Synthesis of 1,1-Linked Galactosyl Mannosides Carrying a Thiazine Ring as Mimetics of Sialyl Lewis X Antigen: Investigation of the Effect of Carboxyl Group Orientation on P−Selectin Inhibition

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This paper describes the synthesis of 1,1-linked galactosyl mannosides as sialyl Lewis X mimetics that contain a spiro-ring to position the carboxylate group in a well-defined orientation. It was found that compound **4** is more active as a P-selectin inhibitor (IC_{50} = 19 μM) than the parent disaccharide **2**, which contains a flexible carboxyl group (IC_{50} = 193 μM). This result is consistent with that observed in the previous NMR study of sialyl Lewis X bound to P-selectin. The chemistry described here should be useful for the development of selective inhibitors of E-, P-, and L-selectins.

Development of small molecules as structural and functional mimics of complex carbohydrates is a subject of current interest.[1] An intensive effort in this regard is the development of simple and active compounds as mimics of the sialyl Lewis X (sLeX) tetrasaccharide (**1**),[2−5] a common ligand for E-, P-, and L-selectins involved in inflammatory reactions and metastasis.[6] Among such mimetics, β-D-galactopyranosyl-(1,1)-α-D-mannopyranoside with a flexible carboxymethyl group (**2**) is more active than sLeX in binding to E-, P-, and L-selectins.[3] We describe here the synthesis of related structures with a fixed orientation of the carboxyl group found in the bound form.

Despite some success in the synthesis of sLeX mimetics based on the functional group requirement for selectin binding (see Figure 1),[7] the bound conformations of sLeX remain unclear. A recent NMR study suggests that the ϕ and ψ angles around the sialyl glycoside are different between the solution conformer and the selectin-bound conformers.[8a−c] Both E- and P-selectins recognize a similar conformer that is different from the predominant solution conformer, and L-selectin recognizes the confor-

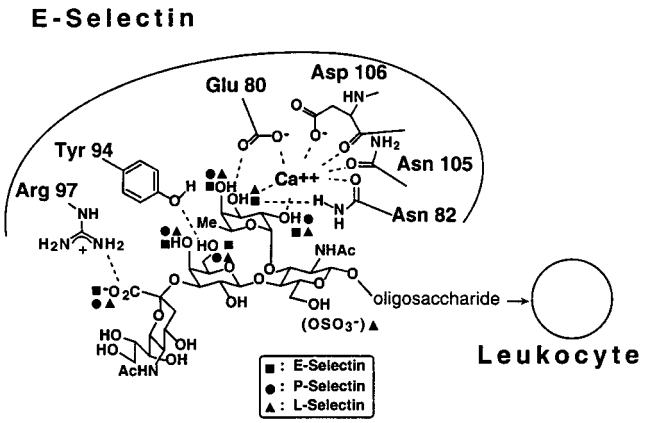

Figure 1. Functional groups of sialyl Lewis X recognized by E-, P-, and L-selectins.

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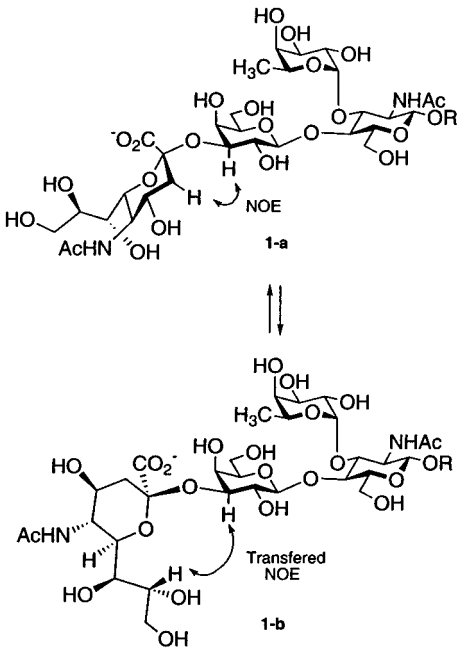


Figure 2. Schematic representation of rotamers around the glycosidic bond between sialic acid and galactose of sialyl Lewis X tetrasaccharide **1**. **1**-a: free and L-selectin-bound conformation determined by NMR. **1**-b: E- and P-selectin-bound conformation determined by transferred NOE.

mation similar to the predominant conformer in solution.[8d] (Figure 2).

Although an investigation of the orientation of the carboxyl group has been addressed and the synthetic mimetic found to be inactive,[9a] the result may be due to the presence of a large group close to the hydroxyl groups that are important for binding. An approach to overcome this problem is the introduction of a tether to support the carboxyl group in the required orientation from an unrecognized side of the galactose moiety. Attachment of a substituent to the α-position of the carboxymethyl group of the sialic acid mimic in order to restrict the orientation of the carboxyl group was found to produce effective E-selectin inhibitors.[9b,c] To introduce additional constraints, we decided to synthesize compounds **3** and **4** with the orientation of carboxyl group fixed by a spiro ring to mimic the conformer bound to L- and E/P-selectins, respectively (Figure 3).

