Synthesis and evaluation of thio-trisaccharides as acceptors for *N*-acetylglucosaminyltransferase-V

Pu-Ping Lu, Ole Hindsgaul, Hong Li, and Monica M. Palcic

Abstract: *N*-Acetylglucosaminyltransferase-V (GlcNAcT-V) transfers a β-linked GlcNAc residue from UDP-GlcNAc to the 6-OH group of the αMan residue in oligosaccharides terminating in the sequence β-D-Glc*p*NAc-(1→2)-α-D-Man*p*-(1→6)-β-D-Glc*p*-OR (**5**, R = $(CH_2)_7CH_3$). The terminal GlcNAc moiety may be replaced by a Glc residue to produce trisaccharide **6**. Two thio analogs (**7**, **8**) of trisaccharide **6**, where the oxygen atoms in the glycosidic linkages between sugar residues were replaced by sulfur, were prepared by multistep chemical synthesis that made use of the key intermediates 1,2-anhydro-3,4,6-tri-*O*-benzyl-α-D-glucose (**10**) and 1,2-anhydro-3,4,6-tri-*O*-benzyl-β-D-mannose (**13**) as donors for the glycosylations. The thio analogs (**7**, **8**) were kinetically evaluated as substrates for GlcNAcT-V and found to be acceptors with two- to three-fold increase in V_{max} but higher K_m values (**7**, K_m = 376 μM; **8**, K_m = 300 μM) than their parent compound **6** (K_m = 111 μM), which has the natural oxygen linkage. The thio analogs **7** and **8** could be quantitatively converted into the expected product tetrasaccharides (**27**, **28**) by incubation with GlcNAcT-V and UDP-GlcNAc. The enzymatic results indicate that GlcNAcT-V tolerates the substitution of the natural oxygen linkage of the acceptor by a sulfur linkage.

Key words: *N*-acetylglucosaminyltransferase-V, enzyme acceptors, trisaccharide analogs, thioglycosides.

Résumé : La *N*-acétylglucosaminyltransférase-V (GlcNAcT-V) permet de transférer un résidu GlcNAc lié en β d'un UDP-GlcNAc à un groupe 6-OH d'un résidu α-Man d'oligosaccharides se terminant par une séquence β-D-Glc*p*NAc-(1→2)-α-D-Man*p*-(1→6)-β-D-Glc*p*-OR (**5**, R = $(CH_2)_7CH_3$). On peut remplacer la portion GlcNAc terminale par un résidu Glc pour produire le trisaccharide **6**. En procédant à une synthèse en plusieurs étapes faisant appel aux intermédiaires clés 1,2-anhydro-3,4,6-tri-*O*-benzyl-α-D-glucose (**10**) et 1,2-anhydro-3,4,6-tri-*O*-benzyl-β-D-mannose (**13**) comme donneurs lors des glycosylations, on a préparé deux analogues thio (**7**, **8**) du trisaccharide **6**, dans lesquels les atomes d'oxygène des liaisons glycosidiques entre les unités de sucre ont été remplacés par des atomes de soufre. On a évalué le comportement cinétique des dérivés thio (**7**, **8**) comme substrats de la GlcNAcT-V et on a trouvé qu'ils sont de meilleurs accepteurs avec des augmentations par des facteurs de 2 à 3 dans les valeurs de V_{max}; les valeurs de K_m sont toutefois plus élevées (**7**, K_m = 376 μM; **8**, K_m = 300 μM) que celle de leur composé parent (**6**, K_m = 111 μM) comportant la liaison oxygénée normale. Par incubation avec de la GlcNAcT-V et du UDP-GlcNAc, on peut transformer quantitativement les analogues thio en tétrasaccharides attendus (**27**, **28**). Les résultats enzymatiques indiquent que la GlcNAcT-V tolère la substitution de la liaison oxygène naturelle de l'accepteur par une liaison sulfurée.

Mots clés : *N*-acétylglucosaminyltransférase-V, accepteurs d'enzyme, analogues trisaccharides, thioglycosides.

[Traduit par la rédaction]

Introduction

UDP-GlcNAc: α-mannoside β (1→6)-*N*-acetylglucosaminyltransferase-V (GlcNAcT-V, EC 2.4.1.155) transfers an *N*-acetyl-D-glucosamine (GlcNAc) residue from uridine 5′-diphospho-GlcNAc (UDP-GlcNAc) to natural glycopeptide acceptors bearing the minimum heptasaccharide sequence **1** (Scheme 1), converting it to the octasaccharide **2** (1, 2). This enzyme plays a key role in the biosynthesis of highly branched asparagine-linked oligosaccharides (1, 2). A strong correlation between an increase in the activity of this enzyme and the increased metastatic potential of several cancer cell lines has been demonstrated (3–5). This enzyme has, therefore, become a target for the development of glycosyltransferase inhibitors.

Knowledge of the detailed substrate specificity of GlcNAcT-V is essential for a rational approach to inhibitor design. We previously found that the much simpler synthetic trisaccharide **3**, a partial structure of **1**, is also an effective substrate for the enzyme, yielding the expected tetrasaccharide **4** (6). The enzyme tolerates the substitution of the βMan residue in **3** by a βGlc residue since trisaccharide **5** was found to be an excellent acceptor (7). The enzyme further tolerates the substitution of the terminal GlcNAc moiety by a βGlc

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This paper is dedicated to Professor William A. Ayer on the occasion of his 65th birthday.

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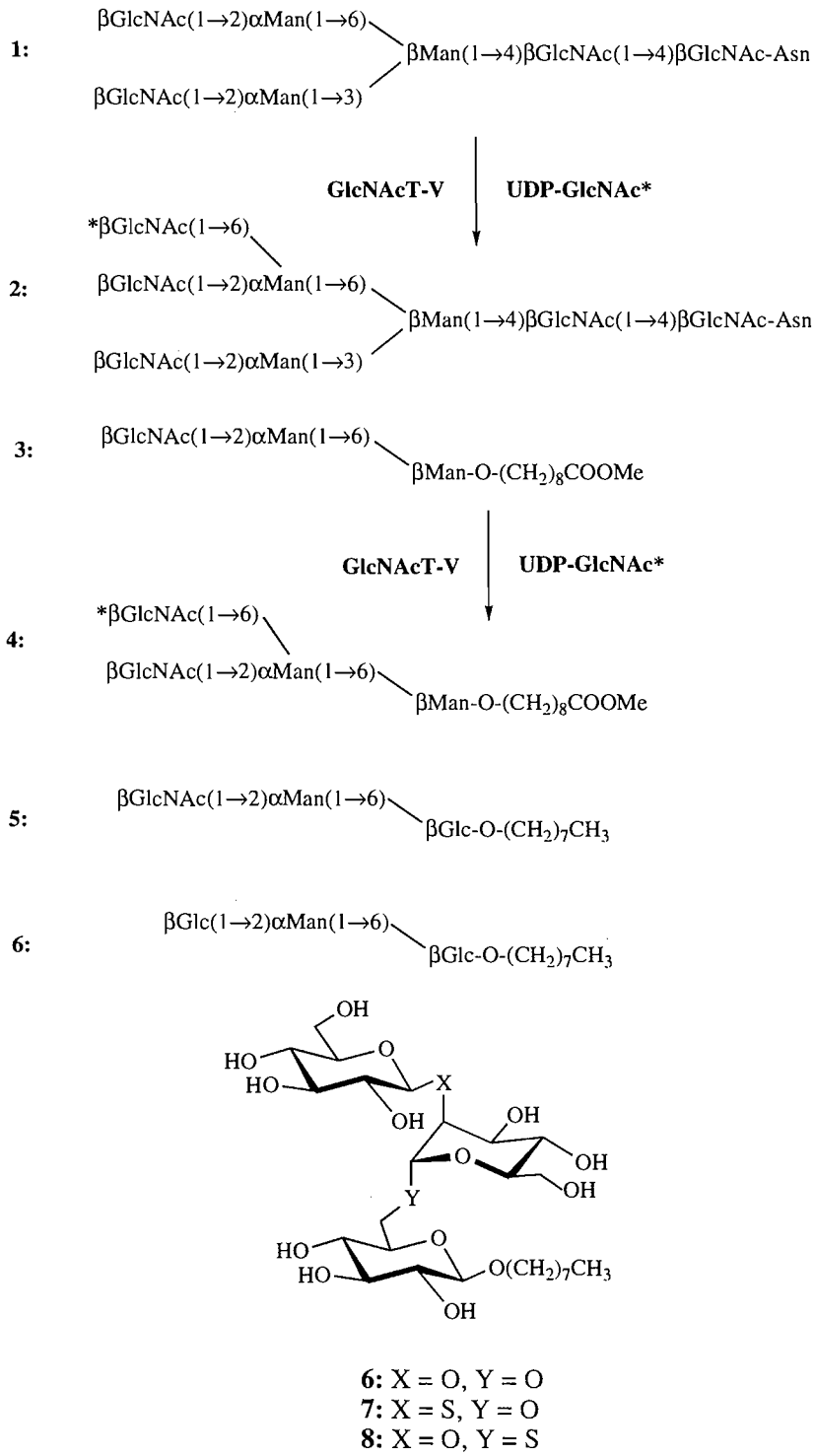
Scheme 1. Glycosylation reactions catalyzed by GlcNAcT-V.



6: X = O, Y = O
7: X = S, Y = O
8: X = O, Y = S

residue, since the trisaccharide **6** was also found to be a good acceptor (8). The aliphatic aglycons in **3** and **5** were incorporated into these structures in order to facilitate the enzyme assay procedures (9). We report here a continuation of these enzyme-specificity studies where the requirement of the natural intra-residue oxygen linkage of **6** in substrate recognition by GlcNAcT-V is probed through the synthesis and enzymatic analysis of the two thio analogs **7** and **8**, which have an intersaccharidic sulfur atom. The use of the 1,2-anhydro sugars (glycal epoxides) as building blocks provided short synthetic routes to these thio-linked trisaccharides.

