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Total synthesis of the aminopropyl functionalized ganglioside GM_1

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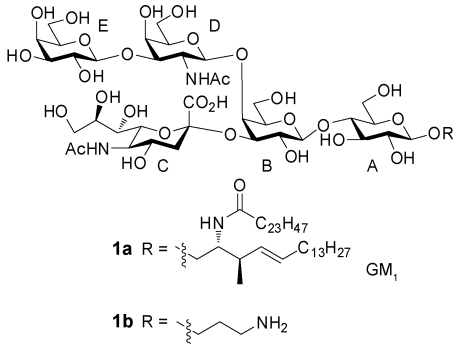
GM_1 is a common ganglioside pentasaccharide present on mammalian cell surface. It has been shown to play important roles in cellular communications and initiation of β-amyloid aggregation. In order to synthesize GM_1, an efficient synthetic route was developed via a [3+2] strategy. The GM_3 trisaccharide acceptor bearing an azido propyl group at the reducing end was prepared using the traditional acetamide protected sialyl thioglycosyl donor, which gave better stereoselectivity than sialyl donors protected with trichloroacetamide or oxazolidinone. The glycosylation of the axial 4-hydroxyl group of GM_3 by the disaccharide donor was found to be highly dependent on donor protective groups. Donor bearing the more rigid benzylidene group gave low glycosylation yield. Replacing the benzylidene with acetates led to productive coupling and formation of the fully protected GM_1 pentasaccharide. Deprotection of the pentasaccharide produced GM_1 functionalized with the aminopropyl side chain, which will be a valuable probe for biological studies.

carbohydrate, chemical synthesis, gangliosides, GM_1

1 Introduction

GM_1 (**1a**), a member of the ganglioside family, is commonly present in vertebrate plasma membranes and especially enriched in nerve tissues [1, 2]. There are many significant biological functions ascribed to GM_1, such as receptor for pathogen binding [3], cell-growth modulators [4], and neurotrophic factor [5]. Furthermore, GM_1 has been proposed to be a nucleating site for initiating β-amyloid aggregation, which is implicated in the development of Alzheimer's diseases (AD) [6–10]. GM_1 level showed significant increase in amyloid positive nerve terminals obtained from the cortex of AD patients [11]. In order to better understand the roles of GM_1 in β-amyloid aggregation and AD development, sufficient quantity of GM_1 is needed. Herein, we report our synthesis of an aminopropyl side chain functionalized GM_1 derivative (**1b**), which can be readily used for bioconjugation.



2 Experimental

2.1 General

All reactions were carried out under nitrogen with anhydrous solvents in flame-dried glassware, unless otherwise noted. All glycosylation reactions were performed in the presence of molecular sieves, which were flame-dried right before the reaction under high vacuum. Glycosylation sol-

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vents were dried using a solvent purification system and used directly without further drying. Chemicals used were of reagent grade as supplied except where noted. Analytical thin-layer chromatography was performed using silica gel 60 F254 glass plates; spots were visualized by UV light (254 nm) and by staining with a yellow solution containing $Ce(NH_4)_2(NO_3)_6$ (0.5 g) and $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$ (24.0 g) in 6% H_2SO_4 (500 mL). Flash column chromatography was performed on silica gel 60 (230–400 mesh). NMR spectra were referenced using Me_4Si (0 ppm), residual $CHCl_3$ (^1H NMR 7.26 ppm, ^{13}C NMR 77.0 ppm). Peak and coupling constant assignments are based on ^1H NMR and ^1H-^1H gCOSY. High-resolution mass spectra were recorded on a Q-TOF Ultima API LC-MS instrument with Waters 2795 Separation Module (Waters Corporation, Milford, MA).