Our initial attempt was to introduce a thiazine ring at C-3 of galactose using the protected 3-ulo compound **6** derived from **5**[10] by Swern oxidation followed by reaction with L- and D-cysteine. Reaction of **6** with

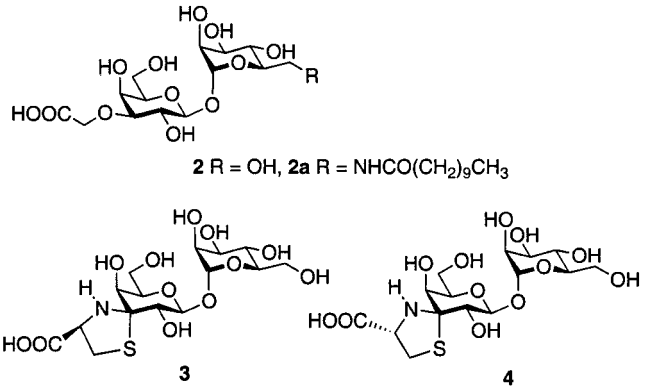
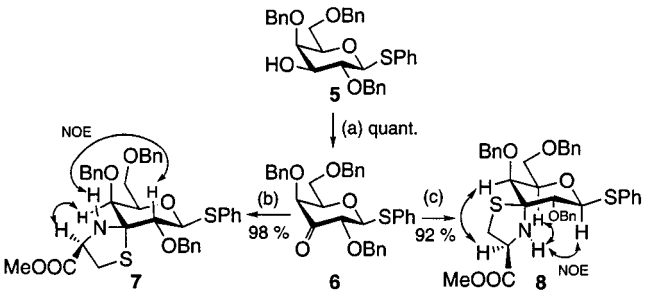


Figure 3. Structures of sLeX mimetic 1,1-linked galactosyl mannoside **2** and its derivatives **3** and **4** with fixed carboxyl group orientation.

Scheme 1[a]



[a] Reagents and conditions: (a) $(COCl)_2$−DMSO−Et_3N/CH_2Cl_2; (b) (i) L-cysteine−*p*TsOH·H_2O/DMF; ii. CH_2N_2/MeOH; (c) (i) D-cysteine−*p*TsOH·H_2O/DMF; (ii) CH_2N_2/MeOH.

L-cysteine in the presence of catalytic amounts of *p*-TsOH afforded the desired spiro compound **7** in high yield. However, reaction of **6** with D-cysteine gave, on the other hand, the undesired product **8** exclusively. The configurations of both compounds were evident from the NOE analyses as shown in Scheme 1. Further attempts to improve the yield of the desired compound failed. The reaction of disaccharide **10** derived from compound **9** was then attempted (Scheme 2), and compound **11** containing a fused L-cysteine residue and a 1:2 mixture of **12** and **13** containing a fused D-cysteine residue were obtained, respectively. The configuration of each compound was also supported by the NOE analysis. The preferred *R*- and *S*-configurations at the galactose C-3 position for the respective reactions with L- and D-cysteine were perhaps due to the steric interaction between the group of L- or D-cysteine and the 2-OH or the 2-OBn group of the iminium intermediate as shown in Figure 4, respectively. In any case, the observation that a single diastereomer is formed with both enantiomers of cysteine in the presence of TsOH is remarkable, given that under acidic deprotection conditions a mixture—presumably of stereoisomers—is formed. Additionally, formation of the thiazine without an acid catalyst also yields mixtures of stereoisomers. It is possible that under some of the conditions described the product distribution is controlled kinetically while under others a thermodynamic mixture is generated. Alternatively, the precise reaction conditions may determine the position of a thermodynamic equilibrium.

Deprotection of the benzyl groups in **11**−**13** was unsuccessful under various hydrogenolysis conditions. Although deprotected materials were obtained when TM-