Results and discussion

Since only small (mg) quantities of **7** and **8** were required, the synthetic plan was to devise short reaction sequences even if yields were found to be low by normal synthetic criteria. The retrosynthetic analysis for the preparation of **7** and **8** is shown in Scheme 2. In designing the synthesis of **7**, we chose 1,2-anhydro-3,4,6-tri-*O*-benzyl-α-D-glucopyranose (**10**) as the central building block. The opening of epoxide **10** by **9** was expected to produce an α/β mixture of disaccharides with OH-2′ free. After conversion of OH-2′ into a leaving group, S_N2 displacement using a protected 1-thio-D-glucose (**11**) was then envisioned to produce the desired trisaccharide. In designing the synthesis of **8**, we chose 1,2-anhydro-3,4,6-tri-*O*-benzyl-β-D-mannopyranose (**13**) as the central building block. The opening of the epoxide **13** by highly nucleophilic **12** would provide a disaccharide with OH-2′ free for further coupling to give the desired trisaccharide.

The three building blocks required for the synthesis of **7** were prepared according to published procedures (**9** (7), **10** (10), **11** (11)). The coupling of **10** with alcohol **9** was carried out in THF at −78°C using trifluoromethanesulfonic acid as catalyst, to afford the desired α-linked disaccharide **15** along with the β-linked disaccharide **16** in 24% and 47% yield, respectively (based on consumed alcohol). Treatment of **15** with excess trifluoromethanesulfonic anhydride in anhydrous pyridine gave the 2′-triflate (**17**) in quantitative yield. The S_N2 displacement of **17** was carried out by using the sodium salt of the 1-thio-D-glucose derivative (**11**) in DMF. The sodium salt was made by treating **11** with sodium hydride in THF. The trisaccharide **18** was obtained in 25% yield. Zemplén deacetylation of **18** yielded **19**. Final debenzylation of **19** by treatment with Na/liquid NH_3 afforded target trisaccharide **7** in 79% yield (Scheme 3).

For the synthesis of **8**, compound **13** was prepared by treatment of 3,4,6-tri-*O*-benzyl-D-mannopyranose (12) with hydrogen chloride in ether, followed by work-up with NH_3 (13). Mesylation of **9**, followed by reaction with potassium thioacetate and deacetylation, provided **12** with the required free 6-thiol group (Scheme 4). Imidate (**14**) was prepared from hemiacetal **23** by reaction with trichloroacetonitrile and sodium hydride in dichloromethane according to standard procedures (14). Compound **23** was prepared by treating **22** (15) with silver carbonate in wet acetone (Scheme 4). The opening of the epoxide **13** by the sodium salt of **12** gave the α-linked disaccharide **24** (63%) with OH-2′ for subsequent coupling. Using *O*-(2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-glucopyranosyl)-trichloroacetimidate (**14**) as donor, glycosylation of **24** afforded the desired β-linked trisaccharide in 88% yield. After Zemplén deacetylation (92%), debenzylation with Na/liquid NH_3 furnished the target trisaccharide in 82% yield (Scheme 5).

Compounds **7** and **8** were characterized by ^1H NMR, ^1H–^1H 2D COSY, ^{13}C NMR (APT), HMQC, and high-resolution FAB mass spectrometry. The anomeric configurations of the mannopyranosyl and glucopyranosyl linkages were further verified by measurement of the ^1J heteronuclear coupling constants (16) of the anomeric carbon atoms for compounds **7** and **8**.

Evaluation of thioglycoside analogs as acceptors for GlcNAcT-V

Compounds **7** and **8** and the unmodified structure **6** were kinetically evaluated as acceptors for GlcNAcT-V using the cloned rat kidney enzyme (17). The enzyme experiments were performed according to a well-established radioactive "Sep-Pak assay" method (9, 18–20). The results of the kinetic evaluations are reported in Table I. Both compounds (**7**, **8**) are relatively good substrates for GlcNAcT-V. The V_{max} values are two- to three-fold higher than those of their parent compound (**6**); however, their binding is likely weaker as reflected in the higher K_m values for the analogs. Trisaccharide **8** was also evaluated with GlcNAcT-V isolated from hamster kidney (18–20). As was previous found (21), both sources of GlcNAcT-V have essentially identical substrate specificity.

To confirm that the thio analogs **7** and **8** were indeed acceptors of GlcNAcT-V, preparative enzymatic syntheses were performed. When the acceptors were incubated with UDP-GlcNAc and GlcNAcT-V, the enzyme transferred a β-D-GlcNAc residue to the 6-OH of the core D-Man*p* unit of the acceptors, converting **7** and **8** into the expected tetrasaccharides **27** and **28**, respectively (Scheme 6). The structures of tetrasaccharides **27** and **28** were confirmed by ^1H NMR and FAB mass spectrometry.

This work indicates that neither of the intersaccharidic oxygen atoms in the trisaccharide acceptor are involved in specific recognition by GlcNAcT-V. The enzyme tolerates the substitution of the natural intersaccharidic oxygen linkages by sulfur. Since thioglycosides are resistant to cleavage by glycosidases, these structures should prove useful as metabolically stable acceptors for assaying GlcNAcT-V.

Experimental

General methods

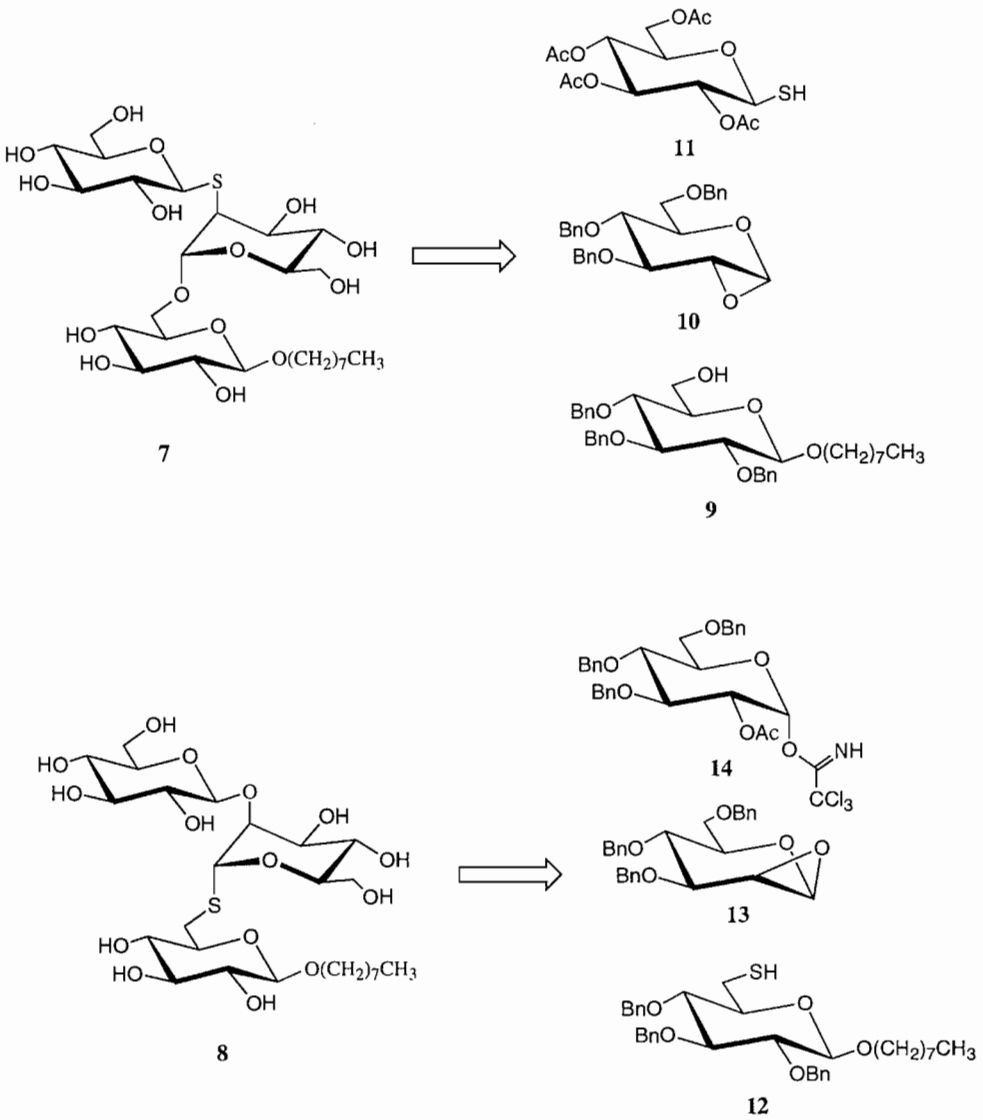
TLC was performed on Silica Gel 60-F254 (E. Merck) with detection by quenching of fluorescence, and (or) by charring

Table 1. Kinetic parameters for GlcNAcT-V acceptor analogs.

Acceptor	GlcNAcT-V	K_m (μM)	V_{max} (pmol/min)	V_{max}, rel[a]
6	Cloned	111±7	2.1	100
7	Cloned	376±16	3.5	167
8	Cloned	300±24	6.1	290
8	Isolated	256±12	9.0	—

[a]The value for compound **6** is arbitrarily set to 100.

Scheme 2. Synthetic strategy for the synthesis of **7** and **8**.

