-O-*ethyl S-(2,3,4,6-tetra*-O-*acetyl-β-*D-*mannopyranosyl) dithiophosphate* (*β*-D-**7**)

To probe the role of the AcO groups, we similarly treated 2,3,4,6-tetra-*O*-benzyl-D-mannopyranose (**5**) with *O*,*O*′-diethyl dithiophosphate (**6**). The α/β ratio of the anomers of **5** in THF and MeCN, as determined by ^{1}H-NMR spectroscopy, is 88 : 12 and 87 : 13, respectively. The disarmed **5** [9] should more readily form an oxycarbenium cation than **1**, and react with lower stereoselectivity. The substitution in THF resulted in a β-D-**8**/α-D-**8** ratio of 3 : 2, in agreement with a shift from a S_N2 towards a S_N1 mechanism. The substitution in MeCN resulted in a β-D-**8**/α-D-**8** ratio of 83 : 17; this result is in keeping with the known participation of MeCN, leading to an α-D-nitrilium cation that leads to β-D-**8** [14][17].

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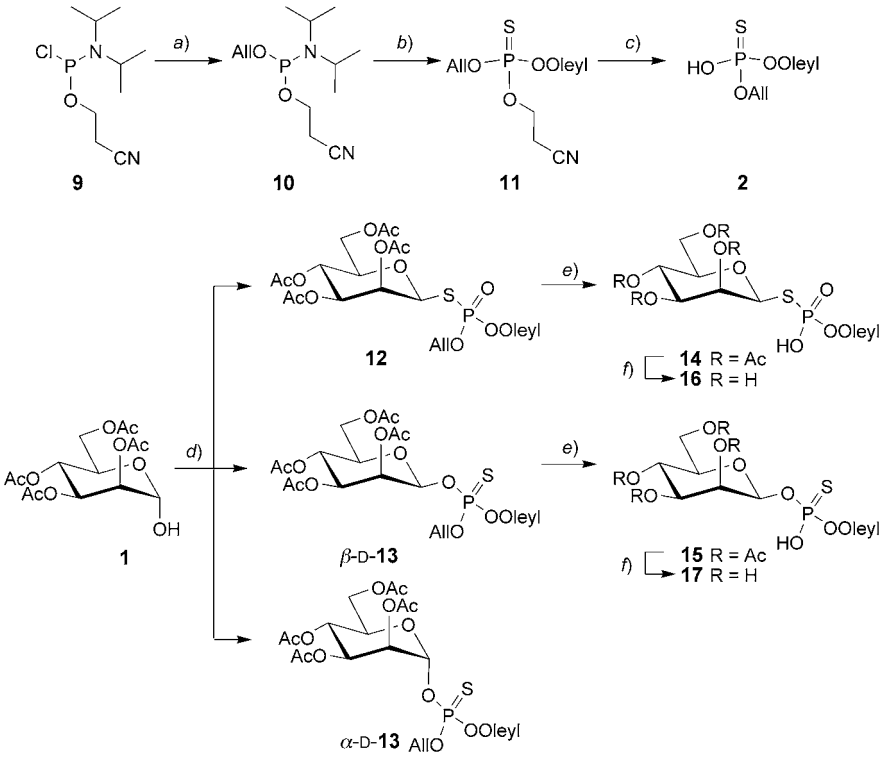
[1]) The crystallographic data have been deposited with the *Cambridge Crystallographic Data Centre* as deposition No. CCDC 249395. Copies of the data can be obtained, free of charge, on application to the CCDC, 12 Union Road, Cambridge CB21EZ, UK (fax. +44(1223)336033; e-mail: deposit@ccdc.cam.ac.uk).

[2]) The *Cambridge Database* contains the structural data of 12 dithiophosphates. Two of them contain a glycosyl residue, namely a 4-[(2-thioxo-1,3,2-dioxaphosphorinan-2-yl)thio] derivative of levoglucosan [15] and a 3′-*O*-(2-thioxo-1,3,2-oxathiaphospholan-2-yl)deoxycytidine [16].

The optimized conditions were used for the synthesis of **12** and **13** (*Scheme 2*). The required monothiophosphoric acid diester **2** was prepared from the chlorophosphoramidite **9** according to the phosphoramidite route [12], by first introducing an allyl substituent (→**10**) [18] [19] and then the oleyl group to provide **11**. Base-induced removal of the 2-cyanoethyl protecting group gave the thiophosphate **2** in a yield of 50% from **9**. The ^{31}P-NMR spectrum ($CDCl_3$) confirmed the presence of a P=S group (61.14 ppm); no resonance was observed at 68 ppm, as expected for the isomer possessing a P=O group. *Mitsunobu* reaction of **1** with **2** afforded the *S*- and *O*-mannopyranosyl phosphates **12** (35%) and α-D-**13**/β-D-**13** 2:1 (25%), all as inseparable 1:1 mixture of diastereoisomers due to the asymmetric P-atom, as shown by ^{31}P-NMR spectroscopy (24.01 and 24.88 ppm for **12**; 67.65 and 67.57 ppm for β-D-**13**; 67.88 and 67.79 ppm for α-D-**13**). The ^{31}P- and ^{1}H-NMR spectra of the crude showed small amounts of α-D-**12**, but only β-D-**12** was isolated. Its configuration was confirmed by the upfield shift of H–C(5) (3.77 and 3.75 ppm) and by ^{1}J(C(1),H) of 165 Hz [12]. The *O*-mannopyranosyl thiophosphate **13** was obtained as a 2:1 β-D/α-D mixture; flash chromatography on *Merck* silica gel *60* (0.015–0.040 mm) gave pure fractions of β-D-**13**

Scheme 2

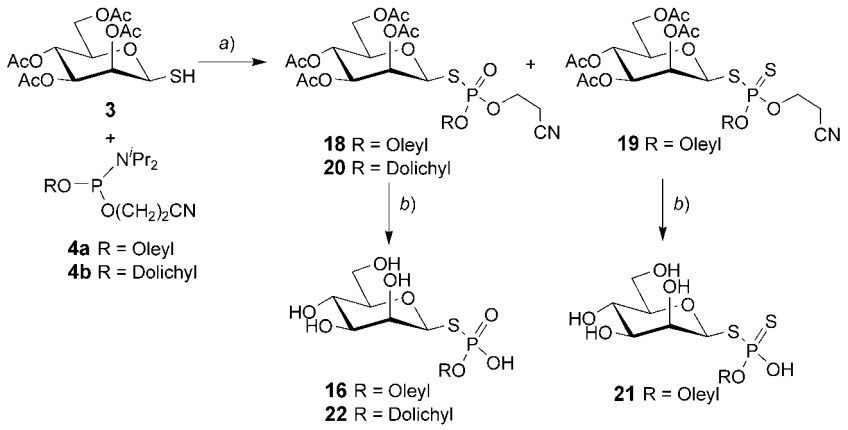


a) AllOH, EtN(i-Pr)$_2$; 90%. *b*) OleylOH, 1*H*-tetrazole, MeCN, then *Beaucage* reagent; 75%. *c*) Bu_4NOH, CH_2Cl_2/H_2O; 95%. *d*) **2**, Diisopropyl azodicarboxylate (DIAD), Ph_3P, THF; 35% of **12** and 25% of α-D-**13**/β-D-**13** 2:1. *e*) $Pd(PPh_3)_4$, Ph_3P, $BuNH_2$, HCOOH, THF; 88% of **14**; 85% of **15**. *f*) NaOMe, MeOH; 85% of **16**; 83% of **17**.

and α-D-**13**. A comparison of the chemical shift for H–C(5) (α-D-**13**: 4.19–4.14; β-D-**13**: 3.78 and 3.76 ppm) confirmed the configurational assignment. Deallylation [20] of **12** and β-D-**13** afforded the hydrogen thiophosphates **14** and **15**, respectively, isolated as their triethylammonium salts. Deacetylation yielded the fully deprotected thiophosphates **16** (85%) and **17** (83%), respectively.

To increase the yield of the *S*-(β-D-mannopyranosyl) thiophosphates and to reduce the number of steps required for the synthesis of the dolichyl derivative **22**, we also examined the phosphitylation of the known β-D-mannopyranosyl thiol **3** [11] (*Scheme 3*). Phosphitylation of **3** with the oleyl phosphoramidite **4a** followed by the *in situ* oxidation with *t*-BuOOH yielded 26% of the expected monothiophosphate **18** besides the dithiophosphate **19** (20%). Again, **18** and **19** were isolated as a 1:1 mixture of diastereoisomers. The unexpected formation of **19** may result from a radical reaction of excess thiol [21] [22]. The S–P=O and S–P=S moieties, and the formation of two diastereoisomers are evidenced by ^{31}P-NMR spectroscopy (24.19 and 24.06 ppm for **18**; 93.14 and 91.16 ppm for **19**).

Scheme 3



a) 1*H*-Tetrazole, MeCN, then *t*-BuOOH; 26% of **18** and 20% of **19**; 30% of **20**. *b*) MeONa, MeOH; 66% of **16**; 73% of **21**; 54% of **22**.

Coupling of the dolichyl phosphoramidite **4b** with **3** at -40 to -20° yielded 45% of the dolichyl derivative **20**, while coupling at room temperature led to decomposition of **4b** [23]. Global deprotection of **18**, **19**, and **20** led to the mono- and dithiophosphates **16**, **21**, and **22** in yields 85–90%. Thus, the stable thiol **3** allowed a stereospecific access to the *S*-(β-D-mannopyranosyl) thiophosphates **16**, **21**, and **22**.