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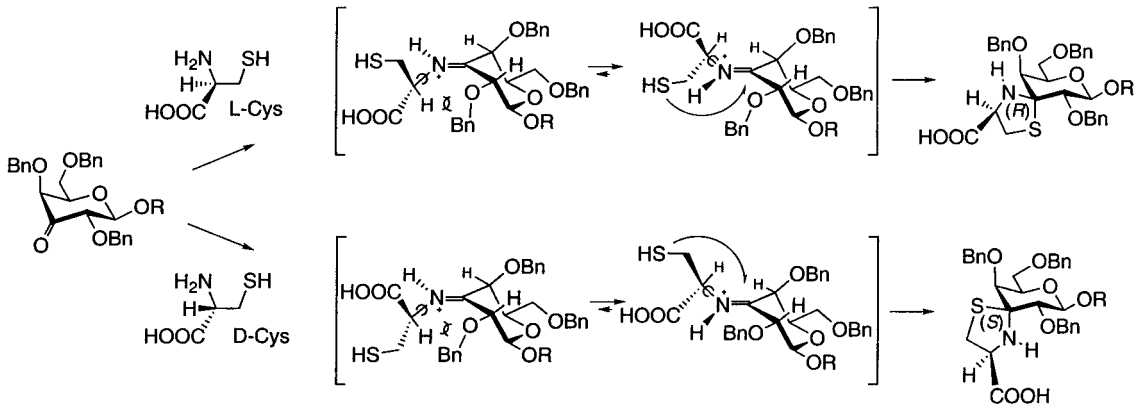
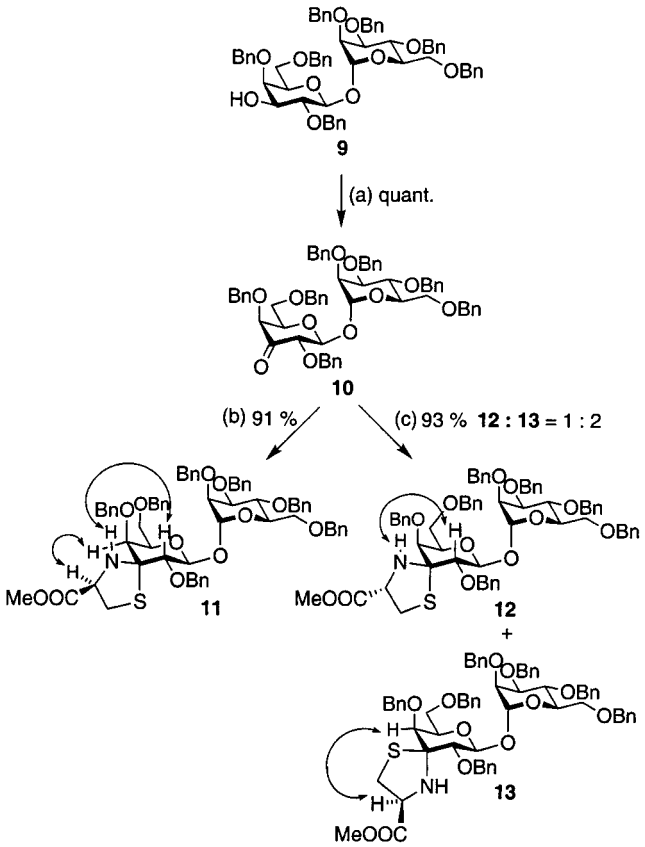


Figure 4. Synthesis of sLex mimics containing a thiazine ring. Other unfavorable rotamers around the N−Ca bond are omitted. Only preferred conformers are shown as the products.

Scheme 2[a]



[a] Reagents and conditions: Identical to those described in Scheme 1.

SOTf and thioanisole were used in TFA,[11] an equilibrium of the acetal through an imine intermediate occurred during the reaction to give a complex mixture leading to low yields.[12] Interestingly, the acylation reaction of the amine in order to stabilize the spiro acetal ring did not proceed at all in our hands.

Finally, we used an unprotected ulo-disaccharide (**14**) to avoid the difficulty of deprotections in the presence of thiazine ring (Scheme 3). Compound **14** obtained by hydrogenolysis under neutral conditions was used without further purification for the coupling with L- or D-cysteine in the absence of an acid catalyst. The reaction proceeded smoothly and yielded spiro compound **3** and approximately a 1:2 mixture of compounds **4** and **16**, which were isolable by silica gel column chromatography. It was found, however, that a slow equilibrium exists between **4** and **16** in water at room temperature (pH 7.4).

Compounds **2**, **3**, and **4** were then tested as inhibitors of P-selectin,[2] and the IC_{50} values were 193, 31, and 19 μM, respectively. Attachment of a long chain hydrophobic group to the mannose 6-position of **2** (see **2a**),[13] however, significantly improves the potency (IC_{50} = 3 μM for **2a**), indicating the importance of the hydrophobic effect on sugar affinity. While both **3** and **4** direct the carboxylate "down", that is, away from the C4′ hydroxyl of the galactose moiety, the two compounds direct the carboxylate in opposite directions. This observation would imply that fixing one of the two dihedrals of the parent compound was vital ("up" versus "down") but fixing the other was essentially unimportant ("left" versus "right"). Note that the flexible dihedrals of the parent compound are interchangeable in this regard. If this is indeed true then the enhancement in activity seems too large to be accounted for by a reduction in conformational entropy. The magnitude of such an enhancement would be no greater that 0.5 kcal mol^{-1}, while the enhancements for both compounds **3** and **4** are greater than 1 kcal mol^{-1} (assuming IC_{50} values are in fact measures of binding). Alternatively, if both dihedrals are important to affinity, then the difference in activity between **3** and **4** is too small, since one should presumably direct the carboxylate in the wrong direction for appropriate interaction with the protein. A more likely basis for the enhancement in affinity observed for both compounds is the increased hydrophobicity of **3** and **4** compared to the reference disaccharide, a concept apparently reinforced by our experiments.

In summary, we have developed a method for the preparation of sLeX mimetics with well-defined carboxyl group orientation for use to address the relationship between the binding activity and the glycosidic torsion angles of the two sugar groups. The strategy may find use in the development of selective inhibitors of selectins.

