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SYNTHESIS OF A SULFATED GLYCOPEPTIDE CORRESPONDING TO THE CARBOHYDRATE-PROTEIN LINKAGE REGION OF PROTEOGLYCANS: β-D-GlcA-(1→3){(SO$_3$Na→4)}-β-D-Gal-(1→3)-β-D-Gal-(1→4)-β-D-Xyl-(1→3)-Ser[1]

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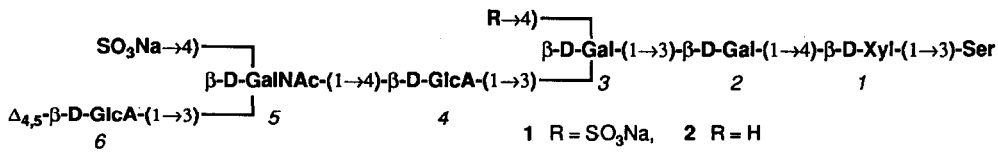
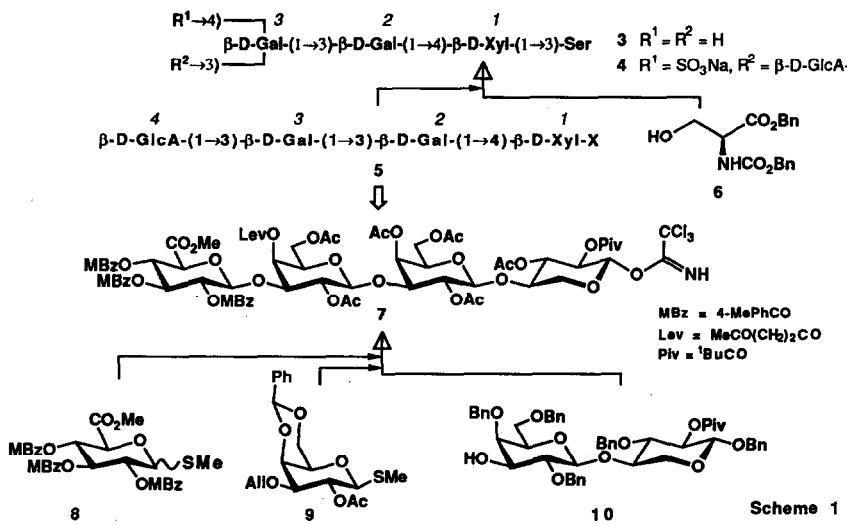
Abstract: A sulfated glycotetraosyl serin **4** was synthesized in a stereocontrolled manner by employing a key glycotetraosyl donor **7** and a serine derivative **6**.

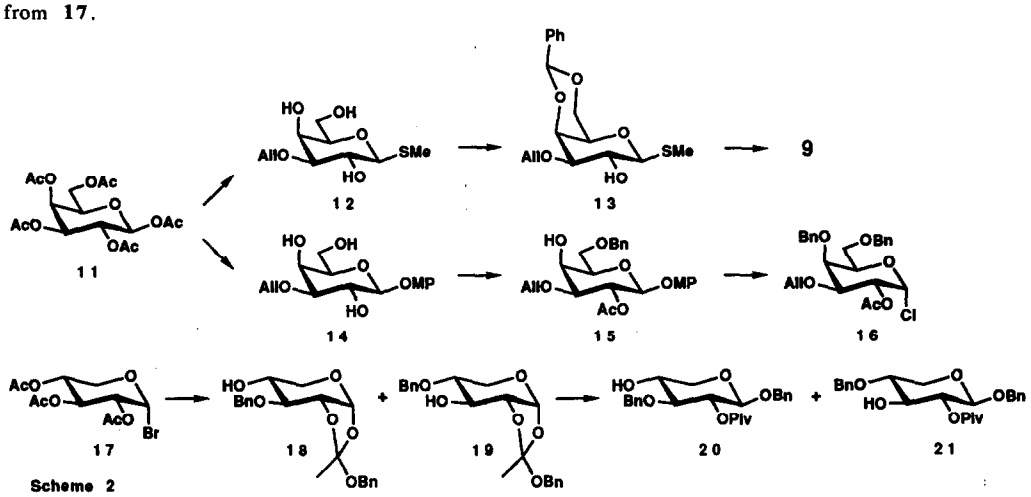
In 1988, sulfated glycohexaosyl serine **1** and **2** were isolated from the linkage region of chondroitin 4-sulfate proteoglycans of Swarm rat chondrosarcoma and characterized by high field ^{1}H-nmr[2]. Presumed function[2] of the O-4 sulfate on Gal residue **3** as a sorting signal for the biosynthesis of chondroitin 4-sulfate might be analysed by providing synthetic substrates such as **4** for emzymic studies. In this paper we describe for the first time a stereocontrolled synthesis of sulfated glycotetraosyl serine **4**. It is to be noted that in the relevant synthetic studies[3] synthesis of glycotriosyl serine **3** has already been described.