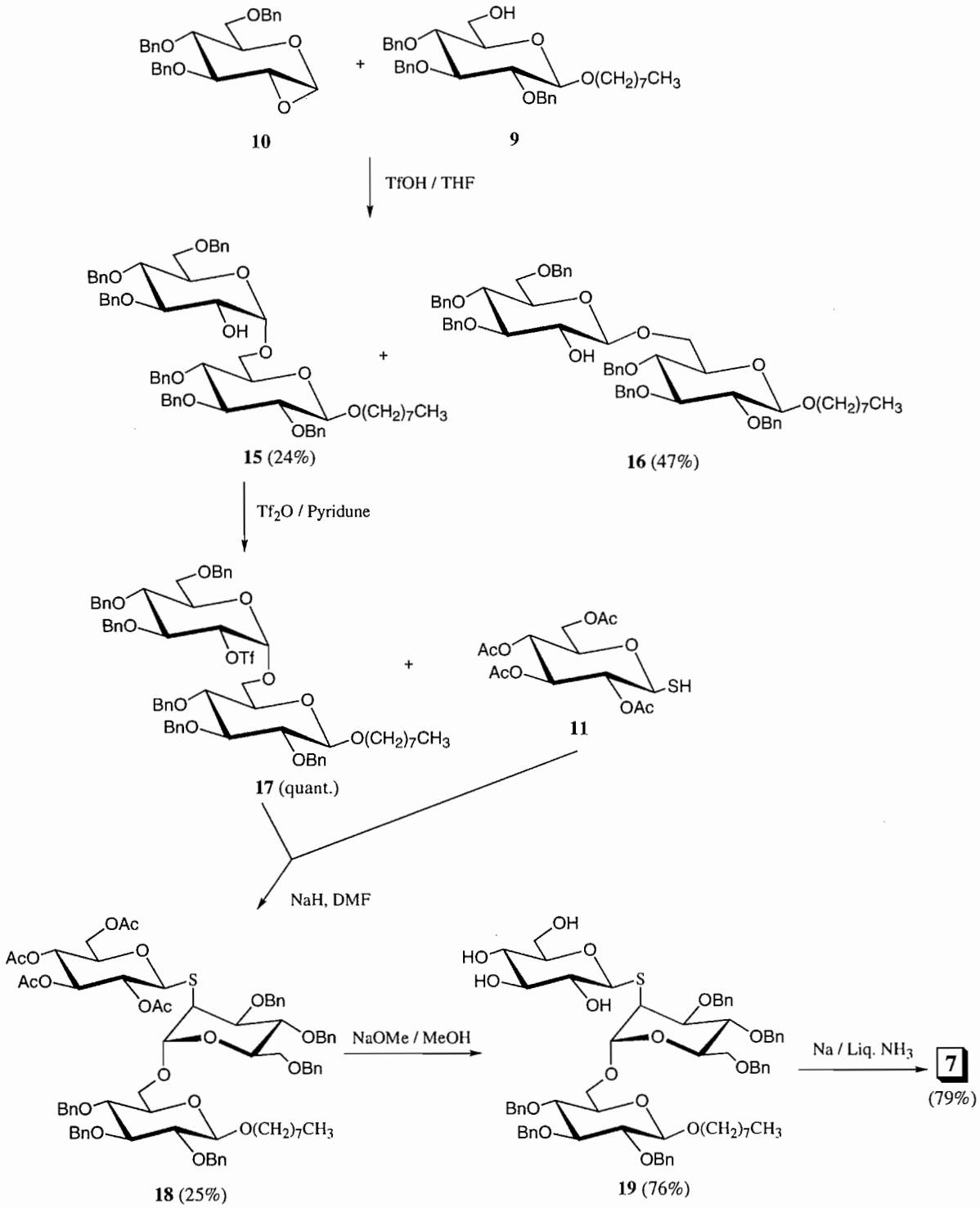


with H_2SO_4. Unless otherwise noted, column chromatography was performed on Silica Gel 60 (E. Merck, 40–63 mm). Iatrobeads were from Iatron Laboratories, Inc. (Japan). C-18 Sep-Pak sample preparation cartridges were from Waters Associates. Millex-GV (0.22 μm) filter units were from Millipore. Optical rotations were measured with a Perkin–Elmer 241 polarimeter at 22°C. IR spectra were recorded with a Nicolet SX-20 FTIR by the spectral services laboratory of the Chemistry Department. ^{1}H NMR spectra were recorded at 360 MHz (Bruker AMR 360), at 400 MHz (Bruker AM 400), or at 500 MHz (Varian UNITY 500) on solutions in $CDCl_3$ (internal Me_4Si, δ 0) or D_2O. ^{13}C NMR spectra were recorded at 75.5 MHz, 100.6 MHz, or 125 MHz, respectively, on the same instruments in $CDCl_3$ (internal Me_4Si δ 0) or D_2O (internal 1,4-dioxane, δ 67.4). The assignments of ^{13}C NMR are tentative. FAB mass spectra (FAB MS) were obtained on a Kratos AEIMS9 instrument using glycerol as matrix. Electrospray ionization mass spectra (ESI MS) were obtained from a Micromass ZabSpec Hybrid Sector-TOF instrument using methanol–toluene as liquid carrier. Elemental analyses were carried out on a Carlo Erba EA1108. For the enzyme experiments, cloned rat kidney GlcNAcT-V was a generous gift from Dr. M. Pierce (17); partially purified GlcNAcT-V was isolated from hamster kidney by a modification (20) of published procedures (18, 19). UDP-^{3}H-GlcNAc was from American Radiolabeled Chemicals, Inc. (specific activity 40–60 Ci/mmol). UDP-GlcNAc (disodium salt) was from Sigma.

Octyl 3,4,6-tri-*O*-benzyl-α-D-glucopyranosyl-(1→6)-2,3,4-tri-*O*-benzyl-β-D-glucopyranoside (15)

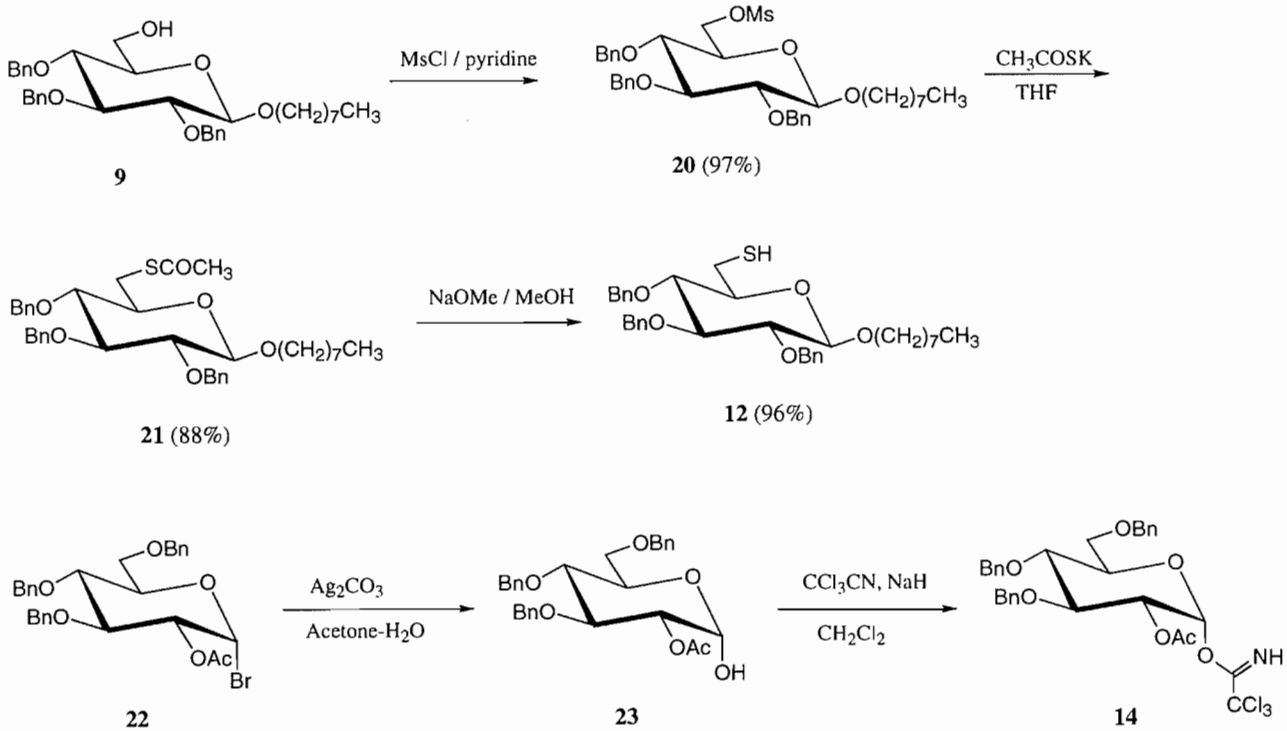
A mixture of **9** (7) (600 mg, 1.07 mmol) and trifluoromethanesulfonic acid (17.6 μL, 0.2 mmol) in dry THF (20 mL) was cooled to −78°C. To the resulting mixture was added dropwise a solution of 1,2-anhydro-3,4,6-tri-*O*-benzyl-α-D-glucopyranose **10** (432 mg, 1.0 mmol) in dry THF (10 mL). The reaction mixture was stirred at −78°C for 2 h, then warmed to room temperature (r.t.) and stirred for 10 h. The reaction mixture was then concentrated and the residue was dissolved in

Scheme 3.



CH_2Cl_2 (20 mL). The CH_2Cl_2 solution was washed with saturated $NaHCO_3$ and water, then dried over anhydrous $MgSO_4$, filtered, and concentrated. The purification of the residue by column chromatography (hexane–EtOAc 2:1) provided **15** (170 mg, 24% based on consumed alcohol), **16** (330 mg, 47%), and recovered alcohol **9** (200 mg, 33%); $[\alpha]_D$ +57.3 (*c* 0.7, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.40–7.10 (m, 30H, Ar-H), 4.97, 4.96, 4.95 (3d, each 1H, J_{gem} 11.0 Hz, $PhCH_2$), 4.95 (d, 1H, $J_{1',2'}$ 3.5 Hz, H-1′), 4.88 (d, 1H J_{gem} 11.0 Hz, $PhCH_2$), 4.84 (d, 2H, J_{gem} 10.8 Hz, $PhCH_2$), 4.78, 4.72 (2d, each 1H, J_{gem} 10.8 Hz, $PhCH_2$), 4.61, 4.57 (2d, each 1H, J_{gem} 11.0 Hz, $PhCH_2$), 4.48 (d, 1H, J_{gem} 10.1 Hz, $PhCH_2$), 4.45 (d, 1H, J_{gem} 11.0 Hz, $PhCH_2$), 4.41 (d, 1H $J_{1,2}$ 7.8 Hz, H-1), 3.82 (ddd, 1H, $J_{2',3'}$ 10.0 Hz, $J_{2',OH}$ 2.0 Hz, H-2′), 3.42 (dd, 1H, $J_{2,3}$

Scheme 4.



