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SCIENCE DIRECT®

Tetrahedron Letters 44 (2003) 7767–7770

TETRAHEDRON LETTERS

A short route to L-iduronic acid building blocks for the syntheses of heparin-like disaccharides

Weijun Ke, Dennis M. Whitfield,* Manjinder Gill, Suzon Larocque and Siu-Hong Yu

Institute for Biological Sciences, National Research Council, Ottawa, ON, Canada K1A 0R6

Received 17 July 2003; revised 20 August 2003; accepted 21 August 2003

Abstract—The effective preparation of differentially protected L-iduronic acid derivatives, as building blocks for the synthesis of heparin-like oligosaccharides, is described in less than nine steps starting from readily available 1,2-*O*-isopropylidene-6,3-D-glucuronolactone. The pivaloyl group was used as a permanent protecting group of hydroxyl groups. Two heparin-like disaccharides with different sulfation pattern have been prepared by using these L-iduronic acid building blocks.
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Glycosaminoglycans (GAGs) and their binding proteins are integral to many physiological processes including inflammation, cell proliferation, and blood coagulation.[1,2] At present there is lack of specific inhibitors suitable for the intervention in GAG-mediated diseases. Cardiotoxins (CTXs) from cobra venom are highly basic, slightly curved, all β-sheet polypeptides capable of inducing general cytotoxic effect on many cell types.[3,4] They cause severe tissue necrosis and local gangrene in humans by an unknown mechanism.[5] In vitro GAG-binding studies of cardiotoxins (CTXs) established CTXs as specific GAG-binding toxins toward different types of GAG chains.[6] The molecular basis of CTX-GAG interactions remain elusive.

We have recently initiated a collaborative project directed to understand at the molecular level the binding specificity of GAGs to the CTXs in order to be able to identify specific inhibitors. To perform structure–activity relationship studies, we decided to synthesize a variety of monosaccharide building blocks, which could be coupled to form oligosaccharides that would serve to determine the key structural features necessary for binding to the cobra toxin proteins (CTXs).

L-Iduronic acid is a typical component of glycosaminoglycans (GAGs), where it plays an important role in various biological processes.[7] The preparation of L-iduronic acid derivatives is much more complicated than that of glucuronic acid derivatives since neither L-iduronic acid nor L-idose are available as starting materials. The preparation of L-ido synthons is a key point in glycoaminoglycan oligosaccharide synthesis and there is a constant need for their efficient preparation.[8] In this paper, we report a short synthetic route for preparing L-iduronic acid building blocks by which two disaccharides with different sulfation patterns were prepared.

After examining the literature and some experimentation, it was decided that the best route to L-iduronic acid building blocks was to start from the commercially available crystalline 1,2-*O*-isopropylidene-6,3-D-glucuronolactone **1**.[9–11] The stereochemistry at *C*-5 was inverted to the *O*-5 pivaloate **2** via triflate intermediate.[9] Then taking advantage of an *O*-5 to *O*-3 migration side reaction noted in Ref. 9, we were able to prepare multigram quantities of methyl ester **3** under the catalysis of the organic base triethylamine at 0°C. We have also shown that acetate and benzoate groups undergo this migration, but for our preparative purposes the pivaloate is the most useful. Hydrolysis of the isopropylidene acetal **3** was then performed in TFA–H_2O 9:1 to afford the L-iduronic acid derivative **4** according to Sinaÿ et al.[12] The key intermediate **5**[13] was then obtained by regio- and stereoselective silylation with dimethylthexylsilyl chloride (TDSCl) and imidazole, (CH_2Cl_2, −20°C) to afford almost exclusively the β-silyl glycoside in 35% yield from **3** (Scheme 1).[14] Analysis of the ^1H NMR (chloroform-d) spectrum of **5** indicate a preferred $^1C_4$ conformation for this compound ($J_{4,5}=1.6$ Hz ($^4C_1$: $J_{4,5}>4.2$ Hz)[10a] and $J_{1,2}=1.0$ Hz). This route is significantly shorter and higher yield-