We tested the oleyl derivatives **16** and **21**, and the dolichyl derivative **22** in an *in vitro* assay for the elongation of [^{3}H]-labeled $Man_6(GlcNAc)_2$-PP-Dol glycolipid (M6 in *Fig. 1*). Detergent-solubilized glycophospholipid was mixed with crude membrane extracts of wild type yeast as the enzyme source. GDP-Mannose was included to allow formation of Dol-P-Man substrate *in vitro*. Under the conditions used (0.4 g/l of **21**,

0.2 g/l of **16**, and 0.4 g/l of **22**), we did not observe a significant inhibitory effect on glycolipid elongation. An analogous experiment with **22**, but without addition of GDP-Man, indicated that the *S*-mannosyl thiophosphate **22** does not replace Dol-P-Man as substrate.

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Experimental Part

General. Immediately before use, THF was destilled from Na and benzophenone, and CH_2Cl_2 from P_2O_5. MeCN was used as obtained from *Fluka*. Dolichol-C_{95} was obtained from the *Polish Academy of Sciences.* Reactions were carried out under Ar, unless stated otherwise. Qual. TLC: precoated silica-gel plates (*Alugram Sil G/UV$_{254}$*); detection by heating with Mostain (400 ml of 10 % H_2SO_4, 20 g of $(NH_4)_6Mo_7O_{24}\cdot 6\,H_2O$), 0.4 g of $Ce(SO_4)_2$). Flash chromatography (FC): silica gel *Fluka 60* (0.04 – 0.063 mm). ATR-IR spectra: Absorption in cm^{-1}. ^{1}H- and ^{13}C-NMR spectroscopy: chemical shifts δ in ppm relative to TMS as external standard, and coupling constants J in Hz. MALDI-MS and HR-MALDI-MS: gentisic acid (=2,5-dihydroxybenzoic acid, DHB) matrix.

*Di-*O*-Ethyl* S-*(2,3,4,6-Tetra-*O*-acetyl-β-*D*-mannopyranosyl) Dithiophosphate* (β-D-**7**). A soln. of Ph_3P (73 mg, 0.28 mmol) in dry THF (1 ml) was cooled to $-10°$, treated with DEAD (0.13 ml, 0.28 mmol), stirred for 10 min, treated with a soln. of **1** (65 mg, 0.19 mmol) in dry THF (1 ml), stirred for 10 min, treated with **6** (0.047 ml, 0.28 mmol), allowed to warm slowly to r.t. over a period of 1.5 h, and evaporated. FC (cyclohexane/$CHCl_3$/AcOEt 1:1:0.1) gave β-D-**7** (93 mg, 94%). M.p. 93 – 95°. R_f (hexane/AcOEt 1:1) 0.70. IR (ATR): 2983*w*, 1745*s*, 1439*w*, 1367*m*, 1216*s*, 1049*s*, 1009*s*, 965*s*, 653*m*. ^{1}H-NMR (300 MHz, $CDCl_3$): 5.54 (*dd*, J = 3.3, 0.9, H–C(2)); 5.22 (*t*, J = 9.9, H–C(4)); 5.17 (*d*, J = 1.2, H–C(1)); 5.11 (*dd*, J = 10.5, 3.6, H–C(3)); 4.30 – 4.06 (*m*, 2 MeCH_2O, 2 H–C(6)); 3.76 (*ddd*, J = 9.9, 5.1, 3.0, H–C(5)); 2.19, 2.08, 2.05, 1.98 (4*s*, 4 AcO); 1.36 (*t*, J = 6.6, Me). ^{13}C-NMR (75 MHz, $CDCl_3$): 170.37, 169.75, 169.44 (3*s*, 4 C=O); 84.71 (*d*, C(1)); 77.17 (*d*, C(5)); 71.75 (*d*, C(3)); 70.76, 70.67 (2*dt*, ^3J(C,P) = 9.7, C(2)); 65.24 (*d*, C(4)); 64.60, 64.34 (2*dt*, ^2J(C,P) = 5.5, CH_2O); 62.52 (*t*, C(6)); 20.88, 20.78, 20.71, 20.63 (4*q*, 4 *Me*C=O); 15.95, 15.84 (2*q*, 2 Me). ^{31}P-NMR (121 MHz, $CDCl_3$): 91.56. HR-ESI-MS: 539.0788 ($[M+Na]^+$, $C_{18}H_{29}NaO_{11}PS_2^+$; calc. 539.0781). Anal. calc. for $C_{18}H_{29}O_{11}PS_2$: C 41.86, H 5.66, S 12.42; found: C 41.98, H 5.73, S 12.36.

Crystal Structure of β-D-**7**. Recrystallization of β-D-**7** in hexane/AcOEt gave crystals suitable for X-ray analysis: $C_{18}H_{29}O_{11}PS_2$ (516.08); monoclinic $P2_1$; a = 7.4467 (2) Å, b = 21.2419 (8) Å, c = 8.9190 (4) Å, β = 112.2210 (13)°; V = 1306.05 (8) Å^3; $D_{calc.}$ = 1.313 mg/m^3; Z = 2. From a crystal of size 0.06 × 0.08 × 0.28 mm, 4071 reflections were measured on an *Enraf Nonius CAD-4* diffractometer with MoK_α radiation (graphite monochromator, λ = 0.71073 Å) at 298 (2) K. R = 0.0671, R_w = 0.1623.

*Di-*O*-Ethyl* S-*(2,3,4,6-Tetra-*O*-benzyl-β-*D*-mannopyranosyl) Dithiophosphate* (β-D-**8**/α-D-**8** 83:17). A soln. of Ph_3P (37 mg, 0.14 mmol) in dry MeCN (1 ml) was treated at $-10°$ with DIAD (0.029 ml, 0.14 mmol), stirred for 10 min, treated with a soln. of **1** (50 mg, 0.093 mmol) in dry MeCN (0.5 ml), stirred for 10 min, treated with **6** (0.023 ml, 0.14 mmol), allowed to warm slowly to r.t. over a period of 1.5 h, and evaporated. FC (cyclohexane/$CHCl_3$/AcOEt 1:1:0.1) gave β-D-**8**/α-D-**8** 83:17 (45 mg, 68%). Colourless oil. R_f (hexane/AcOEt 4:1) 0.30. IR (ATR): 3030*w*, 2864*w*, 1453*m*, 1362*m*, 1208*w*, 1073*s*, 1009*s*, 958*s*, 733*s*, 696*s*, 653*s*. ^{1}H-NMR (300 MHz, $CDCl_3$): 7.43 – 7.17 (*m*, 20 arom. H); α-D-**8**: 5.94 (*dd*, J = 1.8, ^3J(H,P) = 15, C(1)); β-D-**8**: 5.03 (*d*, J = 0.9, H–C(1)); 5.01 (*d*, J = 11.1, PhC*H*); 4.87 (*d*, J = 10.5, PhC*H*); 4.78 (*d*, J = 11.1, PhC*H*); 4.74 (*s*, PhCH_2); 4.58 (*d*, J = 11.4, PhC*H*); 4.57 (*d*, J = 11.1, PhC*H*); 4.48 (*d*, J = 12.0, PhC*H*); α-D-**8**: 4.90 (*d*, J = 9.6, PhC*H*); 4.78 (*d*, J = 11.1, PhC*H*); 4.70 (*d*, J = 11.4, PhC*H*); 4.63 (*d*, J = 11.7, PhC*H*); 4.57 (*d*, J = 11.4, PhC*H*); 4.49 (*d*, J = 11.7, PhC*H*); β-D-**8**/α-D-**8**: 4.24 – 4.04 (*m*, MeCH_2O); 4.02 – 4.12 (*m*, H–C(2)); β-D-**8**: 3.93 (*t*, J = 9.9, H–C(4)); α-D-**8**: 3.79 (*t*, J = 11.1, H–C(4)); β-D-**8**: 3.74 – 3.67 (*m*, H–C(3), 2 H–C(6)); 3.56 (*ddd*, J = 9.3, 7.2, 2.7, H–C(5)); 1.30, 1.23 (2*dt*, J = 7.2, ^4J(H,P) = 0.9, 2 *Me*CH_2O). ^{13}C-NMR (75 MHz, $CDCl_3$): 138.11, 137.87 (2s); 128.44, 128.28, 128.19, 128.12, 127.89, 127.82, 127.63, 127.55, 127.42 (several *d*); 86.57 (*d*, C(1)); 84.24 (*d*, C(3)); 80.18 (*d*, C(2)); 77.30 (*d*, C(5)); 75.16, 74.93 (2*t*, 2 PhCH_2); 74.41 (*d*, C(4)); 73.28, 72.85 (2*t*, 2 PhCH_2); 69.41 (*t*, C(6)); 64.35, 64.21 (2*t*, ^2J(C,P) = 5.5, 2 Me*C*H_2O); 15.98, 15.87 (2*q*, ^3J(C,P) = 3.8, 2 *Me*CH_2O). ^{31}P-NMR (121 MHz, $CDCl_3$): 93.84; 91.52. HR-ESI-MS: 731.2246 ($[M+Na]^+$, $C_{38}H_{45}NaO_7PS_2^+$; calc. 731.2237). Anal. calc. for $C_{38}H_{45}O_7PS_2$: C 64.39, H 6.40; found: C 64.46, H 6.48.

