

TOTAL SYNTHESIS OF A SULFATED GLUCURONYL GLYCOSPHINGOLIPID, IV³GlcA(3-SO₃)_nLcOse₄Cer, A CARBOHYDRATE EPI TOPE OF NEURAL CELL ADHESION MOLECULES¹

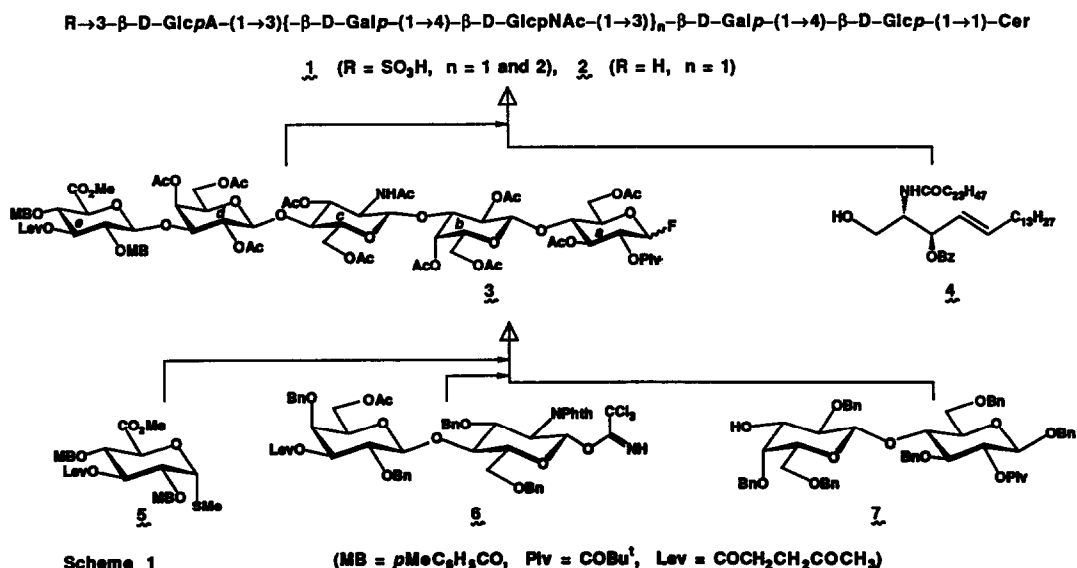
Takahisa Nakano, Yukishige Ito and Tomoya Ogawa*

RIKEN (The Institute of Physical and Chemical Research), Wako-shi, Saitama, 351-01 Japan

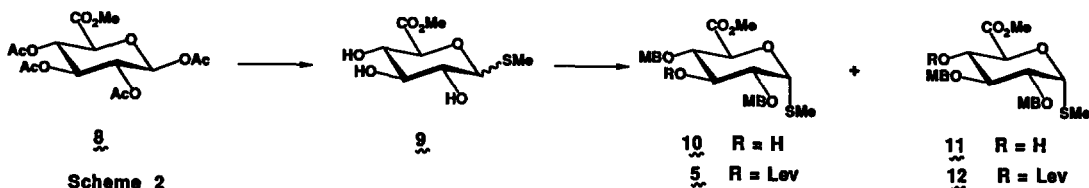
Abstract: A total synthesis of a sulfated glucuronyl glycosphingolipid isolated from human peripheral nervous system was achieved for the first time.

In 1984, Ilyas et al first reported² the presence of acidic glycolipid antigens in human peripheral nerves which was recognized by IgM of patients having peripheral neuropathies and plasma cell abnormalities. The glycolipids were also shown³ to be recognized by an antibody HNK-1 (anti-Leu-7) raised to a membrane antigen from human T cell line HSB-2. Recently⁴ the acidic glycolipids were isolated and chemically characterized. Their structures were proposed as 1. Neural adhesion molecules L1 and N-CAM, J1 glycoprotein, and myelin-associated glycoprotein (MAG) were also shown⁵ to share a carbohydrate epitope of a similar structure recognized by monoclonal antibodies L2 and HNK-1. Although the detailed characterization of the carbohydrate epitope of these glycoproteins remains to be achieved, the epitope seems to be involved in neurone-astrocyte and astrocyte-astrocyte adhesion and acts as a ligand in cell interactions⁶. We describe here a first total synthesis of 1 (n=1) in a stereocontrolled manner.