Based on a retrosynthetic analysis of target molecule **4** shown in scheme 1, we have designed a key glycotetraosyl donor **7** that corresponds to the glycan part **5** of **4** and that should be properly protected so that sulfate group may be introduced at O-4[3] after stereocontrolled coupling with a serine derivative **6**. The key intermediate **7** may be further disconnected into two glycosyl donors **8** and **9**, and a glycosyl acceptor **10**. Since compounds **8** is readily prepared from corresponding triol thioglycoside[4], we first describe the synthesis of compounds **9** and **10**.



Conversion of compound **11** into **12**[5] was efficiently carried out in 3 steps (*1* Bu_3SnSMe, $SnCl_4$ in $(CH_2Cl)_2$[6], *2* NaOMe in 5:4 MeOH-THF, *3* Bu_2SnO in 1:1 toluene-THF reflux, then $CH_2=CHCH_2Br$ (AllBr), Bu_4NBr in THF, reflux[7]; 63% overall). Allyl ether **12** was further transformed into **9**[5] via **13**[5] in 2 steps (*1* $PhCH(OMe)_2$, $TsOH{\cdot}H_2O$ in THF, *2* Ac_2O, DMAP in Py, 77% overall). Another Gal donor **16**[5] that was designed for the synthesis of **10** was obtainable in a conventional manner from **11** via **14**[5] and **15**[5] in 11 steps (*1* 4-MeOPhOH, TMSOTf in CH_2Cl_2 0°, *2* NaOMe in 3:1 MeOH-THF, *3* Bu_2SnO in 1:1 THF-toluene, then AllBr, Bu_4NBr in THF, *4* $PhCH(OMe)_2$, $TsOH{\cdot}H_2O$ in THF, *5* Ac_2O, DMAP in Py, *6* $NaBH_3CN$, powdered molecular sieves 4A (MS4A), HCl in THF[8], *7* BnBr; NaH, in DMF, *8* CAN in 4:1 MeCN-H_2O[9], *9* Ac_2O, DMAP in Py, *10* $NH_2NH_2{\cdot}AcOH$ in DMF[10], *11* CCl_4, $(Me_2N)_3P$ in THF[11]; 22% overall). In the eighth step, a substantial amount of acetyl migration from O-2 to O-1 was observed. A xylosyl derivative **20** with O-2 pivaloyl group was designed so that a key glycosyl donor **7** could be coupled in a β-D-stereoselective manner[12] with the serine derivative **6**. Conversion of **17**[13] into **20**[5] was carried out via **18**[5] in 8 steps (*1* BnOH, Bu_4NBr[14], Et_3N in $(ClCH_2)_2$, 60°, *2* NaOMe in MeOH, *3* Bu_2SnO in toluene reflux, then Bu_4NI; BnBr in THF; reflux, *4* AllBr, NaH in DMF, *5* TMSOTf[15], MS4A in $(CH_2Cl)_2$, *6* NaOMe in MeOH, *7* ^tBuCOCl (PivCl), DMAP in Py, *8* $[Ir(COD)(Ph_2MeP)_2]PF_6$[16], H_2 in THF, 2h, 25°, then I_2 and H_2O; 47% overall). In the third step the desired **18** was obtained along with the minor product **19**[5] in a ratio of 3:1. Because of the difficulty in separating these regio-isomeric compounds at this stage, the mixture was submitted to the subsequent series of reactions and at the last step the desired compound **20** was separated from **21**[5] which was concomitantly obtained in 16% overall yield from **17**.