Allyl 2-Cyanoethyl N,N-*Diisopropylphosphoramidite* (**10**). A soln. of allyl alcohol (0.15 ml, 2.24 mmol) in EtN(i-Pr)$_2$ (0.75 ml, 4.48 mmol) was stirred for 5 min, treated with **9** (0.5 ml, 2.24 mmol) [12], stirred for 1 h at 23°, diluted with CH_2Cl_2 (10 ml), washed with sat. aq. $NaHCO_3$ soln., dried (Na_2SO_4), and evaporated. FC (silica gel; hexane/AcOEt/Et$_3$N 4:1:0.05) gave **10** (520 mg, 90%). Colourless oil. R_f (hexane/AcOEt/Et$_3$N 2:1:0.03) 0.80. IR (ATR): 2967*m*, 2931*w*, 2253*w*, 1646*w*, 1462*w*, 1364*w*, 1182*m*, 1022*s*, 974*s*, 896*m*, 722*m*. ^{1}H-NMR (300 MHz, $CDCl_3$): 5.90 (*ddt*, J = 17.1, 10.0, 6.0, CH_2=C*H*); 5.25 (*dq*, J = 17.1, 1.8), 5.11 (*dq*, J = 10.5, 1.6) (CH_2=CH); 4.22–4.04 (*m*, CH_2=CHCH_2O); 3.89–3.72 (*m*, NCCH$_2$CH_2O); 3.58 (*dsept.*, ^3J(H,P) = 10.5, J = 6.6, (Me$_2$C*H*)$_2$N); 2.61 (*t*, J = 6.3, NCCH_2CH$_2$O); 1.15 (*dd*, J = 6.6, ^4J(H,P) = 2.7, (*Me*CH)$_2$N). ^{13}C-NMR (75 MHz, $CDCl_3$): 135.22 (*dd*, ^3J(C,P) = 7.3, CH_2=*C*H); 117.60 (*s*, CN); 115.60 (*t*, *C*H$_2$=CH); 64.41 (*td*, ^2J(C,P) = 18.2, CH_2=CH*C*H$_2$O); 58.43 (*td*, ^2J(C,P) = 18.8, NCCH$_2$*C*H$_2$O); 43.10 (*dd*, ^2J(C,P) = 12.2 (Me$_2$*C*H)$_2$N); 24.62 (*qd*, ^3J(C,P) = 6.8, (*Me*$_2$CH)$_2$N); 20.47 (*td*, ^3J(C,P) = 6.7, NC*C*H$_2$CH$_2$O). ^{31}P-NMR (121 MHz, $CDCl_3$): 148.3. HR-MALDI-MS: 281.1390 ([M + Na]$^+$, $C_{12}H_{23}N_2NaO_2P^+$; calc. 281.1389).

O-*Allyl* O-*(2-Cyanoethyl)* O-*Oleyl Thiophosphate* (**11**). A soln. of oleyl alcohol (204 mg, 0.76 mmol) in MeCN (1 ml) was treated with **10** (195 mg, 0.76 mmol), followed by 1*H*-tetrazole (4.3 ml, 1.9 mmol), stirred for 1.5 h at 23° and treated with *Beaucage* reagent (3*H*-1,2-benzodithiol-3-one) [24] (152 mg, 0.76 mmol). FC (hexane/AcOEt 5:1) gave **11** (265 mg, 75%). R_f (hexane/AcOEt 6:1) 0.75. IR (neat): 2928*s*, 2856*m*, 2259*w*, 1672*w*, 1465*m*, 1022*s*, 940*m*, 843*m*. ^{1}H-NMR (300 MHz, $CDCl_3$): 5.91 (*ddt*, J = 17.1, 10.0, 6.0, CH_2=CH); 5.39–5.30 (*m*, CH=CH, (*Z*)-CH_2=CH); 5.24 (*dq*, J = 11.4, 1.2, (*E*)-CH_2=CH); 4.55 (*ddt*, J = 5.7, 1.5, ^3J(H,P) = 9.9, CH_2=CHCH_2O); 4.22 (*dt*, J = 6.0, ^3J(H,P) = 10.5, NCCH$_2$CH_2O); 4.05 (*dt*, J = 6.3, ^3J(H,P) = 8.7, CH_2O of oleyl); 2.73 (*t*, J = 6.3, NCCH_2CH$_2$O); 2.08–1.91 (*m*, CH_2CH=CHCH_2); 1.71–1.62 (*m*, MeCH_2); 1.40–1.21 (*m*, 22 H); 0.86 (*t*, J = 6.9, Me). ^{13}C-NMR (75 MHz, $CDCl_3$): 132.42 (*dd*, ^3J(C,P) = 9.1, CH_2=*C*H); 130.19, 129.97 (2*d*, CH=CH); 118.9 (*t*, *C*H$_2$=CH); 116.7 (*s*, CN); 69.18 (*dt*, ^2J(C,P) = 4.9, CH_2O of oleyl and *C*H$_2$=CH*C*H$_2$O); 62.26 (*dt*, ^2J(C,P) = 4.3, NCCH$_2$*C*H$_2$O); 32.12, 30.26, 30.15, 29.98, 29.73, 29.60, 29.53, 29.41, 29.30, 27.43, 25.66, 22.66, 19.73, 19.63 (14*t*); 14.35 (*q*, Me). ^{31}P-NMR (121 MHz, $CDCl_3$): 68.9. HR-MALDI-MS: 480.2674 ([M + Na]$^+$, $C_{24}H_{44}NNaO_3PS^+$; calc. 480.2672).

O-*Allyl Hydrogen* O-*Oleyl Thiophosphate* (**2**). A soln. of **11** (90 mg, 0.2 mmol) in THF/MeOH 1:2 (3 ml) was treated with 0.4M MeONa in MeOH (0.4 ml, 0.16 mmol), stirred for 1.5 h at 23°, diluted with THF (3 ml), and neutralized with *Amberlite IRC50* (H$^+$-form). The resin was filtered off and the filtrate evaporated affording crude **2** (76 mg, 95%), which was used for the next step without further purification. R_f (AcOEt/MeOH/H_2O 7:2:1) 0.65. IR ($CHCl_3$): 2982*s*, 2856*s*, 1650*w*, 1465*w*, 1262*w*, 1100*w*, 1013*s*, 870*w*, 820*w*. ^{1}H-NMR (300 MHz, $CDCl_3$): 7.20 (br. *s*, OH); 5.94 (*ddt*, J = 17.1, 10.5, 5.7, CH_2=C*H*); 5.40–5.21 (*m*, C*H*=CH and (*Z*)-CH_2=CH); 5.23 (*dq*, J = 10.2, 1.2, (*E*)-CH_2=CH); 4.57 (*ddt*, J = 5.1, 1.2, ^3J(H,P) = 9.9, CH_2=CHCH_2O); 4.07 (*dt*, J = 6.7, ^3J(H,P) = 8.4, CH_2O of oleyl); 2.06–1.91 (*m*, CH_2CH=CHCH_2); 1.73–1.63 (*m*, CH_2CH$_2$O); 1.40–1.21 (*m*, 22 H); 0.87 (*t*, J = 6.9, Me). ^{13}C-NMR (75 MHz, $CDCl_3$): 132.27 (*dd*, ^3J(C,P) = 7.9, CH_2=*C*H); 129.8, 129.69 (2*d*, CH_2=*C*H); 118.2 (*s*, CN), 68.6, 68.5 (2*t*, CH_2O of oleyl and *C*H$_2$=CH*C*H$_2$O); 32.0, 30.2, 30.1, 29.9, 29.6, 29.5, 29.4, 27.3, 25.6, 22.8 (several *t*); 14.3 (*q*, Me). ^{31}P-NMR (121 MHz, $CDCl_3$): 60.2. HR-MALDI-MS: 427.2402 ([M + Na]$^+$, $C_{21}H_{41}NaO_3PS^+$; calc. 427.2406).

Treatment of **1** *with* **2**. A soln. of Ph_3P (123 mg, 0.45 mmol) in dry THF (1 ml) was cooled to −10°, treated with DIAD (0.089 ml, 0.45 mmol), stirred for 10 min, treated with a soln. of **1** (105 mg, 0.3 mmol) in dry THF (1 ml), stirred at −10° for 10 min, treated with a soln. of **2** (182 mg, 0.45 mmol) in dry THF (1 ml), and allowed to warm slowly to r.t. over period of 2 h. After evaporation, FC (hexane/AcOEt 5:1 → 1:1) gave *β*-D-**13**/*α*-D-**13** 2:1 (46 mg, 25%) and **12** (64 mg, 35%). FC (cyclohexane/$CHCl_3$/AcOEt 1:1:0.1) on silica gel *60* (0.015–0.040 mm, from *Merck*) of *β*-D-**13**/*α*-D-**13** 2:1 gave pure fractions of *α*-D-**13** (faster moving) and *β*-D-**13**.

Data of O-*Allyl* O-*Oleyl* S-*(2,3,4,6-Tetra*-O-*acetyl-β*-D-*mannopyranosyl) Thiophosphate* (**12**). R_f (hexane/AcOEt 1:1) 0.40. IR: 2928*m*, 2856*w*, 1752*s*, 1456*w*, 1369*m*, 1264*s*, 1223*s*, 1207*m*, 1052*m*, 1012*m*, 987*m*, 598*w*, 564*w*. ^{1}H-NMR (500 MHz, $CDCl_3$; 1:1 mixture of diastereoisomers, assignment based on a DFQCOSY and a HSQC spectrum): 5.94 (*ddt*, J = 17.1, 10.0, 6.0, CH_2=C*H*); 5.53, 5.52 (2*dd*, J = 3.5, 1.2, H−C(2)); 5.37, 5.36 (2*dq*, J = 17.1, 1.5, CH_2=CH); 5.36–5.31 (*m*, CH=CH); 5.30 (*dd*, J = 1.5, ^3J(H,P) = 2.8, H−C(1)); 5.27 (*dq*, J = 10.0, 1.5, CH_2=CH); 5.24, 5.23 (2*t*, J = 10.0, H−C(4)); 5.08, 5.07 (2*dd*, J = 10.5, 1.5, H−C(3)); 4.68–4.50 (*m*, CH_2=CHCH_2O); 4.22, 4.21 (2*dd*, J = 12.4, 5.5, H−C(6)); 4.17–4.03 (*m*, CH_2O, H′−C(6)); 3.77, 3.75 (2*ddd*, J = 9.5, 5.7, 2.4, H−C(5)); 2.21, 2.08, 2.06 (3*s*, 3 AcO); 2.20–1.75 (*m*, CH_2CH=CHCH_2); 1.98 (*s*, AcO); 1.74–1.62 (*m*, OCH$_2$CH_2); 1.40–1.21 (*m*, 22 H); 0.88 (*t*, J = 6.9, Me). ^{13}C-NMR (125 MHz, $CDCl_3$; 1:1 mixture of diastereoisomers, assignment based on a HSQC spectrum): 170.51, 169.95, 169.93, 169.83, 169.82, 169.61, 169.59 (7*s*, 4 C=O); 132.06, 131.86 (2*d*, ^4J(C,P) = 8.0, CH_2=*C*H); 129.98, 129.76, 129.75, 128.02 (4*d*, CH=CH); 118.96, 118.94 (2*t*, *C*H$_2$=CH); 81.99, 81.91 (2*dd*, ^3J(C,P) = 2.8, C(1)); 77.00 (*d*, C(5)); 71.63 (*d*, C(3)); 70.96, 70.89 (2*dd*, ^4J(C,P) = 6.6, C(2)); 68.48, 68.28 (2*td*, ^2J(C,P) = 5.1, CH_2O of oleyl); 68.19, 68.02 (2*td* , ^2J(C,P) = 5.1, CH_2O);

