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# "Armed-Disarmed" Glycosidation Strategy Based on Glycosyl Donors and Acceptors Carrying Phosphoroamidate as a Leaving Group: A Convergent Synthesis of Globotriaosylceramide

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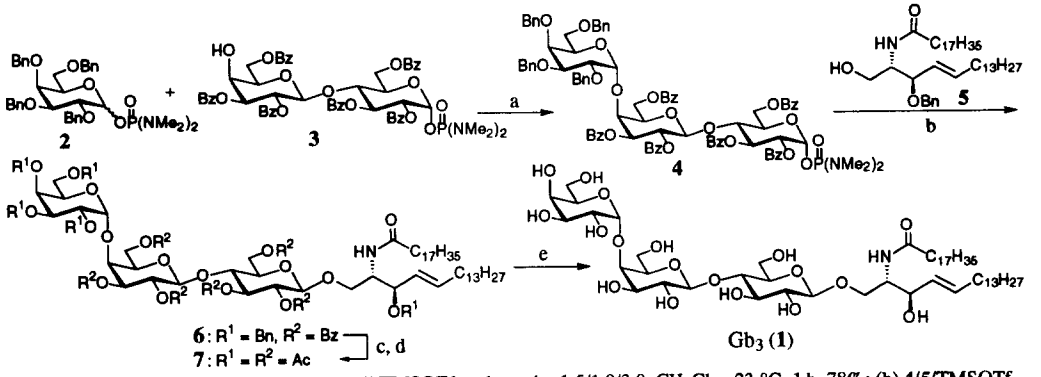
***Abstract:*** *A stereocontrolled synthesis of globotriaosylceramide with three different glycosidic linkages has been accomplished by linear and convergent routes exploiting "armed-disarmed" glycosidation methodology based on glycosyl donors and acceptors carrying tetramethylphosphoroamidate as a leaving group. In particular, the convergent strategy featuring a coupling of a galactosyl-(1→4)-galactosyl donor with a glucosylceramide derivative has proven to be extremely efficient.*


With the advent of the "armed-disarmed" principle proposed by Fraser-Reid,[2] the development of innovative strategies for the synthesis of oligosaccharides has been the subject of intensive investigations in glycotechnology. The "armed-disarmed" glycosidation strategy[2,3] originally based on the electronic or torsional effects of protective groups in a saccharide molecule on anomeric reactivity is currently expanding into "active-latent",[4,5] "one-pot",[6-9] and "orthogonal"[10] glycosidation strategies.[11] Recently, we have reported the chemoselective glycosidation method based on glycosyl donors and acceptors carrying phosphorus-containing leaving groups, wherein the tetramethylphosphoroamidate group plays a pivotal role as an anomeric protective group as well as a leaving group.[12] To test the feasibility of our glycosidation method for the synthesis of biologically important oligosaccharides, we now addressed a synthesis of globotriaosylceramide ($Gb_3$, **1**).