Keywords: glycosylations; carbohydrates; heparin; disaccharides.
* Corresponding author. Tel.: +1-613-993-5265; fax: +1-613-952-9092; e-mail: dennis.whitfield@nrc-cnrc.gc.ca

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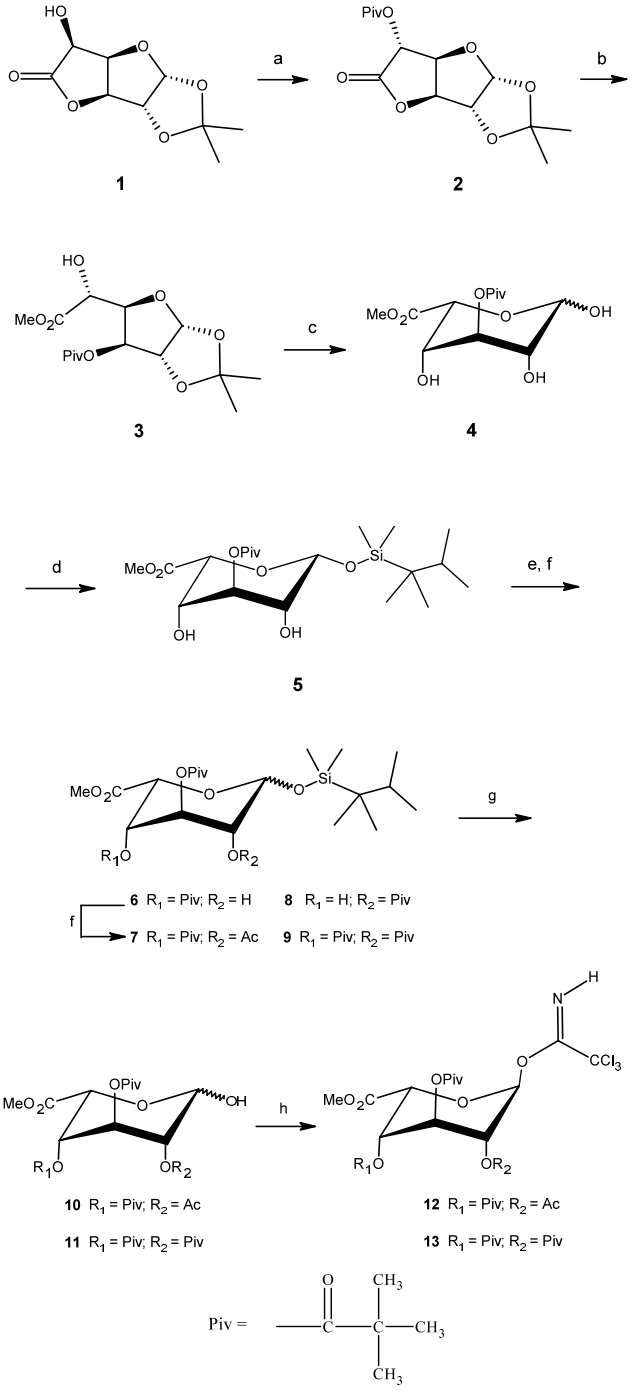
doi:10.1016/j.tetlet.2003.08.092