2.2 Synthesis of fully protected GM_1 pentasaccharide 2

The mixture of donor **3** (216 mg, 0.2 mmol), acceptor **4** (809 mg, 0.6 mmol) and freshly activated MS 4 Å (600 mg) in dry CH_2Cl_2 (6 mL) was stirred for 30 min at room temperature, and cooled down to –70 °C followed by the addition of AgOTf (154 mg, 0.6 mmol) in anhydrous acetonitrile (0.1 mL). After 5 min, *p*-TolSCl (29 μL, 0.2 mmol) was added via a microsyringe directly to the solution without touching the wall of the reaction flask. The orange color of *p*-TolSCl dissipated within a minute. The reaction mixture was stirred for 1.5 h until the temperature reached –20 °C and triethylamine (30 μL) was added. The mixture was diluted with CH_2Cl_2 (50 mL) and filtered through celite. The filtrate was concentrated and purified by flash column chromatography (hexanes:EtOAc = 3:2) to give fully protected GM_1 **2** (267.6 mg, 58%). ^1H NMR (500 MHz, $CDCl_3$) δ 7.95 (s, 2 H), 7.54–6.99 (m, 45 H), 5.52–5.46 (t, 1 H), 5.44 (d, J = 7.0 Hz, 1 H), 5.39 (d, J = 3.0 Hz, 1 H), 5.25–5.20 (m, 2 H), 5.16 (d, J = 10 Hz, 2 H), 5.05–5.00 (m, 1 H), 4.94 (d, J = 10 Hz, 2 H), 4.85–4.76 (m, 3 H), 4.75–4.65 (m, 3 H), 4.63–4.52 (m, 4 H), 4.51–4.37 (m, 5 H), 4.36–4.30 (m, 2 H), 4.26–4.08 (m, 2 H), 4.05–3.76 (m, 11 H), 3.72–3.55 (m, 8 H), 3.54–3.47 (t, 2 H), 3.45–3.39 (m, 2 H), 3.38–3.36 (m, 5 H), 2.11–1.97 (m, 8 H), 1.96 (s, 6H), 1.89 (s, 3 H), 1.88 (s, 3 H), 1.87–1.70 (m, 5 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.9, 170.5, 170.47, 170.2, 168.7, 165.3, 162.2, 139.11, 139.1, 139.0, 138.9, 138.8, 138.5, 138.0, 133.2, 130.5, 130.1, 129.3, 128.6, 128.57, 128.5, 128.49, 128.4, 128.36, 128.2, 128.15, 127.8, 127.77, 127.7, 127.6, 127.5, 126.6, 126.5, 103.7, 102.7, 100.9, 100.6, 100.5, 83.1, 82.5, 81.9, 80.3, 76.8, 76.0, 75.6, 75.5, 75.45, 75.4, 74.0, 73.4, 73.3, 73.1, 73.0, 72.9, 72.3, 71.8, 70.2, 69.4, 69.1, 68.5, 67.7, 67.6, 67.2, 66.7, 66.4, 62.4, 53.1, 51.8, 48.6, 29.9, 25.5, 21.7, 21.0, 20.9, 20.8. ESI-MS: $[M+Na]^+$ $C_{117}H_{132}Cl_3N_5NaO_{37}$ calcd 2326.8, obsd 2327.0.