9 **20** (97%) **21** (88%) **12** (96%) **22** **23** **14**

9.5 Hz, H-2), 0.87 (t, 3H, J_{vic} 7.0 Hz, octyl CH_3); ^{13}C NMR (100.6 MHz) δ: 138.5, 138.4, 138.0 (benzyl C1), 128.4, 128.4, 128.3, 128.1, 128.0, 127.9, 127.8, 127.7, 127.64, 127.59, 127.5 (Ar), 103.5 (C-1), 99.2 (C-1′), 75.7, 75.2, 74.94, 74.86, 74.83, 73.4 (Ph*C*H_2), 70.2 (octyl C1), 68.4 (C-6″), 67.0 (C-6), 31.8, 29.8, 29.4, 29.2, 26.2, 22.6 (octyl C2–C7), 14.1 (octyl CH_3). Anal. calcd. for $C_{62}H_{74}O_{11}$ (995.27): C 74.82, H 7.49; found: C 74.90, H 7.61.

Octyl 3,4,6-tri-*O*-benzyl-2-*O*-trifluoromethanesulfonyl-α-D-glucopyranosyl-(1→6)-2,3,4-tri-*O*-benzyl-β-D-glucopyranoside (17)

To a mixture of compound **15** (115 mg, 0.116 mmol) in dry pyridine (2 mL) at −22°C was added trifluoromethanesulfonic anhydride (85 μL, 0.505 mmol). The reaction mixture was allowed to warm to r.t. and then stirred for 1 h. After addition of ice-cold 1 M HCl (10 mL), the reaction mixture was stirred for 30 min and extracted with CH_2Cl_2. The CH_2Cl_2 solution was washed with saturated $NaHCO_3$ and water, and dried over anhydrous $MgSO_4$. Filtration followed by evaporation of the CH_2Cl_2 gave the title product (130 mg, 100%) as a syrup. ^1H NMR (360 MHz, $CDCl_3$) δ: 7.40–7.05 (m, 30H, Ar-H), 5.175 (d, 1H, $J_{1',2'}$ 3.5 Hz, H-1′), 4.96, 4.95, 4.94, 4.89, 4.85, 4.78, 4.78, 4.76 (8d, each 1H, J_{gem} 11.0 Hz, $PhCH_2$), 4.74 (dd, 1H, $J_{2',3'}$ 9.5 Hz, H-2′), 4.71 (d, 1H, J_{gem} 11.0 Hz, $PhCH_2$), 4.60 (d, 1H, J_{gem} 12.0 Hz, $PhCH_2$), 4.58 (d, 1H, J_{gem} 11.0 Hz, $PhCH_2$), 4.45 (d, 1H, J_{gem} 12.0 Hz, $PhCH_2$), 4.39 (d, 1H, $J_{1,2}$ 7.8 Hz, H-1), 4.05 (dd, 1H, $J_{3',4'}$ 9.5 Hz, H-3′), 0.87 (t, 3H, J_{vic} 7.0 Hz, octyl CH_3).

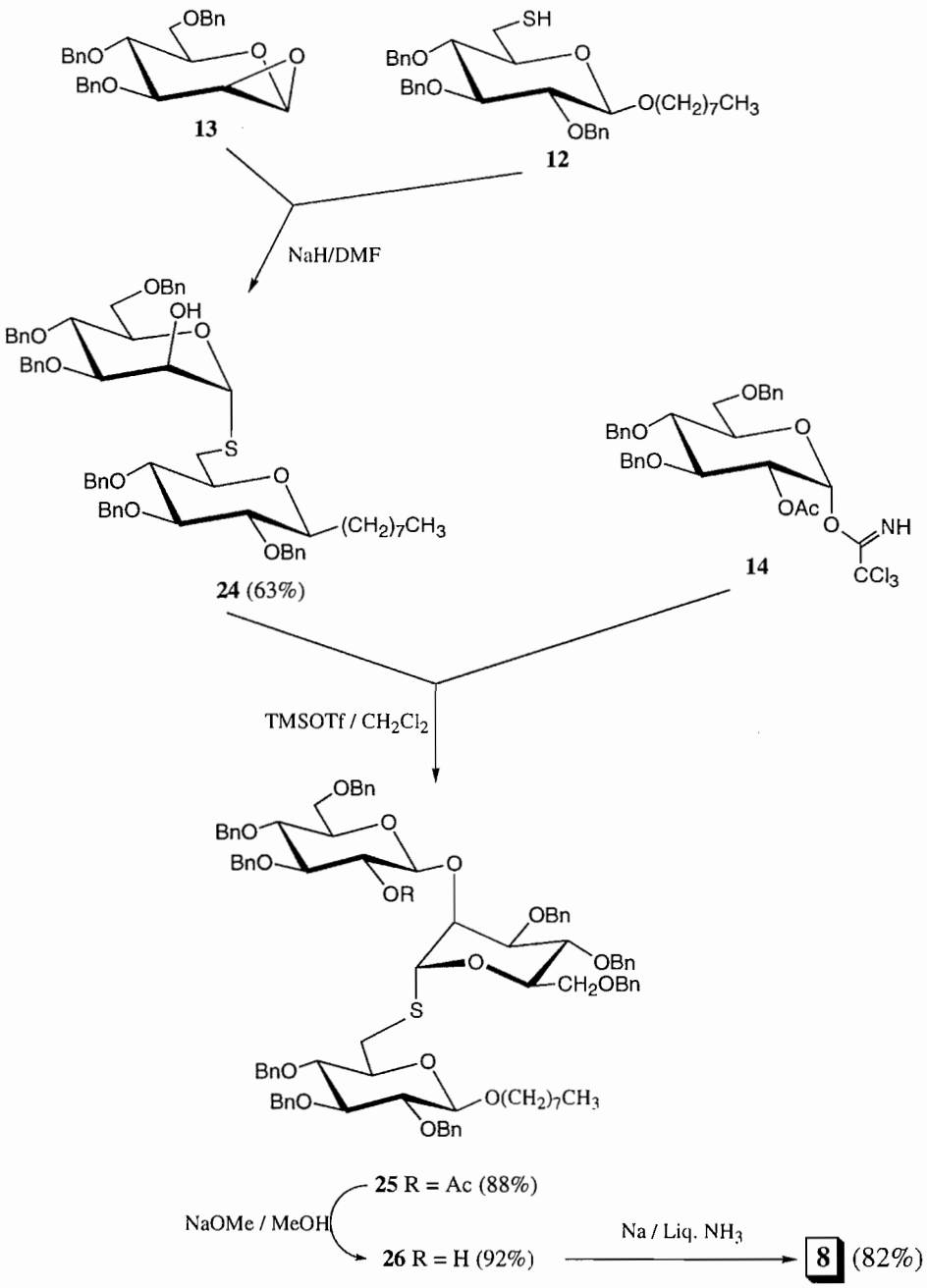
Octyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl-(1→2)-3,4,6-tri-*O*-benzyl-2-deoxy-2-thio-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-benzyl-β-D-glucopyranoside (18)

To a solution of 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranose **11** (11) (105 mg, 0.288 mmol) in dry THF (2 mL) was added sodium hydride (60% suspension in oil, 13 mg, 0.318 mmol). The mixture was stirred, resulting in the evolution of bubbles. After the sodium salt (white solid in THF) was produced, the THF was evaporated using a stream of argon. To the resulting residue was added a solution of **17** (130 mg, 0.115 mmol) in dry DMF (2 mL). The reaction mixture was stirred at r.t. for 12 h, and methanol (2 mL) was added to quench the reaction. The reaction mixture was concentrated to dryness and the residue was dissolved in CH_2Cl_2 (10 mL). The CH_2Cl_2 solution was washed with water, dried over $MgSO_4$, filtered, and concentrated. Purification of the residue by column chromatography using hexane–EtOAc (2:1) as eluant gave the title compound (38 mg, 25%); ^1H NMR (360 MHz, $CDCl_3$) δ: 5.24 (dd, 1H, $J_{2'',3''}$, $J_{3'',4''}$ 9.2 Hz, H-3″), 5.14 (dd, 1H, $J_{1',2'}$ 2.0 Hz, H-1′), 5.10 (dd, 1H, $J_{4'',5''}$ 9.5 Hz, H-4″), 5.06 (dd, 1H, $J_{1'',2''}$ 9.6 Hz, H-2″), 4.98, 4.95, 4.86, 4.82, 4.81, 4.80. 4.78, 4.71 (8d, each 1H, J_{gem} 11.0 Hz, $PhCH_2$), 4.48 (d, 1H, H-1″), 4.40 (d, 1H, $J_{1,2}$ 8.0 Hz, H-1), 4.37 (d, 1H, J_{gem} 10.5 Hz, $PhCH_2$), 4.22 (dd, 1H, J_{gem} 12.5 Hz, $J_{5'',6''a}$ 5.5 Hz, H-6″a), 4.14 (dd, 1H, $J_{5'',6''b}$ 2.0 Hz, H-6″b), 3.38 (dd, 1H, $J_{2,3}$ 9.0 Hz, H-2), 2.01, 1.98, 1.97, 1.96 (4s, each 3H, 4 Ac), 0.87 (t, 3H, J_{vic} 7.0 Hz, octyl CH_3); ^{13}C NMR (75.5 MHz) δ: 174.2, 170.6, 170.1, 169.4 (C═O), 138.5, 138.4, 138.2, 138.1, 137.9 (benzyl C1), 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9, 127.9, 127.8, 127.6, 127.5 (Ar), 103.7 (C-1), 101.3 (C-1″), 87.6 (C-1′), 70.5 (octyl C1), 68.8 (C-6″), 66.1 (C-6), 62.3 (C-6′), 47.0 (C-2′), 31.7, 29.7, 29.5, 29.3, 26.2, 22.7 (octyl C2–C7), 20.7 (*C*H_3CO), 14.10 (octyl CH_3). ESI MS *m/z*: 1363.7 $(M+Na)^+$.