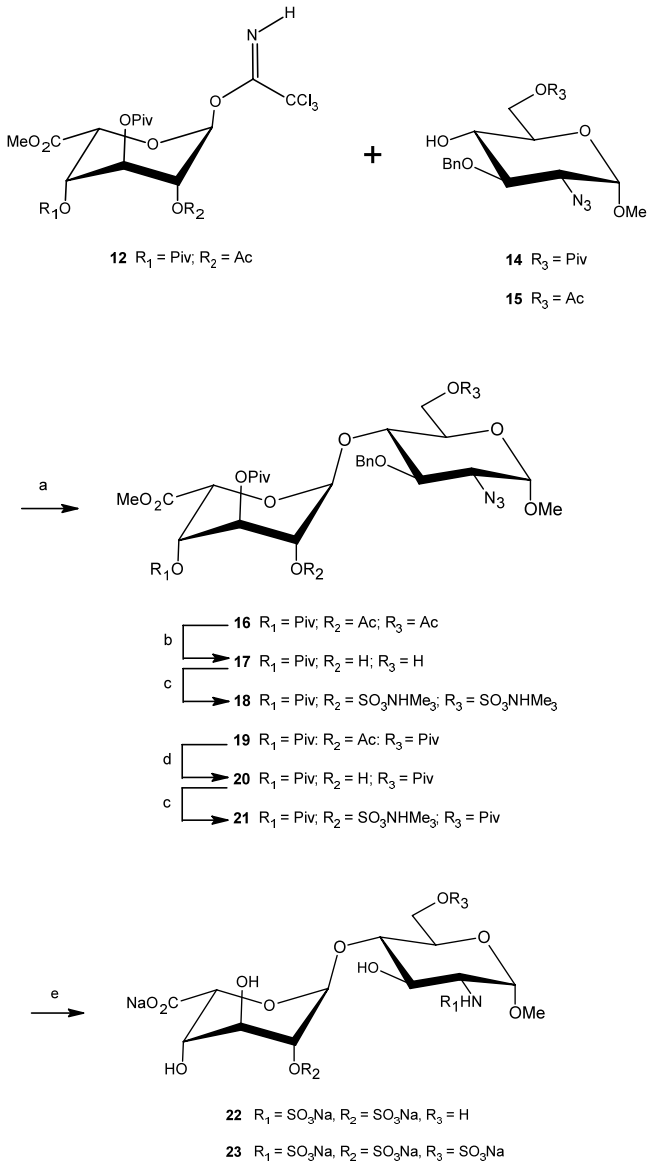
Scheme 1. *Reagents and conditions*: (a) i. Tf_2O, Py; ii. NaOPiv, DMF; (b) Et_3N, MeOH, 0°C, O/N; (c) 90% TFA, rt, 3 h; (d) imidazole, CH_2Cl_2, –20°C, O/N; (e) Piv_2O, $Sc(OTf)_3$, CH_2Cl_2; (f) Ac_2O, $Sc(OTf)_3$, CH_2Cl_2, rt, O/N; (g) CH_3COOH, Bu_4NF, THF, 10–15°C, 5 h; (h) CCl_3CN, DBU, CH_2Cl_2, 0°C, 1 h.

ing than any previously reported route to similar L-iduronic acid derivatives.

The OH-2 and OH-4 of sugar **5** have similar reactivities, but for our purposes they must be differentially protected. It should be noted that conventional *O*-acylation with acyl chlorides or anhydrides in pyridine was very slow and did not allow for successful monoacylation. However, we developed the use of catalytic scandium trifluoromethanesulfonate, $Sc(OTf)_3$, that allowed for acylation to give a mixture of *O*-4, *O*-2 monoacylates and diacylate.[15] For example, pivaloylation of the key intermediate **5** with 1.1 equiv. of pivaloic anhydride at 10°C for 1.5 h gave a mixture of *O*-4 pivaloate **6**, *O*-2 pivaloate **8**, *O*-4 and *O*-2 dipivaloate **9** and traces of unreacted diol **5**. Pure pivaloates **6**, **8** and **9** could easily be separated from the mixture on an MPLC chromatography column eluting with Hex:EtOAc 10:1 (**6**:**8**:**9** = 9:6:5). The ratio of **6**, **8** and **9** could be varied by the reaction temperature, time and even solvent condition. Acetylation of **6** under the catalysis of $Sc(OTf)_3$ at room temperature overnight gave a mixture of α anomer and β anomer of the corresponding *O*-2 acetylated silyl glycoside **7**.[16] Silica gel chromatography afforded pure α-anomer and β-anomer (α:β = 5:8). Glycoside **7** was then deprotected with CH_3COOH and Bu_4NF (THF, 10–15°C) to give the intermediate **10**. Standard protecting group manipulation allowed for the formation of the trichloroacetimidate **12**[17] in 55% yield by treatment of intermediate **10** with CCl_3CN under the catalysis of DBU at 0°C.

