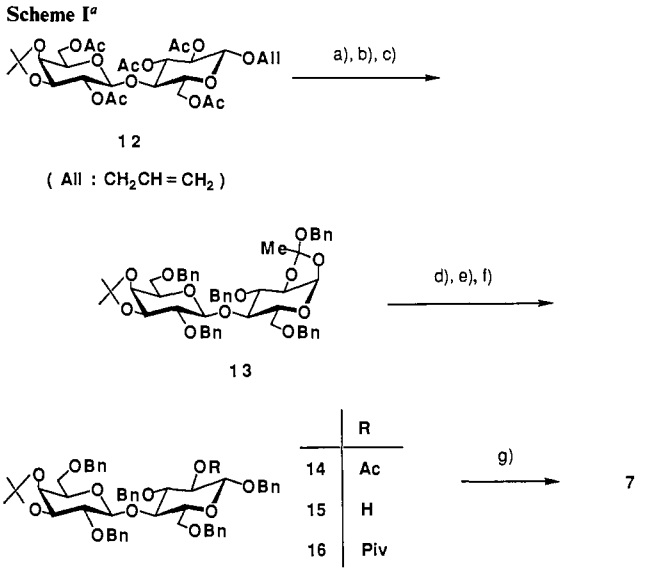
Scheme I[a]



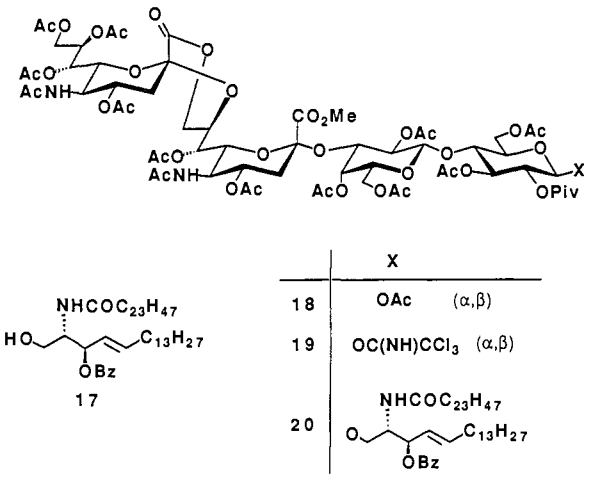
[a](a) $(Ph_3P)_3RhCl$/EtOH–benzene–H_2O,[16] reflux for 20 h; HgO, $HgCl_2$/aqueous acetone,[17] room temperature for 1 h, 68%. (b) CBr_4, $(Me_2N)_3P$/THF, −20 °C to room temperature, 18 h; $PhCH_2OH$, nBu_4NBr, Et_3N/CH_2Cl_2, reflux for 18 h, 63%. (c) NaOMe/MeOH, room temperature for 5 h; $PhCH_2Br$, NaH/DMF, room temperature for 18 h, 86%. (d) TMSOTf/CH_2Cl_2,[18] 0 °C for 1.5 h. (e) NaOMe/MeOH, 60 °C for 18 h, 98%. (f) Me_3CCOCl, 4-DMAP/pyridine, 80 °C for 18 h, 91%. (g) Aqueous CF_3CO_2H, 0 °C for 2.5 h, 92%.