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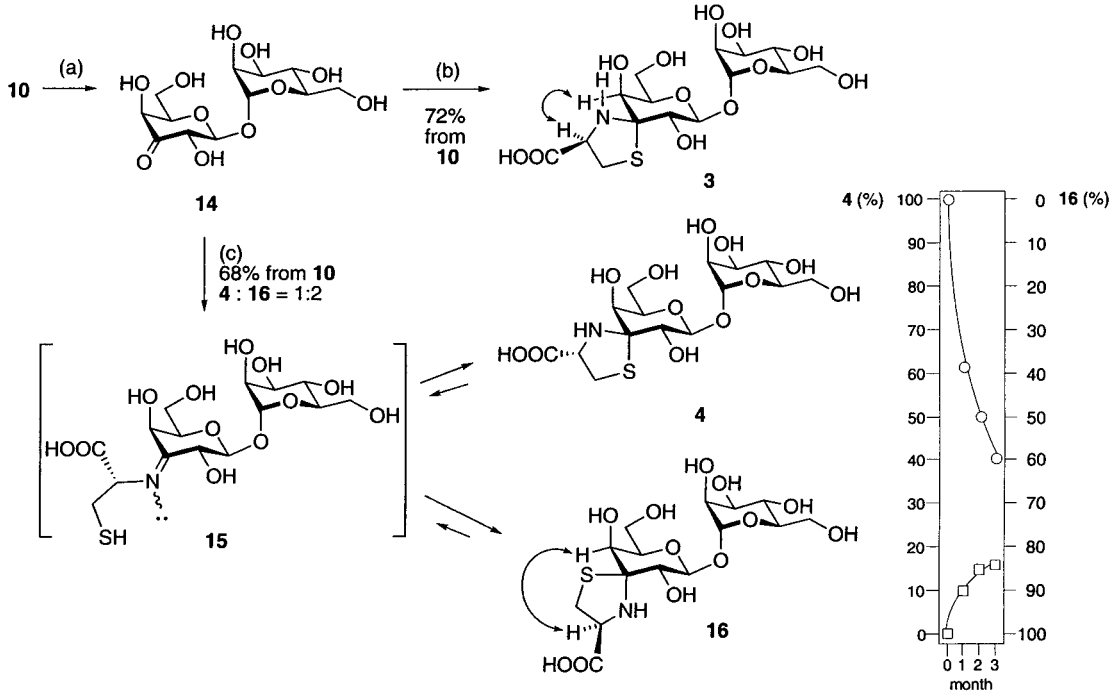
Scheme 3[a]



[a] Reagents and conditions: (a) $Pd(OH)_2-C-H_2/CHCl_3-MeOH$; (b) L-cysteine/DMF; (c) D-cysteine/DMF. Inset: The course of equilibrium between **4** and **16** is shown. The percentage of each compound was estimated by integration of ^1H NMR. □: equilibrium initiated with 100% **4**. ○: equilibrium initiated with 100% **16**. Although several minor signals were detectable in ^1H NMR spectra, the total amount of **4** and **16** was determined to be 100%.

Experimental Section

General Methods. Dried solvents were used for all reactions. Solutions were evaporated under diminished pressure at a bath temperature not exceeding 40 °C. Column chromatography was performed on silica gel. Gel permeation chromatography was performed using Bio Gel P-2. Optical rotations were measured in a 1.0 dm tube at 23 ± 1 °C. ^1H NMR spectra were recorded in $CDCl_3$ or D_2O using Me_4Si (δ 0.00) as the internal standard for solutions in $CDCl_3$ or DOH (δ 4.80 at 25 °C for ^1H NMR). ^{13}C NMR spectra were recorded in $CDCl_3$ or D_2O as indicated. Signals for aromatic carbons were omitted in ^{13}C NMR assignment. The electrospray mass spectra were recorded in MeOH−H_2O (1:1).

Phenyl 2,4,6-Tri-*O*-benzyl-1-thio-*β*-D-xylohexopyranosid-3-ulose (6). To a solution of oxalyl chloride [$(COCl)_2$, 0.84 mL, 9.59 mmol] in dichloromethane (CH_2Cl_2, 50 mL) was added dimethyl sulfoxide (DMSO, 1.02 mL, 14.39 mmol) dropwise at −78 °C. After the mixture was stirred for 30 min, a solution of compound **5** (1.3 g, 2.40 mmol) dissolved in CH_2Cl_2 (30 mL) was added dropwise and the mixture was stirred for 1 h at the temperature, then triethylamine (Et_3N, 2.67 mL, 19.19 mmol) was added and the mixture stirred stirred for 1 h. The mixture was poured into saturated NH_4Cl which was extracted with $CHCl_3$. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. Silica gel column chromatography of the residue using 4:1 hexanes−EtOAc gave **6** (1.28 g, 98.7%): $[\alpha]_D$ +20.5° (*c* 1.91, $CHCl_3$); ^1H NMR (270 MHz, $CDCl_3$) δ 7.60−7.09 (m, 20H), 4.75 (d, 1H, J = 9.8 Hz), 4.54 and 4.40 (AB, 2H, J = 11.4 Hz), 4.53 and 4.47 (AB, 2H, J = 11.9 Hz), 4.32 and 4.29 (AB, 2H, J = 11.9 Hz, 4.33 (d, 1H, J = 9.8 Hz), 3.93 (s, 1H, H-4), and 3.77 (s, 3H, H-5); ^{13}C NMR (68 MHz, $CDCl_3$) δ 204.74, 88.82, 81.87, 79.43, 77.22, 73.50, 73.35, 72.33, and 67.71.

