Small Molecules as Structural and Functional Mimics of Sialyl Lewis X Tetrasaccharide in Selectin Inhibition: A Remarkable Enhancement of Inhibition by Additional Negative Charge and/or Hydrophobic Group

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Received March 24, 1997[⊗]

Abstract: Several sialyl Lewis X (SLe^x) mimics that contain the essential functional groups for receptor interaction and a negative charge or a hydrophobic group have been developed as inhibitors of E-, P-, and L-selectins. Some of the mimics exhibit selectin inhibition activities 10^3-10^4-fold more potent than does the natural ligand tetrasaccharide, with IC_{50} in the low micromolar to high nanomolar range. The syntheses of these mimics are relatively simple, using TMSOTf-Ac_2O mediated *C*-glycosylation with concurrent selective deprotection of the primary benzyl group and enzymatic aldol addition reactions as key steps.

Current interest in the study of selectin−sialyl Lewis X tetrasaccharide (SLe^x) interaction,[1] a recognition process involved in the adhesion leukocytes to vascular endothelial cells during inflammatory responses, has led to an intensive search for small molecules as inhibitors of selectin binding and as potential drug candidates for the treatment of reperfusion injury and other inflammatory disorders.[2] Development of carbohydrate-based therapy is, however, hindered by the complication that most proteins bind carbohydrates with weak affinity[3] and that synthesis of complex carbohydrates is a rather expensive and difficult process. Understanding the molecular basis of protein−carbohydrate interaction is therefore of fundamental importance for development of small molecule inhibitors that are structural and functional mimics of natural ligands and are more effective and accessible than the parent structures.

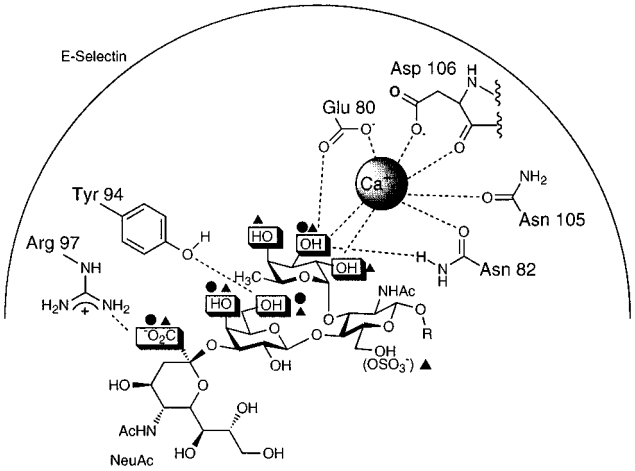

Figure 1. Model showing the structure and functional groups of SLe^x interacting with E-selectin (□), and the groups recognized by P- (●) and L-selectin (▲). Both E- and P-selectins bind SLe^x in a similar manner (as indicated). L-selectin recognized approximately the free form of SLe^x, with the NeuAc-CO_2H pointing above the plane.

Figure 1 indicates the structure and essential functional groups[4] of SLe^x recognized by E-selectin[5] and also the groups recognized by P- and L-selectins.[1e,4] A recent NMR study[5c] suggests that the free and bound states of the Le^x moiety of SLe^x are basically the same in the three selectin−ligand complexes while the NeuAc-carboxylate orientation in the L-selectin complex differs from that of the E- and P-selectin complex. These recognition models have helped the develop-

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⊗ Abstract published in *Advance ACS Abstracts,* August 15, 1997.

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S0002-7863(97)00920-7 CCC: $14.00

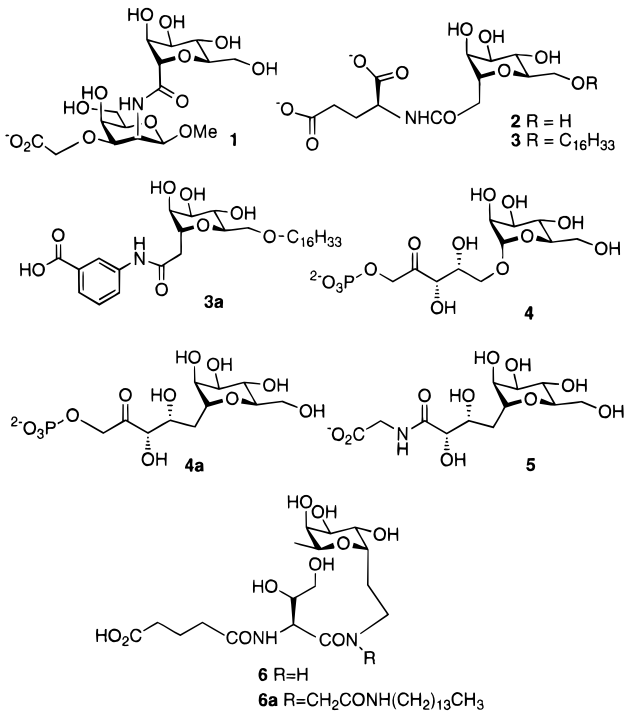


Figure 2. Designated SLe^x mimetics.

Table 1. IC_{50} (μM) Values in Selectin Inhibition[a]

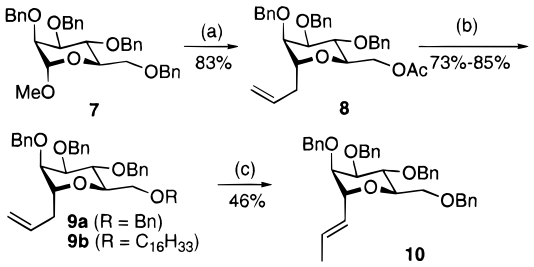
	1	2	3	3a	4	4a	5	6	6a	SLe^x
E-	110	100	40	74	100	800	160	300	37	500 (720)[b]
P-	nd[c]	nd[c]	2.2	5	0.6	5	nd[c]	2500	7	>3000 (8000)[b]
L-	nd[c]	nd[c]	7.6	100	95	40	nd[c]	>3000	190	1300 (3900)[b]

[a] Determined in a cell-free assay (ref 7) based on the polymeric SLe^a interaction with a microtiter plate coated selectin. The values represent the average of three measurements, with ±10% error. [b] The number is taken from the NMR study (ref 5c). [c] Not determined.

ment of small molecules that mimic SLe^x, in interaction with its receptors,[2] and further enhancement of inhibition has been achieved with addition of a hydrophobic group[2b,k] to the mimic or with the use of multivalent strategy.[1b,6]

In this study, we have developed a new series of mannose-based SLe^x mimics (**1**−**4**), as shown in Figure 2, some of which (e.g., **3** and **4**) exhibit the most potent inhibition activity[7] (in the low micromolar range) known to date (Table 1). Compound **1**, a peptide disaccharide, contains the six essential functional groups required for SLe^x recognition, and as expected, its activity is comparable to the natural ligand. Compounds **2** and **3** employ a carboxylate group to replace the Gal residue, with the hope that it may also interact with the nearby Lys111 and Lys113 residues in E- and P-selectins.[2e,3b] Mimetic **3** also contains a long-chain hydrophobic group[8] designed to interact

Scheme 1[a]