65.12, 65.08 (2*d*, C(4)); 62.52, 62.40 (2*t*, C(6)); 31.89 (*t*); 29.75–29.11 (several *t*); 27.20, 27.18, 26.44, 25.37, 22.44, 25.37, 22.67 (7*t*); 20.76, 20.67, 20.57, 20.50 (4*q*, 4 *Me*C=O); 14.10, (*q*, Me). ^{31}P-NMR (121 MHz, $CDCl_3$; 1:1 mixture of diastereoisomers): 24.01, 23.88. HR-MALDI-MS: 757.3365 ($[M+Na]^+$, $C_{35}H_{59}NaO_{12}PS^+$; calc. 757.3357). Anal. calc. for $C_{35}H_{59}O_{12}PS$ (734.35): C 57.20, H 8.09; found: C 57.28, H 8.01.

Data of O-*Allyl* O-*Oleyl* O-*(2,3,4,6-Tetra*-O-*acetyl-β-*D-*mannopyranosyl) Thiophosphate* (*β*-D-**13**). R_f (hexane/AcOEt 1:1) 0.80; R_f (cyclohexane/$CHCl_3$/AcOEt 1:1:0.1; two runs) 0.35. IR (ATR): 3008*m*, 2928*s*, 2856*m*, 1752*s*, 1649*w*, 1456*w*, 1370*m*, 1223*s*, 1162*m*, 1016*s*, 967*s*. ^{1}H-NMR (600 MHz, $CDCl_3$; 1:1 mixture of diastereoisomers, assignment based on a DFQCOSY and a HSQC spectrum): 5.88 (*ddt*, J = 17.0, 10.5, 5.7, CH_2=C*H*); 5.51–5.47 (*m*, H–C(1), H–C(2)); 5.41–5.33 (*m*, CH=CH, (*Z*)-CH_2=CH); 5.23 (*dq*, J = 10.8, 1.2, (*E*)-CH_2=CH); 5.234, 5.229 (2*dt*, J = 9.6, 1.5, H–C(4)); 5.118, 5.107 (2*dd*, J = 9.9, 1.5, H–C(3)); 4.61–4.45 (*m*, OCH_2CH=CH_2); 4.27, 4.25 (2*dd*, J = 12.3, 6.1, H–C(6)); 4.19, 4.18 (2*dd*, J = 12.3, 2.7, H′–C(6)); 4.12–3.91 (*m*, CH_2O); 3.78, 3.76 (2*ddd*, J = 9.5, 4.5, 2.6, H–C(5)); 2.18, 2.08, 2.06 (3*s*, 3 AcO); 2.04–1.95 (*m*, CH_2CH=CHCH_2); 2.00 (*s*, AcO); 1.68–1.62 (*m*, CH_2CH_2O); 1.38–1.23 (*m*, 22 H); 0.86 (*t*, J = 6.9, Me). ^{13}C-NMR (150 MHz, $CDCl_3$, assignment based on a HSQC spectrum): 170.52, 170.50, 169.98, 169.94, 169.79, 169.62 (6*s*, 4 C=O); 132.40, 132.12 (2*dd*, ^4J(C,P) = 9, CH_2=C*H*); 130.01, 129.98, 129.82, 129.77 (4*d*, CH=CH); 118.55, 118.33 (2*t*, CH_2=CH); 94.77 (*d*, C(1)); 73.11 (*d*, C(5)); 70.59 (*d*, C(3)); 68.96, 68.93, 68.82, 68.56, 68.50 (5*t*, CH_2O of oleyl and CH_2=CHCH_2O); 65.64 (*d*, C(4)); 62.28, 62.24 (2*t*, C(6)); 32.62, 31.92, 29.96, 29.91, 29.78, 29.77, 29.71, 29.54, 29.44, 29.42, 29.34, 29.32, 29.26, 29.25, 29.17, 29.11, 27.24, 27.22, 25.46, 25.43, 22.69, 21.73 (several *t*); 20.76, 20.74, 20.68, 20.53 (4*q*, 4 *Me*C=O); 14.11 (*q*, Me). ^{31}P-NMR (121 MHz, $CDCl_3$): 68.65; 68.57. HR-MALDI-MS: 757.3366 ($[M+Na]^+$, $C_{35}H_{59}NaO_{12}PS^+$; calc. 757.3357). Anal. calc. for $C_{35}H_{59}O_{12}PS$ (734.35): C 57.20, H 8.09, S 4.36; found: C 57.45, H 8.17, S 4.17.

Data of O-*Allyl* O-*Oleyl* O-*(2,3,4,6-Tetra*-O-*acetyl-α-*D-*mannopyranosyl) Thiophosphate* (*α*-D-**13**). R_f (hexane/AcOEt 1:1) 0.80. R_f (cyclohexane/$CHCl_3$/AcOEt 1:1:0.1; double eluted) 0.40. ^{1}H-NMR (500 MHz, $CDCl_3$; 1:1 mixture of diastereoisomers, assignment based on a DFQCOSY and a HSQC spectrum): 5.94 (*ddt*, J = 17.2, 11.4, 5.5, CH_2=C*H*); 5.75, 5.73 (2*dd*, J = 1.9, ^3J(H,P) = 5.5, H–C(1)); 5.39, 5.38 (2*dq*, J = 17.2, 1.5, (*Z*)-CH_2=CH); 5.38–5.31 (*m*, CH=CH, H–C(3), H–C(4)); 5.31–5.29 (*m*, H–C(2)); 5.28 (*dq*, J = 11.7, 1.0, (*E*)-CH_2=CH); 4.64–4.54 (*m*, CH_2=CHCH_2O); 4.31, 4.28 (2*dd*, J = 9.1, 4.6, H–C(6)); 4.19–4.14 (*m*, H–C(5)); 4.13–4.04 (*m*, CH_2O, H′–C(6)); 2.17, 2.08 (2*s*, 2 AcO); 2.04, 2.04 (2*s*, AcO); 2.04–1.95 (*m*, CH_2CH=CHCH_2); 2.00, 1.97 (2*s*, AcO); 1.72–1.65 (*m*, CH_2CH_2O); 1.42–1.23 (*m*, 22 H); 0.88 (*t*, J = 6.9, Me). ^{13}C-NMR (125 MHz, $CDCl_3$; 1:1 mixture of diastereoisomers, assignment based on a HSQC spectrum): 170.55, 170.52, 169.81, 169.79, 169.63, 169.56 (6*s*, 4 C=O); 132.22, 132.10 (2*dd*, ^3J(C,P) = 8, CH_2=C*H*); 130.01, 129.98, 129.82, 129.77 (4*d*, CH=CH); 118.92, 118.60 (2*t*, CH_2=CH); 95.40, 95.36 (2*d*, C(1)); 70.35 (*d*, C(5)); 69.18, 69.15, 69.13, 69.09, 69.04, 69.00, (6*t*, OCH_2CH=CH_2, CH_2O); 68.98, 68.89 (2*d*, C(3)); 68.55 (*d*, C(2)); 65.50 (*d*, C(4)); 62.02, 62.01 (2*t*, C(6)); 31.92 (*t*); 29.79, 29.77, 29.76, 29.53, 29.40, 29.34, 29.32, 29.26, 29.12 (several *t*) 27.24, 27.22, 25.46, 25.45, 22.69 (several *t*); 20.78, 20.72, 20.66, 20.61 (4*q*, 4 *Me*C=O); 14.11 (*q*, Me). ^{31}P-NMR (121 MHz, $CDCl_3$; 1:1 mixture of diastereoisomers): 68.88; 68.79. HR-MALDI-MS: 757.3366 ($[M+Na]^+$, $C_{35}H_{59}NaO_{12}PS^+$; calc. 757.3357).