The *O*-2 acetylated donor **12** allows for the preparation of *O*-2-sulfated L-iduronic acid containing oligosaccharides. ^1H NMR analysis of building block **12** revealed a $^1C_4$ conformation as judged by the coupling constant ($J_{4,5} = 2.3$ Hz and $J_{1,2} < 1.0$ Hz). Interestingly, a NOESY experiment showed that donor **12** has an α configuration (a strong NOE between H-1 and H-2, but not to H-5, was observed). Similarly, donor **13**[18] was also obtained from the tripivaloate **9** via the intermediate **11**. ^1H NMR analysis of building block **13** also revealed a predominant $^1C_4$ conformation ($J_{1,2} = 3.1$ Hz, $J_{2,3} = 4.4$ Hz, $J_{3,4} = 4.6$ Hz, $J_{4,5} = 3.5$ Hz). This L-iduronic acid building block can be used to make only *N*-sulfated or *N*-acetylated oligosaccharides as well as for testing the glycosylation chemistry. Other variants can easily be constructed from **5**.

To test the protecting group strategy and the glycosylation chemistry, donor **12** was coupled with acceptor **14** at –20°C under promotion by triethylsilyltrifluoromethanesulfonate (TESOTf) to yield disaccharide **16** in 85% isolated yield (Scheme 2). Similarly, the disaccharide **19** was also obtained by coupling donor **12** with acceptor **15**[19] at –15 to –12°C in a yield of 64%. The key to the deprotection is the selective removal of the *O*-acetyl groups in the presence of *O*-pivaloyl groups. When treating **16** with 7.5% HCl in methanol,[20] the reaction was slow and the yield was not satisfactory due to migration of the pivaloyl group from *O*-4 of iduronic acid to *O*-2. To ensure that the *O*-acetate of disaccharide **16** could be cleaved in the presence of the *O*-pivaloate in an effective way, the organic base DBU was tried and a methanol solution of **16** was treated at –20°C with 0.1 M DBU to give deacetylated disaccharide **17** with the yield of 55%.[21] Interestingly, treatment of disaccharide **19** with DBU under the same condition did not lead to the deacetylated disaccharide **20** effectively. Fortunately, the deacetylated disaccharide **20**



Scheme 2. *Reagents and conditions*: (a) TESOTf, 4 Å MS, CH_2Cl_2, 3 h; (b) 7.5% HCl, MeOH, 0°C, O/N; (c) SO_3:NMe_3, DMF, 60°C, O/N; (d) 0.1 DBU/MeOH, –20°C, 3 h; (e) i. H_2O_2, LiOH, THF, NaOH/H_2O, rt, 36 h, ii. 10% Pd/C, H_2, *t*-BuOH/H_2O, rt, O/N; (iii) SO_3:Py, NaOH/H_2O, pH 9.8, rt, 3 h.

could be obtained in a good yield of 81% by treatment of disaccharide **19** with 7.5% HCl in methanol at room temperature overnight.[20] Then *O*-sulfation of disaccharides **17** and **20** with SO_3:$(CH_3)_3N$ complex in DMF at 60°C overnight led to disaccharides **18** (yield of 88%) and **21** (yield of 83%), respectively. The *O*-sulfated disaccharides were then converted into the known[22] target heparin-like disaccharides **22**[23] and **23**[24] using a series of well known reactions (saponification, hydrogenation and *N*-sulfation).[25]

In conclusion, a simple and convenient synthetic route for preparing L-iduronic acid building blocks was developed. Two heparin-like disaccharides with different sulfation patterns have been prepared by using the L-iduronic acid building block developed by this synthetic route, and their binding properties with CTX A3 are being studied. The detailed syntheses and binding studies will be reported in another paper. Thus, an effective synthetic strategy was developed for preparing heparin-like oligosaccharide chains with defined size, sequence and charge distribution.

