Pergamon

Tetrahedron Letters 41 (2000) 7691–7695

TETRAHEDRON LETTERS

Chemoselective glycosidation strategy based on glycosyl donors and acceptors carrying phosphorus-containing leaving groups: a convergent synthesis of ganglioside GM_3

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Received 13 July 2000; revised 3 August 2000; accepted 4 August 2000

Abstract

A convergent synthesis of ganglioside GM_3 has been achieved by capitalizing on three different phosphorus-containing leaving groups, in which the key features involve a chemo- and regioselective α-glycosidation of sialyl phosphite with partially benzylated galactosyl tetramethylphosphorodiamidate by the aid of trifluoromethanesulfonic acid and a traditionally uncommon coupling of an α-sialyl-(2→3)-galactosyl donor with a glucosylceramide building block which has the β-*O*-linked ceramide prebuilt into the glucose by a diphenyl phosphate method.

Keywords: glycosidation; glycolipids; phosphorous acids and derivatives; phosphoramidates.

With the advent of high-yielding and stereocontrolled glycosidation reactions, an enormous amount of effort is currently being devoted to the device and implementation of innovative strategies for the synthesis of various glycoconjugates as well as oligosaccharides.[1,2] Our efforts in this area have led to the development of a chemo- and stereoselective glycosidation method based on glycosyl donors and acceptors carrying phosphorus-containing leaving groups, in which a tetramethylphosphorodiamidate group plays a pivotal role as an anomeric protecting group as well as a leaving group.[3] The efficiency of this method was demonstrated by a convergent and stereocontrolled synthesis of glycosphingolipid Gb_3.[4] To test the feasibility of our glycosidation strategy for the synthesis of sialic acid-containing glycosphingolipids, we now addressed a synthesis of ganglioside GM_3 (**1**).[5]

Apart from its biological significance,[6] GM_3 (**1**) (α-NeuAc-(2→3)-β-D-Gal-(1→4)-β-D-Glc-ceramide) has presented itself as a target for evaluation of new glycosidation methodologies as well as chemical and enzymatic sialylation reactions. Chemical[7] and chemoenzymatic[8] syntheses of GM_3 have already been achieved by five and four groups, respectively, the majority of which commences with α-sialylation of a lactosyl acceptor followed by coupling with azidosphigosine or ceramide derivatives at a late stage.

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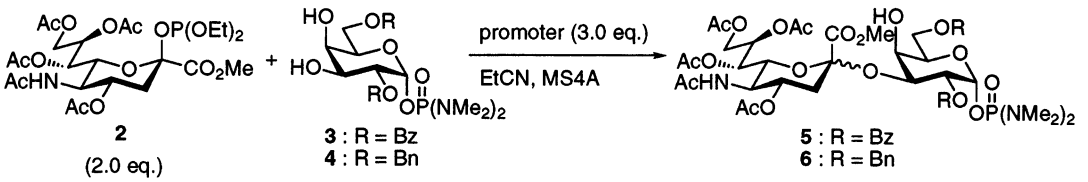
0040-4039/00/$ - see front matter
PII: S0040-4039(00)01343-5

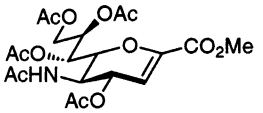
Our convergent strategy to **1** relies on α-selective sialylation of a galactosyl acceptor by means of the chemoselective glycosidation methodology followed by stereocontrolled coupling with a glucosylceramide building block which have a β-*O*-linked ceramide prebuilt into the glucose. In this context, the Schmidt and Wong groups have independently reported the effective use of sialyl phosphites for high-yielding and α-selective sialylation,[7d,9,10] in which the combinational use of TMSOTf as a promoter and MeCN as a solvent is also highlighted. Coupled with our continuing interest in the phosphite method,[11] we explored TMSOTf-promoted coupling of sialyl diethyl phosphite **2** with the partially benzoylated galactosyl tetramethylphosphorodiamidate **3**[12,14] selected as an ideal 'disarmed' acceptor for the subsequent coupling with neighboring group participation. However, the desired reaction did not work well (Table 1, entry 1). Instead, decomposition of **2** occurred to give 2,3-dehydro compound **7** as a major product, probably due to the poor nucleophilicity of **3**.[15]

Table 1
Sialylation of galactosyl tetramethylphosphorodiamidates



2 (2.0 eq.) + **3** : R = Bz, **4** : R = Bn → **5** : R = Bz, **6** : R = Bn

Entry	Acceptor	Promoter	Temp, °C[a]	Time, min	Yield, %	α:β[b]
1[c]	**3**	TMSOTf	-60	60	16	79:21
2[d]	**4**	$BF_3{\cdot}OEt_2$	-45	30	77	55:45
3	**4**	TfOH	-78	90	86	84:16