2.3 Deprotected GM_1 (1b)

The mixture of **2** (231 mg, 0.1 mmol), 1 M NaOH (2 mL, 2 mmol), and THF (15 mL) was stirred at 50 °C overnight and then concentrated to dryness. The resulting residue was diluted with CH_2Cl_2 (100 mL), and the organic phase was washed by H_2O and then dried over Na_2SO_4, filtered, and concentrated to dryness. The resulting residue was dissolved in a mixture of methanol (20 mL) and triethylamine (0.14 mL, 1 mmol). Acetic anhydride (0.094 mL, 1 mmol) was added dropwise, and the mixture was stirred at room temperature for 4 h. The reaction was quenched by adding a few drops of H_2O and then diluted with EtOAc (100 mL). The organic phase was washed with a saturated aqueous solution of $NaHCO_3$ and H_2O, dried over Na_2SO_4, filtered, and concentrated to dryness. Silica gel column chromatography (hexanes:EtOAc = 2:1) afforded the *N*-acetylated product as a white solid. The mixture of the *N*-acetylated product, 1 M PMe_3 in THF (0.2 mL, 0.056 mmol), 0.1 M NaOH (2 mL, 0.5 mmol), and THF (12 mL) was stirred at 60 °C under N_2 overnight. The mixture was concentrated, and the resulting residue was diluted with CH_2Cl_2 (150 mL). The organic phase was washed with H_2O and then dried over Na_2SO_4, filtered, and concentrated to dryness. The resulting residue was purified by quickly passing through a short silica gel column (CH_2Cl_2:MeOH = 10:1). The mixture of the obtained solid and $Pd(OH)_2$ in MeOH/H_2O (10 mL:3 mL) was stirred under H_2 at room temperature overnight and then filtered. The filtrate was concentrated to dryness under vacuum. The aqueous phase was further washed with CH_2Cl_2 (5 mL × 3) and EtOAc (5 mL × 3), and the aqueous phase was dried under vacuum to afford **1b** (acetate salt) as a white solid (68.6 mg, 65% for four steps). ^1H NMR (500 MHz, D_2O) δ 4.71 (d, 1 H, J = 8.5 Hz), 4.50–4.43 (m, 3 H), 4.12–4.05 (m, 3 H), 4.01-3.89 (m, 3 H), 3.85 (d, 1 H, J = 3.5 Hz), 3.81 (dd, 1 H, J = 13.0, 4.0 Hz), 3.79–3.65 (m, 14 H), 3.65–3.50 (m, 7 H), 3.46–3.41 (m, 2 H), 3.32–3.23 (m, 2 H), 3.07 (t, 2 H, J = 6.6 Hz), 2.60 (dd, 1 H, J = 12.5, 4.0 Hz), 1.97 (s, 3 H), 1.94 (s, 3 H), 1.94 (t, 1 H, J = 12 Hz), 1.90–1.84 (m, 2 H). ^{13}C NMR (125 MHz, D_2O) δ 181.6, 175.1, 174.9, 174.2, 104.8, 102.7, 102.6, 102.2, 101.8, 80.5, 78.7, 77.2, 75.0, 74.9, 74.5, 74.2, 73.2, 72.8, 72.6, 72.4, 70.8, 68.8, 68.7, 68.1, 68.0, 63.0, 61.2, 61.1, 60.8, 60.2, 51.7, 51.3, 37.7, 27.1, 23.4, 22.7, 22.2. HRMS: $[M+Na]^+$ $C_{40}H_{69}N_3NaO_{29}$ calcd 1078.3914, obsd 1078.3942.

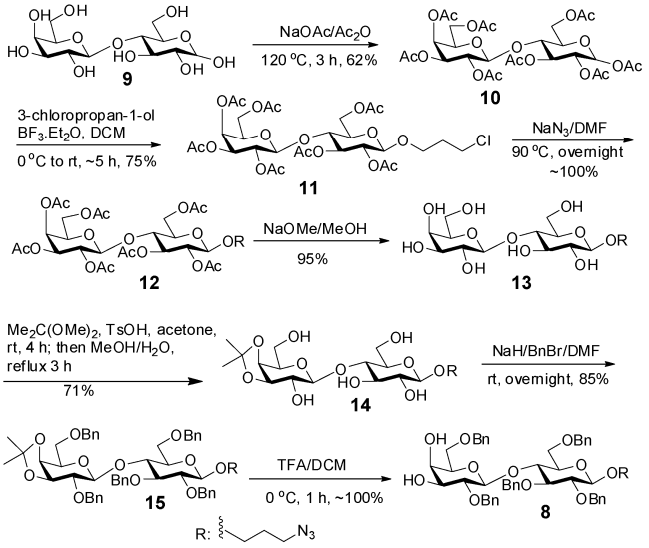
3 Results and discussion

Although synthesis of ganglioside GM_1 and its derivatives had previously been accomplished by several laboratories [12–17], it is still a challenging task. The main difficulty lies in the construction of the bifurcating branches onto the reducing end lactoside. As the sialic acid and the galactose (Gal)-galactosamine (GalN) disaccharide are linked to

