Reactivity-based one-pot total synthesis of fucose GM1 oligosaccharide: A sialylated antigenic epitope of small-cell lung cancer

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The total synthesis of the sialic acid-containing antigenic epitope fucose GM1 (Fuc-GM1) by an improved reactivity-based one-pot synthetic strategy is reported. Based on a thioglycoside reactivity database, three saccharide building blocks, 3, 4, and 5, were designed and prepared to incorporate a descending order of reactivity toward thiophilic activation. Using the reactivity-based one-pot synthetic method, the fully protected Fuc-GM1 glycoside 2 was furnished in a facile manner, which was globally deprotected to give the Fuc-GM1 glycoside 1. In addition, using the promoter system 1-(benzensulfonyl)pyrrolidine/trifluoromethanesulfonic anhydride, the product yield was improved and the reaction time was reduced in comparison with the N-iodosuccinimide/trifluoromethanesulfonic acid- and dimethyl (thiomethyl) sulfonium trifluoromethanesulfonate-promoted systems.

Fucose GM1 (Fuc-GM1) ganglioside was first isolated from bovine thyroid tissue in 1979 (1). It is comprised of a hexasaccharide carbohydrate moiety and a ceramide-reducing end component. Within this carbohydrate framework is a tetrasaccharide (sugars a, b, d, and e) bearing a branched sialic acid residue (c) and a terminal fucose (f) (Fig. 1). It is found specifically in the tumor tissue of small-cell lung cancer (SCLC). SCLC accounts for 20% of lung cancer, which remains one of the leading causes of death in the United States (2). Unlike other cancer antigens, Fuc-GM1 has a more restricted distribution in normal tissue, suggesting that this carbohydrate antigen may be a good target for active immunization. Development of an anti-(Fuc-GM1) vaccine and mAb could potentially be of importance for diagnosis and immunotherapy of these tumors (3, 4). However, one of the barriers preventing effective production of an anticancer vaccine is the limited supply of chemically pure Fuc-GM1 ganglioside. Despite the development of various sialosaccharide synthetic methods (5–8), the first synthesis of Fuc-GM1 glycoside was reported by Allen and Danishefsky (9) two decades after its initial discovery. This elegant strategy incorporated the sulfonamide glycosidation method (10) in conjunction with a [3 + 3] convergent glycosylation, ultimately leading to the target glycoside. It required protecting group and anomeric leaving group manipulations and encountered a problem with stereoselective formation of the β(1,4) glycosidic bond between a bulky trisaccharide donor and the poor nucleophilic trisaccharide acceptor.

We envisioned that the incorporation of our programmable reactivity-based one-pot strategy (8) in the synthesis of the Fuc-GM1 could simplify this complicated synthetic operation. In brief, the reactivity-based one-pot strategy is based on a developed competitive HPLC assay to assess quantitively the reactivity of different thioglycosides, the so-called relative reactivity value (RRV). Such information is then used to guide the reactivity-based one-pot synthesis of an oligosaccharide without protecting group manipulation and intermediate isolation (8). Along with this development, we also report the use of a different thiophilic promoter to improve the efficiency of the reactivity-based one-pot methodology.

