A third caveat concerns molecular mechanical force field and simulation protocol, involving the use of a simple molecular mechanical model and keeping the part of the protein further than 15 Å from the mutation frozen. This "belly approach" appears at least as reasonable and has seemed to work effectively in many different systems. Again, the molecular mechanical parameters are clearly far from perfect, but the use of the same parameters in ΔG_{PN} and ΔG_{PD} may allow for significant cancellation of errors.

One of the most significant and interesting results found in this study has been the fact that the differential stability $\Delta\Delta G$ is mainly a van der Waals rather than electrostic effect. How definite is this conclusion? By using the "slow-growth" procedure, we can unambiguously compute both the electrostatic and van der Waals contribution to the free energy changes. Thus, our calculated result is on firm ground. And it is further supported by our model mutation, in which we zero the partial charges on the O–H group in native and denatured states. Again, this result is consistent with actual mutation calculation in that the electrostatic contribution to the stability of both native and denatured models is nearly identical. It is clear that only the OH and not a CH_3 group can "fit" into the native structure at position 157 and gain more dispersion attraction and avoid exchange repulsion in this position.

One of the most important results of this study is the further demonstration of the power and utility of free energy component analysis.[12] No only can free energy calculations give free energies that can be related to those determined experimentally, but model calculations, such as the zeroing of the charges on the O–H group noted above and mutation of the Asp-159 N–H charges to zero in both Thr and mutant Val native structure, show the power of model calculations using this method to give new insight into protein stability. One of the important functions of theoretical calculation on molecules is to give useful mechanistic insights, and it is clear that the free energy approach fullfills this function in many cases.

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Conclusion

In summary, we have carried out free energy simulations on the Thr-157 → Val-157 mutation in T4 lysozyme. The calculations are successful in reproducing the experimental $\Delta\Delta G$ of protein stability, and this success suggests, at least in this case, a tripeptide model is adequate to represent the denatured protein. It seems counterintuitive that a hydrophilic residue like Thr will provide more stability to the native protein than to the more solvent-exposed denatured protein. Our calculations suggest that, even with the excellent hydrogen-bonding network of Thr-157 in the native protein, the electrostatic hydrogen bonding is no better than in the denatured native protein. Nonetheless, van der Waals energies contribute significantly to the differential stabilization of the Thr-157 than the Val-157 proteins because of greater dispersion attraction or less exchange repulsion of the smaller OH group compared to a CH_3 group. The calculations are less successful in their ability to reproduce all the hydrogen-bonding details of the crystal structures, which is not surprising given the limited time and simple environmental representation used in these simulations and the possibility that surface groups are indeed more mobile in solution than reflected in the crystal.

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Communications to the Editor

Highly Stereoselective Synthesis of Ganglioside GD_3[1]

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Gangliosides, namely, sialic acid containing glycolipids, have attracted much attention because of the numerous biological roles they play in cellular recognition, differentiation, oncogenesis, and so on.[2] In spite of the structural diversity and prevalence in cell surfaces, synthetic studies toward such molecules have been limited to relatively simple ones.[3] This has been mainly due to the low efficiency encountered in the introduction of a sialic acid residue, especially when a secondary alcohol was used as a glycosyl acceptor.[4,5] Recently, we succeeded in obtaining a general solution for this critical problem, which affords α-glycosides of *N*-acetylneuraminic acid (NeuAc), the most representative in a sialic acid family, under nearly complete stereochemical control.[6] Our approach features an efficient use of the stereocontrolling phenylseleno or phenylthio auxiliary residing at the C-3 position of a NeuAc donor such as **9**. With this potent device in hand, our attention has been centered around the synthetic approach toward complex gangliosides with multiple NeuAc residues. Now, we report here the implementation of our strategy to the highly selective synthesis of ganglioside GD_3 (**1**), which has been isolated from various sources such as mammalian retina,[7a] bovine kidney,[7b]