Triethylammonium O-*Oleyl* S-*(2,3,4,6-Tetra*-O-*acetyl-β-*D-*mannopyranosyl) Thiophosphate* (**14**·Et_3N). A soln. of **12** (26 mg, 0.035 mmol) in dry, degassed THF (1.5 ml) was treated with Ph_3P (1.9 mg, 0.007 mmol), $Pd(PPh_3)_4$ (2 mg, 0.0018 mmol), a soln. of $BuNH_2$ (35 µl, 0.35 mmol) and HCOOH (11 µl, 0.35 mmol) in THF (0.5 ml), stirred for 0.5 h at 23°, and evaporated. FC (hexane/AcOEt 3:7 and then AcOEt/MeOH 10:1) afforded yellowish **14** (21 mg, 88%), which was converted into **14**·Et_3N by filtration through silica gel (AcOEt/MeOH/H_2O/Et_3N 7:2:1:0.1). R_f ($CHCl_3$/MeOH 10:1) 0.25. IR (ATR): 2928*s*, 2856*m*, 1750*s*, 1602*w*, 1457*w*, 1370*m*, 1228*s*, 1052*s*, 1012*s*, 573*m*. ^{1}H-NMR (500 MHz, CD_3OD; assignment based on a DFQCOSY and a HSQC spectrum): 5.51 (*dd*, J = 3.5, 1.0, H–C(2)); 5.35–5.52 (*m*, CH=CH); 5.28 (*d*, ^3J(H,P) = 7.2, H–C(1)); 5.25 (*t*, J = 10.0, H–C(4)); 5.10 (*dd*, J = 10.0, 3.5, H–C(3)); 4.25 (*dd*, J = 12.4, 5.1, H–C(6)); 4.11 (*dd*, J = 12.4, 2.2, H′–C(6)); 3.95 (*dt*, J = 6.9 , ^2J(H,P) = 7.2, CH_2O); 3.76 (*ddd*, J = 10.0, 5.0, 2.3, H–C(5)); 3.05 (*q*, J = 4.5, (MeCH_2)$_3$N); 2.18, 2.07, 2.03 (3*s*, 3 AcO); 2.03–1.96 (*m*, CH_2CH=CHCH_2); 1.96 (*s*, AcO); 1.67–1.60 (*m*, CH_2CH_2O); 1.32 (*t*, J = 4.5, *Me*CH_2N); 1.33–1.24 (*m*, 22 H); 0.88 (*t*, J = 7.1, Me). ^{13}C-NMR (125 MHz, $CDCl_3$; assignment based on a HSQC spectrum): 170.70, 170.33, 169.90, 169.68 (4*s*, 4 C=O); 129.93, 129.87 (2*d*, CH=CH); 82.32 (*d*, C(1)); 76.66 (*d*, C(5)); 72.12 (*d*, C(3)); 71.86 (*dd*, ^3J(C,P) = 5.9, C(2)); 66.47 (*dt*, ^2J(C,P) = 5.3, CH_2O); 65.61 (*d*, C(4)); 62.71 (*t*, C(6)); 45.81 (*t*, (MeCH_2)$_3$N); 31.92, 29.82, 29.79, 29.71, 29.57, 29.54, 29.43, 29.36, 29.34, 29.32, 27.26, 27.24, 25.83, 22.69 (several *t*); 20.81, 20.73, 20.70, 20.58 (4*q*, 4 MeC=O); 14.11 (*q*, Me); 8.63 (*q*, (*Me*CH_2)$_3$N). ^{31}P-NMR (121 MHz, $CDCl_3$): 15.16. HR-MALDI-MS: 717.3052 ($[M+Na]^+$, $C_{32}H_{55}NaO_{12}PS^+$; calc. 717.3044).

Triethylammonium O-*Oleyl* O-*(2,3,4,6-Tetra*-O-*acetyl-β*-D-*mannopyranosyl) Thiophosphate* (**15** · Et_3N). A soln. of *β*-D-**13** (20 mg, 0.029 mmol) in dry, degassed THF (1.5 ml) was treated with Ph_3P (1.4 mg, 0.0054 mmol), $Pd(PPh_3)_4$ (1.6 mg, 0.0014 mmol), a soln. of $BuNH_2$ (27 µl, 0.35 mmol) and HCOOH (10 µl, 0.35 mmol) in THF (0.5 ml), stirred for 0.5 h at 23°, and evaporated. FC (hexane/AcOEt 3:7 and then AcOEt/MeOH 10:1) afforded yellowish **15** (16 mg, 85%), which was converted into **15** · Et_3N by filtration through silica gel (AcOEt/MeOH/H_2O/Et_3N 7:2:1:0.1). R_f ($CHCl_3$/MeOH 10:1) 0.20. IR (ATR): 2924*m*, 2853*m*, 1748*s*, 1656*w*, 1437*w*, 1367*m*, 1219*s*, 1083*m*, 1051*s*, 831*m*, 643*m*. ^{1}H-NMR (500 MHz, CD_3OD; 1:1 mixture of diastereoisomers, assignment based on a DFQCOSY and a HSQC spectrum): 5.64, 5.60 (2*dd*, J = 1.0, ^3J(H,P) = 12.0, H–C(1)); 5.48 (br. *d*, J = 3.2, H–C(2)); 5.37–5.30 (*m*, CH=CH); 5.26, 5.24 (2*t*, J = 10.0, H–C(4)); 5.11, 5.10 (2*dd*, J = 10.0, 3.4, H–C(3)); 4.27, 4.25 (2*dd*, J = 12.5, 4.8, H–C(6)); 4.15, 4.14 (2*dd*, J = 12.3, 2.3, H′–C(6)); 3.99–3.80 (*m*, CH_2O); 3.77–3.72 (*m*, H–C(5), CH_2O); 3.09 (*q*, J = 4.5, ($MeCH_2$)$_3$N); 2.16, 2.15 (2*s*, AcO); 2.07, 2.04 (2*s*, 2 AcO); 2.03–1.96 (*m*, CH_2CH=$CHCH_2$); 1.97 (*s*, AcO); 1.67–1.60 (*m*, CH_2CH_2O); 1.35 (*t*, J = 4.5, ($MeCH_2$)$_3$N); 1.31–1.24 (*m*, 22 H); 0.88 (*t*, J = 7.0, Me); ^{13}C-NMR (125 MHz, $CDCl_3$; 1:1 mixture of diastereoisomers, assignment based on a HSQC spectrum): 170.74, 170.71, 170.54, 170.20, 169.89, 169.83, 169.74, 169.73 (8*s*, 4 C=O); 129.91, 129.89, 129.88 (3*d*, CH=CH); 94.77, 94.44 (2*dd*, ^2J(C,P) = 2.6, C(1)); 72.74, 72.63 (2*d*, C(5)); 71.41, 71.18 (2*d*, C(3)); 69.74, 69.43 (2*dd*, ^3J(C,P) = 7.2, C(2)); 66.89, 66.86, 66.81 (3*t*, CH_2O); 65.94, 65.93 (2*d*, C(4)); 62.60, 62.49 (2*t*, C(6)); 45.65 (*t*, ($MeCH_2$)$_3$N); 31.91, 29.82, 29.79, 29.57, 29.54, 29.41, 29.33, 29.32, 27.25, 27.23, 25.83, 25.81 (several *t*); 20.92, 20.82, 20.81, 20.76, 20.60, 20.59 (6*q*, 4 *Me*C=O); 14.13 (*q*, Me); 8.58 (*q*, ($MeCH_2$)$_3$N). ^{31}P-NMR (121 MHz, $CDCl_3$; 1:1 mixture of diastereoisomers): 57.34; 56.40. HR-MALDI-MS: 717.3035 ($[M+Na]^+$, $C_{32}H_{55}NaO_{12}PS^+$; calc. 717.3044).

Hydrogen O-*Oleyl* S-*(β*-D-*Mannopyranosyl) Thiophosphate* (**16**). A soln. of **14** · Et_3N (20 mg, 0.029 mmol) in THF/MeOH 1:2 (0.3 ml) was treated with 0.4M MeONa in MeOH (0.027 ml, 0.011 mmol), stirred at 23° for 1.5 h, diluted with THF (1 ml), and neutralized with *Amberlite IRC50* (H^+-form). The resin was filtered off, and the filtrate was evaporated. A soln. of the residue in EtOH (*ca.* 0.3 ml) was diluted with pentane. The precipitate was filtered off and dried *i.v.* to afford **16** (13 mg, 85%). R_f (AcOEt/MeOH/H_2O 7:2:1) 0.75. R_f ($CHCl_3$/MeOH/H_2O 10:3:1) 0.30. IR (neat): 3276*s* (br.), 2921*m*, 2851*m*, 1465*w*, 1345*w*, 1188*m*, 1049*s*, 1025*s*, 965*m*, 829*m*, 695*m*. ^{1}H-NMR (600 MHz, CD_3OD; assignment based on a DFQCOSY and a HSQC spectrum): 5.44–5.32 (*m*, CH=CH); 4.98 (*dd*, J = 0.9, ^3J(H,P) = 11.7, H–C(1)); 3.96–3.88 (*m*, CH_2O, H–C(2)); 3.84 (*dd*, J = 11.7, 2.4, H–C(6); 3.67 (*dd*, J = 11.7, 5.7, H′–C(6)); 3.54 (*t*, J = 9.6, H–C(4)); 3.47 (*dd*, J = 9.5, 3.3, H–C(3)); 3.28 (*ddd*, J = 9.6, 5.7, 2.3, H–C(5)); 2.08–1.94 (*m*, CH_2CH=$CHCH_2$); 1.68–1.57 (*m*, CH_2CH_2O); 1.44–1.23 (*m*, 22 H); 0.89 (*t*, J = 6.9, Me). ^{13}C-NMR (150 MHz, $CDCl_3$; assignment based on a HSQC spectrum): 131.60, 131.54 (*d*, *C*H=CH); 85.37 (*dd*, ^2J(C,P) = 2.6, C(1)); 83.73 (*d*, C(5)); 76.38 (*d*, C(3)); 75.08 (*dd*, ^3J(C,P) = 6.4, C(2)); 68.24 (*d*, C(4)); 67.08 (*dt*, ^2J(C,P) = 6.2, CH_2O); 63.10 (*t*, C(6)); 32.44, 31.87 (2*t*); 30.55–29.03 (several *t*); 26.93, 25.87, 22.55 (3*t*); 13.27 (*q*, Me). ^{31}P-NMR (121 MHz, $CDCl_3$): 18.64. HR-MALDI-MS: 549.2622 ($[M+H]^+$, $C_{24}H_{48}O_8PS^+$; calc. 549.26215).