Anal. Calcd for $C_{33}H_{32}O_5S$: C, 73.32; H, 5.97; S, 5.93. Found: C, 73.34; H, 5.96; S, 6.05.

Methyl 2′(*S*)-[Phenyl 3(*R*)-2,4,6-tri-*O*-benzyl-1-thio-*β*-D-xylopyranosid-3,5′-spiro[1,4]thiazin]-2′-carboxylate (7). A mixture of compound **6** (233.9 mg, 0.433 mmol), L-cysteine (157.4 mg, 1.299 mmol), *p*-toluenesulfonic acid monohydrate (TsOH, 8.2 mg, 0.043 mmol), and molecular sieves (MS AW-300, 200 mg) in DMF (10 mL) was heated at 60 °C for 3 h, and then pyridine (Pyr, 5 mL) was added to the mixture. The mixture was filtered through a Celite pad, washed with MeOH, and concentrated in vacuo. To a solution of the residue in MeOH (5 mL) was added a solution of diazomethane in ether. The mixture was kept until the starting material was consumed, as measured by TLC, and was concentrated after addition of AcOH and purified on a column of silica gel using 6:1 hexanes−EtOAc to give **7** (280.0 mg, 98.4%): $[\alpha]_D$ −22.1° (*c* 1.28, $CHCl_3$); ^1H NMR (270 MHz, $CDCl_3$) δ 7.60−7.15 (m, 20H), 5.06 and 4.65 (AB, 2H, J = 11.2 Hz,), 4.93 and 4.51 (AB, 2H, J = 11.4 Hz), 4.82 (d, 1H, J = 9.8 Hz), 4.42 and 4.47 (AB, 2H, J = 11.2 Hz), 4.20 (d, 1H, J = 9.8 Hz), 4.02 (br t, 1H, J = 6.3 Hz), 3.76 (ddd, 1H, J = 5.4, 10.4, 12.8 Hz), 3.75 (s, 3H), 3.65 (dd, 1H, J = 9.6, 5.0 Hz), 3.58 (dd, 1H, J = 9.6, 6.9 Hz), 3.62 (s, 1H), 3.17 (dd, 1H, J = 5.4, 10.4 Hz), 2.82 (d, 1H, J = 12.8 Hz), and 2.68 (t, 1H, J = 10.4 Hz); ^{13}C NMR (68 MHz, $CDCl_3$) δ 170.64, 88.93, 86.96, 78.33, 78.02, 77.76, 77.22, 75.78, 75.46, 75.15, 74.81, 73.46, 69.22, 63.60, 52.45, and 39.12.

Anal. Calcd for $C_{37}H_{39}NO_6S_2$: C, 67.57; H, 5.98; N, 2.13; S, 9.75. Found: C, 67.04; H, 5.93; N, 2.14; S, 9.79.

Methyl 2′(*R*)-[Phenyl 3(*S*)-2,4,6-tri-*O*-benzyl-1-thio-*β*-D-xylopyranosid-3,5′-spiro[1,4]thiazin]-2′-carboxylate (8). Compound **8** was synthesized as described for the synthesis of **7** using **6** (51.5 mg, 0.095 mmol), d-cysteine (34.7 mg, 0.286 mmol), TsOH (catalytic amount), MS AW-300 in DMF (2 mL) for acetalization, and diazomethane/MeOH for methylation: column 6:1 hexanes−EtOAc; yield 57.6 mg, 92%; $[\alpha]_D$ +18.9° (*c* 1.26, $CHCl_3$); ^1H NMR (270 MHz, $CDCl_3$) δ 7.64−7.13 (m, 20H), 4.98 and 4.58 (AB, 2H, J = 10.7 Hz), 4.53 and 4.49 (AB, 2H, J = 11.9 Hz), 4.88 (d, 1H, J = 9.9 Hz), 4.86 (s, 2H), 4.34 (t, 1H, J = 7.0 Hz), 4.05 (d, 1H, J = 9.9 Hz), 3.89 (ddd, 1H, J = 5.1, 10.6, 13.4 Hz), 3.76 (s, 3H), 3.67 (d, 2H, J = 7.0 Hz), 3.52 (s, 1H), 3.28 (d, 1H, J = 13.4 Hz), 3.20 (dd, 1H, J = 5.1, 10.6 Hz), and 2.66 (t, 1H, J = 10.6 Hz); ^{13}C NMR (68 MHz, $CDCl_3$) δ 170.80, 87.19, 86.54, 85.55, 80.81, 77.20, 76.75, 76.14, 75.69, 75.60, 73.51, 70.64, 68.59, 65.66, 54.41, 52.44, 47.59, and 39.34.

Anal. Calcd for $C_{37}H_{39}NO_6S_2$: C, 67.57; H, 5.98; N, 2.13; S, 9.75. Found: C, 67.68; H, 6.00; N, 2.10; S, 9.57.