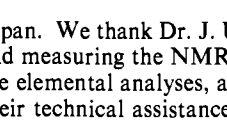
(*c* 1.6), in 78% yield. After deprotection in a standard manner [(1) H_2, $Pd(OH)_2$/MeOH; (2) NaOH/aqueous MeOH], tetrasaccharide **2**, the glycan part of GD_3, was obtained quantitatively; its ^1H NMR spectrum (500 MHz, D_2O) showed characteristic signals at δ 5.207 (d, J = 3.9 Hz, H-1a$_\alpha$), 4.643 (d, J = 7.8 Hz, H-1a$_\beta$), 4.504 (d, J = 7.8 Hz, H-1b), 2.767 (dd, J = 12.5 and 4.6 Hz, H-3d$_{eq}$[21]), 2.669 (dd, J = 12.2 and 4.4 Hz, H-3c$_{eq}$[21]), 2.052, 2.022 (2 s, 2Ac), and 1.727 (t, J = 12 Hz, H-3c,d$_{ax}$). On the other hand, debenzylation followed by acetylation [Ac_2O, pyridine, 4-DMAP] afforded the lactone **18**[22] as an inconsequential mixture of positional isomers with respect to the lactonic linkage. The mixture was, without separation, converted into the corresponding trichloroacetimidate **19**[22] [(1) piperidine, AcOH/THF; (2) CCl_3CN,[23] DBU/CH_2Cl_2; 62%], which was further reacted with the protected ceramide **17**[24] [1.0 equiv of TMSOTf, 4A molecular sieves/$CHCl_3$] to afford the coupled product **20**[22] in 32% yield. After deacetylation [NaOMe/MeOH] and saponification [NaOH/aqueous MeOH], GD_3 (**1**) was obtained in 95% yield. The ^1H NMR spectrum (500 MHz, DMSO-d_6–D_2O, 50:1) of synthetic **1** was in full agreement with the one reported for the natural sample by Yu et al.[25]

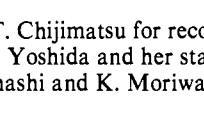
In summary, the first total synthesis of ganglioside GD_3 was achieved in a highly stereo- and regioselective manner.

Acknowledgment. This work was partly supported by Special Coordination Funds of the Science and Technology Agency of Japan. We thank Dr. J. Uzawa and T. Chijimatsu for recording and measuring the NMR spectra, M. Yoshida and her staff for the elemental analyses, and A. Takahashi and K. Moriwaki for their technical assistance.

Supplementary Material Available: Experimental procedures and physical properties for compounds **8**, **6**, and **3** and 500-MHz ^1H NMR spectra of synthetic **1** and **2** (5 pages). Ordering information is given on any current masthead page.







(18) Ogawa, T.; Beppu, K.; Nakabayashi, S. *Carbohydr. Res.* **1981**, *93*, c6–c9.
(19) From **5b**: $Hg(CN)_2$, $HgBr_2/CCl_4$; 30% yield, α:β = 20:1. From **5c**: AgOTf, Cp_2ZrCl_2[20]/CCl_4; 38% yield, α:β = 2.2:1.
(20) Matsumoto, T.; Maeta, H.; Suzuki, K.; Tsuchihashi, G. *Tetrahedron Lett.* **1988**, *29*, 3567–3570.
(21) These assignments may be interchanged.
(22) The assignments of the hydroxy group involved in the lactonic linkage are tentative. For lactonization of sialooligosaccharides, see: Sonnino, S.; Kirshner, G.; Fronza, G.; Egge, H.; Ghidoni, R.; Acquotti, D.; Tettamanti, G. *Glycoconjugate J.* **1985**, *2*, 343–354. Roy, R.; Laferrière, C. A.; Dettman, H. *Carbohydr. Res.* **1989**, *186*, c1–c5.
(23) For a review, see: Schmidt, R. R. *Angew. Chem., Int. Ed. Engl.* **1986**, *25*, 212–235.
(24) Koike, K.; Nakahara, Y.; Ogawa, T. *Glycoconjugate J.* **1984**, *1*, 107–109. Koike, K.; Numata, M.; Sugimoto, M.; Nakahara, Y.; Ogawa, T. *Carbohydr. Res.* **1986**, *158*, 113–123.
(25) Yu, R. K.; Koelner, T. A. W.; Scarsdale, J. N.; Prestegard, J. H. *Chem. Phys. Lipids* **1986**, *42*, 27–48.