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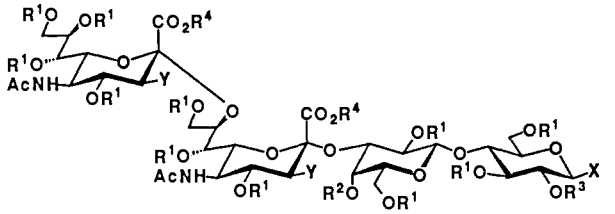
and brain tissues[7c] and is well-known as a human melanoma associated antigen.[8]

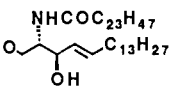
The glycan chain of **1** was planned to be constructed from two disaccharide segments **5** and **7**. As a precursor of **5**, 2,3-dehydro derivative **8** was assumed, which was expected to be obtained by the coupling of the 4,7,9-tri-*O*-benzyl derivative **10** with the bromide **9**.

The synthesis of **10**, $[\alpha]_D$ +6.7° (*c* 0.9),[9] was achieved in four steps from known tetraacetate **11**[10] [(1) NaOMe/MeOH; (2) $PhCH(OMe)_2$, CSA/DMF; (3) $PhCH_2Br$, KOH, CaH_2, *n*-Bu_4NI/DMSO, then CH_2N_2/MeOH-Et_2O; (4) BH_3·NMe_3, $AlCl_3$/THF;[11] 50% overall]. The bromide **9** was then reacted with **10** (1.5 equiv) under well-established conditions[6b] [1.6 equiv of $Hg(CN)_2$, 0.5 equiv of $HgBr_2$, 4A molecular sieves/CCl_4] to afford an anomerically pure disaccharide **8**, $[\alpha]_D$ +12.0° (*c* 0.6), in 64% yield based on **9**. This result is highlighted in comparison with previous efforts toward synthesis of α-D-Neu*p*5Ac-(2→8)-D-Neu*p*5Ac derivatives[4c,d] which have resulted in either low yield or lack of stereoselectivity. In order to derivatize **8** into **5**, further installation of the stereocontrolling phenylthio auxiliary was required. This was achieved by following our addition–epimerization protocol[6b] [(1) NBS/aqueous MeCN; (2) PhSK/*t*-BuOH–THF; (3) DBU/toluene; 80% overall] via a diaxial adduct, and the resultant C-3β hemiketal **6**, $[\alpha]_D$ +29.7° (*c* 1.0), was converted into the bromide **5a**[12] [CBr_4, $(Me_2N)_3P$/THF], the chloride **5b** [CCl_4, $(Me_2N)_3P$/THF; 84%], and the fluoride **5c** [DAST[13]/toluene; 96%; α:β = 45:55].

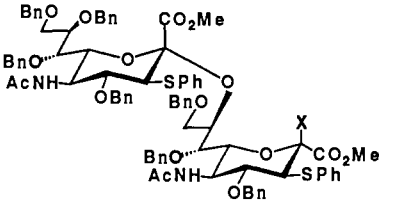
The lactose segment was designed as **7** so as to meet dual demands as follows. First, the C-2a position was protected with a pivaloyl group in order to facilitate the coupling with the ceramide portion.[14] Second, the C-3b position was made to be relatively unhindered by leaving the C-4b OH unprotected, considering the high C-3 vs C-4 regioselectivity observed repeatedly for similar glycosylations.[3a,c,d,4a–c,6a,b] Compound **7**, $[\alpha]_D$ −3.7° (*c* 0.4), was synthesized in a straightforward manner as shown in Scheme I. Thus, the orthoester **13** derived from **12**[15] was transformed into **7**, via **14**, **15**, and **16**. Further glycosylation of **7** into the tetrasaccharide was best accomplished by use of the bromide **5a** [$Hg(CN)_2$, $HgBr_2$/CCl_4], and the α product **3**, $[\alpha]_D$ +14.9° (*c* 1.0), was obtained in 48% yield based on **6**, together with a trace amount of the β-isomer (α:β = 60:1). A corresponding regioisomer could not be detected. On the other hand, the chloride **5b** and the fluoride **5c** were proved to be less efficient for this critical transformation.[19]