[a] Reagents and conditions: (a) allyl-TMS (2 equiv)/MeCN/TMSOTf (0.5 equiv) 0 °C → 25 °C, then Ac_2O, (b) NaOMe (0.3 equiv)/MeOH, 25 °C; NaH (1.2 equiv)/THF RBr (1.2 equiv), 0 °C, 24 h, (c) $PdCl_2$, benzene, 80 °C, 24 h.

with the hydrophobic portion of E- and P-selectins[2k] and therefore to enhance binding. As a result, a dramatic increase in inhibition against all three selectins was observed. Removing the carboxylate from **3** and placing a more constrained benzoic acid in the NeuAc-CO_2H position still shows an impressive activity, indicating the significant effect of the hydrophobic group. Compounds **4** and **4a** were also prepared for evaluation in consideration of our previous result from the related structure **5** which binds more tightly than does SLe^x to E-selectin.[2f] Surprisingly, the phosphate-containing *O*-linked mannoside is 10^4-fold more potent than SLe^x against P-selectin (IC_{50} = 0.6 μM). This remarkable enhancement of inhibition may be due to some additional interactions of the aglycon groups with some other groups of P-selectin. One possibility is the ionic interaction of the phosphate with Lys111 and Lys113 which are close to the NeuAc-CO_2H and the Gal-2-OH groups.[2k] Another possibility is that a nucleophile in the active site may interact with the carbonyl group, as some Le^x-3′-phosphate derivatives did not significantly improve the affinity.[2l] The *C*-linked phosphate **4a**, structurally related to **5**, was found less active than **4**, though much more potent than SLe^x against P- and L-selectins. For comparison, the inhibition activities of **6** and **6a** prepared previously[2g] were also included.

The synthesis of these mimics are relatively simple as indicated in Schemes 1−4. Of particular interest is the synthesis of key intermediate **8** from **7** via a one-pot TMSOTf-Ac_2O mediated *C*-allylation with a concurrent debenzylation/acetylation of the 6-benzyl group, which provides an easy access to *C*-glycosides with alkyl substituents at the C-6 position. This reaction was found to be also applicable to other benzylated hexoses.[9] Another interesting reaction is the one-step enzymatic aldol reaction to give **4** (Scheme 4) and **4a** using the *O*- and *C*-linked aldehyde substrates, respectively. The other diastereomers of **4** with different aglycon configurations were also prepared using different aldolases,[10] but all the products exhibit weaker inhibition than **4** against P-selectin.

In conclusion, this study describes the synthesis of several new molecules that are structural and functional mimics of SLe^x in selectin inhibition. It appears that very tight-binding inhibitors, with an increase in binding energy of ∼6 kcal/mol (it is noted that IC_{50} values do not always correspond to binding energy), can be developed via incorporation of one or two new groups to the carbohydrate mimic to have additional electrostatic and/or hydrophobic interactions with the receptor. Work is in

(5) For the SLe^x structure bound to E-selectin, see: (a) Scheffler, K.; Ernst, B.; Katopodis, A.; Magnani, J. L.; Wang, W. T.; Weisemann, R.; Peters, T. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 1841−1844. (b) Cooke, R. M.; Hale, R. S.; Lister, S. G.; Shah, G.; Weir, M. P. *Biochemistry* **1994**, *33*, 10591−10596. For SLe^x bound to E-, P-, and L-selectin, see: (c) Poppe, L.; Brown, G. S.; Philo, J. S.; Nikrad, P. V.; Shah, B. H. *J. Am. Chem. Soc.* **1997**, *119*, 1727. The dissociation constants determined for SLe^x bound to E-, P-, and L-selectins are 0.72, 7.8, and 3.9 mM, respectively. For the functional groups of E-selectin involved in SLe^x binding based on modeling, see: (d) Kogan, T. P.; Revelle, B. M.; Tapp, S.; Scott, D.; Beck, P. J. A. *J. Biol. Chem.* **1995**, *270*, 14047−14055.

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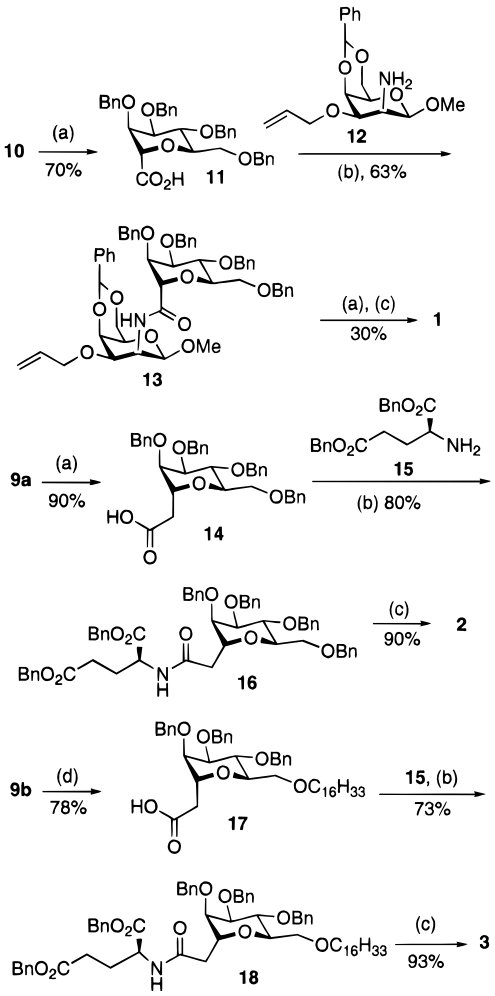
(8) When the long-chain hydrophobic group was changed to aryl hydrophobic group, it was also shown a better activity than that of SLe^x (unpublished result).

(9) Hung, S.-C; Lin, C.-C.; Wong, C.-H. *Tetrahedron Lett.* **1997**, in press.

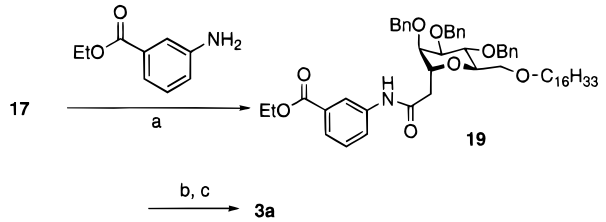
(10) Both fuculose 1-phosphate and rhamnulose 1-phosphate aldolases were used to prepare the *O*-linked diastereomers.

Scheme 2[a]



[a] Reagents and conditions: (a) O_3/78 °C, DMS/23 °C, Jones reagent/0 °C; (b) EDC/HOBt (1.2 equiv), NMM, 0 °C; (c) H_2, Pd−C (Degussa type, 10% w/w), HOAc−H_2O (8:2 v/v); (d) i. OsO_4, NMO; ii. $NaIO_4$, iii. Jones reagent.

Scheme 3[a]



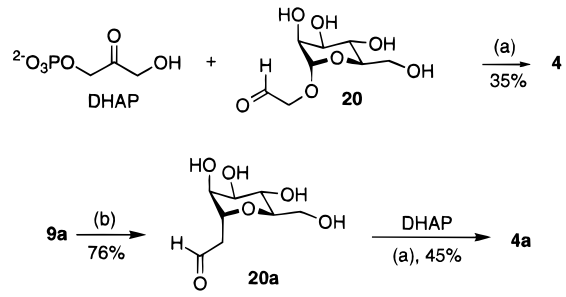
[a] Reagents and conditions: (a) EDC/HOBt (1.2 equiv), NMM, 0 °C, 63%; (b) LiOH, 87%; (c) H_2, Pd−C (Degussa type, 10 % w/w), HOAc−H_2O (8:2 v/v), 91%.

progress to investigate the mechanisms of these mimics binding and to prepare polyvalent inhibitors to further enhance the inhibition.