Bromochlorofluoromethane and Deuteriobromochlorofluoromethane of High Optical Purity

Thomas R. Doyle and Otto Vogl*

Polytechnic University, 333 Jay Street
Brooklyn, New York 11201
Received July 5, 1989

Bromochlorofluoromethane (**1**) has been of considerable interest for nearly one century because of the chirality engendered by the all-halogen pendant group. Two synthetic approaches have been made to prepare **1**. One method involves the direct separation of racemic **1** into its antipodes. The second method involves the synthesis of optically active intermediates, which then undergo stereoselective reactions in the final steps to prepare optically active **1**.[1-3]

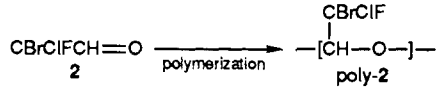
Hargreaves[3] obtained (+)-**1** and (−)-**1** with $[\alpha]^{19}_D$ = +0.20° and −0.13° (in cyclohexane) respectively by treating (+)- and (−)-BrClFCCOCH$_3$ with KOH; **1** was also prepared with an $[\alpha]^{19}_D$ of +0.13° (neat) upon complexation of **1** with brucine[4] and was shown to have an enantiomeric excess of 4.3 ± 1%. The enantiomeric excess was demonstrated by ^1H NMR spectroscopy of a diastereomeric inclusion complex of **1** with a chiral tailor-made cryptophane.[5] Extrapolation of this rotation value to enantiomeric purity gave a maximum rotation for **1** of α^{25}_D = +3.0 ± 0.5° and an α^{25}_{365} of +6.2 ± 1°.

We wished to prepare **1** of high enantiomeric purity because of our interest in the synthesis and polymerization of optically active bromochlorofluoroacetaldehyde (**2**) to chiral poly-**2**, a polymer that was expected to have optical activity based on the contribution not only from the chiral bromochlorofluoromethyl

(1) Swarts, F. *Bull. Cl. Sci., Acad. R. Belg.* **1893**, [3]*26*, 102; **1896**, [3]*31*, 28; *Memoires Couronnes* **1896**, *54*, 1–26.
(2) Berry, K. L.; Sturtevant, J. M. *J. Am. Chem. Soc.* **1942**, *64*, 1599–1600.
(3) Hargreaves, M. K.; Modarai, B. *J. Chem. Soc. D* **1969**, *No. 1*, 16. Hargreaves, M. K.; Modarai, B. *J. Chem. Soc. C* **1971**, *No. 5*, 1013–1015.
(4) Wilen, S. H.; Bunding, K. A.; Kascheres, C. M.; Wieder, M. J. *J. Am. Chem. Soc.* **1985**, *107*, 6997–6998.
(5) Canceill, J.; Lacombe, L.; Collet, A. *J. Am. Chem. Soc.* **1985**, *107*, 6993–6996.

0002-7863/89/1511-8510$01.50/0

unit attached to the polyacetal main chain but also from the rigid helical polymer chain conformation which causes optical activity based on macromolecular asymmetry.[6]



For the preparation of **2**, we started with 1-chloro-1,2,2-trifluoroethylene and obtained, after a sequence of five synthesis steps, bromochlorofluoroacetic acid (**3**) in about 25% yield.[7] **3** could be separated into the optical antipodes by fractional crystallization of its strychnine salt from methanol;[1] no melting point.

The determination of the optical purity was attempted by ^{19}F NMR spectroscopy of the strychnine salt of **3** in nonpolar solvents, but neither the salt nor the salt in the presence of shift reagents (chiral or achiral) gave satisfactory results. However, it was possible to determine the optical purity of **3** by esterification of the acid with borneol and employing ^{19}F NMR spectroscopy for the determination of the optical purity. The chemical shift of the diasteromeric esters gave fluorine shift values at about −63.8 ppm (relative to fluorotrichloromethane). The ^{19}F peaks of the diastereomers were separated by 0.13 ppm. The borneol ester of racemic **3** gave a 50/50 integration of the ^{19}F spectra for the diastereomers.[7]