Octyl β-D-glucopyranosyl-(1→2)-3,4,6-tri-*O*-benzyl-2-deoxy-2-thio-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-benzyl-β-D-glucopyranoside (19)

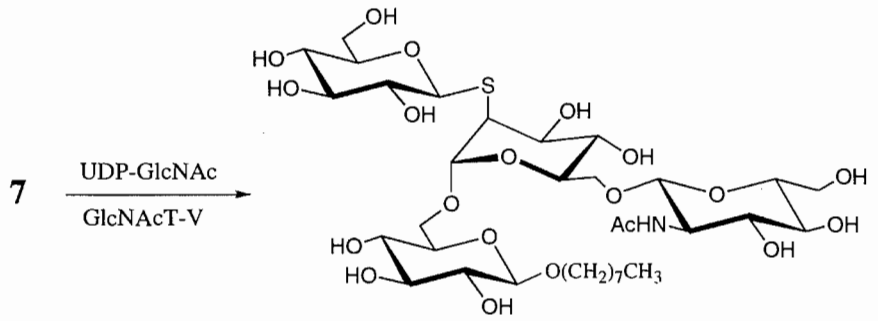
Compound **18** (30 mg, 22.4 μmol) was treated with methan-

Scheme 5.



olic NaOMe (0.05 N, 3 mL) at r.t. for 12 h. Neutralization with Amberlite IR-120 (H^+) resin, removal of the resin by filtration, and concentration left a residue that was purified by column chromatography (5% MeOH in CH_2Cl_2) and preparative TLC (5% MeOH in CH_2Cl_2) to give compound **19** as a syrup (20 mg, 76%); $[\alpha]_D$ +30.9 (*c* 1.3, CH_2Cl_2); ^1H NMR (400 MHz, $CDCl_3$) δ: 5.10 (d, 1H, $J_{1',2'}$ 2.0 Hz, H-1′), 4.97, 4.96, 4.88, 4.81, 4.79, 4.78, 4.74, 4.70 (8d, each 1H, J_{gem} 11.0 Hz, $PhCH_2$), 4.42 (d, 1H, J_{gem} 12.5 Hz, $PhCH_2$), 4.39 (d, 1H, $J_{1,2}$ 8.0 Hz, H-1), 4.29 (d, 1H, $J_{1'',2''}$ 9.5 Hz, H-1″), 4.16 (dd, 1H, $J_{2',3'}$ 4.0 Hz, $J_{3',4'}$ 8.5 Hz, H-3′), 3.94 (dt, 1H, J_{gem} 10.0 Hz, J_{vic} 6.2 Hz, octyl C1-H_a), 3.45 (dd, 1H, H-2′), 2.58 (d, 1H, *J* 1.0 Hz, OH), 2.52 (s, 1H, OH), 2.51 (d, 1H, *J* 1.2 Hz, OH), 2.32 (dd, 1H, *J* 6.0, 5.5 Hz, C6″-OH), 0.87 (t, 3H, J_{vic} 7.0 Hz, octyl CH_3); ^{13}C NMR (100.6 MHz) δ: 138.4, 138.2, 138.0, 137.0 (benzyl C1), 128.7, 128.6, 128.4, 128.4, 128.3, 128.1, 127.9, 127.70, 127.66, 127.5 (Ar), 103.7 (C-1), 101.9 (C-1″), 85.1 (C-1′), 75.8, 74.9, 74.8, 74.6, 73.7, 73.3 ($PhCH_2$), 71.99 (octyl C_1), 68.5 (C-6′), 65.7 (C-6), 62.4 (C-6″), 45.9 (C-2′), 31.8, 29.68, 29.42, 29.24, 26.12, 22.64 (octyl C2–C7), 14.07 (octyl CH_3): FAB MS *m/z*: 1211.3 $(M+K)^+$, 1196.4 $(M+Na)^+$.

Scheme 6. Enzymatic synthesis of **27** and **28**.



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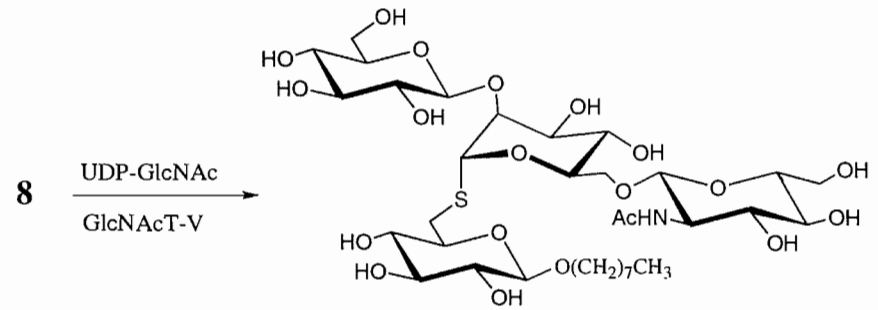

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Octyl β-D-glucopyranosyl-(1→2)-2-deoxy-2-thio-α-D-mannopyranosyl-(1→6)-β-D-glucopyranoside (7)

A solution of **19** (13 mg, 11.1 μmol) in THF (0.5 mL) was added to a mixture of liquid NH_3 (10 mL, distilled over Na) and *tert*-butanol (50 μL) at −78°C. Small pieces of sodium were added until the reaction mixture remained blue, and the mixture was stirred at −78°C for 4 h. Ammonium chloride (solid) was then added until the blue color disappeared. The liquid NH_3 was allowed to evaporate slowly and the remaining solution was concentrated to dryness. The residue was dissolved with Milli-Q water. The aqueous solution was loaded onto a C-18 Sep-Pak cartridge. The cartridge was washed with water (2 × 10 mL) followed by methanol (10 mL). The methanolic eluate was concentrated to dryness. The residue was dissolved with Milli-Q water, passed through a Millex filter, and the filtrate was lyophilized to give **7** (5.5 mg, 79%); $[\alpha]_D$ +1.1 (*c* 0.7, CH_3OH); ^1H NMR (500 MHz, D_2O) δ: 5.15 (d, 1H, $J_{1',2'}$ 1.0 Hz, H-1′), 4.55 (d, 1H, $J_{1'',2''}$ 10.0 Hz, H-1″), 4.45 (d, 1H, $J_{1,2}$ 8.0 Hz, H-1), 4.18 (dd, 1H, $J_{2',3'}$ 4.5 Hz, $J_{3',4'}$ 9.5 Hz, H-3′), 3.91 (dd, 1H, J_{gem} 11.2 Hz, $J_{5,6a}$ 5.4 Hz, H-6a), 3.88 (dd, 1H, J_{gem} 12.5 Hz, $J_{5',6'a}$ 2.2 Hz, H-6′a), 3.84 (dd, 1H, J_{gem} 11.9 Hz, $J_{5'',6''a}$ 2.5 Hz, H-6″a), 3.78 (dd, 1H, $J_{5,6b}$ 2.0 Hz, H-6b), 3.72 (dd, 1H, $J_{5',6'b}$ 6.9 Hz, H-6′b), 3.67 (dt, 1H, J_{gem} 10.5 Hz, J_{vic} 7.0 Hz, octyl C1-H_b), 3.63 (dd, 1H, H-2′), 3.58 (m, 1H, H-5), 3.51 (dd, 1H, $J_{4',5'}$ 9.6 Hz, H-4′), 3.48 (dd, 1H, $J_{2'',3''}$, $J_{3'',4''}$ 9.6 Hz, H-3″), 3.45 (dd, 1H, $J_{2,3}$, $J_{3,4}$ 9.5 Hz, H-3), 3.42 (dd, 1H, $J_{4'',5''}$ 9.5 Hz, H-4″), 3.36 (dd, 1H, H-2″), 3.25 (dd, 1H, H-2), 1.62 (p, 2H, J_{vic} 7.0 Hz, octyl C2-H), 1.38–1.24 (m, 10H, octyl CH_2), 0.86 (t, 3H, J_{vic} 7.0 Hz, octyl CH_3); ^{13}C NMR (125.7 MHz, D_2O) δ: 103.2 (C-1, $J_{C\text{-}1,H\text{-}1}$ 160.4 Hz), 101.3 (C-1′, $J_{C\text{-}1',H\text{-}1'}$ 174.3 Hz), 86.0 (C-1″, $J_{C\text{-}1'',H\text{-}1''}$ 156.4 Hz), 80.8 (C-3), 77.84 (C-5″), 76.9 (C-3″), 74.9 (C-5), 74.0 (C-5′), 73.8 (C-2), 73.1 (C-2″), 71.6 (octyl C1), 70.4 (C-4), 70.2 (C-4″), 69.7 (C-3′), 68.6 (C-4′), 66.7 (C-6), 61.6 (C-6′), 61.4 (C-6″), 51.1 (C-2′), 31.9, 29.6, 29.3, 29.2, 25.9, 22.9 (octyl C2–C7), 14.3 (octyl CH_3): FAB MS *m/z*: 671.0 $(M+K)^+$, 655.0 $(M+Na)^+$, 633.0 $(M+H)^+$.