Hydrogen O-*Oleyl* O-*(β*-D-*Mannopyranosyl) Thiophosphate* (**17**). A soln. of **15** · Et_3N (15 mg, 0.022 mmol) in THF/MeOH 1:2 (0.3 ml) was treated with 0.4M MeONa in MeOH (0.02 ml, 0.008 mmol), stirred at 23° for 1.5 h, diluted with THF (1 ml), and neutralized with *Amberlite IRC50* (H^+-form). The resin was filtered off, and the filtrate was evaporated. A soln. of the residue in EtOH (*ca.* 0.4 ml) was diluted with pentane. The precipitate was filtered off and dried *i.v.* to afford **17** (10 mg, 83%). R_f ($CHCl_3$/MeOH 10:1) 0.20. IR (ATR): 3302*s* (br.), 2922*s*, 2852*s*, 1631*w*, 1464*w*, 1375*w*, 1145*m*, 1068*s*, 1015*s*, 840*m*, 670*m*, 630*m*. ^{1}H-NMR (600 MHz, CD_3OD; 1:1 mixture of diastereoisomers, assignment based on a DFQCOSY and a HSQC spectrum): 5.35–5.33 (*m*, CH=CH); 5.28, 5.26 (2*dd*, J = 1.0, ^3J(H,P) = 6.5, H–C(1)); 4.00–3.89 (*m*, CH_2O, H–C(2)); 3.85, 3.84 (2*dd*, J = 12.4, 2.5, H–C(6)); 3.70, 3.68 (2*dd*, J = 12.5, 5.6, H′–C(6)); 3.58, 3.54 (2*t*, J = 9.5, H–C(4)); 3.56, 3.55 (2*dd*, J = 9.5, 3.5, H–C(3)); 3.76 (*ddd*, J = 9.5, 5.7, 2.4, H–C(5)); 2.06–1.98 (*m*, CH_2CH=$CHCH_2$); 1.64–1.60 (*m*, CH_2CH_2O); 1.38–1.29 (*m*, 22 H); 0.90 (*t*, J = 7.0, Me). ^{13}C-NMR (125 MHz, $CDCl_3$; 1:1 mixture of diastereoisomers, assignment based on a HSQC spectrum): 130.94, 130.87 (2*d*, CH=CH); 97.68, 97.27 (2*dd*, ^2J(C,P) = 4.5, C(1)); 78.96, 78.88 (2*d*, C(5)); 75.18, 75.09 (2*d*, C(3)); 72.90, 72.85 (2*dd*, ^3J(C,P) = 6.9, C(2)); 68.32, 68.22 (2*d*, C(4)); 67.50, 67.45 (2*td*, ^2J(C,P) = 6.8, CH_2O); 62.92, 62.91 (2*t*, C(6)); 33.65, 33.09 (2*t*); 31.70, 31.69, 31.67, 31.64, 30.94, 30.88, 30.81, 30.69, 30.57, 30.55, 30.46, 30.40, 30.36, 30.24 (several *t*); 28.22, 28.16, 27.00, 23.76 (4*t*); 14.47 (*q*, Me). HR-MALDI-MS: 571.2432 ($[M+Na]^+$, $C_{24}H_{47}NaO_8PS^+$; calc. 571.2441).

2-Cyanoethyl Oleyl N,N-*Diisopropylphosphoramidite* (**4a**). A soln. of oleyl alcohol (130 mg, 0.48 mmol) in CH_2Cl_2 (2 ml) was treated with EtN(i-Pr)$_2$ (0.16 ml, 0.96 mmol), followed after 5 min by **9** (0.11 ml, 0.48 mmol), stirred for 1 h at 23°, diluted with CH_2Cl_2 (7 ml), washed with sat. aq. $NaHCO_3$ soln., dried (Na_2SO_4), and evaporated. FC (silica gel, hexane/AcOEt/Et_3N 4:1:0.05) gave **4a** (180 mg, 80%). Colourless oil. R_f (hexane/AcOEt/Et_3N 2:1:0.03) 0.80. IR (ATR): 2963*m*, 2924*s*, 2854*m*, 2250*w*, 1462*w*, 1363*w*, 1183*w*, 1051*m*, 975*s*, 893*m*,

715*s*. ^{1}H-NMR (300 MHz, $CDCl_3$): 5.36–5.30 (*m*, CH=CH); 3.90–3.73 (*m*, CH_2O); 3.70–3.51 (*m*, $(Me_2CH)_2N$); 2.62 (*td*, $J = 6.6$, ^4J(P,H) = 0.6, $NCCH_2CH_2O$); 2.03–1.96 (*m*, $CH_2CH=CHCH_2$); 1.62–1.55 (*m*, CH_2CH_2O); 1.40–1.21 (*m*, 22 H); 1.18 (*dd*, $J = 6.6$, ^4J(H,P) = 3.0, $(Me_2CH)_2N$); 0.87 (*t*, $J = 7.2$, Me). ^{13}C-NMR (75 MHz, $CDCl_3$): 129.77, 129.67 (CH=CH); 117.51 (*s*, CN); 63.72 (*dt*, ^2J(C,P) = 17.0, OCH_2); 58.30 (*dt*, ^2J(C,P) = 18.8, $NCCH_2CH_2O$); 43.98 (*dd*, ^2J(C,P) = 12.2, $(Me_2CH)_2N$); 31.97, 31.32, 31.23 (3*t*); 29.83–29.31 (several *t*); 27.27, 26.00, 24.77, 24.66, 24.56, 22.76 (6*t*); 20.43 (*q*, ^4J(C,P) = 6.8, $(Me_2CH)_2N$); 14.21 (*q*, Me). ^{31}P-NMR (121 MHz, $CDCl_3$): 147.32. HR-ESI-MS 491.3740 ($[M+Na]^+$, $C_{27}H_{53}N_2NaO_2P^+$; calc. 491.3737).

2-Cyanoethyl Dolichyl N,N-*Diisopropylphosphoramidite* (**4b**). A soln. of dolichol alcohol (20 mg, 0.015 mmol) in CH_2Cl_2 (0.2 ml) was treated with EtN(i-Pr)$_2$ (6 µl, 0.03 mmol), followed after 5 min by **9** (4 µl, 0.015 mmol), stirred for 1 h at 23°, diluted with CH_2Cl_2 (5 ml), washed with sat. aq. $NaHCO_3$ soln. (3 ml), dried (Na_2SO_4), and evaporated. FC (silica gel, hexane/AcOEt/Et_3N 5:1:0.05) gave **4b** (19 mg, 83%). Colourless oil. R_f (hexane/AcOEt/Et_3N 2:1:0.03) 0.85. IR (neat): 2961*m*, 2925*s*, 2853*m*, 2723*w*, 2487*w*, 1447*w*, 1376*w*, 1011*s*, 792*s*, 703*w*. ^{1}H-NMR (300 MHz, $CDCl_3$): 5.20–5.06 (*m*, 18 C=CH); 3.88–3.53 (*m*, 2 CH_2O, $(Me_2CH)_2N$); 2.63 (*t*, $J = 6.6$, $NCCH_2CH_2O$); 2.12–1.96 (*m*, CH_2CH_2O, 17 $CH_2MeC=CHCH_2$, $Me_2C=CHCH_2$); 1.68 (br. *s*, 17 Me, $CH_2CHMeCH_2$); 1.60 (br. *s*, $Me_2C=CH$, MeCH); 1.18 (*dd*, $J = 6.6$, ^4J(H,P) = 3.0, $(Me_2CH)_2N$); 0.89 (*d*, $J = 6.6$, *Me*CH). ^{13}C-NMR (300 MHz, $CDCl_3$): 135.12, 135.84, (2*d*, 7 $CH_2MeC=CH$); 131.17 (*d*, $Me_2C=CH$); 125.29, 124.99, 124.32, 124.14 (4*s*, 18 CH=*C*); 115.34 (*s*, CN); 61.95 (*dt*, ^2J(C,P) = 17.5, CH_2O); 58.34 (*dt*, ^2J(C,P) = 18.2, $NCCH_2CH_2O$); 43.14, 42.98, 39.82, 38.38, 37.46 (5*t*); 32.30, 32.09 (2*t*); 29.41(*d*); 26.87–25.82 (several *t*); 25.34 (*q*); 24.83–24.63 (several *t*); 23.56 (*q*, several Me); 20.43, 19.52, 17.82 (3*q*); 16.12 (*q*, *Me*CH). ^{31}P-NMR (121 MHz, $CDCl_3$): 147.45; 147.35. HR-MALDI-MS: 1536.3152 ($[M+H]^+$). HR-ESI-MS 1536.3137 ($[M+H]^+$, $C_{104}H_{174}N_2O_2P^+$; calc. 1536.3127).

Treatment of **3** *with* **4a**. A soln. of **3** [11] (80 mg, 0.22 mmol) in MeCN (1 ml) was treated with **4a** (160 mg, 0.33 mmol), 0.45M 1*H*-tetrazole in MeCN (1.2 ml, 0.55 mmol), stirred for 1.5 h at 23°, treated with 5.5M *t*-BuOOH in decane (0.4 ml, 5.5M in decane, 2.2 mmol), and stirred for 1 h. FC (hexane/AcOEt 5:1 → 1:1) gave **18** (49 mg, 30%) and **19** (43 mg, 26%).