Chart I

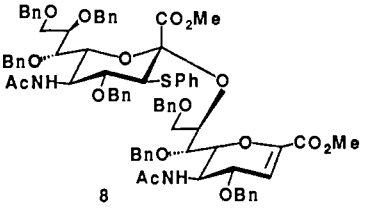


	Y	R^1	R^2	R^3	R^4	X
1	H	H	H	H	H	
2	H	H	H	H	H	OH (α,β)
3	SPh	Bn	H	Piv	Me	OBn
4	H	Bn	Ac	Piv	Me	OBn

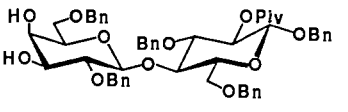
(Piv: $COCMe_3$)



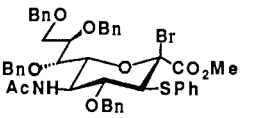
	X
5a	Br
b	Cl
c	F
6	OH



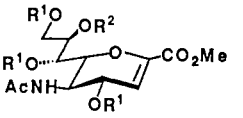
8



7



9



	R^1	R^2
10	Bn	H
11	Ac	Ac

Acetylation of **3** followed by the reductive removal of the phenylthio groups [Ph_3SnH, AIBN/benzene] gave **4**, $[\alpha]_D$ −0.5°

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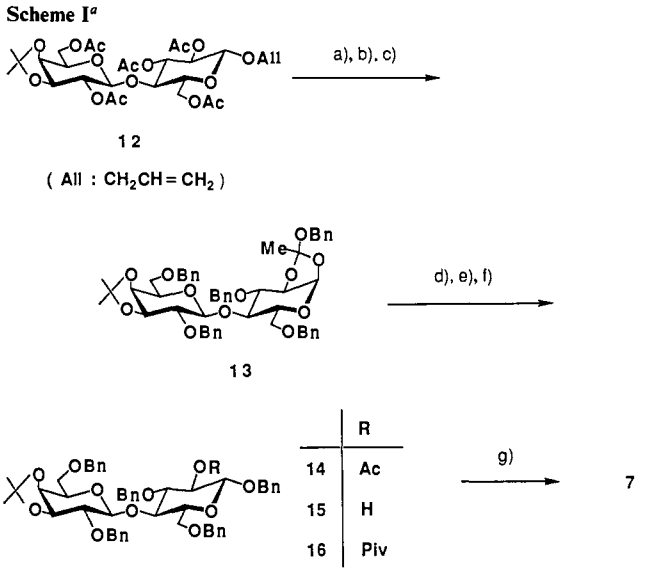
Scheme I[a]



[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.

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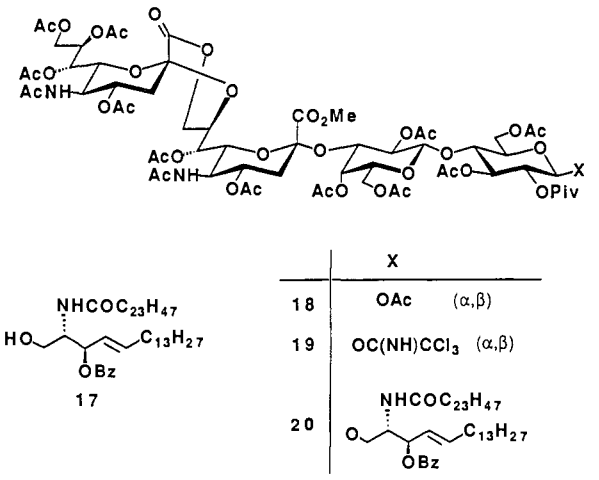
Supplementary Material Available: Experimental procedures and physical properties for compounds **8**, **6**, and **3** and 500-MHz ^1H NMR spectra of synthetic **1** and **2** (5 pages). Ordering information is given on any current masthead page.



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(19) From **5b**: $Hg(CN)_2$, $HgBr_2/CCl_4$; 30% yield, $\alpha:\beta$ = 20:1. From **5c**: AgOTf, Cp_2ZrCl_2[20]/CCl_4; 38% yield, $\alpha:\beta$ = 2.2:1.
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Bromochlorofluoromethane and Deuteriobromochlorofluoromethane of High Optical Purity

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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

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