Acknowledgements

This research was supported by NSC Grant 89-2311-B-007-045 and an NSC-NRC Joint Research Project Grant (N-001). Ken Chan is thanked for obtaining the mass spectra. Suzon Larocque is thanked for her assistance in performing the NMR studies. This is NRC paper # 42477.

References

1. Jackson, R. L.; Busch, S. J.; Cardin, A. D. *Physiol. Rev.* **1991**, *71*, 481–539.
2. (a) Conrad, H. E. *Heparin Binding Proteins*; Academic Press: New York, 1998; (b) Capila, I.; Linhardt, R. *Angew. Chem.* **2002**, *114*, 426–450; *Angew. Chem., Int. Ed.* **2002**, *41*, 390–412.
3. Dufton, M. J.; Hider, R. C. In *Snake Venom*; Harvey, A. L., Ed.; Pergamon Press: New York, 1991; pp. 259–272.
4. Fletcher, J. E.; Jiang, M.-S. *Toxicon* **1993**, *31*, 669–695.
5. *Snake Venom: Handbook of Experimental Pharmacology*; Lee, C. Y., Ed.; Springer-Verlag: Berlin, 1979; Vol. 52.
6. (a) Sue, S.-C.; Brisson, J.-R.; Chang, S.-C.; Huang, W.-N.; Lee, S.-C.; Jarrell, H. C.; Wu, W. *Biochemistry* **2001**, *40*, 10436–10446; (b) Vyas, A. A.; Pan, J.-J.; Patel, H. V.; Vyas, K. A.; Chiang, C.-M.; Sheu, Y.-C.; Hwang, J.-K.; Wu, W. *J. Biol. Chem.* **1997**, *172*, 9661–9670; (c) Vyas, K. A.; Patel, H. V.; Vyas, A. A.; Wu, W. *Biochemistry* **1998**, *37*, 4527–4534.
7. (a) Fransson, L. A. In *The Polysaccharides*; Aspinall, G. O., Ed.; Academic Press, 1985; Vol. 3, pp. 337–415; (b) Kjellen, L.; Lindahl, U. *Annu. Rev. Biochem.* **1991**, *60*, 443–475; (c) *Heparin*; Lane, D. A.; Lindahl, U., Eds.; CRC Press: Florida, 1989.
8. Pellissier, H. *Org. Prep. Proc. Int.* **2002**, *34*, 443–465.
9. Ojeda, R.; de Paz, J. L.; Martin-Lomas, M.; Lassaletta, J. M. *Synlett* **1999**, 1316–1318.
10. (a) Whitfield, D. M.; Birnbaum, G. I.; Pang, H.; Baptista, J.; Sarkar, B. *J. Carbohydr. Chem.* **1991**, *10*, 329–348; (b) Haller, M.; Boons, G.-J. *J. Chem. Soc., Perkin Trans. 1* **2001**, 814–822; (c) Haller, M. F.; Boons, G.-J. *Eur. J. Org. Chem.* **2002**, 2033–2038.
11. (a) Orgueira, H. A.; Bartolozzi, A.; Schell, P.; Litjens, R. E. J. N.; Palmacci, E. R.; Seeberger, P. H. *Chem. Eur. J.* **2003**, *9*, 140–169; (b) Medakovié, D. *Carbohydr. Res.* **1994**, *353*, 299–300; (c) Dilhas, A.; Bonnaffé, D. *Carbohydr. Res.* **2003**, *338*, 681–686; (d) Kornilov, A. V.; Sukhova, E. V.; Nifantiev, N. E. *Carbohydr. Res.* **2001**, *336*, 309–313.
12. Jacquinet, J.-C.; Petitou, M.; Duchaussoy, P.; Lederman, I.; Choay, J.; Torri, G.; Sinaÿ, P. *Carbohydr. Res.* **1984**, *130*, 221–241.