The plot of the optical rotation as a function of optical purity of **3** (as determined by ^{19}F NMR spectroscopy of its borneol ester) resulted in a linear relationship. The pure antipode was calculated to have an $[\alpha]^{22}_D$ of 15.5 ± 0.2° (diethyl ether, 6.50 g/dL). The least soluble salt fraction yielded **3** with an $[\alpha]^{22}_D$ of +10.3° (diethyl ether, 6.86 g/dL) (path length 10 mm), and the most soluble salt fraction yielded **3** with an $[\alpha]^{22}_D$ of −6.3° (diethyl ether, 6.17 g/dL) (path length 10 mm). The enantiomeric excess was 66% (83/17 mixture of the (+) antipode) and 42% (29/71 mixture of the (−) antipode), respectively.

The strychnine salt of **3** was thermally decarboxylated in a heterogeneous medium between 100 and 120 °C when ethylene glycol or deuterium oxide was used as the decarboxylation medium; **1** was obtained in 50–70% yield in ethylene glycol and in 30% yield in deuterium oxide. Elemental anal. Calcd for CHBrClF: C, 8.15; H, 0.69. Found: C, 8.19; H, 0.70. NMR: ^1H, 7.35 and 7.95 ppm (J_{FH} = 54 Hz); ^{13}C, 84.65 and 98.17 ppm (J_{FC} = 304.43 Hz); ^{19}F, −80.09 and −80.74 ppm (J_{FH} = 54.99 Hz).

$$BrClFCCOO^-, \text{strychnine}^+H \xrightarrow[HOCH_2CH_2OH \text{ (or } D_2O)]{100\text{–}120\ ^\circ C} \underset{\mathbf{1}}{BrClFCH \text{ (or } BrClFCD)} + CO_2$$

The least soluble strychnine salt of (+)-**3** was decarboxylated in ethylene glycol, and **1** was obtained with an optical rotation of α^{22}_D = +1.80 ± 0.04° (neat) (path length 1 dm). The most soluble strychnine salt of (−)-**3** after decarboxylation gave **1** with an optical rotation of α^{22}_D = −0.92 ± 0.04° (neat) (path length 1 dm). When the extrapolated values of Collet[5] for optical purity (3.0 ± 0.5°) were used, the optical purity of (+)-**1** was 60% and that of (−)-**1** was 31%. Using our determination of the optical purity of **3** by the borneol ester method[7] and assuming that no racemization had occurred during this decarboxylation procedure of **3** to **1**, we estimate a maximum value of α^{22}_D of 2.75 ± 0.05° as the value of the pure antipode of **1** (probably in the range of 2.7° and 3.5°). This value is in good agreement with the value of α^{25}_D = 3.0 ± 0.5° obtained previously.[4,5] The extrapolated value of ref 4 and 5 for optically pure **1** has the disadvantage of using **1** with a very low optical excess of the antipodes for the extrapolation.

We have also decarboxylated (−)-**3** (enantiomeric purity 34%) in deuterium oxide and obtained (−)-**1** with a deuterium purity (by ^{19}F spectroscopy) as high as 96%. The optical rotation of deuterated (−)-**1** was α^{22}_D = −1.35 ± 0.05°, α^{22}_{365} = −2.35 ± 0.05° (neat, path length 2 mm), which corresponds to the optical rotation of deuterated **1** of α^{22}_D = −4.0 ± 0.3° or α^{22}_{365} = 7.0 ± 0.5° for 100% optical purity. NMR: ^{13}C, 81.72, 83.22, 84.72, 95.16, 96.66, and 98.17 ppm (J_{CF} = 302.97 Hz and J_{CD} = 33.82 Hz); ^{19}F, −82.09, −82.18, −82.31 ppm (J_{FD} = 8.3 Hz).

Our results indicate that decarboxylations of the strychnine salts of **3** in protic (deuterio) media result in the formation of **1** with a high degree of selectivity in retention (or inversion) of the configuration. We believe that **1** of 100% optical purity could be obtained by decarboxylation of optically pure **3**; with our present accuracy limit, we can only predict an optical purity of at least 80%.