Experimental Section

3-(6-*O*-Acetyl-2,3,4-tri-*O*-benzyl-α-D-mannopyranosyl)propene (8). To a solution of methyl 2,3,4,6-tetra-*O*-benzyl-α-glycoside (**7**) (0.69 mmol) in CH_3CN (1.4 mL) was added allyltrimethylsilane (1.45 mmol) at 0 °C under argon. To the mixture was added TMSOTf (0.35 mmol), and the reaction was kept stirring under the same temperature overnight, then warmed up to 25 °C. The mixture was added acetic anhydride (1 mL) drop by drop. After 5 min, the reaction was diluted with CH_2Cl_2 (10 mL) and the resulting solution was quenched by saturated $NaHCO_3$. The aqueous layer was extracted with CH_2Cl_2 (2 × 10 mL), and the combined organic layers were washed with brine, dried with $MgSO_4$, filtered, evaporated, and purified by column chromatography to provide acetate **8** in 83% yield: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.35−7.28 (m, 15 H), 5.72 (ddt, J = 17.0, 10.3, 6.9 Hz, 1 H), 5.04−5.00 (m, 2 H), 4.75 (d, J = 11.2 Hz, 1 H), 4.63−4.53 (m, 5 H), 4.39 (dd, J = 11.6, 6.4 Hz, 1 H), 4.25 (dd, J = 11.6, 2.8 Hz, 1 H), 4.07 (ddd, J = 10.5, 5.9, 2.2 Hz, 1 H), 3.83−3.75 (m, 3 H), 3.62 (dd, J = 4.4, 2.7 Hz, 1 H), 2.38−2.27 (m, 2 H), 2.05 (s, 3 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.90, 138.07, 137.92, 133.94, 128.43, 128.39, 128.31, 127.99, 127.95, 127.87, 127.83, 127.77, 127.67, 117.27, 76.93, 74.94, 74.73, 73.98, 72.25, 72.12, 72.02, 71.51, 63.20, 34.32, 20.90; HRMS (FAB, M + Cs) calcd for $C_{32}H_{36}O_6Cs$ 649.1566, found 649.1589.

Scheme 4[a]



[a] Reagents and conditions: (a) FDP aldolase (200 U), pH = 6.7, 30 h, 25 °C; (b) O_3/78 °C, DMS/23 °C.

3-(6-*O*-Hexadecanyl-2,3,4-tri-*O*-benzyl-α-D-mannopyranosyl)propene (9b). To a solution of **8** (516 mg, 1 mmol) in anhydrous MeOH (5 mL) was added NaOMe (16.2 mg, 0.3 mmol) at 25 °C under argon. After 2 h, the mixture was neutralized with Dowex 50 × 8−100 acidic resin and the solution was filtered, washed with MeOH, and evaporated to yield the desired alcohol compound (450.2 mg, 95%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37−7.28 (m, 15 H), 5.68 (ddt, J = 17.2, 10.2, 7.0 Hz, 1 H), 5.05−4.98 (m, 2 H), 4.83 (d, J = 11.1 Hz, 1 H), 4.67−4.56 (m, 5 H), 4.03 (ddd, J = 9.0, 6.3, 3.1 Hz, 1 H), 3.85 (t, J = 8.0 Hz, 1 H), 3.81 (dd, J = 11.6, 6.1 Hz, 1 H), 3.78 (dd, J = 8.0, 3.1 Hz, 1 H), 3.72 (dd, J = 11.6, 3.1 Hz, 1 H), 3.64 (t, J = 3.1 Hz, 1 H), 3.61 (ddd, J = 8.0, 6.1, 3.1 Hz, 1 H), 2.36 (ddd, J = 14.8, 9.0, 7.0 Hz, 1 H), 2.22 (ddd, J = 14.8, 7.0, 6.3 Hz, 1 H), 2.1 (bs, 1 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.12, 138.05, 133.79, 128.43, 128.39, 128.33, 128.00, 127.97, 127.81, 127.74, 127.69, 117.60, 77.93, 75.18, 74.51, 73.84, 73.46, 72.16, 71.91, 62.15, 34.11; HRMS (FAB, M + Na) calcd for $C_{30}H_{34}O_5Na$ 497.2304, found 497.2315.

To a solution of above compound (1.00 g, 2.11 mmol) in DMF (7 mL) was added 95% NaH (0.10 g, 2.74 mmol) at 0 °C under argon. After 30 min, to the mixture was added 1-bromohexadecane (0.84 mL, 2.74 mmol) followed by tetra-*n*-butylammonium iodide (37.4 mg, 0.11 mmol) and the resulting solution was warmed up to 25 °C overnight. The reaction was quenched by H_2O (10 mL), then extracted with EtOAc (3 × 10 mL), and the combined organic layers were washed with brine, dried with $MgSO_4$, filtered, evaporated, and purified by column chromatography (hexane to EtOAc/hexane = 1/20) to yield ether **9b** (1.13 g, 77%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36−7.27 (m, 15 H), 5.79−5.68 (m, 1 H), 5.02−4.98 (m, 2 H), 4.74 (d, J = 11.4 Hz, 1 H), 4.61−4.54 (m, 5 H), 4.05 (dt, J = 7.5, 4.8 Hz, 1 H), 3.86 (t, J = 6.9 Hz, 1 H), 3.79−3.75 (m, 2 H), 3.71−3.63 (m, 2 H), 3.61 (dd, J = 4.5, 3.2 Hz, 1 H), 3.48−3.38 (m, 2 H), 2.39−2.26 (m, 2 H), 1.58−1.52 (m, 2 H), 1.25 (bs, 26

H), 0.88 (t, $J = 6.5$ Hz, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.36, 138.19, 138.13, 134.27, 128.34, 128.31, 128.27, 128.24, 127.97, 127.90, 127.84, 127.64, 127.56, 117.08, 76.84, 75.01, 74.82, 73.81, 73.46, 72.24, 71.93, 71.57, 71.36, 69.67, 34.55, 31.88, 29.65, 29.61, 29.51, 29.32, 26.13, 22.65, 14.09; HRMS (FAB, M + Cs) calcd for $C_{46}H_{66}O_5Cs$ 831.3965, found 831.3984.

***trans*-1-(2,3,4,6-Tetra-*O*-benzyl-α-D-mannopyranosyl)propene (10).** To a solution of the terminal olefin **9a** (500 mg, 0.887 mmol) in benzene (50 mL) was added $PdCl_2$ (catalytic), and the solution was heated to reflux for 24 h. The reaction mixture was filtered through Celite and evaporated, and the crude oil was purified by silica gel chromatography (EtOAc: hexane, 1:9 to 1:1), giving the internal olefin **10** in 46% yield (30 mg): ^{13}C NMR ($CDCl_3$, 100 MHz) δ 138.34, 138.29, 138.26, 129.66, 128.27, 128.25, 127.92, 127.87, 127.74, 127.57, 127.50, 127.39, 126.90, 78.60, 76.03, 75.20, 74.50, 73.88, 73.59, 73.27, 91.99, 71.56, 69.42, 18.07; HRMS calcd for $C_{37}H_{40}O_5$-Cs (M + Cs), 697.1930, found 697.1954.