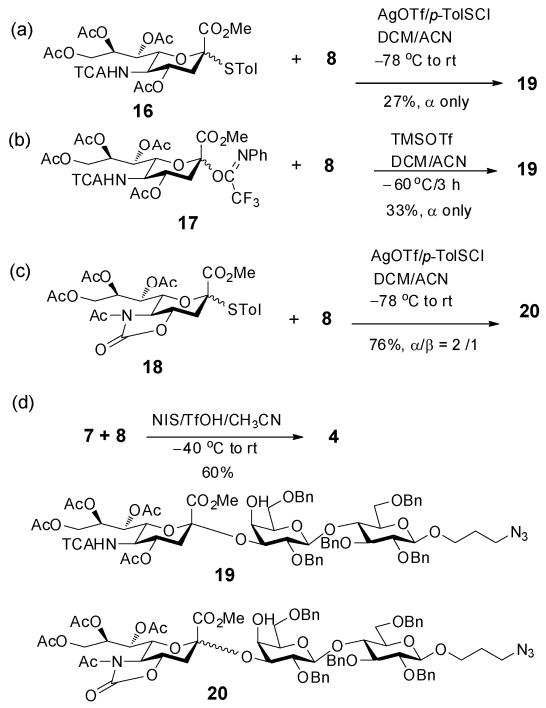
neighboring hydroxyl groups, the installation of one branch can pose serious steric hindrance for introduction of the other. We decided to attach the sialic acid first as sialyl donors are known to have lower anomeric reactivities than common pyranosyl donors due to the presence of the electron withdrawing carbonyl group at the anomeric center [18, 19] (Figure 1). Based on this consideration, the key step of our synthesis will be the coupling of the Gal^E-$GalN^D$ disaccharide fragment **3** with a selectively protected sialyllactose block **4** (GM_3 chain).

To synthesize the GM_3 trisaccharide, the lactosyl diol **8** bearing multiple benzyl ethers as the protective groups was selected as the acceptor, which should have good nucleophilicity due to the electron donating nature of the multiple benzyl ether groups present. The synthesis of lactose acceptor **8** began with the commercially available lactose **9** (Scheme 1). Acetylation of **9** in the presence of sodium acetate and acetic anhydride gave the per-acetylated lactose **10**, which underwent $BF_3{\cdot}Et_2O$ promoted glycosylation with 3-chloropropan-1-ol to provide the desired β-lactoside **11** [20]. S_N2 displacement of the chloride with sodium azide followed by Zemplen deacetylation and regioselective isopropylidene formation produced lactoside **14**. Global protection of the free hydroxyl groups as benzyl ethers and acid mediated isopropylidene removal led to the desired lactosyl diol acceptor **8** [21] in 85% yield for the two steps.

Sialylation of the lactosyl disaccharide **8** was tested next. Besides the aforementioned low reactivity of sialyl donor, another difficulty in sialylation is stereochemical control as the naturally existing sialyl linkage is the thermodynamically less favored α linkage [19]. Recently, it was discovered that the installation of an electron withdrawing protective group including TCA and oxazolidinone on the 5-N position significantly enhanced the reaction yield and stereoselectivity [22, 23]. Thus, we investigated three sialyl donors **16** [24], **17** [24] and **18** [25] with their 5-*N*-acetyl group substituted with more electron withdrawing protective groups. As shown in Scheme 2, donor **16** gave excellent stereoselectivity, albeit with a low yield (Scheme 2(a)). Changing the anomeric leaving group from STol in **16** to trifluoroacetimidate (donor **17**) did not improve the situation much (Scheme 2(b)). On the other hand, the usage of the oxazolidinone protected sialyl donor **18** gave 76% yield



Scheme 1 Synthesis of the lactosyl acceptor **8**.



Scheme 2 Synthesis of GM_3 trisaccharide acceptor.