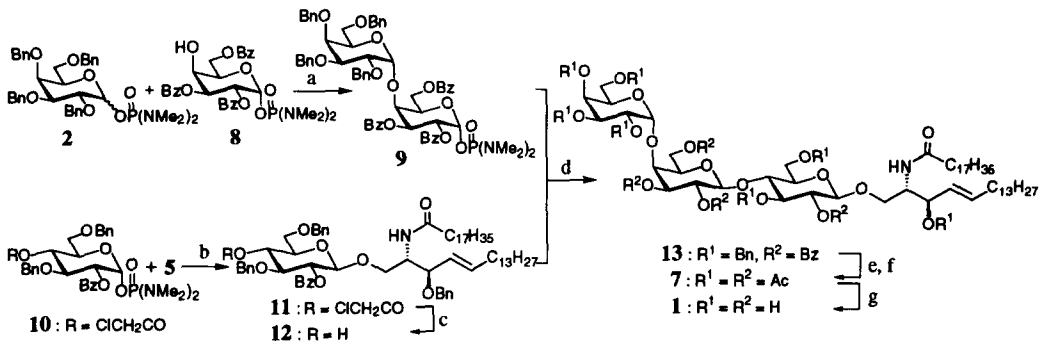
$Gb_3$ (**1**), known as $P^k$ antigen in the P blood-group system,[13] has been shown to be highly expressed in Burkitt lymphoma cell-lines,[14] human teratocarcinoma,[15] human embryonal carcinoma,[16] and other types of tumor cells,[17] and it is also closely related to Fabry's disease which is due to a deficiency of α-galactosidase activity.[18] Recently, this molecule has been recognized as a cell-surface receptor for Shiga-like toxin and verotoxin.[19] Owing to its biological significance as well as a common structure, α-D-Gal-(1→4)-β-D-Gal-(1→4)-β-D-Glc-ceramide, shared by various *globo*-series glycosphingolipids, total syntheses of $Gb_3$ have already been reported by four groups.[20-23] However, these total syntheses incur the problem of stereocontrol of the α-galactosidic linkage, except for the synthesis of Nicolaou and his coworkers,[22] as well as requiring extra steps to convert an anomeric protective group of glycosyl acceptors after glycosidation into a leaving group for the next coupling. Furthermore, the yields in coupling of a trisaccharide donor with a ceramide derivative are far from satisfactory as is usual for most glycosphingolipid syntheses;[24] in this respect, the "azidosphingosine glycosylation procedure" developed by Schmidt[25] has proven to be the method of choice, though this method requires two additional steps involving azide reduction and amide formation. Herein, we wish to report a stereocontrolled synthesis of $Gb_3$ (**1**) by linear and convergent routes based on the "armed-disarmed" methodology, wherein the convergent strategy featuring a coupling of an α-galactosyl-(1→4)-galactosyl donor with a β-glucosylceramide derivative has proven to be extremely efficient.



**Scheme 1.** *Reagents and conditions*: (a) **2**/**3**/TMSOTf molar ratio=1.5/1.0/3.0, $CH_2Cl_2$, -23 °C, 1 h, 78%; (b) **4**/**5**/TMSOTf molar ratio=1.0/1.1/2.0, $CH_2Cl_2$, 0 °C, 1 h, 45%; (c) Na, liq. $NH_3$, THF, -78 to -20 °C, 1 h, then MeOH; (d) $Ac_2O$, pyridine, 50% (3 steps); (e) NaOMe, MeOH, 91%.

Our initial approach was conventionally centered on the "armed-disarmed" coupling of a galactosyl donor with a lactosyl acceptor followed by attachment of the ceramide appendage (Scheme 1). Toward this end, chemoselective glycosidation of the fully benzylated galactosyl tetramethylphosphoroamidate **2**[26] (α:β=90:10) with the partially benzoylated α-lactosyl tetramethylphosphoroamidate **3**[27] was explored. We previously observed that trimethylsilyl trifluoromethanesulfonate (TMSOTf)-promoted glycosidations of **2** with primary alcohols or less congested secondary alcohols in propionitrile at -78 °C exhibited high levels of β-selectivity due probably to the intermediacy of an α-nitrilium ion whereas use of other solvents such as $CH_2Cl_2$, ether, and toluene displayed poor to modest selectivities.[26] Thus, we were gratified to find that TMSOTf-promoted coupling of the "armed" donor **2** with the "disarmed" acceptor **3** in $CH_2Cl_2$ at -23 °C proceeded smoothly to afford exclusively the desired trisaccharide **4**[29,30] in 78% yield, no products arising from self-condensation of **3** being detected. The complete α-selectivity might be attributed to the extremely poor nucleophilicity of the axial hydroxyl group at C4' in **3** wherein, apart from the steric factor, the adjacent electron-withdrawing benzoyl groups decrease the electron density at the oxygen atom so as to favor the axial attack of the alcohol on the oxocarbenium ion generated from **2**. The crucial coupling of the "disarmed" trisaccharide **4** with the ceramide derivative **5**[31] was achieved by the aid of TMSOTf in $CH_2Cl_2$ at 0 °C to give the protected $Gb_3$ **6** in 45% yield and with complete stereocontrol as expected from the neighboring group participation of the 2-*O*-benzoyl group. Orthoester formation was not detected; however, cleavage of the acid-labile α-galactosidic linkage concurrent with the formation of **6** was found to reduce the product yield.[33] Deprotection of the benzyl and benzoyl groups was effected in one-pot by treatment of **6** with sodium in liquid ammonia followed by the action of methanol. In order to facilitate the isolation, the resultant product was protected as the peracetate **7** which, upon methanolysis, furnished the target $Gb_3$ (**1**), $[\alpha]_D^{25}$ +23.9 (*c* 0.98, pyridine) [lit.,[22b] $[\alpha]_D^{23}$ +24.1 (*c* 0.44, pyridine)], in 46% overall yield from **6**.