1-(2,3,4,6-Tetra-*O*-benzyl-α-D-mannopyranosyl)formic Acid (11). To a solution of olefin **10** (230 mg, 0.407 mmol) in CH_2-Cl_2 (20 mL) at −78 °C was bubbled O_3 until a blue color persisted. To remove residual O_3, pure O_2 was bubbled through until the solution turned clear. DMS (1.0 mL) was added, and the reaction mixture was warmed to 23 °C and stirred for 24 h. The reaction mixture was concentrated under reduced pressure. The crude oil was used directly without further purification.

The aldehyde prepared above was dissolved in acetone (5 mL) and cooled to 0 °C. Jones reagent was added dropwise until an orange color persisted. ^{i}PrOH (1 mL) was added to quench any excess Jones reagent, and the reaction mixture was then partitioned between EtOAc (50 mL) and 1 N HCl (50 mL). The aqueous layer was extracted with EtOAc (50 mL), and the combined organic phases were dried ($MgSO_4$), concentrated under reduced pressure, and purified by silica gel flash chromatography (EtOAc:Hexane:HOAc, 3:1:0.01), giving the carboxylic acid **11** in 70% yield (two steps, 634 mg): HRMS calcd for $C_{35}H_{36}O_7Cs$ (M + Cs) 701.1515, found 701.1528.

Methyl 3-Allyl-2-amino-4,6-benzylidene-β-D-talopyranoside (12). To a solution of methyl β-D-galactopyranoside (10 g, 51.5 mmol) in CH_3CN (250 mL) was added benzaldehyde dimethyl acetal (15.6 mL, 103 mmol) followed by CSA (1.19 g, 5.15 mmol). After 30 min, Et_3N (1 mL) was added and the solvent was removed under reduced pressure and the crude solid was recrystallized from hot MeOH, affording the benzylidene acetal in good yield (85%): ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.55−7.35 (m, 5 H), 5.57 (s, 1 H), 4.36 (dd, $J = 1.4$, 12.4 Hz, 1 H), 4.23 (dd, $J = 1.2$, 3.8 Hz, 1 H), 4.22 (d, $J = 7.4$ Hz, 1 H), 4.10 (dd, $J = 1.9$, 12.5 Hz, 1 H), 3.60 (s, 3 H), 3.78−3.67 (m, 2 H), 3.51 (dd, $J = 1.6$, 3.0 Hz, 1 H), 3.49 (d, $J = 5.48$ Hz, 1 H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 138.0, 130.03, 129.0, 127.15, 104.38, 102.06, 75.75, 73.14, 72.18, 69.53, 67.06, 57.54; IR (neat) 3385 (br), 2966, 2872, 1466, 1551, 1403, 1366, 1173, 1078 cm^{-1}; MS calcd for $C_{14}H_{18}O_6Na$ (M + Na) 305.

To a solution of the above galactose benzylidene acetal (4.7 g, 16.7 mmol) in toluene (55 mL) was added Bu_2SnO (4.56 g, 18.3 mmol), and the solution was dehydrated using a Dean−Stark trap (130 °C, 2 h). The reaction mixture was cooled to 70 °C, and TBAI (4.3 g, 11.7 mmol) was added followed by allyl bromide (2.18 mL, 25 mmol). The solution was stirred at 130 °C for 24 h before being cooled to 23 °C and partitioned between EtOAc (200 mL) and saturated $NaHCO_3$ (200 mL). The aqueous layer was extracted with EtOAc (2 × vol), and the combined organic layers were dried ($MgSO_4$), concentrated under reduced pressure, and chromatographed (1:1 to 100% EtOAc/hexane), giving the product in low yield (35%): ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.60−7.25 (m, 5 H), 6.02−5.92 (m, 1 H), 5.55 (s, 1 H), 5.33 (m, 1 H), 5.22 (m, 1 H), 4.36 (dd, $J = 1.5$, 12.4 Hz, 1 H), 4.27 (d, 1 H), 4.26 (m, 1 H), 4.22 (m, 1 H), 4.09 (dd, $J = 1.9$, 12.4 Hz, 1 H), 3.95 (ddd, $J = 1.8$, 7.7, 9.7 Hz, 1 H), 3.59 (s, 3 H), 3.48 (dd, $J = 3.6$, 9.7 Hz, 1 H), 3.43 (m, 1 H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 138.56, 135.60, 129.66, 128.81, 127.12, 118.45, 104.48, 101.68, 79.42, 73.41, 70.99, 70.32, 69.71, 67.02, 57.33; IR (neat) 3419, 2966, 2871, 1450, 1401, 1370, 1079, 1050, 812 cm^{-1}; HRMS calcd for $C_{17}H_{22}O_6Na$ (M + Na) 345.1314, found 345.1316. Anal. Calcd for $C_{17}H_{22}O_6$: C, 63.34; H, 6.87. Found: C, 63.20; H, 6.85.

To a solution of $(COCl)_2$ (209 μL, 2.40 mmol) in CH_2Cl_2 (4 mL) at −78 °C was added DMSO (341 μL, 78.1 mmol). The reaction mixture was warmed to 0 °C for 5 min and then recooled to −78 °C. Methyl 3-allyl-4,6-benzylidene-2-hydroxy-β-D-galactopyranoside (704 mg, 2.19 mmol) was dissolved in CH_2Cl_2 (4 mL) and added slowly dropwise. The reaction mixture was stirred for 30 min, and DIPEA was added (1.48 mL, 10.9 mmol). The reaction mixture was warmed to 23 °C, diluted with CH_2Cl_2 (100 mL), washed with saturated $NaHCO_3$ (50 mL), and dried ($MgSO_4$). The crude product was used directly in the next step without further purification.

To a solution of the above ketone in MeOH (20 mL) was added NH_4OAc until the solution was saturated. Sodium cyanoborohydride (116 mg, 2.19 mmol) was added, and the reaction mixture was stirred for 48 h. The reaction mixture was partitioned between EtOAc (100 mL) and saturated $NaHCO_3$ (50 mL), and the aqueous layer was further extracted with EtOAc (2 × 50 mL). The combined organic layers were dried ($MgSO_4$), concentrated under reduced pressure, and chromatographed (5% MeOH/CH_2Cl_2), giving the amine **12** in good yield (77% two steps): ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.66−7.25 (m, 5 H), 5.99−5.88 (m, 1 H), 5.46 (s, 1 H), 5.32−5.26 (m, 1 H), 5.21−5.18 (m, 1 H), 4.36 (dd, $J = 1.4$, 12.5 Hz, 1 H), 4.25 (d, $J = 1.4$ Hz, 1 H), 4.20 (d, $J = 3.8$ Hz, 1 H), 4.21−4.07 (m, 1 H), 4.08 (dd, $J = 2.0$, 12.5 Hz, 1 H) 3.54 (s, 3 H), 3.54−3.48 (m, 1 H), 3.29 (d, $J = 1.3$ Hz, 1 H), 3.21 (d, $J = 4.0$ Hz, 1 H), 2.19 (s, 1 H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 138.41, 135.42, 129.71, 128.90, 127.0, 118.67, 103.01, 101,-97, 75.67, 73.76, 69.93, 69.51, 67.41, 57.22, 51.86; IR (neat) 3376, 3310, 2867, 1748, 1687, 1587, 1542, 1451, 1402, 1366 cm^{-1}; HRMS calcd for $C_{17}H_{24}O_5NNa$ (M + Na) 344.11474, found 345.1476.