Octyl 2,3,4-tri-*O*-benzyl-6-*O*-methanesulfonyl-β-D-glucopyranoside (20)

To a solution of **9** (213 mg, 0.370 mmol) in pyridine at −25°C was added dropwise methanesulfonyl chloride (292 μL, 3.79 mmol). The reaction mixture was warmed to r.t. over 30 min and stirred at r.t. for 4 h. The mixture was then poured into ice-cold 0.5 N aqueous HCl and extracted with CH_2Cl_2. The CH_2Cl_2 solution was washed by saturated $NaHCO_3$ and water. Concentration gave **20** (234 mg, 97%). ^1H NMR (360 MHz, $CDCl_3$) δ: 7.35–7.10 (m, 15H, Ar-H), 4.96, 4.94, 4.89, 4.80, 4.71, 4.62 (6d, each 1H, J_{gem} 11.0 Hz, $PhCH_2$), 4.46 (dd, 1H, J_{gem} 11.5 Hz, $J_{5,6a}$ 1.0 Hz, H-6a), 4.41 (d, 1H, $J_{1,2}$ 7.8 Hz, H-1), 4.33 (dd, 1H, $J_{5,6b}$ 4.0 Hz, H-6b), 3.90 (dt, 1H, J_{gem} 10.0 Hz, J_{vic} 6.5 Hz, octyl C1-H_a), 3.67 (ddd, 1H, $J_{4,5}$ 9.5 Hz, H-5), 3.59 (dt, 1H, octyl C1-H_b), 3.42 (dd, 1H, $J_{2,3}$ 9.2 Hz, H-2), 3.03 (s, 3H, mesyl CH_3), 1.63 (p, 2H, J_{vic} 7.0 Hz, octyl C2-H), 1.41–1.19 (m, 10 H, octyl CH_2), 0.88 (t, 3H, J_{vic} 7.0 Hz, octyl CH_3).

Octyl 6-*S*-acetyl-2,3,4-tri-*O*-benzyl-6-deoxy-6-thio-β-D-glucopyranoside (21)

A mixture of **20** (230 mg, 0.359 mmol) and potassium thioacetate (433 mg, 3.79 mmol) in dry DMF (15 mL) was stirred at r.t. for 16 h. TLC showed incomplete reaction. The reaction mixture was then stirred at 40°C for an additional 2 h and concentrated to dryness. The residue was dissolved in CH_2Cl_2,

washed with water, and concentrated. The residue was purified by silica gel chromatography using hexane–EtOAc (6:1) as eluant to afford **21** (195 mg, 88%). ^{1}H NMR (360 MHz, $CDCl_3$) δ: 7.35–7.10 (m, 15H, Ar-H), 4.95, 4.93, 4.88, 4.78, 4.70, 4.64 (6d, each 1H, J_{gem} 11.0 Hz, $PhCH_2$), 4.36 (d, 1H, $J_{1,2}$ 8.0 Hz, H-1), 3.91 (dt, 1H, J_{gem} 9.8 Hz, J_{vic} 7.0 Hz, octyl C1-H_a), 3.63 (dd, 1H, $J_{2,3}$, $J_{3,4}$ 9.5 Hz, H-3), 3.60 (dd, 1H, J_{gem} 14.0 Hz, $J_{5,6a}$ 2.7 Hz, H-6a), 3.57 (dt, 1H, octyl C1-H_b), 3.46–3.31 (m, 3H, H-2, H-4, H-5), 2.97 (dd, 1H, $J_{5,6b}$ 7.5 Hz, H-6b), 2.36 (s, 3H, CH_3CO), 1.65 (p, 2H, J_{vic} 7.0 Hz, octyl C2-H), 0.89 (t, 3H, J_{vic} 7.0 Hz, octyl CH_3); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 194.9 (C=O), 138.5, 138.4, 137.8 (benzyl C1), 128.5, 128.4, 128.34, 128.26, 128.1, 127.9, 127.6 (Ar), 103.5 (C-1), 75.7, 75.1, 74.8 (Ph*C*H_2), 31.8 (C-6), 31.1 (octyl C2), 30.5 (*C*H_3CO), 29.8, 29.4, 29.2, 26.2, 22.7 (octyl C3–C7), 14.1 (octyl CH_3). Anal. calcd. for $C_{37}H_{48}O_6S$ (620.84): C 71.58, H 7.79, S 5.16; found: C 71.27, H 8.15, S 5.52.

Octyl 2,3,4-tri-*O*-benzyl-6-deoxy-6-thio-β-D-glucopyranoside (12)

Compound **21** (133 mg, 0.214 mmol) was treated with methanolic NaOMe (0.05 N, 45 mL) at r.t. for 30 min. Neutralization with Amberlite IR-120 (H^+) resin, removal of the resin by filtration, and concentration gave **12** (124 mg, 96%); ^{1}H NMR (360 MHz, $CDCl_3$) δ: 7.40–7.10 (m, 15H, Ar-H), 4.96, 4.95, 4.89, 4.79, 4.72, 4.62 (6d, each 1H, J_{gem} 11.0 Hz, $PhCH_2$), 4.42 (d, 1H, $J_{1,2}$ 8.0 Hz, H-1), 3.95 (dt, 1H, J_{gem} 9.6 Hz, J_{vic} 6.5 Hz, octyl C1-H_a), 3.66 (dd, 1H, $J_{2,3}$, $J_{3,4}$ 9.5 Hz, H-3), 3.56 (dt, 1H, octyl C1-H_b), 3.47 (dd, 1H, $J_{4,5}$ 9.5 Hz, H-4), 3.43 (dd, 1H, $J_{2,3}$ 9.5 Hz, H-2), 3.37 (ddd, 1H, $J_{5,6a}$ 2.8 Hz, $J_{5,6b}$ 7.5 Hz, H-5), 2.89 (ddd, 1H, J_{gem} 14.0 Hz, $J_{6a,SH}$ 9.5 Hz, H-6a), 2.63 (ddd, 1H, $J_{6b,SH}$ 7.6 Hz, H-6b), 1.70 (dd, 1H, SH, D_2O exchangeable), 1.66 (p, 2H, J_{vic} 7.0 Hz, octyl C2-H), 0.88 (t, 3H, J_{vic} 7.0 Hz, CH_3).

2-*O*-Acetyl-3,4,6-tri-*O*-benzyl-α-D-glucose (23)

To a solution of 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-glucopyranosyl bromide (**22**) (15) (313 mg, 0.563 mmol) in acetone (4 mL) at 0°C was added water (1 drop) and silver carbonate (192 mg, 0.698 mmol). The reaction mixture was vigorously stirred, warmed to r.t., and stirred overnight. The reaction mixture was filtered through a Celite bed that was washed with acetone. The acetone filtrate was concentrated, and the residue was purified by chromatography using hexane–EtOAc (2:1) as eluant to afford **23**. Compound **23** was not characterized but was used directly for the preparation of imidate **14**.

***O*-(2-*O*-Acetyl-3,4,6-tri-*O*-benzyl-α-D-glucopyranosyl)-trichloroacetimidate (14)**

To a solution of **23** (64 mg, 0.130 mmol) in dry CH_2Cl_2 was added NaH (60% suspension in oil, 5.2 mg, 0.130 mmol) and trichloroacetonitrile (60 μL). The reaction mixture was stirred at r.t. for 2 h and was concentrated. The residue was purified by chromatography using hexane–ether–Et_3N (50:50:1) as eluant to afford imidate **14** (50 mg, 60%); ^{1}H NMR (360 MHz, $CDCl_3$) δ: 8.58 (s, 1H, NH), 7.30–7.10 (m, 15H, Ar-H), 6.53 (d, 1H, $J_{1,2}$ 3.5 Hz, H-1), 5.07 (dd, 1H, $J_{2,3}$ 9.6 Hz, H-2), 4.86 (d, 1H, J_{gem} 11.5 Hz, $PhCH_2$), 4.84 (d, 1H, J_{gem} 10.8 Hz, $PhCH_2$), 4.77 (d, 1H, J_{gem} 11.5 Hz, $PhCH_2$), 4.64 (d, 1H, J_{gem} 11.8 Hz, $PhCH_2$), 4.58 (d, 1H, J_{gem} 10.8 Hz, $PhCH_2$), 4.50 (d, 1H, J_{gem} 11.8 Hz, $PhCH_2$), 4.10 (dd, 1H, $J_{3,4}$ 9.5 Hz, H-3), 4.02 (ddd, 1H, $J_{4,5}$ 9.5 Hz, H-5), 3.88 (dd, 1H, H-4), 3.82 (dd, 1H, J_{gem} 11.2 Hz, $J_{5,6a}$ 3.5 Hz, H-6a), 3.70 (dd, 1H, $J_{5,6b}$ 2.0 Hz, H-6b), 1.92 (s, 3H, Ac).

Octyl 3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-benzyl-6-deoxy-6-thio-β-D-glucopyranoside (24)