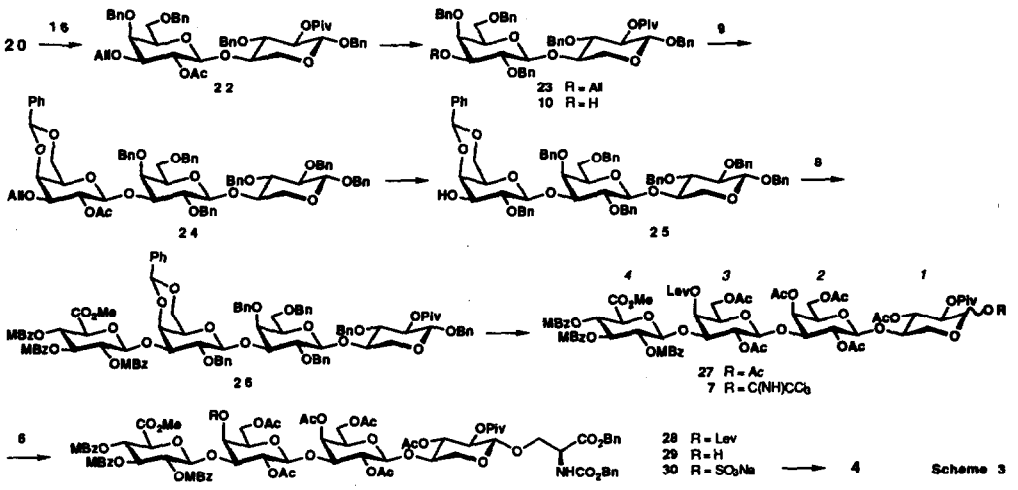


Scheme 2

Silver triflate[17]-MS4A promoted glycosylation of **20** with **16** in 1:1 CH_2Cl_2-toluene at -50° gave 75% of **22**[5] together with 14% of the α-anomer. Conversion of **22** into the glycosyl acceptor **10**[5] was carried out via **23**[5] in 3 steps (*1* LiOH, 30% H_2O_2 in THF[18], *2* BnBr, KI, Ag_2O in DMF, *3* $[Ir(COD)(Ph_2MeP)_2]PF_6$, H_2 in THF, then I_2, H_2O 90% overall). $CuBr_2$-n-Bu_4NBr-AgOTf-MS4A[19] promoted glycosylation of **10** with **9** in $(CH_2Cl)_2$ proceeded in a stereoselective manner to afford 86% of **24**[5], which was then converted into the glycotriosyl acceptor **25**[5] in 3 steps (*1* 30% H_2O_2, LiOH in THF, *2* BnBr, KI, Ag_2O in DMF, *3* $[Ir(COD)(Ph_2MeP)_2]PF_6$, H_2 in THF, then H_2O, $NaHCO_3$, I_2, 79% overall). Chain extension of **25** by GlcA donor **8**[5] was performed in the presence of $CuBr_2$-n-Bu_4NBr-AgOTf-MS4A in CH_2Cl_2 to afford 80% of **26**[5] that was converted into a key glycotetraosyl donor **7**[5] via **27**[5] (α and β-anomer in a ratio of 1:1) in 7 steps (*1* Camphor sulfonic

acid (CSA) in 3:1 MeOH-CH_2Cl_2, *2* AcCl in Py, -5°, *3* Lev_2O, DMAP in 4:1 Py-$(CH_2Cl)_2$, *4* 10% Pd-C, H_2 in 2:1 MeOH-EtOAc, *5* Ac_2O, DMAP in Py, *6* piperidine•AcOH in THF[20], *7* CCl_3CN, DBU in CH_2Cl_2[21]; 40% overall). Crucial coupling between **6** and **7** was achieved in a stereocontrolled manner in the presence of $BF_3•OEt_2$ in $(CH_2Cl)_2$ at -23° to give 75% of **28**[5]. Lev group at O-4[3] of **28** was chemoselectively removed by treatment with $NH_2NH_2•AcOH$[22] in 1:5 toluene-EtOH at -5° to give 90% of **29**[5] which was then sulfated with $Me_3N•SO_3$ in DMF at 50° to give 97% of **30**. Deprotection of **30** into the target glycotetraosyl serine **4** was carried out in 3 steps (*1* Pd, H_2 in 1:1 EtOAc-MeOH, *2* LiOH in 10:3 THF-H_2O, -5°, then purification by Sephadex LH-20 in 5:5:1 $CHCl_3$-MeOH-H_2O, *3* 5:1 MeOH-*M*aq.NaOH, -5°, then purification by Sepahdex G-10 in H_2O, 97% overall). The assigned structure for synthetic **4** was deduced from the unambiguous synthetic sequence and was confirmed by the ^{1}H-nmr data that was found to be in agreement with those for the relevant natural samples[2].