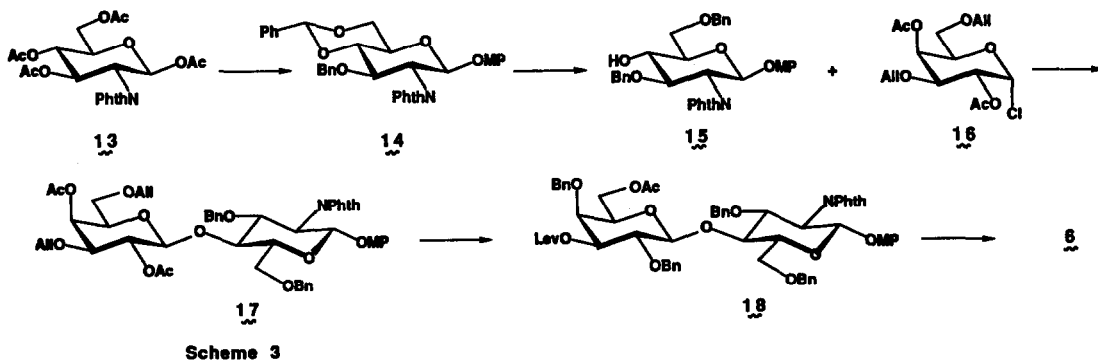
Retrosynthetic analysis of 1 led us to design a suitable glycopentaosyl donor 3 which, after being coupled with a ceramide derivative 4⁷, may be regioselectively deprotected to introduce a sulfate function at O-3e. The donor 3 may, in turn, be obtainable from mono- and di- saccharide derivatives (5, 6) and a known lactose derivative 7⁸.



The glucuronyl donor **5**⁹ was prepared from the known tetraacetate **8**¹⁰ via **9**⁹ and **10**⁹ in 4 steps (1 Bu₃SnSMe¹¹, SnCl₄ in (ClCH₂)₂, 2 MeONa in MeOH, 3 Bu₂SnO¹² reflux in toluene, then MeBzCl, Et₃N in THF, 4 Lev₂O, DMAP in Py, 34% overall). Although separation of an 8:1 mixture of the α -anomers **10** and **11**⁹ from the corresponding β -anomers was achieved by chromatography, **10** could not be separated from the isomer **11**. However, levuloyl ester **5** was successfully separated by SiO₂ chromatography in 5:1 hexane-EtOAc from the regioisomer **12**⁹.