To a solution of **12** (124 mg, 0.214 mmol) in dry THF (5 mL) was added sodium hydride (60% suspension in oil, 13 mg, 0.321 mmol). The resulting mixture was stirred at r.t. for 30 min. After the sodium salt (white precipitate) was produced, THF was removed using a stream of argon. The residuum sodium salt was dissolved with DMF (2 mL) and was added dropwise to a solution of 1,2-anhydro-3,4,6-tri-*O*-benzyl-β-D-mannose **13** (13) (85 mg, 0.195 mmol) in DMF (1 mL). The reaction mixture was stirred at r.t. for 1 h. Methanol (1 mL) was added to the reaction mixture; the reaction mixture was then concentrated and the residue was purified by column chromatography using hexane–EtOAc (3:1) as eluant to give **24** (94 mg, 63%); $[\alpha]_D$ +83.4 (*c* 0.5, CH_2Cl_2); ^{1}H NMR (360 MHz, $CDCl_3$) δ: 5.44 (d, 1H, $J_{1',2'}$ 1.5 Hz, H-1′), 4.96, 4.94, 4.85, 4.82, 4.76, 4.71 (6d, each 1H, J_{gem} 11.0 Hz, $PhCH_2$), 4.62 (d, 1H, J_{gem} 12.2 Hz, $PhCH_2$), 4.57, 4.51 (2d, each 1H, J_{gem} 11.0 Hz, $PhCH_2$), 4.44 (d, 1H, J_{gem} 12.2 Hz, $PhCH_2$), 4.38 (d, 1H, $J_{1,2}$ 8.0 Hz, H-1), 4.14 (ddd, 1H, $J_{2',3'}$ 3.2 Hz, $J_{2',OH}$ 2.7 Hz, H-2′), 4.12 (ddd, 1H, H-5′), 3.93 (dt, J_{gem} 10.0 Hz, J_{vic} 6.5 Hz, octyl C1-H_a), 3.91 (dd, 1H, $J_{4',5'}$, $J_{3',4'}$ 9.5 Hz, H-4′), 3.85 (dd, 1H, H-3′), 3.75 (dd, 1H, J_{gem} 11.8 Hz, $J_{5',6'a}$ 4.5 Hz, H-6′a), 3.63 (dd, 1H, $J_{2,3}$, $J_{3,4}$ 9.5 Hz, H-3), 3.61 (dd, 1H, $J_{5',6'b}$ 2.0 Hz, H-6′b), 3.50 (dt, 1H, octyl C1-H_b), 3.48 (dd, 1H, $J_{4,5}$ 9.5 Hz, H-4), 3.48 (m, 1H, H-5), 3.41 (dd, 1H, H-2), 3.07 (dd, J_{gem} 14.0 Hz, $J_{5,6a}$ 2.5 Hz, H-6a), 2.78 (dd, 1H, $J_{5,6b}$ 6.0 Hz, H-6b), 2.57 (d, 1H, C2′-OH), 0.87 (t, 3H, J_{vic} 7.0 Hz, octyl CH_3); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ: 138.6, 138.5, 138.4, 138.2, 137.7 (benzyl C1), 128.6, 128.4, 128.3, 128.1, 128.0, 127.91, 127.86, 127.6, 127.5 (Ar), 103.5 (C-1), 84.5 (C-1′), 75.6, 75.2, 75.1, 74.8, 73.4, 72.0 (Ph*C*H_2), 70.2 (octyl C1), 68.9 (C-6′), 31.9 (C-6), 31.8, 29.8, 29.4, 29.3, 26.2, 22.7 (octyl C2–C7), 14.1 (octyl CH_3). Anal. calcd. for $C_{62}H_{74}O_{10}S$ (1011.33): C 73.63, H 7.38, S 3.17; found: C 73.18, H 7.44, S 3.30.

Octyl 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-β-D-glucopyranosyl-(1→2)-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-benzyl-6-deoxy-6-thio-β-D-glucopyranoside (25)

A mixture of **14** (48 mg, 75.4 μmol), **24** (66 mg, 65.2 μmol), powdered molecular sieve 4Å (60 mg), and CH_2Cl_2 (3 mL) was stirred at r.t. for 30 min. To the mixture was added a solution of TMSOTf (1.45 μL, 7.5 μmol) in CH_2Cl_2 (150 μL) under argon. After stirring for 1 h, Et_3N (0.1 mL) was added and the mixture was filtered through Celite and concentrated. The residue was purified by chromatography using hexane–EtOAc (3:1) as eluant to afford **25** (44 mg, 88% based on consumed alcohol) and recovered alcohol **24** (32 mg, 49%); $[\alpha]_D$ +37.7 (*c* 1.2, CH_2Cl_2); ^{1}H NMR (360 MHz, $CDCl_3$) δ: 5.40 (d, 1H, $J_{1',2'}$ 1.2 Hz, H-1′), 5.15 (dd, 1H, $J_{1'',2''}$ 8.5 Hz, $J_{2'',3''}$ 9.0 Hz, H-2″), 4.47 (d, 1H, H-1″), 4.39 (d, 1H, $J_{1,2}$ 8.0 Hz, H-1), 4.27 (dd, 1H, $J_{2',3'}$ 3.2 Hz, H-2′), 4.08 (ddd, 1H, $J_{4',5'}$ 9.6 Hz, $J_{5',6'a}$ 6.4 Hz, $J_{5',6'b}$ 2.0 Hz, H-5′), 3.97 (dt, 1H, J_{gem} 10.0 Hz, J_{vic} 6.5 Hz, octyl C1-H_a), 3.82 (dd, 1H, $J_{3',4'}$ 9.5 Hz, H-3′), 3.62 (dd, 1H, $J_{3,4}$ 9.5 Hz, H-3), 3.53 (dt, 1H, octyl C1-H_b), 3.44 (dd, $J_{2,3}$

9.2 Hz, H-2), 3.10 (dd, 1H, J_{gem} 14.2 Hz, $J_{5,6a}$ 2.2 Hz, H-6a), 2.82 (dd, 1H, $J_{5,6b}$ 5.4 Hz, H-6b), 1.92 (s, 3H, Ac), 0.86 (t, 3H, J_{vic} 7.0 Hz, octyl CH_3); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ: 169.4 (C=O), 138.58, 138.56, 138.5, 138.33, 138.27, 138.1, 138.0, 137.9 (benzyl C1), 128.4, 128.37, 128.34, 128.28, 128.2, 128.1, 127.99, 127.97, 127.87, 127.81, 127.69, 127.66, 127.6, 127.4 (Ar), 103.7 (C-1), 99.0 (C-1″), 84.6 (C-1′), 75.6, 75.3, 75.1, 75.0, 74.83, 74.81, 73.6, 72.9, 70.5 (PhCH$_2$), 70.3 (octyl C1), 69.7 (C-6″), 69.5 (C-6′), 31.82 (C-6), 31.81, 29.8, 29.5, 29.3, 26.2, 22.6 (octyl C2–C7), 20.9 (CH_3CO), 14.1 (octyl CH_3). Anal. calcd. for $C_{91}H_{104}O_{16}S$ (1485.99): C 73.56, H 7.05, S 2.16; found: C 73.59, H 7.14, S 2.10.

Octyl 3,4,6-tri-*O*-benzyl-β-D-glucopyranosyl-(1→2)-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-benzyl-6-deoxy-6-thio-β-D-glucopyranoside (26)

Compound **25** (24 mg, 16.2 μmol) was treated with methanolic NaOMe (0.05 N, 25 mL) at r.t. for 8 h. Neutralization with Amberlite IR-120 (H^+) resin, removal of the resin by filtration, and concentration left a residue that was purified by column chromatography (hexane–EtOAc 2:1) to give **26** as a syrup (21 mg, 92%); ^1H NMR (400 MHz, $CDCl_3$) δ: 5.54 (d, 1H, $J_{1',2'}$ 1.8 Hz, H-1′), 4.44 (d, 1H, $J_{1'',2''}$ 7.8 Hz, H-1″), 4.37 (d, 1H, $J_{1,2}$ 8.0 Hz, H-1), 4.33 (dd, 1H, $J_{2',3'}$ 3.2 Hz, H-2′), 4.12 (dd, 1H, $J_{3',4'}$, $J_{4',5'}$ 9.5 Hz, H-4′), 4.03 (ddd, 1H, $J_{5',6'a}$ 4.5 Hz, $J_{5',6'b}$ 2.0 Hz, H-5′), 3.98 (dt, 1H, J_{gem} 10.0 Hz, J_{vic} 6.5 Hz, octyl C1-H$_a$), 3.88 (dd, 1H, H-3′), 3.43 (dd, 1H, $J_{2,3}$ 9.0 Hz, H-2), 3.38 (br s, 1H, C2″-OH), 3.06 (dd, 1H, J_{gem} 14.5 Hz, $J_{5,6a}$ 2.0 Hz, H-6a), 2.83 (dd, 1H, $J_{5,6b}$ 5.0 Hz, H-6b), 0.85 (t, 3H, J_{vic} 7.0 Hz, octyl CH_3); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ: 139.0, 138.6, 138.52, 138.47, 138.3, 138.2, 138.0, 137.97, 137.7 (benzyl C1), 128.46, 128.34, 128.28, 128.25, 128.01, 127.93, 127.87, 127.81, 127.76, 127.64, 127.58, 127.54, 127.42 (Ar), 103.7 (C-1), 100.6 (C-1″), 84.5 (C-1′), 75.6, 75.1, 75.1, 75.0, 74.9, 74.8, 73.5, 73.3, 71.7 (PhCH$_2$), 70.4 (octyl C1), 69.5 (C-6″), 68.7 (C-6′), 32.4 (C-6), 31.8, 29.8, 19.5, 29.2, 26.2, 22.6 (octyl C2–C7), 14.1 (octyl CH_3).

Octyl β-D-glucopyranosyl-(1→2)-α-D-mannopyranosyl-(1→6)-6-deoxy-6-thio-β-D-glucopyranoside (8)