While a stereocontrolled assembly of the building blocks **2**, **3** and **5** was accomplished by a linear route without a single protective group manipulation, there was great room for improvement in the direct coupling of the trisaccharide donor **4** and the ceramide derivative **5** as well as the multi-step preparation of the lactosyl acceptor **3**. To overcome these problems, we envisaged a convergent strategy involving coupling of an α-galactosyl-(1→4)-galactosyl donor with a β-glucosylceramide derivative (Scheme 2). Toward a second-generation synthesis of **1**, coupling of the fully benzylated galactosyl tetramethylphosphoroamidate **2** with the partially benzoylated α-galactosyl tetramethylphosphoroamidate **8**[34] was carried out in the presence of TMSOTf in $CH_2Cl_2$ at -45 °C to furnish the disaccharide **9**[36] as mainly the α-anomer (α:β=97:3) in 85% yield. The virtually complete α-selectivity might also be accounted for by the foregoing steric and electronic factors



**Scheme 2.** *Reagents and conditions*: (a) **2/8**/TMSOTf molar ratio=1.5/1.0/3.0, $CH_2Cl_2$, -46 °C, 2 h, 85% (α:β=97:3) (72% after separation from β-anomer); (b) **10/5**/TMSOTf molar ratio=1.0/1.1/2.0, $CH_2Cl_2$, 0 °C, 1 h, 74%; (c) $H_2NC(S)NH_2$, 2,6-lutidine, EtOH, 70 °C, 2 h, 99%; (d) **9/12**/TMSOTf molar ratio=1.0/1.1/2.0, $CH_2Cl_2$, 0 °C, 1 h, 80%; (e) Na, liq. $NH_3$, THF, -78 to -20 °C, 1 h, then MeOH; (f) $Ac_2O$, pyridine, 63% (3 steps); (g) NaOMe, MeOH, 88%.

imparted on the acceptor alcohol. On the other hand, the β-glucosylceramide acceptor **12** was highly efficiently synthesized by glycosidation of the appropriately protected α-glucosyl tetramethylphosphoroamidate **10**[37] with the ceramide derivative **5** followed by selective removal of the chloroacetyl group from **11** with thiourea (73% overall yield). Indeed, TMSOTf-promoted coupling of the "disarmed" disaccharide **9** with **12** in $CH_2Cl_2$ at 0 °C was found to proceed uneventfully to give the protected $Gb_3$ **13** in 80% yield. Deblocking of all the protective groups in **13** under the foregoing conditions completed the convergent synthesis of **1**.

In summary, we have accomplished a stereocontrolled synthesis of $Gb_3$ (**1**) by linear and convergent routes based on the "armed-disarmed" methodology, wherein the dual role of the tetramethylphosphoroamidate group as an anomeric protective group as well as a leaving group is crucial to the success of the present strategies. In particular, the second-generation synthesis which features a high-yielding coupling with a glucosylceramide derivative in place of the conventionally used coupling with azidosphigosine or ceramide derivatives should provide a new and facile access to a variety of biomedically important glycosphingolipids.[39]