Methyl 2-[*N*-[(2,3,4,6-Tetra-*O*-benzyl-α-D-mannopyranosyl)carbonyl]amino-3-allyl-4,6-*O*-dibenzylidinetalopyranoside (13). To a solution of compound **12** (65 mg, 0.203 mmol), mannose carboxylic acid **11** (150 mg, 0.264 mmol), NMM (45 μL, 0.407), and HOBt (41.1 mg, 0.305 mmol) in CH_2Cl_2 (3 mL) at 0 °C was added EDC (60.1 mg, 0.305 mmol). The reaction mixture was warmed to 23 °C and stirred for 24 h. The reaction mixture was diluted with EtOAc (50 mL) and washed successively with a 5% citric acid solution (20 mL) and saturated $NaHCO_3$ (20 mL). The solvent was removed under reduced pressure and dried ($MgSO_4$), and the crude oil was purified by silica gel chromatography (EtOAc:hexane, 1:3 to 3:1), giving the coupled product **13** in good yield (63%, 41 mg): ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.62−7.08 (m, 25 H), 5.85−5.70 (m, 1 H), 5.50 (s, 1 H), 5.16 (dd, $J = 1.3$, 17.2 Hz, 1 H), 4.97 (dd, $J = 0.72$, 9.6 Hz, 1 H), 4.88−4.27 (m, 4 H), 4.22 (d, $J = 3.2$ Hz, 1 H), 4.16 (t, $J = 9.6$ Hz, 1 H), 4.09 (dd, $J = 1.6$, 12.5 Hz, 1 H), 4.00−3.93 (m, 1 H), 3.84−3.75 (m, 3 H), 3.64−3.51 (m, 6 H), 3.40 (dd, $J = 2.5$, 11.4 Hz, 1 H), 3.32 (s, 1 H), 2.64 (dd, $J = 1.2$, 11.2 Hz, 1 H); HRMS calcd for $C_{52}H_{57}O_{11}NCs$ (M + Cs) 1004.2986, found 1004.2951.

Methyl 2-[*N*-[(2,3,4,6-Hydroxy-α-D-mannopyranosyl)carbonyl]amino]-3-carboxymethyl-4,6-hydroxytalopyranoside (1). To a solution of olefin **13** (100 mg, 0.114 mmol) in CH_2Cl_2 (10 mL) at −78 °C was bubbled O_3 until a blue color persisted. To remove residual O_3, pure O_2 was bubbled through until the solution turned clear. DMS (1.0 mL) was added, and the reaction mixture was warmed to 23 °C and stirred for 4 h. The reaction mixture concentrated under reduced pressure. The crude oil was used directly without further purification.

The aldehyde prepared above was dissolved in acetone (5 mL) and cooled to 0 °C. Jones reagent was added dropwise until a orange color persisted. ^{i}PrOH (1 mL) was added to quench any excess Jones reagent, and the reaction mixture was then partitioned between EtOAc (50 mL) and 1 N HCl (50 mL). The aqueous layer was extracted with EtOAc (50 mL), and the combined organic phases were dried ($MgSO_4$), concentrated under reduced pressure, and purified by silica gel flash chromatography (EtOAc:HOAc, 95:5), giving the carboxylic acid in 33% yield (two steps, 634 mg): ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.54−7.10 (m, 25 H), 5.48 (s, 1 H), 4.77−4.26 (m, 5 H), 4.09−3.93 (m, 3 H), 3.94 (d, J = 9.6 Hz, 1 H), 3.77 (s, 1 H), 3.64 (d, J = 7.2 Hz, 1 H), 3.53 (s, 3 H), 3.57−3.47 (m, 1 H), 3.31 (s, 1 H), 2.64 (d, J = 8.4 Hz, 1 H), 2.78 (d, J = 8.4 Hz, 1 H); HRMS calcd for $C_{51}H_{55}O_{13}$ NCs (M + Cs) 1022.2728, found 1022.2768.

To a solution of the above protected mimic (33 mg, 0.037 mmol) in 80% HOAc/H_2O (10 mL) was added a catalytic amount of Pd/C (Degussa type, 10% by wt). The solution was flushed with hydrogen for 30 min, then stirred for 24 h under a H_2 atmosphere. The reaction mixture was filtered through Celite and evaporated down under reduced pressure. The crude oil was further evaporated with H_2O (2 × 15 mL) and finally lyophilized, giving mimic **1** as a white hygroscopic solid: ^{1}H NMR (D_2O, 400 MHz) δ 8.02 (d, J = 7.6 Hz, 1 H), 4.58 (s, 2 H), 4.51 (s, 1 H), 4.48 (s, 1 H), 4.24−4.16 (m, 2 H), 4.07 (s, 1 H), 3.84−3.73 (m, 6 H), 3.62 (dd, J = 3.2, 4.0 Hz, 1 H), 3.55−3.45 (m, 5 H).

2-(2,3,4,6-Tetra-*O*-benzyl-α-D-mannopyranosyl)acetic Acid (14). To a solution of olefin **9a** (679 mg, 1.20 mmol) in CH_2Cl_2:MeOH (8 mL:4 mL) at −78 °C was bubbled O_3 in O_2 until a blue color persisted. To remove residual O_3, pure O_2 was bubbled through until the solution turned clear. DMS (1.7 mL, 24.0 mmol) was added, and the reaction mixture was warmed to 23 °C and stirred for 24 h. The reaction mixture was evaporated and partitioned between a saturated $NaHCO_3$ solution (50 mL) and EtOAc (5 mL). The aqueous phase was extracted with EtOAc (2 × 30 mL), and the combined organic phases were dried and concentrated under reduced pressure. The crude oil was used directly without further purification.

The aldehyde prepared above was dissolved in acetone (5 mL) and cooled to 0 °C. Jones reagent was added dropwise until a orange color persisted, which indicated the oxidation had gone to completion. ^{i}PrOH (1 mL) was added to quench any excess Jones reagent, and the reaction mixture was then partitioned between EtOAc (50 mL) and 1 N HCl (50 mL). The aqueous layer was extracted with EtOAc (50 mL), and the combined organic phases were dried ($MgSO_4$), concentrated under reduced pressure, and purified by silica gel flash chromatography (EtOAc:hexane:HOAc, 3:1:0.01), giving the carboxylic acid **14** in excellent yield (90%, 634 mg): ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.35−7.15 (m, 25 H), 4.02 (m, 1 H), 3.86 (dd, J = 7.3, 10.0 Hz, 1 H), 3.78 (dd, J = 2.7, 4.9 Hz, 1 H), 3.72 (dd, J = 3.2, 3.2 Hz, 1 H), 3.66−3.62 (m, 1 H), 3.61 (dd, J = 2.8, 7.6 Hz, 1 H), 2.77 (dd, J = 4.2, 15.8 Hz, 1 H), 2.55 (dd, J = 8.7, 15.8 Hz, 1 H); IR (neat) 3059, 2866, 1719, 1496, 1453, 1437, 1363, 1266, 1156, 1119 cm^{-1}; HRMS calcd for $C_{36}H_{38}O_7Cs$ (M + Cs) 715.1672, found 715.1680. Anal. Calcd for $C_{36}H_{38}O_7$: C, 74.20; H, 6.78. Found: C, 74.01; H, 6.50.