In summary, sulfated glycotetraosyl serine **4** was synthesized for the first time by employing a key glycotetraosyl donor **7**.



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References and Notes

1 Part 87 in the series "Synthetic Studies on Cell-Surface Glycans". For part 86, see T. Slaghek, Y. Nakahara, and T. Ogawa, *Tetrahedron Lett.*, submitted.

2 K. Sugawara, I. Yamashina, P. De Waard, H. Van Halbeek, and J. F. G. Vliegenthart, *J. Biol. Chem.*, **263**, 10168 (1988).

3 B. Lindberg and B.-G. Silvander, *Acta Chem. Scand.*, **19**, 530 (1965); B. Erbing, B. Lindberg, and T. Norberg, *Acta Chem. Scand. B*, **32**, 308 (1978); P. J. Garegg, B. Lindberg, and T. Norberg, *ibid.*, **33**, 449 (1979); H. G. Garg, T. Hasenkamp, and H. Paulsen, *Carbohydr. Res.*, **151**, 225 (1986); G. Ekborg, M. Klinger, L. Rodén, J. W. Jensen, J. S. Schutzbach, D. H. Huang, N. R. Krishna, and G. M. Anantharamaiah, *Glycoconjugate J.*, **4**, 255 (1987); H. Paulsen and M. Brenken, *Liebigs Ann. Chem.*, 649 (1988); G. Ekborg, T. Curenton, N. R. Krishna, and L. Rodén, *J. Carbohydr. Chem.*, **9**, 15 (1990); S. Rio, J.-M. Beau, and J.-C. Jacquinet, *Carbohydr. Res.*, **219**, 71 (1991).

4 T. Nakano, Y. Ito, and T. Ogawa, *Tetrahedron Lett.*, **31**, 1597 (1990).

5 Physical data for new compounds are given below, values of $[\alpha]_D$ and $\delta_{H,C}$ were measured at 25°±3° for solutions in $CHCl_3$ and $CDCl_3$, respectively, unless noted otherwise. **4**: R_F 0.28 in