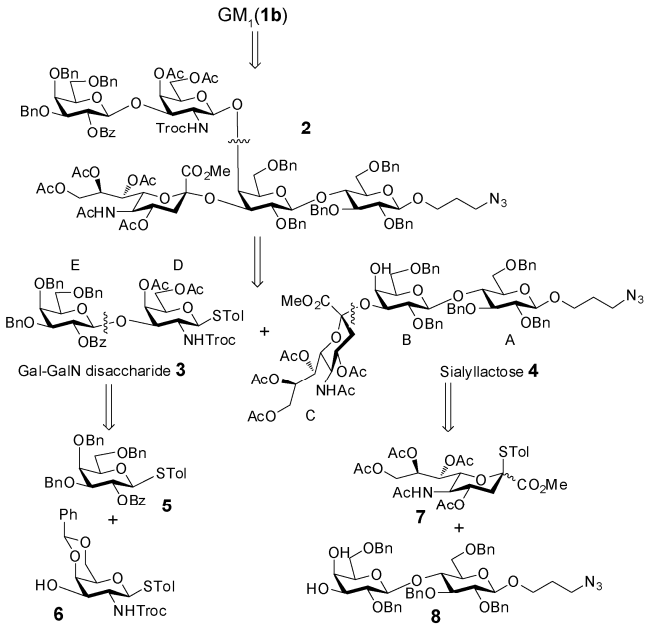


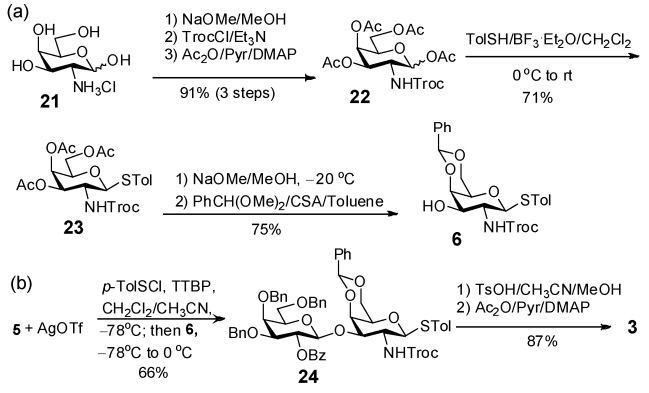
Figure 1 Retrosynthetic analysis for synthesis of GM_1 **1b**.

of the trisaccharide as a 2:1 mixture of α:β anomers, which were difficult to separate (Scheme 2(c)). Instead of further optimizing the glycosylation reactions using these donors, we examined the 5-*N*-acetamide donor **7**[24], as it was simpler to prepare than donors **16**–**18**. Interestingly, donor **7** gave 60% yield and exclusive α selectivity in coupling with the lactose acceptor **8** (Scheme 2(d)).

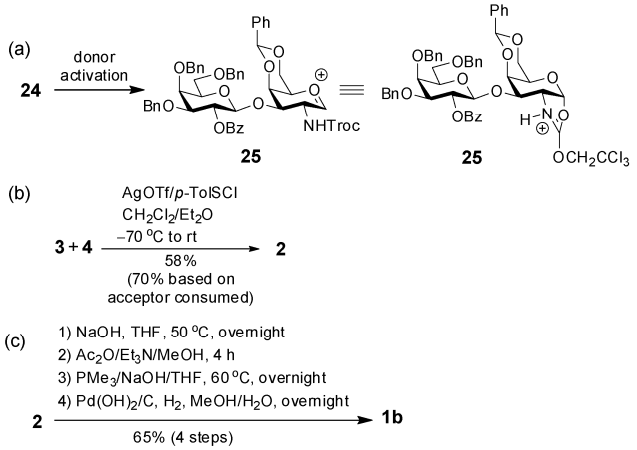
After establishing a viable route to GM_3, we assessed the formation of the second branch. The Gal-GalN disaccharide building block **3** was accessed by the reaction of galactosyl donor **5** [26] and galactosaminyl acceptor **6** [27]. To prepare compound **6**, the amino group of GalN **21** was protected by the trichloroethoxocarbonyl (Troc) group followed by peracetylation to give **22** (Scheme 3(a)). The anomeric acetate in **22** was replaced with *p*-toluenethiol as promoted by $BF_3{\cdot}Et_2O$ to yield compound thioglycoside **23**. Zemplen reaction using sodium methoxide to remove all the acetyl groups in **23** followed by benzylidenation of the newly liberated 4,6-hydroxyl groups led to the galactosylaminyl acceptor **6**. It was important to maintain low temperature for this reaction to avoid the possible side reaction of Troc with sodium methoxide. As both **5** and **6** are thioglycosides, the glycosylation of **6** by **5** was performed under the pre-activation condition [28] to avoid the activation of the acceptor or the product. Treatment of donor **5** with AgOTf/*p*-TolSCl [27] at −78 °C in the presence of a sterically hindered base tri-^{t}butylpyrimidine (TTBP) [27] cleanly activated the donor within a few minutes. Addition of acceptor **6** to the activated donor solution led to the formation of disaccharide **24** in 66% yield (Scheme 3(b)).