Data of O-*(2-Cyanoethyl)* O-*Oleyl* S-*(2,3,4,6-Tetra*-O-*acetyl-β*-D-*mannopyranosyl) Thiophosphate* (**18**). R_f (hexane/AcOEt 1:1) 0.34. IR (neat): 2924*m*, 2854*m*, 2255*w*, 1746*s*, 1434*w*, 1368*m*, 1211*s*, 1047*s*, 995*s*, 769*m*, 699*m*. ^{1}H-NMR (300 MHz, $CDCl_3$; 1:1 mixture of diastereoisomers, assignment based on a DFQCOSY and a HSQC spectrum): 5.52, 5.49 (2*dd*, $J = 2.7$, 1.2, H–C(2)); 5.41–5.28 (*m*, CH=CH); 5.27, 5.23 (2*dd*, ^3J(H,P) = 12.3, $J = 1.2$, H–C(1)); 5.22 (*t*, $J = 9.9$, H–C(4)); 5.12, 5.06 (2*dd*, $J = 9.9$, 3.6, H–C(3)); 4.37–4.05 (*m*, 2 H–C(6), 2 CH_2O); 3.76, 3.74 (2*ddd*, $J = 10.2$, 5.4, 2.4, H–C(5)); 2.80 (*t*, $J = 6.6$, $NCCH_2CH_2O$); 2.21, 2.09, 2.06, 1.98 (4*s*, 4 AcO); 2.09–1.92 (*m*, $CH_2CH=CHCH_2$); 1.76–1.65 (*m*, CH_2CH_2O); 1.40–1.21 (*m*, 22 H); 0.88 (*t*, $J = 6.3$, Me). ^{13}C-NMR (75 MHz, $CDCl_3$; 1:1 mixture of diastereoisomers, assignment based on a HSQC spectrum): 170.31, 169.80, 169.83, 169.42 (4*s*, 4 C=O); 130.35, 130.12, 129.89, 129.66 (4*d*, CH=CH); 116.19 (*s*, CN); 81.98, 81.69 (2*d*, C(1)); 77.20 (*d*, C(5)); 71.55 (*d*, C(3)); 70.90, 70.65 (2*dd*, ^3J(C,P) = 8.2, C(2)); 69.95, 68.90 (2*dt*, ^2J(C,P) = 6.1, CH_2O); 64.95 (*d*, C(4)); 62.28, 62.09 (2*t*, C(6)); 61.76, 61.65 (2*td*, ^2J(C,P) = 4.9, $CNCH_2CH_2O$); 32.69, 31.99 (2*t*); 30.19–29.19 (several *t*); 27.31, 25.51, 25.44, 22.79 (4*t*); 20.89, 20.79, 20.68, 20.62, 19.74, 19.64, 19.48 (7*q*, 4 *Me*C=O); 14.25 (*q*, Me). ^{31}P-NMR (121 MHz, $CDCl_3$; 1:1 mixture of diastereoisomers): 24.15; 24.03. HR-MALDI-MS: 770.3321 ($[M+Na]^+$, $C_{35}H_{58}NNaO_{12}PS^+$; calc. 770.3310). Anal. calc. for $C_{35}H_{58}NO_{12}PS$ (747.34): C 56.21, H 7.82, N 1.87; found: C 55.92, H 8.00, N 1.72.

Data of O-*(2-Cyanoethyl)* O-*Oleyl* S-*(2,3,4,6-Tetra*-O-*acetyl-β*-D-*mannopyranosyl) Dithiophosphate* (**19**). R_f (hexane/AcOEt 1:1) 0.75. IR (neat): 2924*s*, 2854*m*, 2255*w*, 1748*s*, 1463*w*, 1367*m*, 1216*s*, 1047*s*, 990*s*, 659*m*. ^{1}H-NMR (400 MHz, $CDCl_3$; 1:1 mixture of diastereoisomers, assignment based on a DFQCOSY and a HSQC spectrum): 5.52, 5.57 (2*dd*, $J = 3.6$, 0.9, H–C(2)); 5.40–5.31 (*m*, CH=CH); 5.24, 5.23 (2*t*, $J = 10.2$, H–C(4)); 5.21, 5.15 (2*dd*, ^3J(C,P) = 14.4, $J = 1.2$, H–C(1)); 5.12, 5.10 (2*dd*, $J = 10.2$, 3.9, H–C(3)); 4.38–4.03 (*m*, 2 H–C(6), 2 CH_2O); 3.77, 3.74 (2*ddd*, $J = 10.2$, 5.1, 2.4, H–C(5)); 2.79 (*t*, $J = 6.6$, $NCCH_2CH_2O$); 2.19, 2.09, 2.04, 1.99 (4*s*, 4 AcO); 2.09–1.92 (*m*, $CH_2CH=CHCH_2$); 1.76–1.65 (*m*, CH_2CH_2O); 1.42–1.21 (*m*, 22 H); 0.87 (*t*, $J = 6.9$, Me). ^{13}C-NMR (100 MHz, $CDCl_3$; 1:1 mixture of diastereoisomers, assignment based on a HSQC spectrum): 170.51, 170.47, 169.89, 169.86, 169.64, 169.61 (6*s*, 4 C=O); 130.46, 130.26, 130.00, 129.80 (4*d*, CH=CH); 116.48, 116.39 (2*s*, CN); 85.06 (br. *d*), 84.06 (*dd*, ^2J(C,P) = 2.3) (C(1)); 77.49, 77.26 (2*d*, C(5)); 71.73, 71.68 (2*d*, C(3)); 70.63, 70.48 (2*dd*, ^3J(C,P) = 8.6, C(2)); 69.18, 69.01 (2*td*, ^2J(C,P) = 6.5, CH_2O); 65.16, 66.00 (2*d*, C(4)); 62.29, 62.15 (2*t*, C(6)); 61.91, 61.68 (2*td*, ^2J(C,P) = 4.0, $NCCH_2CH_2O$); 32.62, 32.59, 31.91 (3*t*); 29.94–29.07 (several *t*); 27.33 (*t*); 25.56 (*t*); 22.81(*t*); 21.00 (3*t*); 20.86, 20.81, 20.67, 20.59, 20.57, 20.52, 19.45, 19.36 (8*q*, 4 *Me*C=O); 14.27 (*q*, Me). ^{31}P-NMR (121 MHz, $CDCl_3$; 1:1 mixture of diastereoisomers): 93.12; 91.14. HR-MALDI-MS: 786.3090 ($[M+Na]^+$, $C_{35}H_{58}NNaO_{11}PS_2^+$; calc. 786.3081). Anal. calc. for $C_{35}H_{58}NO_{11}PS_2$ (763.32): C 55.03, H 7.65, N 1.83; found: C 55.29, H 7.90, N 1.75.

O-*(2-Cyanoethyl)* O-*Dolichyl* S-*(2,3,4,6-Tetra*-O-*acetyl-β*-D-*mannopyranosyl) Thiophosphate* (**20**). A soln. of **3** (6.5 mg, 0.018 mmol, dried for 2 h *i.v.*) in CH_2Cl_2 (0.3 ml) was cooled to $-40°$, treated with **4b** (27 mg, 0.018 mmol, dried for 2 h *i.v.*) and 0.45M 1*H*-tetrazole in MeCN (0.1 ml, 0.045 mmol), warmed to $-10°$, and stirred for 1.5 h. Then, the mixture was cooled to $-20°$ and treated dropwise with 5.5M *t*-BuOOH in decane (0.4 ml, 2.2 mmol), stirred for 1 h, and evaporated. FC (hexane/acetone 97:3 → 9:1) gave **20** (13 mg, 40%). R_f (hexane/AcOEt 1:1) 0.45. IR (neat): 2961*m*, 2915*m*, 1854*m*, 1751*s*, 1662*w*, 1447*m*, 1374*m*, 1220*s*, 1050*m*, 1000*m*, 833*m*, 756*s*. ^{1}H-NMR (600 MHz, $CDCl_3$; 1:1 mixture of diastereoisomers, assignment based on a DFQCOSY and a HSQC spectrum): 5.54, 5.52 (2*dd*, $J = 3.4$, 1.2, H–C(2)); 5.34, 5.26 (2*dd*, ^3J(H,P) = 12.0, $J = 1.2$, H–C(1)); 5.25 (*t*, $J = 9.9$, H–C(4)); 5.16–5.04 (*m*, 18 C=CH, H–C(3)); 4.37–4.10 (*m*, 2 H–C(6), 2 CH_2O); 3.77, 3.75 (2*ddd*, $J = 9.9$, 5.1, 2.8, H–C(5)); 2.80 (*t*, $J = 6.6$, $NCCH_2CH_2O$); 2.22, 2.09 (2*s*, 2 AcO); 2.10–1.94 (*m*, 17 $CH_2MeC{=}CHCH_2$, $Me_2C{=}CHCH_2$, AcO); 1.98 (*s*, AcO); 1.68 (br. *s*, 17 Me, $CH_2CHMeCH_2$); 1.60 (br. *s*, $Me_2C{=}CH$, MeCH); 0.92 (*d*, $J = 6.6$, *Me*CH). ^{13}C-NMR (150 MHz, $CDCl_3$; one diastereoisomer only, assignment based on a HSQC spectrum): 170.46, 169.96, 169.83, 169.58 (4*s*, 4 C=O); 135.38–134.95 (several *s*, 17 $CH_2MeC{=}CH$); 131.25 ($Me_2C{=}CH$); 125.14–124.19 (several *d*, 18 CH=C); 116.14 (*s*, CN); 81.83 (*dd*, ^3J(C,P) = 2.6, C(1)); 77.49 (*d*, C(5)); 71.60 (*d*, C(3)); 70.84 (*dd*, ^4J(C,P) = 7.8, C(2)); 67.27 (*td*, ^2J(C,P) = 6.2, CH_2O); 65.01 (*d*, C(4)); 62.27 (*t*, CH_2O); 62.09 (*t*, C(6)), 61.70 (*t*, ^2J(C,P) = 4.5, $NCCH_2CH_2O$); 40.06, 39.77, 39.74, 37.20, 37.05, 37.01 (6*t*); 32.26, 32.23, 32.01 (2*t*, several CH_2); 29.10 (*d*); 26.80–26.36 (several *t*); 25.69 (*q*); 25.12 (*t*); 23.43 (*q*, several Me); 20.76, 20.66, 20.54, 20.49 (4*q*, 4 *Me*C=O); 19.60, 19.54, 19.13, 17.68, 16.01 (5*q*); 15.97 (*q*, *Me*CH). ^{31}P-NMR (121 MHz, $CDCl_3$; 1:1 mixture of diastereoisomers): 24.10; 23.98. HR-MALDI-MS: 1815.2680 $[M + Na]^+$, $C_{112}H_{178}NNaO_{12}PS^+$; calc. 1815.2699).