5:2:2:2, Me_2CO-AcOH-MeOH-H_2O; δ_H (D_2O) 4.781 (d, 3.1Hz, 4^3), 4.765 (d, 7.9Hz, 1^4), 4.685 (d, 7.9Hz, 1^3), 4.524 (d, 7.9Hz, 1^2), 4.447 (d, 7.6Hz, 1^2), 4.173 (d, 3.4Hz, 4^2), 4.050 (dd, 3.1 and 10.1Hz, β^{Ser}). **7**: R_F 0.47 in 1:2 toluene-EtOAc; $[\alpha]_D$ +27.2° (c 0.9); δ_H 8.630 (s, NH), 6.418 (d, 3.7Hz, 1^1), 3.693 (s, OMe), 2.204 (s, Lev), 1.128 (s, Piv). **8**: A mixture of α and β anomers in a ratio of 5:3. R_F 0.28 in 5:1 hexane-EtOAc; δ_H 5.875 (d, 5.2Hz, 1α), 4.717 (d, 9.8Hz, 1β), 2.181 (s, SMe). **9**: R_F 0.29 in 2:1 hexane-EtOAc; $[\alpha]_D$ +27.3° (c 0.3); mp 109-110° (Et_2O-hexane); δ_H 5.530 (s, PhCH), 5.433 (t, 9.8Hz, 2); 4.323 (d, 9.8Hz, 1), 2.242 (s, SMe), 2.109 (s, Ac). **10**: R_F 0.22 in 7:3 hexane-EtOAc; $[\alpha]_D$ -40.2° (c 0.5); δ_H 4.527 (d, 6.1Hz, 1^1), 4.404 (d, 7.6Hz, 1^2), 1.142 (s, Piv). **12**: R_F 0.11 in 1:1 toluene-EtOAc; $[\alpha]_D$ +11.0° (c 1.0, MeOH); m.p. 99-100° (MeOH-Et_2O); δ_H(CD_3OD) 4.227 (d, 9.6Hz, 1), 2.190 (s, SMe). **13**: R_F 0.65 in 10:1 $CHCl_3$-MeOH; $[\alpha]_D$ +37.7° (c 0.4); mp 179-180° (hexane-EtOAc); δ_H 5.526 (s, PhCH), 4.305 (d, 9.5Hz, 1), 2.250 (s, SMe). **14**: R_F 0.61 in 4:1 $CHCl_3$-MeOH; mp 139-140° (Et_2O); δ_H ($DMSOd_6$) 4.685 (d, 7.8Hz, 1), 3.684 (s, OMe). **15**: R_F 0.50 in 2:1 toluene-EtOAc; mp 89.5-90° (hexane); δ_H 5.377 (dd, 7.9 and 9.8Hz, 2), 4.820 (d, 7.9Hz, 1), 2.103 (s, Ac). **16**: R_F 0.63 in 2:1 toluene-EtOAc; $[\alpha]_D$ +117.7° (c 0.3); δ_H 6.376 (d, 4.0Hz, 1), 5.349 (dd, 4.0 and 10.1Hz, 2), 2.120 (s, Ac). **18**: R_F 0.59 in 1:1 EtOAc-toluene; δ_H 5.475 (d, 3.3Hz, 1), 1.780 (s, CMe). **19**: R_F 0.59 in 1:1 EtOAc-toluene; δ_H 5.475 (d, 3.3Hz, 1), 4.780 (s, CMe). **19**: R_F 0.59 in 1:1 EtOAc-toluene; δ_H 5.604 (d, 4.9Hz, 1), 1.758 (s, C-Me). **20**: R_F 0.45 in 2:1 hexane-EtOAc; δ_H 4.826 (dd, 7.0 and 8.2Hz, 2), 4.498 (d, 7.0Hz, 1), 1.182 (s, Piv). **21**: R_F 0.26 in 2:1 hexane-EtOAc; δ_H 5.079 (dd, 5.8 and 7.3Hz, 2), 4.553 (d, 5.8Hz, 1), 1.159 (s, Piv). **22**: R_F 0.38 in 7:3 hexane-EtOAc; $[\alpha]_D$ -40.4° (c 0.3); mp 123.5-124° (Et_2O-hexane); δ_H 4.445 (d, 7.3Hz, 1^1), 4.436 (d, 7.9Hz, 1^2), 2.079 (s, Ac), 1.121 (s, Piv); α-isomer: R_F 0.52, $[\alpha]_D$ +25.3° (c 0.3); mp 134-135° (Et_2O-hexane); δ_H 5.230 (d, 3.7Hz, 1^2). **23**: R_F 0.27 in 7:3 hexane-EtOAc; $[\alpha]_D$ +2.7° (c 0.5); mp 74.5-75° (Et_2O-hexane); δ_H 4.512 (d, 6.4Hz, 1^1), 4.400 (d, 7.6Hz, 1^2), 1.135 (s, Piv). **24**: R_F 0.48 in 1:1 hexane-EtOAc; $[\alpha]_D$ -35.3° (c 0.3); δ_H 5.543 (s, PhCH), 4.901 (d, 7.9Hz, 1^3), 4.497 (d, 6.4Hz, 1^1), 4.395 (d, 7.6Hz, 1^2), 1.924 (s, Ac), 1.131 (s, Piv). **25**: R_F 0.36 in 1:1 hexane-EtOAc; $[\alpha]_D$ -25.3° (c 0.5); δ_H 5.554 (s, PhCH), 4.862 (d, 7.6Hz, 1^3), 4.530 (d, 6.1Hz, 1^1), 4.421 (d, 7.3Hz, 1^2), 1.148 (s, Piv). **26**: R_F 0.43 in 2:1 toluene-EtOAc; $[\alpha]_D$ -16.5° (c 0.7); δ_H 5.631 (s, PhCH), 5.381 (d, 7.0Hz, 1^4), 4.855 (d, 7.5Hz, 1^3), 4.490 (d, 6.1Hz, 1^1), 4.378 (d, 7.6Hz, 1^2), 3.644 (s, OMe), 1.132 (s, Piv). **27**: a 1:1 mixture of α- and β-anomer; R_F 0.26 in 1:1 toluene-EtOAc; δ_H 6.230 (d, 3.7Hz, $1^1\alpha$), 5.633 (d, 7.6Hz, $1^1\beta$), 4.382 and 4.375 (2s, 7.9Hz, 1^2), 4.360 (d, 7.9Hz, 1^3), 3.692 (s, OMe), 1.140 and 1.120 (2s, Piv). **28**: R_F 0.40 in 1:1 toluene-EtOAc; δ_H 5.483 (d, 3.7Hz, 4^3), 5.292 (d, 2.8Hz, 4^2), 4.905 (d, 7.7Hz, 1^4), 4.377 (d, 7.9Hz, 1^3), 4.334 (d, 7.0Hz, 1^1), 4.319 (d, 7.9Hz, 1^2), 3.694 (s, OMe), 2.202 (s, Lev). **29**: R_F 0.66 in 2:1 EtOAc-hexane; δ_H 5.317 (d, 3.4Hz, 4^2), 5.005 (d, 7.0Hz, 1^4), 4.389 (d, 7.9Hz, 1^3), 4.335 (d, 7.3Hz, 1^1), 4.331 (d, 8.2Hz, 1^2), 4.133 (bs, 4^3), 3.661 (s, OMe), 1.115 (s, Piv). **30**: R_F 0.55 in 15:1 $CHCl_3$-MeOH; δ_H(CD_3OD) 5.307 (d, 3.4Hz, 4^3), 5.206 (d, 7.6Hz, 1^4), 5.009 (d, 3.1Hz, 4^2), 4.514 and 4.530 (2d, 8.0Hz, 1^2 and 1^3), 4.486 (d, 7.0Hz, 1^1), 3.657 (s, OMe), 1.155 (s, Piv).