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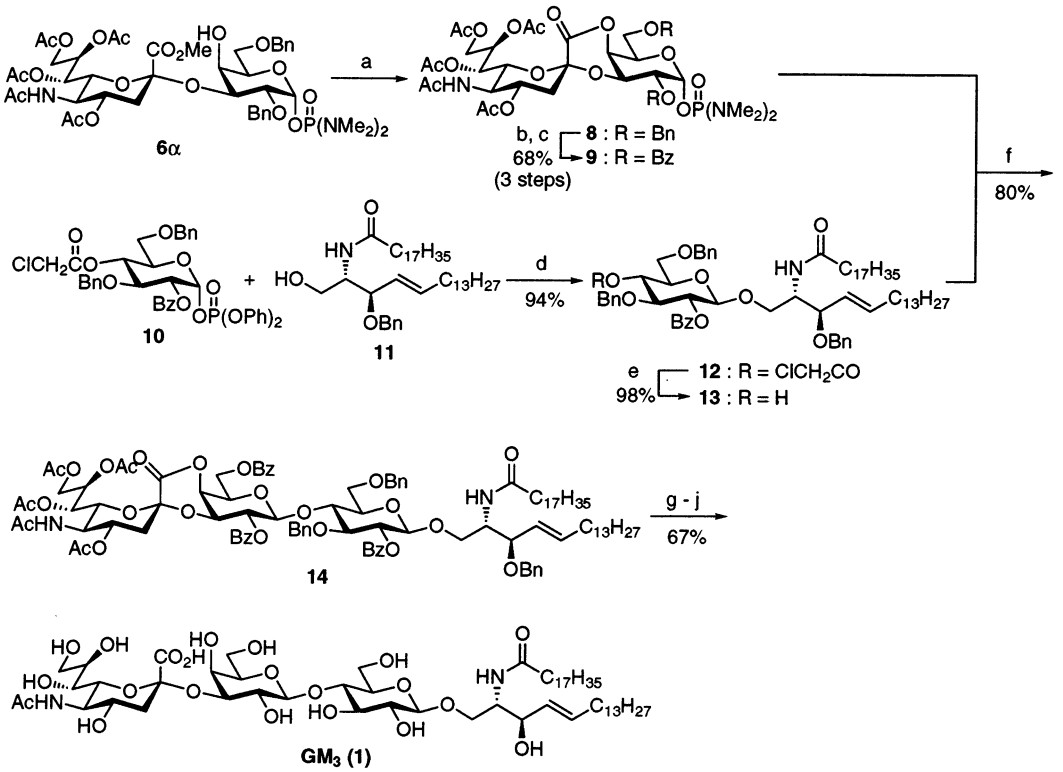
[a] The limit temperature allowing for smooth reaction. [b] The ratio was determined by ^{31}P NMR in $CDCl_3$ at 109 MHz using 85% H_3PO_4 as an external standard: **5**, δ_P=19.4 (α), 19.7 (β); **6**, δ_P=19.8 (α), 20.0 (β). [c] In the absence of MS4A. [d] Using 3.5 eq. of $BF_3{\cdot}OEt_2$.

In an attempt to enhance the reactivity of the acceptor alcohol, benzoyl protection on **3** was switched to benzyl ether protection. As might be expected, however, the protective group interchange presented another critical problem; the 2,6-di-*O*-benzyl-protected galactosyl phosphorodiamidate **4**[16] as well as sialyl phosphite **2** could be activated by the use of TMSOTf in EtCN at −78°C. Thus, we surveyed other promoters which could selectively activate the phosphite group in **2** while leaving the phosphorodiamidate in **4** intact. Based on our findings that glycosidation of the fully benzylated glycosyl diethyl phosphites could be effected with the aid of $BF_3{\cdot}OEt_2$ at −78°C whereas the corresponding tetramethylphosphorodiamidates were unreactive at below −10°C,[3] we first examined coupling of **2** with **4** under the influence of $BF_3{\cdot}OEt_2$. Indeed, $BF_3{\cdot}OEt_2$-promoted coupling at −45°C displayed complete chemo- and regioselectivity to give disaccharide **6** in 77% yield, but did not exhibit a reasonable level of stereoselectivity (entry 2), although the use of EtCN as a solvent was expected to favor α-selective sialylation.[18]