Materials and Methods

General. All chemicals were purchased and used without further purification. Dichloromethane (CH2Cl2) was distilled over calcium hydride. Propionitrile (EtCN) was treated with activated molecular sieves (MS) (AW-300) overnight before use. MS used in glycosylation were crushed and flame-activated before use. Reactions were monitored with analytical TLC on silica gel 60 F254 plates and visualized under UV (254 nm) and/or by staining with acidic ceric ammonium molybdate. Flash column chromatography was performed on silica gel (35–75 μm) or iatro beads. 1H-NMR spectra were recorded on a Bruker DRX-500 (500 MHz) or DRX-600 (600 MHz) spectrometer at 20°C. Chemical shift (in ppm) was determined relative to either CDCl3 (δ = 77.00 ppm) or acetone in deuterated water (δ = 2.05 ppm). Coupling constants in Hz were measured from one-dimensional spectra. 13C-Attached Proton Test (13C-Apt) NMR spectra were obtained by using the same Bruker NMR spectrometers (125 or 150 Hz) and were calibrated with CDCl3 (δ = 77.00 ppm). Peracetylated lactosyl acetate 11 is commercially available and monosaccharide building blocks 3 (8), 6 (8), 9 (11), 14 (12), and 15 (8) are known compounds. Experimental details for the synthesis of the key thioglycoside building blocks, 4, 5, and 10, the protected Fuc-GM1 2 and the unprotected Fuc-GM1 1 are described. The synthesis of the remaining reaction intermediates 7, 8, 12, 13, and 16 and all of the characterization data for 1, 2, 4, 5, 7, 8, 10, 12, 13, and 16 together with their NMR spectra are reported separately in the Supporting Text and Figs. 4–23, which are published as supporting information on the PNAS web site, www.pnas.org.
Disaccharide Building Block (4). Disaccharide precursor 10 (0.27 g, 0.27 mmol) was dissolved in dry pyridine (3 ml), and 1 M hydrazine hydrate (NH₂NH₂·H₂O) in pyr/AcOH mixture (vol/vol = 3:2) (0.81 ml, 0.81 mmol) was added. The reaction mixture was stirred at room temperature for 4 h and then penta-2,4-dione (1 ml) was added. The solvent was removed under reduced pressure, and the residue was dissolved in CH₂Cl₂, washed with H₂O and brine, dried (Na₂SO₄), concentrated, and purified by flash column chromatography purification (hexane/EtOAc, 2:1) to provide the desired disaccharide 4 (0.218 mg, 81%) as a white glassy solid.

Trisaccharide Building Block (5). Sialyl donor 14 (1.4 g, 1.9 mmol), lactoside acceptor 13 (2.8 g, 2.6 mmol), and MS (3 g) were stirred in dry EtCN (15 ml) at room temperature for 2 h under Ar. The reaction mixture was then cooled to −45°C and 0.5 M trimethylsilyl trifluoromethanesulfonate in EtCN (380 µl, 0.19 mmol) was added. The reaction mixture was stirred at −30°C overnight, and then quenched with triethylamine. The reaction mixture was then filtered and concentrated under reduced pressure. The residue was diluted with CH₂Cl₂ and washed with saturated aqueous (sat. aq.) NaHCO₃, H₂O, and brine and dried (Na₂SO₄). The resulting crude mixture was purified by flash column chromatography (gradient eluent hexane/EtOAc, 1:1 → 1:1.5) to yield 5 (1.6 g, 53%) as a colorless glassy solid.

Disaccharide Building Block Precursor (10). Galactosaminyl acceptor 8 (0.3 g, 0.55 mmol), galactosaminyl donor 9 (0.47 g, 0.72 mmol), and MS (0.75 g) were suspended in dry CH₂Cl₂ (3 ml) for 1 h at room temperature under Ar and then cooled to −45°C. N-iodosuccinimide (NIS) (0.16 g, 0.72 mmol) and 0.5 M trifluoromethanesulfonic acid (TfOH) in Et₂O (144 µl, 0.072 µmol) were added. The reaction mixture was stirred at −45°C for 3 h and quenched with sat. aq. NaHCO₃ and solid Na₂S₂O₃. The reaction mixture was filtered and washed with sat. aq. NaHCO₃, H₂O, and brine, dried (Na₂SO₄), and concentrated for flash column chromatography purification (hexane/EtOAc, 2:1) to yield 10 (0.27 g, 45%) as a white glassy solid.