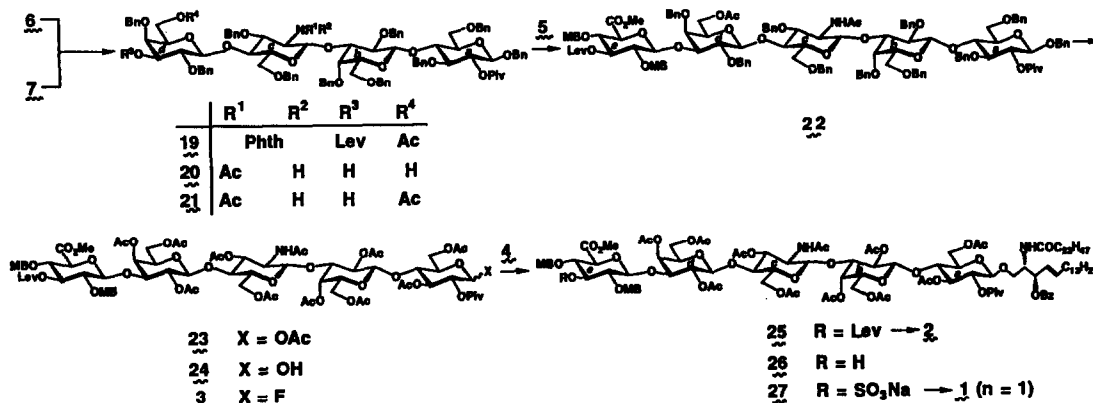


A lactosamine derivative **6** was prepared as follows. Conversion of **13**¹³ into **15**⁹ was achieved via **14**⁹ in 6 steps (1 4-MeOPhOH, TMSOTf in (ClCH₂)₂, 2 MeONa in MeOH, 3 PhCH(OMe)₂, TsOH·H₂O in DMF, 4 BnBr, NaH in DMF, 5 TsOH in MeOH-dioxane, 6 (Bu₃Sn)₂O in toluene, then BnBr and Bu₄NBr¹⁴, 32% overall). Silver(I) triflate promoted glycosylation of **15** with **16**¹⁵ in (ClCH₂)₂ in the presence of powdered molecular sieves 4A(MS4A) afforded 91% of **17**⁹ which was further converted into the designed glycosyl donor **6** via **18** in 7 steps (1 LiOH, 30% H₂O₂ in THF¹⁶, 2 Ag₂O, KI, BnBr in DMF, 3 (Ph₃P)₃Rh(I)Cl, DABCO in 10:5:1 CH₃CN-EtOH-H₂O^{15,17}, then HgCl₂-HgO in 10:1 Me₂CO-H₂O, 4 AcCl in Py at 0°, 5 Lev₂O, DMAP in Py at 25°, 6 CAN in 1:1.3:1 MePh-MeCN-H₂O¹⁸, 7 CCl₃CN, DBU in (ClCH₂)₂¹⁹, 33% overall).



Glycosylation of **7** with **6** in the presence of TMSOTf and MS4A in (ClCH₂)₂ gave 86% of **19**⁹ which was then converted into **21**⁹ via **20**⁹ in 3 steps (1 NH₂NH₂·H₂O in EtOH, 2 Ac₂O in MeOH, 3 AcCl in Py, 94% overall). Copper(II) bromide-Bu₄NBr-AgOTf²⁰ and MS4A promoted glycosylation of **21** with **5** did afford 58% of **22**⁹ which was converted into a glycosyl fluoride **3** via **23**⁹ and **24**⁹ in 4 steps (1 Pd-C, H₂ in MeOH, 2 Ac₂O, DMAP in Py, 3 piperidine-AcOH in THF, 4 DAST²¹ in (ClCH₂)₂, 60% overall). Crucial coupling between **3** and **4** was achieved in the presence of SnCl₂-AgOTf-MS4A²² in CHCl₃ to give 55% of **25**, the structure of which was confirmed by complete deprotection to **2**⁹ (1 LiOH in aq. THF at -15°-0°, 2 MeONa in 1:1 MeOH-THF at 25°, 86%).

Finally, **25** was converted into the target **1**⁸ via **26**⁹ and **27**⁹ in 3 steps (1 NH₂NH₂·AcOH in EtOH, 2 Me₃N·SO₃ in DMF at 55°, then Dowex 50 (Na⁺), 3 LiOH in aq. THF at -15°-0°, then MeONa in 1:1 MeOH-THF at 25°, overall 58%). The ¹H-n.m.r. data for synthetic **1**(n=1) were in reasonable agreement with those⁴ of natural **1**(n=1).



Scheme 4

In conclusion, a first total synthesis of the sulfated glucuronyl neolactotetraosyl ceramide **1**(n=1) was successfully achieved in a regio- and stereocontrolled manner by employing a properly protected glycopentaosyl donor **3** as a key intermediate.