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27. Compound **3** was prepared from octa-*O*-acetyl-β-D-lactose by the following sequence: (1) *p*-methoxyphenol, TMSOTf, $(CH_2Cl)_2$, 2 h, 63%;[28] (2) $Et_3N$, MeOH, reflux, 14 h, 97%; (3) $Me_2C(OMe)_2$, TsOH, DMF, 1 h; (4) BzCl, DMAP, pyridine, $CH_2Cl_2$, 1 h, 68% (2 steps); (5) TFA-$H_2O$ (9:1), $CH_2Cl_2$, 30 min, 97%; (6) BzCN, $Et_3N$, DMF, -20 to 0 °C, 30 min, 88%; (7) $ClCH_2C(O)Cl$, pyridine, acetone, 1.5 h, 90%; (8) cerium(IV) ammonium nitrate, MeCN-$H_2O$ (4:1), 2 h, 85%; (9) *n*-BuLi, THF, -78 °C, 15 min, then $(Me_2N)_2P(O)Cl$, HMPA, -10 °C, 3 h, 55%; (10) $H_2NC(S)NH_2$, 2,6-lutidine, EtOH, 60 °C, 2 h, 71%.
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29. All new compounds exhibited satisfactory spectral (500 MHz $^1H$ NMR, 125 MHz $^{13}C$ NMR, and 109 MHz $^{31}P$ NMR) and high resolution mass spectral characteristics.
30. **4**: $^1H$ NMR ($CDCl_3$) δ 6.12 (dd, *J* = 3.3, 8.0 Hz, *H*1), 5.02 (d, *J* = 7.9 Hz, *H*1'), 4.81 (d, *J* = 3.4 Hz, *H*1"); $^{13}C$ NMR ($CDCl_3$) δ 101.71 101.26 (*C*1' and *C*1"), 91.81 (d, $J_{C\text{-}P}$ = 3.8 Hz, *C*1); $^{31}P$ NMR ($CDCl_3$) δ 19.66.
31. Compound **5** was prepared from (4*S*,α*R*)-3-*tert*-butoxycarbonyl-2,2-dimethyl-α-(1*E*-pentadecenyl)-1,3-oxazolidine-4-methanol[32] by the following sequence: (1) BnBr, NaH, $Bu_4NI$, THF-DMF (3:1), 12 h, 99%; (2) 6 N HCl-AcOEt (1:1), 40 h, 77%; (3) $C_{17}H_{35}C(O)Cl$, NaOAc, THF, 30 min, 91%.
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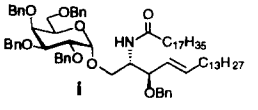
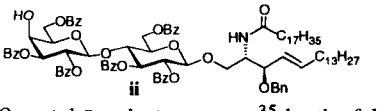
33. The reduced yield is due to the formation of 22% of α-galactosylceramide (**i**) and 11% of 4'-deprotected-β-lactosylceramide (**ii**).



i



ii

34. Compound **8** was prepared from 2,3,4,6-tetra-*O*-acetyl-D-galactopyranose[35] by the following sequence: (1) *n*-BuLi, THF, -78 °C, 15 min, then $(Me_2N)_2P(O)Cl$, HMPA, -10 °C, 2 h, 62 %; (2) $Et_3N$, MeOH, 1 d; (3) BzCl, pyridine, -40 to 0 °C, 6 h, 63% (2 steps).
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36. **9**: $^1H$ NMR ($CDCl_3$) δ 6.13 (dd, *J* = 3.5, 7.9 Hz, *H*1), 4.97 (d, *J* = 3.4 Hz, *H*1'); $^{13}C$ NMR ($CDCl_3$) δ 101.01 (*C*1'), 92.90 (d, $J_{C\text{-}P}$ = 3.8 Hz, *C*1); $^{31}P$ NMR ($CDCl_3$) δ 19.75 (α), 19.67 (β). The anomeric ratio was determined by $^{31}P$ NMR using 85% $H_3PO_4$ as an external standard.
37. Compound **10** was prepared from allyl 3-*O*-benzyl-4,6-*O*-benzylidene-D-glucopyranoside[38] by the following sequence: (1) BzCl, DMAP, pyridine, $CH_2Cl_2$, 2 h, 97%; (2) $NaBH_3CN$, satd. HCl in $Et_2O$, THF, 3A MS, 0 °C, 30 min, 96%; (3) $ClCH_2C(O)Cl$, pyridine, $CH_2Cl_2$, 0 °C, 2 h, 97%; (4) $PdCl_2$, NaOAc, AcOH-$H_2O$ (9:1), 80 °C, 1 h, 76%; (5) *n*-BuLi, THF, -78 °C, 15 min, then $(Me_2N)_2P(O)Cl$, HMPA, -20 °C, 2 h, 66%.
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