2,3,4,6-Tetra-*O*-benzyl-α-D-mannopyranosyl-(1−1)-2,4,6-tri-*O*-benzyl-*β*-D-xylopyranosid-3-ulose (10). Compound **10** was synthesized as described for the synthesis of **6** using **9** (412.8 mg, 0.424 mmol), $(COCl)_2$ (0.11 mL, 1.274 mmol), DMSO (0.15 mL, 2.123 mmol), and Et_3N (0.3 mL, 2.123 mmol) in CH_2Cl_2 (20 mL): column 4:1 hexanes−EtOAc; yield 410 mg, quant; $[\alpha]_D$ −5.3° (*c* 0.66, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) *δ* 7.34−7.15 (m, 35H), 5.15 (d, 1H, *J* = 1.8 Hz), 4.68 (s, 2H), 4.88 and 4.49 (AB, 2H, *J* = 11.0 Hz), 4.59 (d, 1H, *J* = 7.8 Hz), 4.30 (d, 1H, *J* = 7.8 Hz), 4.68−4.32 (m, 10H), 4.08 (brd d, 1H, *J* = 2.6, 9.3 Hz), 4.04 (t, 1H, *J* = 9.3 Hz), 3.91 (dd, 1H, *J* = 3.0, 9.3 Hz), 3.85 (s, 1H), 3.74 (dd, 1H, *J* = 1.8, 3.0, 2.9 Hz), 3.73 and 3.58 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) *δ* 203.85, 102.86, 99.47, 82.40, 80.79, 79.74, 74.95, 74.70, 74.21, 73.65, 73.42, 73.34, 73.20, 72.65, 72.48, 72.42, 68.86, and 67.13.

Anal. Calcd for $C_{61}H_{62}O_{11}$: C, 75.44; H, 6.43. Found: C, 75.47; H, 6.64.

Methyl 2′(*S*)-[2,3,4,6-Tetra-*O*-benzyl-α-D-mannopyranosyl-(1−1)-3(*R*)-2,4,6-tri-*O*-benzyl-*β*-D-xylopyranosid-3,5′-spiro[1,4]thiazin]-2′-carboxylate (11). Compound **11** was synthesized as described for the synthesis of **7** using **10** (202.0 mg, 0.208 mmol), L-cysteine (75.7 mg, 0.625 mmol), TsOH (4 mg), and MS AW-300 (200 mg) in DMF (5 mL) for acetalization and diazomethane/MeOH for methylation: column 5:1 hexanes−EtOAc; yield 206.7 mg, 91.3%; $[\alpha]_D$ +7.2° (*c* 0.92, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) *δ* 7.38−7.16 (m, 35H), 5.21 (d, 1H, *J* = 1.8 Hz), 4.89 and 4.51 (AB, 2H, *J* = 11.4 Hz), 4.82 and 4.46 (AB, 2H, *J* = 11.3 Hz), 4.67 (d, 1H, *J* = 7.9 Hz), 4.72−4.29 (m, 10H), 4.11 (brd, 1H), 4.07 (t, 1H, *J* = 9.4 Hz), 3.96 (d, 1H, *J* = 6.3 Hz), 3.95 (dd, 1H, *J* = ca. 2.0, 9.4 Hz), 3.90 (d, 1H, *J* = 7.9 Hz), 3.75 (overlapped 4H), 3.71 (dd, 1H, *J* = 10.9, 3.5 Hz), 3.68 (dd, 1H, *J* = 1.8, 3.1 Hz), 3.59 (dd, 1H, *J* = 10.9, 1.5 Hz), 3.50 (s, 1H), 3.44 (d, 2H, *J* = 6.3 Hz), 3.20 (dd, 1H, *J* = 5.5, 10.1 Hz), 2.84 (d, 1H, *J* = 12.8 Hz), and 2.69 (t, 1H, *J* = 10.1 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) *δ* 170.72, 104.36, 99.64, 85.72, 79.48, 77.90, 76.19, 75.06, 74.89, 74.84, 74.70, 73.20, 73.13, 72.48, 72.43, 72.28, 68.77, 63.24, 52.42, and 39.47.

Anal. Calcd for $C_{65}H_{69}NO_{12}S$: C, 71.74; H, 6.39; N, 1.29; S, 2.95. Found: C, 71.70; H, 6.41; N, 1.20; S, 2.79.

Methyl 2′(*R*)-[2,3,4,6-Tetra-*O*-benzyl-α-D-mannopyranosyl-(1−1)-3(*R*)-2,4,6-tri-*O*-benzyl-*β*-D-xylopyranosid-3,5′-spiro[1,4]thiazin]-2′-carboxylate (12) and Methyl 2′-(*R*)-[2,3,4,6-Tetra-*O*-benzyl-α-D-mannopyranosyl-(1−1)-3(*S*)-2,4,6-tri-*O*-benzyl-*β*-D-xylopyranosid-3,5′-spiro-[1,4]thiazin]-2′-carboxylate (13). Compounds **12** and **13** were obtained under the conditions described for the synthesis of **7** using **10** (514.3 mg, 0.530 mmol), D-cysteine (192.5 mg, 1.589 mmol), TsOH (10 mg), MS AW-300 (500 mg) in DMF (15 mL) for acetalization, and diazomethane/MeOH for methylation: column gradient 8:1 → 6:1 hexanes−EtOAc; yield **13** (174.3 mg, 30.2%) and **12** (365.8 mg, 63.5%).