Acknowledgment. We thank Dr. J. Uzawa and Mrs. T. Chijimatsu for recording and measuring the n.m.r. spectra and Mrs. M. Yoshida and her staff for the elemental analyses. We also thank Ms. A. Takahashi and Ms. K. Moriwaki for their technical assistance.

Reference and Notes

- Part 74 in the series "Synthetic Studies on Cell-Surface Glycans". For part 73, see M. Sugimoto, K. Fujikura, S. Nunomura, Y. Ito, and T. Ogawa, *Tetrahedron Lett.*, in press.
- A. A. Ilyas, R. H. Quarles, T. D. McIntosh, M. J. Dobersen, B. D. Trapp, M. C. Dalakas, and R. O. Brady, *Proc. Natl. Acad. Sci. U.S.A.*, **81**, 1225 (1984).
- A. A. Ilyas, R. H. Quarles, and R. O. Brady, *Biochem. Biophys. Res. Commun.*, **122**, 1206 (1984); T. Abo, M. D. Cooper and C. M. Balch, *J. Immunol.*, **129**, 1752 (1982).
- D. K. H. Chou, A. A. Ilyas, J. E. Evans, C. Costello, R. H. Quarles, and F. B. Jungalwala, *J. Biol. Chem.*, **261**, 11717 (1986); T. Ariga, T. Kohriyama, L. Freddo, N. Latov, M. Saito, K. Kon, S. Ando, M. Suzuki, M. E. Hemling, K. L. Rinehart, Jr., S. Kusunoki, and R. K. Yu, *ibid.*, **262**, 848 (1987).
- E. Nobile-Ogazio, A. P. Hays, N. Latov, G. Perman, J. Golier, M. E. Shy, and L. Freddo, *Neurology*, **34**, 1336 (1984); J. Kruse, R. Mailhammer, H. Wernecke, A. Faissner, I. Sommer, C. Goridis, and M. Schachner, *Nature*, **311**, 153 (1984).
- G. Keilhauer, A. Faissner, and M. Schachner, *Nature*, **316**, 728 (1985); V. Künemund, F. B. Jungalwala, G. Fischer, D. H. Chou, G. Keilhauer, and M. Schachner, *J. Cell. Biol.*, **106**, 213 (1988).
- K. Koike, M. Numata, M. Sugimoto, Y. Nakahara, and T. Ogawa, *Carbohydr. Res.*, **158**, 113 (1986).
- S. Sato, S. Nunomura, T. Nakano, Y. Ito, and T. Ogawa, *Tetrahedron Lett.*, **29**, 4097 (1988).
- Physical data for new compounds are given below. Values of [α]_D and δ_HC were measured at 25°±3° for solutions in CHCl₃ and CDCl₃, respectively, unless noted otherwise. **1**(n=1): δ_H (49:1 (CD₃)₂SO-D₂O, 60°) 5.541 (dt, 15.3 and 6.6 Hz, 5Cer), 5.372 (dd, 15.3 and 7.0 Hz, 4Cer), 4.675 (d, 8.2 Hz, 1e), 4.514 (d, 7.6 Hz, 1c), 4.310 (d, 7.6 Hz, 1d), 4.280 (d, 7.3 Hz, 1b), 4.172 (d, 7.9 Hz, 1a), 3.995 (t, 9.1 Hz, 3e), 1.827 (s, NAc), 0.856 (t, 6.8 Hz, 2CH₂Me). **2**: δ_H (49:1 (CD₃)₂SO-D₂O, 60°) 5.555 (dt,