6 T. Ogawa and M. Matsui, *Carbohydr. Res.*, **54**, C17 (1977).
7 J. Alais, A. Maranduba, and A. Veyrieres, *Tetrahedron Lett.*, **24**, 2383 (1983); A. Maranduba and A. Veyrieres, *Carbohydr. Res.*, **135**, 330 (1985).
8 P. J. Garegg, H. Hultberg, and S. Wallin, *Carbohydr. Res.*, **108**, 97 (1982).
9 T. Fukuyama, A. A. Laird, and L. M. Hotchkiss, *Tetrahedron Lett.*, **26**, 6291 (1985).
10 G. Excoffier, D. Gagnaire, and J.-P. Utille, *Carbohydr. Res.*, **39**, 368 (1975).
11 I. M. Downie, H. Heaney, and G. Kemp, *Angew. Chem. Int. Ed. Engl.*, **14**, 370 (1975).
12 H. Kunz and A. Harrens, *Liebigs Ann. Chem.*, 41 (1982); J. Vlahov and G. Snatzke, *ibid.*, 570 (1983); R. R. Shmidt and P. Zimmermann, *Angew Chem. Int. Ed. Engl.*, **25**, 725 (1986); S. Sato, S. Nunomura, T. Nakano, Y. Ito, and T. Ogawa, *Tetrahedron Lett.*, **29**, 4097 (1988); S. Sato, Y. Ito, and T. Ogawa, *ibid.*, **29**, 5267 (1988).
13 B. Capon, P. M. Collins, A. A. Levy, and W. G. Overend, *J. Chem. Soc.*, 3242 (1964).
14 R. U. Lemieux, K. B. Hendriks, R. V. Stick, and K. James, *J. Am. Chem. Soc.*, **97**, 4056 (1975).
15 T. Ogawa, K. Beppu, and S. Nakabayashi, *Carbohydr. Res.*, **93**, C6 (1981).
16 L. M. Haines and E. Singleton, *J. Chem. Soc., Dalton Trans.*, 1891 (1972); J. J. Oltvoort, C.A.A. van Boeckel, J. H. De Koning, and J. H. van Boom, *Synthesis*, 305 (1981).
17 S. Hanessian and J. Banoub, *Carbohydr. Res.*, **53**, C13 (1977).
18 E. J. Corey, S. Kim, S. Yoo, K. C. Nicolaou, L. S. Melvin, Jr., P. J. Brunelle, J. R. Flack, E. J. Trybulski, R. Lett, and R. W. Sheldrake, *J. Am. Chem. Soc.*, **100**, 4620 (1978).
19 S. Sato, M. Mori, Y. Ito, and T. Ogawa, *Carbohydr. Res.*, **155**, C6 (1986).
20 T. Nakano, Y. Ito, and T. Ogawa, *Tetrahedron Lett.*, **31**, 1597 (1990).
21 R. R. Schmidt and J. Michel, *Angew. Chem. Int. Ed. Engl.*, **19**, 731 (1980).
22 H. J. Koeners, J. Verhoeven, and J. H. van Boom, *Rec. Trav. Chim. Pays-Bas.*, **100**, 65 (1981).

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