The union of the Gal-GalN disaccharide **24** and GM_3 trisaccharide **4** was then explored, which failed to produce the desired GM_1 pentasaccharide. We hypothesized this was because upon donor activation, the Troc moiety in donor **24** participates in stabilizing the oxacarbenium ion through the formation of a five membered oxazoline ring (Scheme 4(a)). In order to accommodate this, the pyranosyl ring of GalN needs to undergo conformational changes. However, the presence of the benzylidene ring on the GalN conformationally rigidifies the ring [29], thus hindering conformational changes in the pyranosyl ring and lowering the reactivity of the activated donor towards the sterically hindered GM_3 acceptor. To overcome this difficulty, the benzylidene group was removed from disaccharide **24** and the free hydroxyl groups were acetylated (disaccharide **3**). Gratifyingly, glycosylation of donor **3** with GM_3 trisaccharide **4** proceeded to give the fully protected GM_1 pentasaccharide **2** in 58% yield (70% based on acceptor consumed) (Scheme 4(b)), with the correct molecular weight of 2327.0 $[M+Na]^+$ given by mass spectrometry. NMR spectra of compound **2** showed broad peaks, which was presumably due to the presence of multiple conformations at room temperature.

The deprotection of pentasaccharide **2** began with the removal of the Troc, acyl and ester protecting groups using 1 M NaOH in THF overnight (Scheme 4(c)). The newly liberated amine was selectively acetylated in the presence of multiple hydroxyl groups by acetic anhydride in methanol. Staudinger reduction of the azide group and subsequent catalytic hydrogenation over Pearlman's catalyst gave the fully deprotected GM_1 analog **1b** in 65% overall yield for all deprotection steps. The α linkage between sialic acid and the lactose unit was confirmed by the NMR coupling constant between C_1 and H_{3ax} of sialic acid ($^3J(C_1,H_{3ax})$ = 5.7 Hz) [27]. The β linkages for the rest of the glycosidic bonds were supported by the one bond coupling constants between the respective anomeric carbon and proton (163.5 Hz, 162 Hz, 162 Hz, 164 Hz) [27]. Correlations of the anomeric carbon of the $GalN^D$ unit (102.6 ppm) with H_4 of Gal^B (4.06 ppm) and the anomeric carbon of the sialic acid unit (101.8 ppm) with H_3 of Gal^B (4.08 ppm) were found in HMBC NMR spectrum, thus confirming the regiochemistry of **1b**.



Scheme 3 Synthesis of (a) the galactosaminyl acceptor **6**; and (b) Gal-GalN disaccharides **24** and **3**.



Scheme 4 (a) Formation of the 5-membered oxazoline ring by activated donor **24** was presumably difficult due to the presence of the rigid benzylidene ring, thus lowering the reactivity of activated **24** towards acceptor **4**. (b) Formation of the fully protected GM_1 pentasaccharide **2**. (c) Deprotection of pentasaccharide **2**.

4 Conclusions

In conclusion, a stereo- and regio-controlled total synthesis of aminopropyl functionalized GM_1 was achieved. Compared to previous synthesis, our method only employed a single type of glycosyl donors, i.e., thioglycosides, which simplified overall building block design. With the aminopropyl side chain, our GM_1 analog can be readily conjugated to liposomes and nanoparticles. This will be very useful for deciphering the role of GM_1 ganglioside in the induction of β-amyloid aggregation as well as pathogen infection. The results from those studies will be reported in due course.

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