- 15.0 and 6.1 Hz, *5Cer*), 5.372 (dd, 15.0 and 7.3 Hz, *4Cer*), 4.681 (d, 7.6 Hz, *1e*), 4.368 (d, 7.6 Hz, *1c*), 4.317 (d, 7.6 Hz, *1d*), 4.279 (d, 7.3 Hz, *1b*), 4.174 (d, 7.6 Hz, *1a*), 1.832 (s, *NAC*), 0.854 (t, 6.7 Hz, *2CH₂Me*). 3 (a 3:1 mixture of β and α): δ_{H} 5.698 (dd, 53.1 and 2.4 Hz, *1a\alpha*), 5.561 (t, 9.5 Hz, *3e*), 5.487 (t, 9.5 Hz, *4e*), 5.313 (dd, 52.5 and 5.5 Hz, *1a\beta*), 3.691 (s, *OMe*), 2.411 and 2.402 (2 s, *2PhMe*), 1.187 (s, *tBu\beta*), 1.183 (s, *tBu\alpha*). 5: $[\alpha]_{\text{D}}$ +102.5° (c 0.9); δ_{H} 5.811 (d, 5.5 Hz, *1*), 5.771 (t, 9.5 Hz, *3*), 5.463 (t, 9.5 Hz, *4*), 5.338 (dd, 9.5 and 5.5 Hz, *2*), 4.905 (d, 9.5 Hz, *5*). 6: $[\alpha]_{\text{D}}$ +57.7° (c 1.0); δ_{H} 8.522 (s, *NH*), 6.392 (d, 8.8 Hz, *1a*), 4.817 (dd, 10.3 and 3.3 Hz, *3b*), 2.154 and 1.988 (2s, *2COMe*). 9: (a 5:3 mixture of α and β isomers); δ_{H} 5.364 (d, 5.5 Hz, *1\alpha*), 4.549 (d, 9.2 Hz, *5\alpha*), 4.398 (d, 9.5 Hz, *1\beta*), 3.831 (s, *OMe\alpha*), 3.819 (s, *OMe\beta*), 2.229 (s, *SMe\beta*), and 2.156 (s, *SMe\alpha*). 10: δ_{H} 5.747 (d, 5.2 Hz, *1*), 5.397 (t, 8.9 Hz, *4*), 5.286 (dd, 8.9 and 5.2 Hz, *2*), 4.870 (d, 8.9 Hz, *5*), 4.365 (dt, 3.4 and 8.9 Hz, *3*). 11: δ_{H} 5.759 (d, 5.2 Hz, *1*), 5.699 (t, 9.5 Hz, *3*), 5.397 (dd, 9.5 and 5.2 Hz, *2*), 4.779 (d, 9.5 Hz, *5*), 4.152 (dt, 3.8 and 9.5 Hz, *4*). 12: δ_{H} 5.821 (t, 9.0 Hz, *3*), 5.814 (d, 5.3 Hz, *1*), 5.421 (t, 9.0 Hz, *4*), 5.380 (dd, 9.0 and 5.3 Hz, *2*), 4.852 (d, 9.0 Hz, *5*). 14: $[\alpha]_{\text{D}}$ +72.3° (c 0.8); δ_{H} 5.742 (d, 8.3 Hz, *1*), 5.643 (s, *CHPh*), 3.683 (s, *OMe*). 15: $[\alpha]_{\text{D}}$ +56.1° (c 0.8); Acetate of 15: δ_{H} 5.647 (d, 8.2 Hz, *1*), 5.190 (dd, 9.8 and 8.5 Hz, *4*), 3.691 (s, *OMe*), 1.978 (s, *Ac*). 17: $[\alpha]_{\text{D}}$ +47.2° (c 0.6); δ_{H} 5.615 (d, 8.3 Hz, *1a*), 5.408 (d, 2.7 Hz, *4b*), 5.068 (dd, 10.0 and 8.1 Hz, *2b*), 4.565 (d, 8.1 Hz, *1b*), 3.697 (s, *OMe*), 2.063 and 2.055 (2s, *2Ac*). 18: $[\alpha]_{\text{D}}$ +41.8° (c 1.6); δ_{H} 5.609 (d, 8.4 Hz, *1a*), 4.856 (dd, 10.3 and 2.9 Hz, *3b*), 4.511 (d, 7.7 Hz, *1b*), 2.151 and 1.983 (2s, *2COMe*). 