Our attention was next turned to the use of TfOH, which had been once employed by Wong and co-workers for glycosidation of fully benzylated glycosyl phosphites.[19] Prior to the real coupling, we found that sialylation of methyl 2,3,4-tri-*O*-benzyl-α-D-glucopyranoside with **2** in EtCN could be promoted with the aid of TfOH at −78°C to give the corresponding sialosides

in 84% yield with the α:β ratio of 86:14. To the best of our knowledge, this is the first example that demonstrates the effectiveness of TfOH as a promoter for sialylation with sialyl phosphites. In a separate experiment, we also confirmed that phosphorodiamidate **4** remained intact under such conditions when kept at below −25°C. In the event, coupling of **2** with **4** in EtCN in the presence of TfOH proceeded chemo- and regioselectively at −78°C to give disaccharide **6** in 86% yield with the α:β ratio of 84:16 (entry 3),[20] from which the desired α-anomer **6α** could be easily separated by column chromatography. It is worthy of note that the beneficial effect of EtCN on α-selectivity was markedly pronounced with TfOH compared with $BF_3{\cdot}OEt_2$ (entry 2 vs 3).[21]

With the chemoselective α-sialylation secured, the stage was now set for the installation of glucosylceramide building block **13**[4] (Scheme 1). To this end, the benzyl groups in **6α** were deblocked by catalytic hydrogenolysis. However, subsequent benzoylation was found to stop at the monobenzoylation stage.[22] It was thought that the sialyl moiety was too demanding as a steric presence to allow benzoylation of the hydroxyl group at C2. Thus, we revised our scenario so as to diminish the supposed steric hindrance by forming a rigid lactone system. In the event, lactonization of **6α** with DBU and debenzylation was followed by smooth benzoylation to provide disaccharide donor **9** in 68% yield.[23] Compound **13** was prepared by a slight modification of the previously reported protocol,[4] in which the chemical yield was improved by employing diphenyl phosphate **10** as a glycosyl donor instead of the corresponding tetramethylphosphorodiamidate (94% vs 74%). Coupling of **9** and glucosylceramide **13** under



Scheme 1. *Reagents and conditions*: (a) DBU, CH_2Cl_2, 0°C, 1 h; (b) H_2, $Pd(OH)_2/C$, THF, 2 h; (c) Bz_2O, pyridine, DMAP; (d) **10**:**11**:TMSOTf molar ratio = 1.1:1.0:1.5, CH_2Cl_2, 0°C, 1 h; (e) $H_2NC(S)NH_2$, 2,6-lutidine, EtOH, 70°C, 2 h; (f) **9**:**13**:TMSOTf molar ratio = 1.1:1.0:3.0, CH_2Cl_2, 0°C, 1 h; (g) NaOMe, MeOH, then H_2O; (h) Na, liq. NH_3, THF, −78 to −33°C, 15 min, then MeOH; (i) Ac_2O, pyridine; (j) NaOMe, MeOH, then H_2O

promotion of TMSOTf in CH_2Cl_2 at 0°C proceeded uneventfully to give the protected GM_3 **14** in 80% yield and with complete stereocontrol.[24] Deprotection of the acyl protecting groups and lactone ring-opening were effected in one-pot by treatment of **14** with NaOMe in MeOH followed by the action of water. Debenzylation of the product in the crude form with sodium in liquid ammonia, followed by peracetylation (for the purpose of purification),[25] per-deacetylation and saponification, furnished the target GM_3 (**1**), $[\alpha]_D^{23}$ +1.92 (*c* 0.2, 1:1 $CHCl_3$–MeOH) [lit.,[7b] $[\alpha]_D$ +1.8 (*c* 0.2, 1:1 $CHCl_3$–MeOH)] in 67% yield from **14**, which exhibited identical ^1H NMR data with those reported for this natural product.[26]

In summary, we have accomplished a convergent synthesis of GM_3 based on the chemoselective glycosidation methodology, in which the anomeric reactivity of the glycosyl donor and acceptor carrying different phosphorus-containing leaving groups can be regulated by a judicious choice of the protecting groups as well as reaction conditions. We have also demonstrated the effective use of TfOH as a promoter for sialylation with sialyl phosphites. Further extension of the present method to the synthesis of more complex gangliosides is in progress.

Acknowledgements

This research was partially supported by Takeda Science Foundation and a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Sports and Culture, Japan. H.S. is grateful to the JSPS for a graduate fellowship.

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12. Compound **3** was prepared from allyl 2,6-di-*O*-benzoyl-α-D-galactopyranoside[13] by the following sequence: (1) $PhCH(OMe)_2$, CSA, $CHCl_3$, 5 h, 65%; (2) $Pd(PPh_3)_4$, DABCO, EtOH–H_2O (9:1), reflux, 6 h; (3) I_2, pyridine,

THF–H_2O (4:1), 30 min, 80% (two steps); (4) *n*-BuLi, THF, −78°C, then $ClPO(NMe_2)_2$, HMPA, −30°C, 2 h, 73%; (5) H_2, $Pd(OH)_2$/C, EtOH, 24 h, 85%.

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20. Under these conditions, 6-*O*-benzoyl-α-D-galactopyranosyl *N*,*N*,*N*′,*N*′-tetramethylphosphorodiamidate also remained intact, but the reaction with **2** gave a complex mixture of products.
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