The benzyl ether protecting groups in **26** (18 mg, 12.5 μmol) were removed by treatment with Na/liquid NH_3, and then purified as described for the preparation of **7**. Lyophilization from water gave **8** as a white powder (6.5 mg, 82%); $[\alpha]_D$ +54.6 (*c* 0.5, CH_3OH); ^1H NMR (500 MHz, D_2O) δ: 5.50 (d, 1H, $J_{1',2'}$ 1.0 Hz, H-1′), 4.50 (d, 1H, $J_{1'',2''}$ 8.0 Hz, H-1″), 4.46 (d, 1H, $J_{1,2}$ 8.0 Hz, H-1), 4.24 (dd, 1H, $J_{2',3'}$ 3.5 Hz, H-2′), 4.01 (ddd, 1H, $J_{4',5'}$ 9.5 Hz, $J_{5',6'a}$ 3.2 Hz, $J_{5',6'b}$ 6.0 Hz, H-5′), 3.90 (dd, 1H, J_{gem} 12.0 Hz, $J_{5'',6''a}$ 2.0 Hz, H-6″a), 3.89 (dd, 1H, J_{gem} 12.0 Hz, $J_{5',6'a}$ 6.5 Hz, H-6′a), 3.87 (dd, 1H, $J_{5',6'b}$ 2.5 Hz, H-6′b), 3.87 (dd, 1H, $J_{3',4'}$ 9.5 Hz, H-3′), 3.85 (dt, 1H, J_{gem} 10.5 Hz, J_{vic} 6.5 Hz, octyl C1-H$_a$), 3.75 (dd, 1H, $J_{5'',6''b}$ 5.4 Hz, H-6″b), 3.74 (dd, 1H, $J_{3',4'}$, $J_{4',5'}$ 9.5 Hz, H-4′), 3.71 (dt, 1H, octyl C1-H$_b$), 3.61 (ddd, 1H, $J_{4,5}$ 9.5 Hz, $J_{5,6a}$ 2.7 Hz, $J_{5,6b}$ 8.0 Hz, H-5), 3.49 (ddd, 1H, $J_{4'',5''}$ 9.5 Hz, H-5″), 3.47 (dd, $J_{2,3}$, $J_{3,4}$ 9.5 Hz, H-3), 3.43 (dd, 1H, H-4″), 3.39 (dd, 1H, H-4), 3.36 (dd, 1H, $J_{2'',3''}$ 9.5 Hz, H-2″), 3.26 (dd, 1H, $J_{2,3}$ 9.5 Hz, H-2), 3.20 (dd, J_{gem} 14.4 Hz, $J_{5,6a}$ 2.7 Hz, H-6a), 2.80 (dd, 1H, $J_{5,6b}$ 8.0 Hz, H-6b), 1.64 (p, 2H, J_{vic} 7.0 Hz, octyl C2-H), 1.40–1.25 (m, 10H, 5 octyl CH_2), 0.87 (t, 3H, J_{vic} 7.0 Hz, octyl CH_3); ^{13}C NMR (100.6 MHz) δ: 103.2 (C-1, $J_{C\text{-}1,H\text{-}1}$ 160.6 Hz), 101.8 (C-1″, $J_{C\text{-}1'',H\text{-}1''}$ 161.1 Hz), 82.7 (C-1′, $J_{C\text{-}1',H\text{-}1'}$ 167.9 Hz, C-1′), 79.4 (C-2′), 76.8 (C-3), 76.5 (C-5″), 76.3 (C-3″), 74.9 (C-5), 74.1 (C-2), 73.9 (C-5′), 73.5 (C-2″), 73.1 (C-4), 71.8 (octyl C1), 71.1 (C-3′), 70.1 (C-4″), 67.9 (C-4′), 61.5 (C-6″), 61.2 (C-6′), 32.4 (C-6), 31.9, 29.7, 29.3, 29.2, 25.9, 22.8 (octyl C2–C7), 14.2 (octyl CH_3); FAB MS *m/z*: 671.1 $(M+K)^+$, 655.2 $(M+Na)^+$, 633.3 $(M+H)^+$. Exact FAB MS for $C_{26}H_{48}O_{15}SNa$, theoretical MS: 655.2612; found: 655.2620 (std. deviation 1.3).

Evaluation of trisaccharide analogs as acceptors for GlcNAcT-V

Materials

The cloned rat kidney GlcNAcT-V was from Dr. M. Pierce. Partially purified GlcNAcT-V was prepared by a modification (20) of published procedures (18, 19). The activity of cloned GlcNAcT-V was 10 mU/mL with **5** as acceptor (protein content = 0.286 mg/mL, specific activity = 35 mU/mg). The activity of isolated GlcNAcT-V was 1.0 mU/mL with **5** as acceptor. Enzyme was stored at 4°C in 50 mM MES buffer, pH 6.5, containing 300 mM NaCl, and 5 mM EDTA.

Methods

A standard assay contained final concentrations of 40 μM **5** as acceptor, 550 μM UDP GlcNAc including UDP-^{3}H-GlcNAc (~200 000 dpm) as donor substrates, 5–9 μU cloned GlcNAcT-V (0.5–0.9 μL), in 25 mM sodium cacodylate buffer, pH 6.5, 0.05% Triton X-100, 10% glycerol, 5 mM EDTA, and 0.5 mg/mL BSA, in a total volume of 20 μL. Assay tubes were incubated for 30–60 min at 37°C, then quenched with 0.5 mL water. Radiolabeled product was separated from unreacted donor on Sep-Pak C-18 reversed-phase cartridge and quantitated by liquid scintillation counting in 10 mL of Ecolite cocktail (ICN) (9, 18–20).

Kinetics

Kinetic parameters for acceptors were determined with the radiochemical assay described above. Assays contained buffer, donor, ^{3}H donor, and enzyme concentrations as for the standard assay. Assays were performed in duplicate with six different acceptor concentrations. Tubes were incubated at 37°C for 60 or 30 min and processed on C-18 Sep-Pak cartridges as for the standard assay. Kinetic data (K_m and V_{max}) listed in Table 1 were obtained by fitting the initial rate data to the Michaelis-Menten equation using nonlinear regression analysis (Sigma Plot 4.10) (9).

Preparative enzymatic synthesis of octyl β-D-glucopyranosyl-(1→2)-[2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→6)]-2-deoxy-2-thio-α-D-mannopyranosyl-(1→6)-β-D-glucopyranoside (27)

A mixture of **7** (0.49 mg, 0.774 μmol), UDP-GlcNAc (2 mg, 3 μmol), cloned rat kidney GlcNAcT-V (17) (0.5 mL, 1.48 mU/mL), and buffer (170 mM sodium cacodylate buffer, pH 6.5, 70% glycerol, 35 mM EDTA, and 3.5 mg/mL BSA, in a total volume of 200 μL) was incubated at r.t. for 48 h. The mixture was diluted with Milli-Q water and loaded onto a C-18 Sep-Pak cartridge. The cartridge was eluted with water (3 × 10 mL) followed by methanol (10 mL). The methanolic eluate was concentrated to dryness, and the residue was dissolved in Milli-Q water and passed through a Millex filter. Compound

27 was obtained as a white powder in 100% yield after lyophilization of the filtrate. ^{1}H NMR (500 MHz, D_2O) δ: 5.13 (d, 1H, $J_{1',2'}$ 0.5 Hz, H-1′), 4.53 (d, 1H, $J_{1'',2''}$ 10.5 Hz, H-1″), 4.45 (d, 1H, $J_{1,2}$ 8.0 Hz, H-1), 4.52 (d, 1H, $J_{1''',2'''}$ 8.0 Hz, H-1‴), 4.17 (dd, 1H, $J_{2',3'}$ 4.5 Hz, $J_{3',4'}$ 9.8 Hz, H-3′), 3.61 (dd, 1H, H-2′), 3.38 (dd, 1H, $J_{2'',3''}$ 9.5 Hz, H-2″), 3.26 (dd, 1H, $J_{2,3}$ 9.5 Hz, H-2), 2.09 (s, 3H, Ac), 1.63 (p, 2H, J_{vic} 7.0 Hz, octyl C2-H), 1.40–1.24 (m, 10H, octyl CH_2), 0.86 (t, 3H, J_{vic} 7.0 Hz, octyl CH_3); FAB MS *m/z*: 858.1 $(M+Na)^+$.

Preparative enzymatic synthesis of octyl β-D-glucopyranosyl-(1→2)-[2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→6)]-α-D-mannopyranosyl-(1→6)-6-deoxy-6-thio-β-D-glucopyranoside (28)

A mixture of **8** (0.50 mg, 0.789 μmol), UDP-GlcNAc (1.5 mg, 2.3 μmol), partially purified GlcNAcT-V (1.5 mL, 1 mU/mL) from hamster kidneys (18–20), and buffer (150 mM sodium cacodylate buffer, pH 6.5, 60% glycerol, 30 mM EDTA, and 3 mg/mL BSA, in a total volume of 500 μL) was incubated at r.t. for 72 h. The mixture was diluted with Milli-Q water and loaded onto a C-18 Sep-Pak cartridge. The cartridge was eluted with water (3 × 10 mL) followed by methanol (10 mL). The methanolic eluate was concentrated to dryness, and the residue was purified by chromatography on Iatrobeads using $CHCl_3$–MeOH–H_2O (65:35:8) as eluant to give the crude product. The crude product was dissolved in Milli-Q water and was loaded onto a C-18 Sep-Pak cartridge. The cartridge was washed with water (10 mL) followed by methanol (10 mL). The methanolic eluate was concentrated to dryness, the residue was dissolved in Milli-Q water and passed through a Millex filter. Compound **28** was obtained as a white powder in 100% yield after lyophilization of the filtrate. ^{1}H NMR (500 MHz, D_2O) δ: 5.48 (d, 1H, $J_{1',2'}$ 1.0 Hz, H-1′), 4.52 (d, 1H, $J_{1'',2''}$ 8.4 Hz, H-1″), 4.48 (d, 1H, $J_{1''',2'''}$ 7.6 Hz, H-1‴), 4.45 (d, 1H, $J_{1,2}$ 8.1 Hz, H-1), 4.22 (dd, 1H, $J_{2',3'}$ 3.5 Hz, H-2′), 4.15 (dd, 1H, J_{gem} 11.0 Hz, $J_{5',6'a}$ 2.4 Hz, H-6′a), 4.11 (ddd, 1H, $J_{4',5'}$ 9.5 Hz, $J_{5',6'b}$ 4.8 Hz, H-5′), 3.94 (d, 1H, J_{gem} 12.0 Hz, $J_{5'',6''a}$ 2.0 Hz, H-6″a), 3.83 (dd, 1H, $J_{3',4'}$ 9.5 Hz, H-3′), 3.61 (ddd, 1H, $J_{4,5}$ 9.6 Hz, $J_{5,6a}$ 3.0 Hz, $J_{5,6b}$ 8.0 Hz, H-5), 3.26 (dd, 1H, $J_{2,3}$ 9.5 Hz, H-2), 3.17 (dd, 1H, J_{gem} 14.3 Hz, $J_{5,6a}$ 3.0 Hz, H-6a), 2.80 (dd, 1H, $J_{5,6b}$ 8.0 Hz, H-6b), 2.07 (s, 3H, Ac), 1.64 (p, 2H, J_{vic} 7.0 Hz, octyl C2-H), 0.87 (t, 3H, J_{vic} 7.0 Hz, octyl CH_3); FAB MS *m/z*: 874.2 $(M+K)^+$, 858.2 $(M+Na)^+$, 836.3 $(M+H)^+$.

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