13. **5**: Colorless syrup, $[\alpha]_D = +27.2$ (c 1.0 $CHCl_3$); ^1H NMR (399.89 MHz; $CDCl_3$, 25°C): δ 5.22 (br t, 1H), 4.93 (d, $J = 1.0$ Hz, 1H), 4.32 (d, $J = 1.6$ Hz, 1H), 3.87 (m, 1H), 3.77 (s, 3H), 3.59 (m, 1H), 3.14 (br, 1H), 1.63 (m, 1H), 1.20, 1.20 (2×s, 2×9H), 0.85 (m, 12H), 0.25, 0.19 (2×s, 2×3H); ^{13}C NMR (50.32 MHz; $CDCl_3$, 22.1°C): δ 175.88, 168.35, 93.49, 74.64, 69.00, 68.63, 66.99, 52.24, 38.75, 33.93, 27.02, 24.98, 20.16, 19.89, 18.54, 18.34, −2.02, −3.69; MS: $m/z = 435.2$ $[M+H]^+$. ^1H and ^{13}C NMR for all other compounds were obtained under the same conditions unless otherwise noted.
14. Wetter, H.; Oertle, K. *Tetrahedron Lett*. **1985**, *26*, 5515–5518.
15. Mehta, S.; Whitfield, D. *Tetrahedron Lett*. **1998**, *39*, 5907–5910.
16. **7**: α-anomer: colorless syrup, $[\alpha]_D = +38.5$ (c 1.01 $CHCl_3$); ^1H NMR: δ 5.12 (br t, 1H), 5.03 (d, $J = 1.8$ Hz, 1H), 4.98 (m, 1H), 4.90 (m, 1H), 4.49 (d, $J = 2.5$ Hz, 1H), 3.73 (s, 3H), 2.06 (s, 3H), 1.61 (m, 1H), 1.23, 1.19 (2×s, 2×9H), 0.85 (m, 12H), 0.22, 0.15 (2×s, 2×3H); ^{13}C NMR: δ 176.67, 175.28, 169.40, 167.17, 93.08, 72.86, 67.90, 66.72, 66.08, 52.21, 38.81, 33.92, 27.10, 26.95, 25.00, 20.87, 20.11, 19.89, 18.55, 18.41, −2.01, −3.33; MS: $m/z = 401.3$ $[M-OSithexyl]^+$; β-anomer: colorless syrup, $[\alpha]_D = -52.9$ (c 1.01 $CHCl_3$); ^1H NMR: δ 5. 39 (d, $J = 2.6$ Hz 1H), 5.10 (br t, 1H), 5.07 (br t, 1H), 4.87 (d, $J = 3.3$ Hz, 1H), 4.70 (br t, 1H), 3.75 (s, 3H), 2.03 (s, 3H), 1.63 (m, 1H), 1.21, 1.18 (2×s, 2×9H), 0.86 (m, 12H), 0.19, 0.17 (2×s, 2×3H); ^{13}C NMR: δ 176.69, 176.45, 169.33, 168.50, 92.93, 69.43, 67.46, 67.16, 67.10, 52.40, 38.81, 38.78, 33.82, 27.06, 26.97, 25.04, 20.85, 20.04, 20.01, 18.48, −2.46, −3.21; MS: $m/z = 561.4$ $[M+H]^+$.
17. **12**: White solid, $[\alpha]_D = -49.7$ (c 1.88 $CHCl_3$); ^1H NMR: δ 8.86 (s, 1H), 6.49 (s, 1H), 5.13 (m, 2H), 4.97 (m, 1H), 4.89 (d, $J = 2.3$ Hz, 1H), 3.74 (s, 3H), 2.07 (s, 3H), 1.23, 1.18 (2×s, 2×9H); ^{13}C NMR: δ 176.48, 176.26, 168.92, 167.33, 159.80, 94.00, 90.62, 68.12, 66.12, 52.62, 38.89, 38.85, 27.08, 26.93, 20.72; MS: $m/z = 401.3$ $[M-OTCI]^+$.