19: $[\alpha]_{\text{D}}$ -3.8° (c 1.7); δ_{H} (2DCOSY) 5.369 (d, 8.5 Hz, *1c*), 5.021 (dd, 9.2 and 8.0 Hz, *2a*), 4.856 (dd, 10.3 and 3.3 Hz, *3d*), 4.526 (d, 7.7 Hz, *1d*), 4.286 (d, 8.0 Hz, *1a*), 4.262 (dd, 10.8 and 8.5 Hz, *2c*), 3.788 (dd, 10.3 and 7.7 Hz, *2d*), 3.395 (t, 9.2 Hz, *3a*), 2.153 and 1.935 (2s, *2COMe*), 1.082 (s, *tBu*). 20: $[\alpha]_{\text{D}}$ -5.7° (c 0.9); δ_{H} 5.092 (dd, 9.5 and 8.1 Hz, *2a*), 1.418 (s, *NAC*), 1.118 (s, *tBu*). 21: $[\alpha]_{\text{D}}$ -12.7° (c 0.7); δ_{H} 5.090 (dd, 9.5 and 8.1 Hz, *2a*), 1.921 (s, *OAc*), 1.419 (s, *NAC*), 1.116 (s, *tBu*). 22: $[\alpha]_{\text{D}}$ -17.9° (c 0.9); δ_{H} (2DCOSY) 5.622 (t, 9.9 Hz, *3e*), 5.494 (t, 9.9 Hz, *4e*), 5.359 (dd, 9.9 and 7.7 Hz, *2e*), 5.219 (d, 7.7 Hz, *2e*), 4.884 (d, 7.7 Hz, *1c*), 4.174 (d, 9.9 Hz, *5e*), 3.873 (2d, 2.6 Hz, *4bd*), 3.672 (s, *OMe*), 2.421 and 2.340 (2s, *2PhMe*), 1.953 and 1.888 (2s, *2COMe*), 1.388 (s, *NAC*), 1.112 (s, *tBu*). 23: δ_{H} 6.285 (0.5 H, d, 3.7 Hz, *1a\alpha*), 5.691 (0.5 H, d, 8.1 Hz, *1a\beta*), 5.563 (t, 9.5 Hz, *3e*), 5.485 (t, 9.5 Hz, *4e*), 5.457 (d, 7.3 Hz, *1e*), 5.457 and 5.288 (2d, 3.3 Hz, *4bd*), 5.201 (dd, 9.5 and 7.3 Hz, *2e*), 4.837 (d, 7.3 Hz, *1c*), 4.189 (d, 9.5 Hz, *5e*), 3.690 (s, *OMe*), 2.411 and 2.403 (2s, *2PhMe*), 1.130 and 1.117 (2s, *tBu*). 24: δ_{H} 3.687 (s, *OMe*), 2.411 and 2.403 (2s, *2PhMe*), 1.174 (s, *tBu*). 25: $[\alpha]_{\text{D}}$ +12.4° (c 0.6); δ_{H} (2DCOSY) 5.871 (dt, 14.7 and 7.0 Hz, *5Cer*), 5.744 (d, 9.5 Hz, *NHCer*), 5.563 (t, 9.5 Hz, *3e*), 5.535 (t, 7.9 Hz, *3Cer*), 5.483 (t, 9.5 Hz, *4e*), 5.352 (d, 8.6 Hz, *NHCer*), 5.202 (dd, 9.5 and 7.3 Hz, *2e*), 5.175 (t, 9.5 Hz, *3a*), 4.834 (d, 7.3 Hz, *1e*), 4.539 (d, 7.6 Hz, *1c*), 4.409 (d, 7.9 Hz, *1a*), 4.369 (d, 7.9 Hz) and 4.274 (d, 7.6 Hz, *1bd*), 3.690 (s, *OMe*), 2.413 and 2.403 (2s, *2PhMe*), 1.139 (s, *tBu*). 26: $[\alpha]_{\text{D}}$ +11.6° (c 0.9); δ_{H} 3.710 (s, *OMe*), 2.418 and 2.411 (2s, *2PhMe*), 2.122, 2.122, 2.111, 2.098, 2.092, 2.018, 2.016, 1.978, 1.911, 1.882 and 1.798 (11s, *11Ac*), 1.140 (s, *tBu*), 0.879 (t, 6.8 Hz, *2CH₂Me*). 