Data for compound **12**: $[\alpha]_D$ +56.9° (*c* 1.25, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) *δ* 7.34−7.16 (m, 35H), 5.20 (d, 1H, *J* = 1.7 Hz), 4.97 and 4.66 (AB, 2H, *J* = 11.6 Hz), 4.87 and 4.50 (AB, 2H, *J* = 10.5 Hz), 4.65 and 4.47 (AB, 2H, *J* = 11.6 Hz), 4.67 (d, 1H, *J* = 7.9 Hz), 4.62 and 4.52 (AB, 2H, *J* = 11.4 Hz), 4.60 and 4.36 (AB, 2H, *J* = 11.6 Hz), 4.36 and 4.32 (AB, 2H, *J* = 11.7 Hz), 4.13 (dd, 1H, *J* = 4.4, 6.3 Hz), 4.09 (brd, 1H), 4.05 (t, 1H, *J* = 9.3 Hz), 3.93 (dd, 1H, *J* = 3.2, 9.3 Hz), 3.77 (t, 1H, *J* = 6.6 Hz), 3.74 (s, 1H), 3.72 (dd, 1H, *J* = 10.5, 2.3 Hz), 3.70 (d, 1H, *J* = 6.1 Hz), 3.69 (s, 3H), 3.64 (dd, 1H, *J* = 1.7, 3.2 Hz), 3.60 (dd, 1H, *J* = 10.5, 1.5 Hz), 3.47 (dd, 1H, *J* = 9.9, 7.0 Hz), 3.44 (dd, 1H, *J* = 9.9, 6.0 Hz), 3.18 (dd, 1H, *J* = 6.3, 10.5 Hz), 3.08 (dd, 1H, *J* = 10.5, 4.4 Hz), and 2.34 (brd, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) *δ* 172.83, 103.77, 99.51, 83.01, 82.20, 80.62, 79.63, 75.14, 75.07, 74.98, 74.89, 74.38, 73.18, 73.13, 72.41, 72.26, 68.86, 68.76, 64.63, 52.30, and 37.59.

Anal. Calcd for $C_{65}H_{69}NO_{12}S$: C, 71.74; H, 6.39; N, 1.29; S, 2.95. Found: C, 71.81; H, 6.43; N, 1.19; S, 3.14.

Data for compound **13**: $[\alpha]_D$ +32.4° (*c* 0.61, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) *δ* 7.36−7.15 (m, 35H), 5.12 (d, 1H, *J* = 1.5 Hz), 4.92 and 4.54 (AB, 2H, *J* = 10.4 Hz), 4.86 and 4.48 (AB, 2H, *J* = 11.3 Hz), 4.83 and 4.65 (AB, 2H, *J* = 11.4 Hz), 4.74 (d, 1H, *J* = 9.0 Hz), 4.61 and 4.40 (AB, 2H, *J* = 12.2 Hz), 4.58 and 4.49 (AB, 2H, *J* = 11.6 Hz), 4.53 (s, 2H), 4.37 (s, 2H), 4.21 (dd, 1H, *J* = 5.9, 8.4 Hz), 4.09 (ddd, 1H, *J* = 1.5, 4.0, 10.0 Hz), 4.00 (t, 1H, *J* = 9.6 Hz), 3.89 (dd, 1H, *J* = 3.2, 9.3 Hz), 4.85 (ddd, 1H, *J* = 5.2, 10.7, 13.1 Hz), 3.79 (s, 3H), 3.77 (d, 1H, *J* = 9.0 Hz), 3.72 (dd, 1H, *J* = 9.1, 4.3 Hz), 3.61 (dd, 1H, *J* = 9.1, 2.0 Hz), 3.59 (d, 1H, *J* = 1.5 Hz), 3.56 (dd, 1H, *J* = 9.0, 8.4 Hz), 3.48 (dd, 1H, *J* = 9.0, 5.9 Hz), 3.39 (s, 1H), 3.18 (dd, 1H, *J* = 5.6, 10.5 Hz), 3.18 (d, 1H, *J* = 13.1 Hz), and 2.66 (t, 1H, *J* = 10.5, 4.4 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) *δ* 170.94, 101.40, 99.20, 85.31, 80.59, 79.80, 78.84, 76.23, 75.86, 75.07, 74.96, 74.84, 73.36, 73.13, 73.02, 72.38, 72.33, 72.29, 69.00, 68.46, 65.65, 52.43, and 39.30.

Anal. Calcd for $C_{65}H_{69}NO_{12}S$: C, 71.74; H, 6.39; N, 1.29; S, 2.95. Found: C, 71.82; H, 6.48; N, 1.20; S, 2.85.