18. **13**: White solid, $[\alpha]_D = -57.3$ (c 1.0 $CHCl_3$); ^1H NMR: δ 8.81 (s, 1H), 6.51 (d, $J = 3.1$ Hz, 1H), 5.26 (br t, 1H), 5.21 (br t, 1H), 5.07 (br t, 1H), 4.89 (d, $J = 3.5$ Hz, 1H), 3.75 (s, 3H), 1.22, 1.21, 1.19 (3×s, 3×9H); ^{13}C NMR: δ 176.57, 176.46, 176.06, 167.19, 159.77, 94.56, 90.54, 69.13, 67.80, 67.49, 67.09, 52.62, 38.99, 38.92, 27.22; MS: $m/z = 443.3$ $[M-OTCI]^+$.
19. Tabeur, C.; Machetto, F.; Mallet, J.-M.; Duchaussoy, P.; Petitou, M.; Sinaÿ, P. *Carbohydr. Res*. **1996**, *281*, 253–276.
20. (a) Coleman, R. S.; Grant, E. B. *J. Am. Chem. Soc*. **1994**, *116*, 8795–8796; (b) Pinto, B. M.; Morissette, D. G.; Bundle, D. R. *J. Chem. Soc., Perkin Trans. 1* **1987**, 9–14.
21. *Protective Groups in Organic Synthesis*, 3rd ed.; Greene, T. W.; Wuts, P. G. M., Eds.; John Wiley & Sons: New York, 1999; pp. 179–188.
22. Jaurand, G.; Tabeur, C.; Petitou, M. *Carbohydr. Res*. **1994**, *255*, 295–301.
23. **22**: White solid. ^1H NMR (D_2O): δ 5.13 (br s, 1H), 4.97 (d, $J = 3.3$ Hz, 1H), 4.72 (d, $J = 2.3$ Hz, 1H), 4.25 (br t, 1H), 4.04 (br t, 1H), 4.01 (br t, 1H), 3.91 (m, 2H), 3.84 (br t, 1H), 3.82 (br t, 1H), 3.74 (br t, 1H), 3.45 (s, 3H), 3.25 (dd, $J = 3.3$, 10.1 Hz, 1H).
24. **23**: White solid. ^1H NMR (D_2O): δ 5.16 (br s, 1H), 5. 04 (d, $J = 3.5$ Hz, 1H), 4.74 (d, $J = 2.7$ Hz, 1H), 4.34 (br t, 2H), 4.26 (br t, 1H), 4.05 (br t, 1H), 4.00 (m, 2H), 3.75 (br t, 1H), 3.69 (br t, 1H), 3.44 (s, 3H), 3.30 (dd, $J = 3.5$, 9.9 Hz, 1H).
25. (a) Lei, P. S.; Duchaussoy, P.; Sisun, P.; Mallet, J.-M.; Petitou, M.; Sinaÿ, P. *Bioorg. Med. Chem*. **1998**, *6*, 1337–1346; (b) Tabeur, C.; Mallet, J.-M.; Bono, F.; Herbert, J.-M.; Petitou, M.; Sinaÿ, P. *Bioorg. Med. Chem*. **1999**, *7*, 2003–2012; (c) Kovensky, J.; Duchaussoy, P.; Bono, F.; Salmivirta, M.; Sizun, P.; Herbert, J.-M.; Petitou, M.; Sinaÿ, P. *Bioorg. Med. Chem*. **1999**, *7*, 1567–1580.