27: $[\alpha]_{\text{D}}$ +7.7° (c 0.4, MeOH); δ_{H} (CD₃OD) 5.780 (dt, 15.3 and 7.0 Hz, *5Cer*), 5.466 (t, 7.0 Hz, *3Cer*), 5.374 (d, 3.1 Hz) and 5.257 (d, 3.6 Hz, *H-4bd*), 4.890 (d, 7.6 Hz, *1e*), 4.533 (d, 7.9 Hz), 4.523 (d, 7.6 Hz), 4.368 (d, 8.2 Hz), 4.343 (d, 7.9 Hz, *1abcd*), 4.213 (d, 9.8 Hz, *5e*), 3.532 (s, *OMe*), 2.403 and 2.389 (2s, *2PhMe*).
- 10 G. N. Bollenback, J. W. Long, D. G. Benjamin, and J. A. Lindquist, *J. Am. Chem. Soc.*, **77**, 3310 (1955).
- 11 T. Ogawa and M. Matsui, *Carbohydr. Res.*, **54**, C17 (1977).
- 12 D. Wagner, J. Verheyden and J. G. Moffatt, *J. Org. Chem.*, **39**, 24 (1974); C. Auge, S. David and A. Veyrieres, *J. Chem. Soc., Chem. Commun.*, 375 (1976); M. A. Nashed and L. Anderson, *Tetrahedron Lett.*, 3503 (1976); R. M. Munavu and H. H. Szmant, *J. Org. Chem.*, **41**, 1832 (1976).
- 13 R. U. Lemieux, T. Takeda, and B. Y. Chung, *ACS Symp. Ser.*, **39**, 90 (1976).
- 14 T. Ogawa and M. Matsui, *Carbohydr. Res.*, **62**, C1 (1978); T. Ogawa, K. Katano, K. Sasajima, and M. Matsui, *Tetrahedron*, **37**, 2779 (1981); A. Veyrieres, *J. Chem. Soc., Perkin Trans 1*, 1626 (1981).
- 15 Y. Ito and T. Ogawa, *Agr. Biol. Chem.*, **50**, 3227 (1986).
- 16 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).
- 17 E. J. Corey and W. J. Suggs, *J. Org. Chem.*, **38**, 3224 (1973); R. Gigg and C. D. Warren, *J. Chem. Soc., C*, 1903 (1968).
- 18 T. Fukuyama, A. A. Laird, and L. M. Hotchkiss, *Tetrahedron Lett.*, **26**, 6291 (1985).
- 19 R. R. Schmidt and J. Michel, *Angew. Chem. Int. Ed. Engl.*, **19**, 731 (1980); R. R. Schmidt, J. Michel, and M. Roos, *Liebigs Ann. Chem.*, 1343 (1984).
- 20 S. Sato, M. Mori, Y. Ito, and T. Ogawa, *Carbohydr. Res.*, **155**, C6 (1986).
- 21 Wm. Rosenbrook, Jr., D. A. Riley, and P. A. Lartey, *Tetrahedron Lett.*, **26**, 3 (1985); G. H. Posner and S. R. Haines, *ibid.*, **26**, 5 (1985).
- 22 T. Mukaiyama, Y. Murai, and S. Shoda, *Chem. Lett.*, 431 (1981); T. Mukaiyama, Y. Hashimoto, and S. Shoda, *ibid.*, 935 (1985).