Transformation of **18** *into* **16**. A soln. of **18** (30 mg, 0.043 mmol) in THF/MeOH 1:2 (2 ml) was treated with 0.4M of MeONa in MeOH (0.1 ml) and stirred at 23° for 1.5 h, diluted with THF (3 ml), neutralized with *Amberlite IRC50* (H^+-form) and filtered. The filtrate was evaporated. A soln. of **16** in EtOH (*ca.* 0.3 ml) was treated with pentane. The precipitate was filtered off and dried *i.v.* to afford **16** (15 mg, 66%).

Hydrogen O-*Oleyl* S-*(β*-D-*Mannopyranosyl) Dithiophosphate* (**21**). A soln. of **19** (43 mg, 0.056 mmol) in THF/MeOH 1:2 (2 ml) was treated with 0.4M MeONa in MeOH (0.3 ml), stirred at 23° for 1.5 h, diluted with THF (3 ml), and neutralized with *Amberlite IRC50* (H^+-form). The resin was filtered off, and the filtrate was evaporated. A soln. of **21** in EtOH (*ca.* 0.4 ml) was treated with pentane. The precipitate was filtered off and dried *i.v.* to afford **21** (22 mg, 73%). R_f (AcOEt/MeOH/H_2O 7:2:1) 0.75. R_f ($CHCl_3$/MeOH/H_2O 10:3:1) 0.4. IR (neat): 3276*m* (br.), 2921*m*, 2851*m*, 1465*w*, 1345*w*, 1282*w*, 1188*m*, 1049*s*, 1025*s*, 965*m*, 829*w*, 695*m*. ^{1}H-NMR (600 MHz, CD_3OD; 1:1 mixture of diastereoisomers, assignment based on a DFQCOSY and a HSQC spectrum): 5.39–5.31 (*m*, CH=CH); 5.06, 5.00 (2*d*, $J = 1.0$, ^3J(H,P) = 12.6, H–C(1)); 4.01–3.85 (*m*, CH_2O, H–C(2)); 3.84, 3.82 (2*dd*, $J = 11.6$, 2.3 H–C(6)); 3.70, 3.66 (2*dd*, $J = 11.9$, 5.2, H′–C(6)); 3.58, 3.53 (2*t*, $J = 9.6$, H–C(4)); 3.49, 3.48 (2*dd*, $J = 9.5$, 3.3, H–C(3)); 3.29, 3.27 (2*ddd*, $J = 9.6$, 5.1, 2.1, H–C(5)); 2.08–1.94 (*m*, $CH_2CH{=}CHCH_2$); 1.68–1.60 (*m*, CH_2CH_2O); 1.43–1.25 (*m*, 22 H); 0.90 (*t*, $J = 6.6$, Me). ^{13}C-NMR (150 MHz, CD_3OD; 1:1 mixture of diastereoisomers, assignment based on a HSQC spectrum): 131.61, 131.54, 130.95, 130.87 (CH=CH); 87.64, 86.50 (2*d*, *C*(1)); 82.81, 82.57 (2*d*, C(5)); 76.53, 76.43 (2*d*, C(3)); 74.97 (*dd*, ^3J(C,P) = 6.2, C(2)); 68.32, 68.18 (2*d*, C(4)); 68.04, 67.80 (2*dt*, ^3J(C,P) = 6.2, CH_2O); 63.15, 63.00 (2*t*, C(6)); 33.35, 32.78 (2*t*); 31.27–29.93 (several *t*); 27.90, 27.85, 26.86, 26.79, 23.48 (5*t*), 14.23 (*q*, Me). ^{31}P-NMR (121 MHz, $CDCl_3$; 1:1 mixture of diastereoisomers): 73.27, 71.34. HR-MALDI-MS (neg. mode): 541.24281 ($[M-H]^-$, $C_{24}H_{47}O_7PS_2^-$; calc. 541.2434).

O-*Dolichyl Hydrogen* S-*(β*-D-*Mannopyranosyl) Thiophosphate* (**22**). A soln. of **20** (15 mg, 0.008 mmol) in THF/MeOH 1:2 (0.3 ml) was treated with 0.4M MeONa in MeOH (0.02 ml), stirred at 23° for 1.5 h, diluted with THF (1 ml), and neutralized with *Amberlite IRC50* (H^+-form). The resin was filtered off, and the filtrate was evaporated to afford **22** (7 mg, 54%). R_f (AcOEt/MeOH/H_2O 7:2:1) 0.75. R_f ($CHCl_3$/MeOH/H_2O 10:3:1) 0.65. IR (ATR): 3600–3000*m* (br.), 2957*m*, 2922*s*, 2853*m*, 1442*m*, 1429*m*, 1259*m*, 1230*m*, 1059*m*, 1023*m*, 799*m*. ^{1}H-NMR (600 MHz, $CDCl_3$/CD_3OD/D_2O, 6:1:0.1; assignment based on a DFQCOSY and a HSQC spectrum): 5.06–5.04 (*m*, 18 C=CH); 4.86 (br. *d*, ^3J(H,P) = 11.3, H–C(1)); 3.88–3.85 (*m*, H–C(2)); 3.83–3.79 (*m*, CH_2O, H–C(6)); 3.58 (*dd*, $J = 12.1$, 6.3, H′–C(6)); 3.48 (*dd*, $J = 9.5$, 3.0, H–C(3)); 3.44 (*t*, $J = 9.3$, H–C(4)); 3.28–3.26 (*m*, H–C(5)); 2.10–1.94 (*m*, 18 $CH_2MeC{=}CHCH_2$, $Me_2C{=}CHCH_2$); 1.68 (br. *s*, 17 Me, $CH_2CHMeCH_2$); 1.52 (br. s, $Me_2C{=}CH$, MeCH); 0.81 (*d*, $J = 7.2$, *Me*CH). ^{13}C-NMR (150 MHz, $CDCl_3$/CD_3OD/D_2O, 6:1:0.1; assignment based on a HSQC spectrum): 134.41–133.99 (several *s*, 17 $CH_2MeC{=}CH$); 130.29 (s, $Me_2C{=}CH$); 124.38–123.24 (several *d*, 18 CH=C); 82.24 (*dd*, ^2J(C,P) = 2.5, C(1)); 79.87 (*d*, C(5)); 73.48 (*d*, C(3)); 72.21 (*dd*, ^3J(C,P) = 13.2, C(2)); 65.87 (*d*, C(4)); 63.88 (*dt*, ^2J(C,P) = 6.2, CH_2O); 60.34, 59.71 (*t*, C(6)); 39.08, 38.78, 38.76, 36.65, 36.59, 36.54 (6*t*); 31.29, 31.26, 31.16, 31.02 (4*t*, several CH_2); 28.71 (*t*), 28.43, 28.39 (2*d*); 25.81, 25.70, 25.67, 25.61, 25.56, 25.47, 25.36 (several *t*); 24.63 (*q*); 24.18 (*t*); 22.41 (*q*, several Me); 18.00 (*q*, *Me*CH). ^{31}P-NMR

(121 MHz, $CDCl_3/CD_3OD/D_2O$, 6:1:0.1): 18.73. ESI-MS (neg. mode): 1570.72 $[M-H]^-$. HR-MALDI-MS: 1594.2020 ($[M+Na]^+$, $C_{101}H_{167}NaO_8PS^+$; calc. 1594.2012).

Isolation of [^{3}H]Man$_6$(GlcNAc)$_2$-PP-Dol (Substrate). Yeast lacking the Alg9 mannosyltransferase were labeled with [^{3}H]mannose [25][26]. Workup of lipid-linked oligosaccharides was stopped after the $CHCl_3$MeOH/H_2O 10:10:3 extraction and the glycolipids were stored at $-20°$.

Preparation of Yeast Membrane Extracts. Microsomal membranes were prepared from 200 OD_{546} of wild type yeast according to [27] with the following modification: the pelleted cells were washed and lysed in membrane buffer containing 50 mM HEPES pH 7.5, 3 mM $MgCl_2$, 1 mM DTT, and protease inhibitors (1 mM PMSF and 1 μg/ml E-64). Lysis buffer supplemented with 35% (*v*/*v*) glycerol was used as storage buffer. Protein concentration was determined by the BCA method [28].

In vitro *Assay for the Elongation of [^{3}H]Man$_7$(GlcNAc)$_2$-PP-Dol.* 100 μl of [^{3}H]Man$_7$(GlcNAc)$_2$-PP-Dol (*ca.* 16000 cpm) isolated as described before in substrate buffer (70 mM HEPES/NaOH, pH 6.5, 84 mM NaCl, 5.8 mM $MgCl_2$, 4.2 mM $CaCl_2$, 4.2 mM $MnCl_2$, 1 mM DTT, 0.7 mM GDP-Man, 0.14 mM CTP, 0.42% (*w*/*v*) NP40), with or without inhibitor, were added to yeast membranes (100 μg of protein) in 20 μl of storage buffer and mixed by pipetting and mild vortexing. The reaction was stopped after incubation at 25° for 10 min by addition of 800 μl of $CHCl_3$/MeOH 1:1 (*v*/*v*) and thorough vortexing. The supernatant obtained by centrifugation for 5 min at 16000 × g at 4° was dried at 37° under N_2, subjected to mild acid hydrolysis and analysed by HPLC as described in [26].

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