Dibenzyl *N*-[(2,3,4,6-Tetra-*O*-benzyl-α-D-mannopyranosyl)acetyl]-L-glutamate (16). To a solution of carboxylic acid **14** (50 mg, 0.086 mmol), H-Glu-$(OBn)_2$·pTsOH (0.095 mmol), HOBt (12.8 mg, 0.095 mmol), and NMM (10.3 μL, 0.095 mmol) in CH_2Cl_2 (500 μL) at 0 °C was added EDC (18 mg, 0.095 mmol). The reaction was allowed to stir for 24 h before being diluted with CH_2Cl_2 (50 mL) and washed successively with a 5% citric acid solution (25 mL), saturated $NaHCO_3$ solution (25 mL), and brine (25 mL). The organic phase was dried ($MgSO_4$), concentrated under reduced pressure, and purified by silica gel chromatography (EtOAc:hexane, 1:1), giving the coupled product **16** in good yield (80%): ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.35−7.15 (m, 35 H), 5.10 (s, 2 H), 5.03 (m, 2 H), 4.60 (ddd, J = 5.3, 8.3, 10.8 Hz, 1H), 4.51−4.35 (m, 8 H), 4.29 (ddd, J = 3.1, 8.7, 8.7 Hz, 1 H), 4.04−3.98 (m, 1 H), 3.84 (dd, J = 7.9, 10.2 Hz, 1 H), 3.72 (dd, J = 3.0, 5.1 Hz, 1 H), 3.63 (dd, J = 3.7, 5.2 Hz, 1H), 3.57 (dd, J = 3.0, 7.1 Hz, 1 H), 3.55 (dd, J = 4.7, 10.2 Hz, 1 H), 2.60 (dd, J = 3.3, 16.0 Hz, 1 H), 2.52 (dd, J = 9.0, 15.9 Hz, 1 H), 2.40−2.25 (m, 2 H), 2.16−2.07 (m, 1 H), 1.90−1.81 (m, 1 H); ^{13}C NMR ($CDCl_3$, 400 MHz) δ 171.36, 170.66, 138.07, 137.88, 137.78, 137.71, 130.32, 128.54, 128.47, 128.45, 128.39, 128.35, 128.32, 128.18, 128.14, 128.09, 128.04, 127.97, 127.94, 127.85, 127.78, 127.63, 75.25, 72.31, 74.24, 73.13, 72.80, 72.47, 71.46, 67.97, 67.73, 66.98, 53.45, 37.22, 36.73; IR (neat) 3336, 3062, 3029, 2919, 2861, 1741, 165, 1515, 1453, 1208, 1096 cm^{-1}; HRMS calcd for $C_{55}H_{58}O_{10}N$ (M + H) 892.4061, found 892.4095.

***N*-[(α-D-Mannopyranosyl)acetyl]-L-glutamic Acid (2).** To a solution of the benzyl-protected glycine aminoglycoside **16** (33 mg, 0.045 mmol) in 80% HOAc/H_2O was added a catalytic amount of Pd/C (Degussa type, 10% by weight). The solution was flushed with hydrogen for 30 min then stirred for 24 h under a H_2 atmosphere. The reaction mixture was filtered and evaporated down under reduced pressure. The crude oil was further evaporated with H_2O (2 × 5 mL) and finally lyophilized, giving mimic **2** as a white hygroscopic solid 990% yield): ^{1}H NMR (D_2O, 400 MHz) δ 4.37−4.42 (m, 1 H), 4.32 (ddd, J = 1.8, 5.0, 5.0 Hz, 1 H), 3.87 (t, J = 2.9 Hz, 1 H), 3.79 (dd, J = 3.3, 9.0 Hz, 1 H), 3.71−3.77 (m, 2 H), 3.67 (dd, J = 9.2, 9.2 Hz, 1 H), 3.53−3.60 (m, 1 H), 2.80 (dd, J = 10.8, 14.8 Hz, 1 H), 2.55 (dd, J = 5.1, 14.9 Hz, 1 H), 2.46 (dd, J = 7.0, 7.0 Hz, 2H), 2.11−2.20 (m, 1 H), 1.90−2.02 (m, 1 H); ^{13}C NMR (D_2O/DMSO, 100 MHz) δ 192, 187, 181.8, 84.2, 83.7, 79.9, 76.2, 70.0, 44.3, 35.1; HRMS calcd for $C_{13}H_{22}O_{10}N$ (M + H), 352.1244, found 352.1238.

Dibenzyl *N*-[(6-*O*-Hexadecanyl-2,3,4-tri-*O*-benzyl-α-D-mannopyranosyl)acetyl]-L-glutamate (18). To a solution of **9b** (1.13g, 1.62 mmol) in 1/1 acetone/H_2O (10 mL) was added NMO (0.24 g, 2.05 mmol) followed by a solution of OsO_4 in ^{t}BuOH (0.2 g, 2.5% w/w) at 25 °C. The solution was stirred for 16 h, and the reaction was quenched by the addition of $Na_2S_2O_3$ (0.2 g), Florisil (1 g), and H_2O (10 mL). The mixture was acidified to pH = 1 with 1 N HCl, and the resulting solution was extracted with EtOAc (3 × 20 mL). The combined organic layers were washed with saturated $NaHCO_3$ and brine, dried with $MgSO_4$, filtered, and evaporated to yield a 1/1 mixture of diastereomeric diols (1.2173 g).

To a solution of above diols (1.2173 g) in THF (8 mL) was added $NaIO_4$ (0.61 g) in one portion. The stirred slurry was added H_2O (8 mL) over 5 min, and the mixture was kept stirring for 2 h. The resulting solution was extracted with EtOAc (3 × 20 mL), and the combined organic layers were washed with

brine, dried with $MgSO_4$, filtered, and evaporated to yield the crude aldehyde (1.1090 g).

The above aldehyde (1.1090 g) was dissolved in acetone (7 mL), and the solution was cooled to 0 °C. The mixture was added Celite (0.8 g) in one portion followed by the addition of Jones reagent drop by drop until a orange color persisted, which indicated the oxidation had gone to completion. ^{i}PrOH (1 mL) was added to quench any excess Jones reagent, and the reaction mixture was then partitioned between EtOAc (50 mL) and 1 N HCl (50 mL). The aqueous layer was extracted with EtOAc (2 × 50 mL), and the combined organic phases were dried with ($MgSO_4$), concentrated, and purified by silica gel flash chromatography (EtOAc/hexane/AcOH, 3/1/0.01) to yield the carboxylic acid **17** (0.904 g, 78% in three steps): HRMS (FAB, M + Cs) calcd for $C_{45}H_{64}O_7Cs$ 849.3706, found 849.3729.