2′(*S*)-[α-D-Mannopyranosyl-(1−1)-3(*R*)-*β*-D-xylopyranosid-3,5′-spiro[1,4]thiazin]-2′-carboxylic Acid (3). Compound **10** (82.7 mg, 0.085 mmol) was treated with 20% $Pd(OH)_2$−C (ca. 20 mg) in a 4:1 mixture of MeOH−$CHCl_3$ (1 mL) under H_2 atmosphere for 2 h. The reaction mixture was filtered and the filtrate containing **14** was directly poured into a mixture of L-cysteine (30.9 mg, 0.256 mmol) and MS AW 300 (ca. 50 mg) in DMF (1 mL), and the resulting mixture was stirred at 60 °C for 3 h. A residue obtained after filtration and concentration in vacuo was purified on a column of Bio gel P2 eluted with water to afford **3** (27 mg, 71.5 mg): ^{1}H NMR (500 MHz, D_2O) *δ* 5.15 (d, 1H, *J* = 1.4 Hz), 4.58 (d, 1H, *J* = 8.3 Hz), 4.04 (dd, 1H, *J* = 1.4, 3.2 Hz), 3.98 (dd, 1H, *J* = 5.0, 6.9 Hz), 3.93 (ddd, 1H, *J* = 2.3, 6.4, 10.1 Hz), 3.89 (d, 1H, *J* = 8.3 Hz), 3.87 (dd, 1H, *J* = 3.2, 9.6 Hz), 3.83 (s, 1H), 3.77 (dd, 1H, *J* = 6.4, 10.1 Hz), 3.66 (t, 1H, *J* = 10.1 Hz), 3.34 (dd, 1H, *J* = 6.0, 10.1 Hz), and 2.77 (t, 1H, *J* = 10.1 Hz); ^{13}C NMR (125 MHz, D_2O) *δ* 178.36, 104.13, 102.22, 86.12, 75.59, 74.59, 72.18, 71.24, 70.17, 69.78, 67.69, 66.16, 62.44, 61.92, 40.57; ESI MS *m*/*z* calcd for $C_{15}H_{25}NO_{12}S$ 443.1, negative mode:442.1 $[M - H]^-$, positive mode 466.1 $[M + Na]^+$.

2′(*R*)-[α-D-Mannopyranosyl-(1−1)-3(*R*)-*β*-D-xylopyranosid-3,5′-spiro[1,4]thiazin]-2′-carboxylic Acid (4) and 2′-(*R*)-[α-D-Mannopyranosyl-(1−1)-3(*S*)-*β*-D-xylopyranosid-3,5′-spiro[1,4]thiazin]-2′-carboxylic Acid (16). Compounds **4** and **16** were obtained under the conditions described for the synthesis of **3** using **10** (92.3 mg, 0.095 mmol), $Pd(OH)_2$ (ca. 20 mg), D-cysteine (34.6 mg, 0.285 mmol), and MS AW-300. A mixture of diastereomers obtained after column chromatography on Bio gel P2 (elution: water) was further purified using Iatro Beads (elution: 2:2:1 EtOAc−EtOH−H_2O) to afford **4** (21 mg, 49.9%) and **16** (10 mg, 23.7%).

Data for compound **4**: ^{1}H NMR (500 MHz, D_2O) *δ* 5.18 (s, 1H), 4.80 (signal overlapped with DOH), 4.13 (ddd, 1H, *J* = 1.0, 3.4, 6.8 Hz), 4.05 (dd, 1H, *J* = 1.8, 3.2 Hz), 3.93 (ddd, 1H, *J* = 1.9, 5.9, 10.0 Hz), 3.87 (d, *J* = 9.3 Hz), 3.85 (dd, 1H, *J* = 3.3, 9.6 Hz), 3.76 (dd, 1H, *J* = 5.8, 10.3 Hz), 3.61 (s, 1H), 3.24 (dd, 1H, *J* = 5.8, 10.3 Hz), and 2.73 (t, 1H, *J* = 10.3 Hz); ^{13}C NMR (125 MHz, D_2O) *δ* 177.53, 101.76, 101.34, 85.90, 75.58, 73.65, 72.08, 70.24, 69.94, 69.89, 68.45, 66.86, 61.68, 61.11, and 39.02.

Data for compound **16**: ^{1}H NMR (500 MHz, D_2O) *δ* 5.12 (d, 1H, *J* = 1.8 Hz), 4.54 (d, 1H, *J* = 8.2 Hz), 4.17 (t, 1H, *J* = 6.8 Hz), 4.03 (dd, 1H, *J* = 1.8, 3.3 Hz), 3.82 (s, 1H), 3.65 (t, 1H, *J* = 9.9 Hz), 3.28 (dd, 1H, *J* = 6.8, 10.5 Hz), and 3.14 (dd, 1H, *J* = 6.8, 10.5 Hz); ^{13}C NMR (125 MHz, D_2O) *δ* 179.04, 103.09, 101.49, 84.52, 76.18, 73.71, 71.67, 70.41, 69.88, 67.21, 66.85, 61.45, 61.10, and 38.51; ESI MS *m*/*z* calcd for $C_{15}H_{25}NO_{12}S$ 443.1, negative mode 442.1 $[M - H]^-$, positive mode 466.1 $[M + Na]^+$.

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Supporting Information Available: Copies of the ^{1}H NMR for compounds **2**−**4** and **16**. This material is available free of charge via the Internet at http://pubs.acs.org.

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