Acknowledgment. We thank J. Bartus for his assistance in the measurements of the optical rotations and his advice on experimental procedures, Paul Resnick, and Koichi Hatada and his research group for their assistance with the ^{19}F NMR spectroscopy of the borneol esters. We also appreciate the comments of Mark Green.

(6) Corley, L. S.; Vogl, O. *Polym. Bull. (Berlin)* **1980**, *3*, 211–217. Doyle, T. R.; Vogl, O. *Polym. Bull. (Berlin)* **1985**, *14*, 535–540. Vogl, O.; Corley, L. S.; Harris, W. J.; Jaycox, G. D.; Zhang, J. *Makromol. Chem., Suppl.* **1985**, *13*, 1–12.

(7) Doyle, T. R. Ph.D. Dissertation, Polytechnic University, Brooklyn, NY 11201, 1989. Hatada, K.; Ute, K.; Nakano, T.; Okamoto, Y.; Doyle, T. R.; Vogl, O. *Polymer J. (Tokyo)* **1989**, *21*, 171–177.

Crystal Structure of Holoenolase Refined at 1.9 Å Resolution: Trigonal-Bipyramidal Geometry of the Cation Binding Site

Lukasz Lebioda* and Boguslaw Stec†

Department of Chemistry
University of South Carolina
Columbia, South Carolina 29208
Received July 24, 1989

We have determined and refined, using crystallographic restrained least squares, the structure of the enolase–Zn^{2+} complex at 1.9-Å resolution. The final crystallographic *R* factor was 14.9%, and bond lengths in the molecule have a root-mean-square deviation from ideal values of 0.015 Å. The ligands of the Zn^{2+} cation are all oxygen atoms which form an almost regular trigonal bipyramid with the monodentate carboxylic groups of Asp246 and Asp320 in the axial positions and the monodentate carboxylic group of Glu295 and two water molecules in the equatorial positions. The enolase–Zn^{2+} complex has 80% of the activity of the physiological enolase–Mg^{2+} complex,[1,2] so it is most probable that the Zn^{2+} and Mg^{2+} complexes are isostructural. The structure of the active ternary complex enolase–Mg^{2+}–2-phosphoglycerate/phosphoenolpyruvate also shows a trigonal bipyramid coordination geometry for Mg^{2+} cation. A search through the Cambridge Crystallographic Database[3] did not find any all-oxygen trigonal-bipyramidal coordination geometry for Mg^{2+} ion and only two for Zn^{2+} ion. Thus the metal-ion environment in the active site of enolase is unusual and most probably critical for the catalytic process. It can be speculated that five-coordinated Mg^{2+} or Zn^{2+} ions are well suited for participation in enolase catalysis. They should strongly polarize the ligand, the H_2O molecule or the hydroxyl group of 2-phosphoglycerate; on the other hand, the ion environment should be relatively unstable to allow easy dissociation of substrate/product molecules.

The structure of holoenolase is very similar to that of apoenolase,[4,5] with an average deviation between the main-chain atoms of 0.19 Å and 0.31 Å between the side-chain atoms.

Enolase catalyzes the dehydration of 2-phospho-D-glycerate to phosphoenolpyruvate. All known enolases exhibit an absolute

† Permanent address: SLAFiBS, Jagiellonian University, Cracow, Poland.

(1) Wold, F.; Ballou, C. E. *J. Biol. Chem.* **1957**, *227*, 313.
(2) Elliot, J. I.; Brewer, J. M. *J. Inorg. Biochem.* **1984**, *20*, 39.
(3) Allen, F. H.; Kennard, O.; Taylor, R. *Acc. Chem. Res.* **1983**, *16*, 146. January 1989 edition of *Cambridge Structural Database*.
(4) Lebioda, L.; Stec, B.; Brewer, J. M. *J. Biol. Chem.* **1989**, *264*, 3685.
(5) Stec, B.; Lebioda, L. *J. Mol. Biol.* In press.

0002-7863/89/1511-8511$01.50/0