To a solution of carboxylic acid **17** (260 mg, 0.363 mmol) in CH_2Cl_2 (3 mL) were added BnO-Glu(OBn)-NH_2·*p*-TsOH (200 mg, 0.399 mmol), triethylamine (56 μL, 0.399 mmol), HOBt (54.0 mg, 0.399 mmol), and EDC (76.4 mg, 0.399 mmol) at 25 °C under argon. The reaction was allowed to stir for 24 h before being diluted with CH_2Cl_2 (20 mL) and washed successively with a 1 N hydrochloric acid solution (15 mL), saturated $NaHCO_3$ solution (15 mL), and brine (15 mL). The organic phase was dried with $MgSO_4$, concentrated, and purified by silica gel chromatography to yield **18** (271 mg, 73%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.33−7.22 (m, 25 H), 7.17 (d, J = 7.9 Hz, 1 H), 5.14 (s, 2 H), 5.07, 5.04 (ABq, J = 12.3 Hz, 2 H), 4.66 (dt, J = 7.9, 5.2 Hz, 1 H), 4.54−4.44 (m, 6 H), 4.29 (dt, J = 8.3, 3.3 Hz, 1 H), 3.98−3.95 (m, 1 H), 3.78−3.71 (m, 3 H), 3.61−3.58 (m, 2 H), 3.39−3.29 (m, 2 H), 2.61 (dd, J = 15.8, 3.2 Hz, 1 H), 2.52 (dd, J = 15.8, 8.7 Hz, 1 H), 2.43−2.30 (m, 2 H), 2.23−2.16 (m, 1 H), 1.98−1.91 (m, 1 H), 1.49 (t, J = 6.1 Hz, 2 H), 1.25 (bs, 26 H), 0.88 (dt, J = 6.8, 2.7 Hz, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.26, 171.35, 170.49, 137.81, 137.67, 135.70, 135.27, 128.46, 128.40, 128.32, 128.28, 128.22, 128.08, 128.04, 128.02, 127.78, 127.73, 127.69, 75.03, 74.27, 73.94, 73.89, 72.48, 72.28, 71.37, 68.31, 67.49, 66.90, 66.23, 51.37, 37.44, 31.82, 30.13, 29.61, 29.57, 29.45, 29.26, 27.02, 26.00, 22.59, 14.05; HRMS (FAB, M + Cs) calcd for $C_{64}H_{83}NO_{10}Cs$ 1158.5071, found 1158.5116.

***N*-[(6-*O*-Hexadecanyl-α-D-mannopyranosyl)acetyl]-L-glutamic Acid (3).** To a mixture of benzyl ether **18** (250 mg, 0.24 mmol) in HOAc/THF/H_2O (12 mL, 4/1/1) was added a catalytic amount of Pd/C (Degussa type, 10% by wt). The solution was flushed with hydrogen for 30 min, then stirred for 24 h under a H_2 atmosphere. The reaction mixture was filtered and coevaporated with toluene under reduced pressure. The crude produce was recrystallized from chloroform and hexane to yield mimetic **3** as a white solid (130 mg, 93%): ^{1}H NMR (400 MHz, CD_3OD/$CDCl_3$, 1/1) δ 4.52−4.48 (m, 1 H), 4.30−4.26 (m, 1 H), 3.76−3.69 (m, 6 H), 3.50 (t, J = 7.0 Hz, 2 H), 2.67 (dd, J = 14.9, 8.2 Hz, 1 H), 2.57 (dd, J = 14.9, 5.8 Hz, 1 H), 2.44−2.40 (m, 2 H), 2.26−2.22 (m, 1 H), 2.05−1.98 (m, 1 H), 1.59−1.56 (m, 2 H), 1.27 (bs, 26 H), 0.89 (t, J = 6.9 Hz, 3 H); HRMS (FAB, M + Na) calcd for $C_{29}H_{53}NO_{10}Na$ 598.3567, found 598.3576.

Ethyl 3-{*N*-[(6-*O*-Hexadecanyl-2,3,4-tri-*O*-benzyl-α-D-mannopyranosyl)acetyl]amino}benzoate (19). The preparation for the coupling of **17** and ethyl 3-aminobenzoate was followed by the above procedure to afford **19** in 63% yield: ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.09 (s, 1 H), 8.05 (t, J = 1.5 Hz, 1 H), 7.84 (d, J = 6.5 Hz, 1 H), 7.75 (dt, J = 6.5, 1.5 Hz, 1 H), 7.34−7.22 (m, 16 H), 4.60 (d, J = 12.1 Hz, 1 H), 4.56−4.48 (m, 5 H), 4.39−4.35 (m, 1 H), 4.35 (q, J = 7.1 Hz, 2 H), 4.16 (dt, J = 9.0, 3.5 Hz, 1 H), 4.00 (t, J = 10.0 Hz, 1 H), 3.80 (t, J = 3.7 Hz, 1 H), 3.63 (dd, J = 8.2, 2.9 Hz, 1 H), 3.59 (dd, J = 4.4, 3.2 Hz, 1 H), 3.41−3.38 (m, 1 H), 3.38 (t, J = 6.9 Hz, 2 H), 2.79 (dd, J = 16.0, 2.3 Hz, 1 H), 2.71 (dd, J = 16.0, 9.5 Hz, 1 H), 1.39−1.36 (m, 2 H), 1.37 (t, J = 7.1 Hz, 3 H), 1.26−1.12 (m, 26 H), 0.88 (t, J = 7.0 Hz, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.21, 166.25, 138.35, 137.69, 137.58, 137.44, 130.87, 128.64, 128.46, 128.42, 128.10, 127.90, 127.74, 124.90, 121.40, 75.39, 74.73, 74.55, 73.35, 72.80, 72.41, 71.77, 71.51, 67.75, 66.93, 60.88, 38.43, 31.86, 29.64, 29.60, 29.55, 29.49, 29.35, 29.31, 29.17, 25.82, 22.64, 14.27, 14.08; HRMS (FAB, M + Cs) calcd for $C_{54}H_{73}NO_8Cs$ 996.4391, found 996.4353.

3-{*N*-[(6-*O*-Hexadecanyl-α-D-mannopyranosyl)]acetyl]-amino}benzoic Acid (3a). An ice-cold solution of 0.25 M LiOH in 75% $MeOH_{(aq)}$ (5.3 mL) was added to a solution of **19** (230 mg, 0.27 mmol) in tetrahydrofuran (5 mL), and the mixture was vigorously stirred for 2 days at 4 °C. The resulting solution was acidified with 1 N HCl to pH 1−2 and extracted with EtOAc (3 × 10 mL). The combined organic layers were washed with brine, dried with $MgSO_4$, filtered, evaporated, and purified by a short column chromatography to yield free acid compound 193 mg (87%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.19 (s, 1 H), 8.06 (t, J = 1.7 Hz, 1 H), 8.02 (ddd, J = 7.9, 1.7, 1.2 Hz, 1 H), 7.78 (dt, J = 7.9, 1.2 Hz, 1 H), 7.37−7.23 (m, 16 H), 4.60 (d, J = 12.0 Hz, 1 H), 4.57−4.49 (m, 5 H), 4.40 (dt, J = 8.8, 2.5 Hz, 1 H), 4.17 (dt, J = 9.1, 3.4 Hz, 1 H), 4.00 (t, J = 9.9 Hz, 1 H), 3.81 (t, J = 3.7 Hz, 1 H), 3.65 (dd, J = 8.1, 2.9 Hz, 1 H), 3.61 (dd, J = 4.5, 3.2 Hz, 1 H), 3.43−3.41 (m, 1 H), 3.39 (t, J = 7.0 Hz, 2 H), 2.83 (dd, J = 16.2, 2.5 Hz, 1 H), 2.70 (dd, J = 16.2, 9.6 Hz, 1 H), 1.43−1.37 (m, 2 H), 1.31−1.13 (m, 26 H), 0.87 (t, J = 7.0 Hz, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.86, 169.36, 138.60, 137.68, 137.59, 137.39, 129.78, 128.89, 128.48, 128.45, 128.17, 127.95, 127.82, 127.79, 125.60, 125.43, 121.82, 75.41, 74.65, 74.46, 73.48, 72.74, 72.48, 71.81, 71.58, 67.83, 67.12, 38.64, 31.89, 29.68, 29.64, 29.60, 29.54, 29.41, 29.34, 29.20, 25.85, 22.66, 14.11; HRMS (FAB, M + Cs) calcd for $C_{52}H_{69}NO_8Cs$ 968.4078, found 968.4060.

The above acid was hydrogenated by following the previously described method to give compound **3a** in 91% yield: ^{1}H NMR (400 MHz, CD_3OD/$CDCl_3$ = 1/1) δ 8.16 (s, 1 H), 7.87 (d, J = 7.6 Hz, 1 H), 7.78 (d, J = 7.6 Hz, 1 H), 7.40 (t, J = 7.6 Hz, 1 H), 4.39−4.36 (m, 1 H), 4.10 (s, 1 H), 3.82−3.64 (m, 5 H), 3.47 (t, J = 7.1 Hz, 2 H), 2.81 (dd, J = 15.1, 9.9 Hz, 1 H), 2.67 (dd, J = 15.1, 4.6 Hz, 1 H), 1.44−1.18 (m, 28 H), 0.89 (t, J = 6.8 Hz, 3 H); HRMS (FAB, M + Cs) calcd for $C_{31}H_{51}NO_8Cs$ 698.2669, found 698.2684.

Compound 20. D-Mannose (1500 mg, 8.3 mmol) was added to 10 mL of allyl alcohol together with a catalytic amount (10 mg) of camphorsulfonic acid. The mixture was heated to 90 °C overnight, and then the excess allyl alcohol was evaporated *in vacuo*. Flash chromatography of the residue (EtOAc:MeOH, 6:1 to 4:1) gave 1400 mg (76%) of α-*O*-allylmannopyranoside: ^{13}C NMR (CD_3OD, δ, ppm) 136.31, 118.28, 101.48, 75.47, 73.55, 72.94, 69.67, 69.36, 63.68.

To a solution of the above compound (482 mg, 2.2 mmol) in CH_2Cl_2:MeOH (1:4) was bubbled ozone at −78 °C in the presence of a catalytic amount of $NaHCO_3$. After the blue color appeared, indicating that the solution was saturated with ozone, an excess (3 equiv) of Ph_3P was added. After stirring overnight, an extractive workup was performed (CH_2Cl_2/water), the aqueous portion was evaporated, and the residue showed the presence of only one aldehyde as the dihydrate as judged by ^{13}C NMR (CD_3OD, δ, ppm): 100.13, 88.22, 72.73, 70.36, 69.84, 66.66, 60.85.

Compound 20a. The ozonolysis of **9a** was performed as described above. The aldehyde product (2 mmol, 1.1 g) was

dissolved in a 3:1 mixture of THF and water and hydrogenated overnight at 1 atm in the presence of a catalytic amount of Pd−C. After filtration through Celite and evaporation of the solvent, the deprotected aldehyde **20a** (440 mg, 98%) was isolated: ^{13}C NMR (D_2O) major 88.36 (hydrate), 75.05, 74.13, 71.45, 70.68, 67.38, 61.30, 35.65 (CH_2); minor 204.35 (aldehyde), 74.70, 72.38, 70.68, 70.49, 67.29, 60.99, 42.23 (CH_2).

Enzymatic Aldol Reaction. An aldehyde (1.2−1.5 mmol) was dissolved in a solution of dihydroxyacetone phosphate (DHAP, 1 mmol, 3 mL of a 330 mM solution). The pH was adjusted to 6.7 by adding NaOH and 200 U of FDP aldolase (Sigma) was added. The progress of the reaction was followed by DHAP consumption (UV assay) and by ^{31}P-NMR spectroscopy. After DHAP had been consumed and ^{31}P-NMR showed the appearance of a new product, the pH was adjusted to 8 and 1 g of $BaCl_2 \cdot 2H_2O$ (4.4 mmol) in 5 mL of water was added slowly. The cloudy mixture was kept in an ice bath for 15 min, and the precipitates were removed by centrifugation. Two volumes of acetone were added to the supernate and the mixture stored at 0 °C for 1 h. The precipitates were collected by centrifugation, and the supernatant was discarded. The pellet was treated with Dowex-50 H^+ until the solid was completely dissolved (ca. 30 min), and the resin was filtered off. The pH of the filtrate was adjusted to 7.0 by adding NaOH. Lyophilization of the solution yielded a mixture of the phosphonates that were further separated by ion exchange (HCO_3^- column, 400 mM of $Et_3NH \cdot HCO_3$), converted to the H^+ form (Dowex-50 H^+) and lyophilized to yield the product.

Compound 4: ^{1}H NMR (D_2O, 400 MHz) δ 4.72 (d, $J = 1.7$ Hz, 1H), 4.69 (dd, $J = 18.4$, 6.7 Hz, 1H), 4.54 (dd, $J = 18.4$, 6.7 Hz, 1H), 4.41 ($J = 3$ Hz, 1H), 4.15 (m, $J = 8.7$, 5.7, 3 Hz, 1H), 3.83 (dd, $J = 3.3$, 1.7 Hz, 1H), 3.75−3.65 (m, 3H, 3.60 (m, 2H), 3.55−3.45 (m, 2H) ppm; ^{13}C NMR (D_2O, 100 MHz) δ 206.07, 96.49, 76.71, 73.72, 71.11, 70.94, 70.71, 69.18, 68.04, 65.71, 61.73 ppm.

Compound 4a: ^{1}H NMR (D_2O, 400 MHz) δ 4.55 (dd, $J = 18.7$, 6 Hz, 1H), 4.45 (dd, $J = 18.7$, 6, 1H), 4.33 (d, $J = 2$ Hz, 1H), 4.28 (ddd, J-10.4, 3, 2 Hz, 1H), 4.20 (ddd, $J = 11.5$, 3.6, 2 Hz, 1H), 3.77 (dd, $J = 3.3$, 9.3 Hz, 1H), 3.74 (dd, $J = 12.1$, 5.8 Hz, 1H), 3.67 (dd, $J = 9.3$, 3.3 Hz, 1H), 3.56 (dd, $J = 12.1$, 5.8 Hz, 1H), 3.48 (t, $J = 9.3$ Hz, 1H), 3.42 (ddd, $J = 9.3$, 5.8, 2.2 Hz, 1H), 1.92 (ddd, $J = 14.7$, 11.5, 3 Hz, 1H), 1.60 (ddd, $J = 14.7$, 10.4, 3.6 Hz, 1H) ppm; ^{13}C NMR (D_2O, 100 MHz) δ 212.60, 78.13, 75.11, 73.97, 72.09, 70.86, 68.40, 68.20, 67.65, 61.47, 30.49 ppm.

Acknowledgment. This research was supported by the NSF and Novartis Pharma AG.

JA970920Q