NIS/TfOH Promoted One-Pot Synthesis of Protected Fuc-GM₁ (2). Fucosyl donor 3 (0.158 g, 0.29 mmol), disaccharide building block 4 (0.22 g, 0.225 mmol), and MS (0.75 g) were stirred in dry CH₂Cl₂ (3 ml) at room temperature for 1 h under Ar and cooled to −78°C. This was followed by the addition of NIS (66 mg, 0.29 mmol) and 0.5 M TfOH in Et₂O (58 µl, 0.29 mmol). The reaction mixture was stirred at −70°C for 2 h. After consumption of donor 3 (TLC, hexane/EtOAc, 2.5:1), the trisaccharide acceptor 5 (0.69 g, 0.45 mmol) in dry CH₂Cl₂ (2 ml) was added followed by freshly prepared dimethyl (thiomethyl) sulfonium trifluoromethanesulfonate (DMTST) (0.29 g, 1.125 mmol) in CH₂Cl₂ (1 ml). The reaction mixture was stirred at 0°C overnight and quenched with sat. aq. NaHCO₃ and solid Na₂S₂O₃, filtered, washed with sat. aq. Na₂S₂O₃, sat. aq. NaHCO₃, H₂O, and brine, dried (Na₂SO₄), and concentrated. The crude mixture was purified twice by flash column chromatography (toluene/EtOAc, 1:2, followed by toluene/acetone, 4:1) to provide Fuc-GM₁ 2 (0.23 g, 36%) as a white glassy solid.

Benzensulfinylpyrperidine (BSP)/Trifluoromethanesulfonic Anhydride (Tf₂O) Promoted One-Pot Synthesis of Protected Fuc-GM₁ (2). Fucosyl donor 3 (0.092 g, 0.17 mmol), disaccharide building block 4 (0.16 g, 0.16 mmol), BSP (0.018 g, 0.088 mmol), and MS (0.75 g) were stirred in dry CH₂Cl₂ (2 ml) at room temperature for 1 h under Ar. The reaction mixture was cooled to −70°C, followed by the addition of Tf₂O (16 µl, 0.086 mmol), and the temperature was increased gradually from −70°C to −10°C within 1 h. After the donor 3 was consumed, the reaction mixture was cooled to −70°C, which was followed by the addition of the trisaccharide acceptor 5 (0.34 g, 0.22 mmol). Subsequently the second portion of BSP (0.017 mg, 0.08 mmol) and Tf₂O (15 µl, 0.088 mmol) was added. The reaction temperature was increased gradually from −70°C to 0°C in 1 h and the mixture was stirred at 0°C for an additional 4 h. The reaction was quenched with triethylamine.

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**Fig. 2.** Retrosynthetic analysis of the Fuc-GM₁ glycoside.
(0.2 ml) and diluted with CH₂Cl₂. The reaction mixture was filtered and washed sequentially with sat. aq. Na₂SO₃, sat. aq. NaHCO₃, H₂O, and brine, dried (Na₂SO₄), filtered, and concentrated. The crude mixture was purified twice by flash column chromatography (toluene/EtOAc, 1:2, followed by toluene/acetone, 4:1). The protected Fuc-GM₁ (0.21 g, 47%) was obtained as a white glassy solid.

Deprotected Fuc-GM₁ (1). Protected Fuc-GM₁ (80 mg, 29 µmol) was dissolved in acetic anhydride/CH₂Cl₂ mixture (vol/vol = 1:1, 2 ml). To the solution was added freshly activated Zn dust (1 g, washed with 1 M HCl, H₂O, MeOH, and Et₂O, then dried under vacuum for 10 min). After 5 h, the reaction mixture was filtered and the solvent was removed under reduced pressure. The residue was diluted with CH₂Cl₂ and subsequently washed with sat. aq. NaHCO₃ and brine, dried (Na₂SO₄), and concentrated. The residue was purified by flash column chromatography (toluene/acetone, 3:1). The resulting N-acetamido product was dissolved in MeOH/CH₂Cl₂ (vol/vol = 1:1, 2 ml) and sodium methoxide (NaOMe) (25% wt/vol in MeOH, 50 µl) was added. The mixture was stirred for 10 h at room temperature and neutralized with Amberlite IR-50 resin. After removal of the resin by filtration, the solution was concentrated to give the deacetylated residue, which was dissolved in MeOH with 10% (wt/vol) formic acid (3 ml). To the reaction mixture, Pd-black (70 mg) was added and the mixture was stirred under H₂ (1 atm) at room temperature for 18 h. The reaction mixture was filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography on Iatro beads (CH₂Cl₂/pH11005, 3:4:1) to yield the deprotected Fuc-GM₁ (15 mg, 44%) as a white powdery solid after lyophilization.

Results and Discussion

Design of Sugar Building Blocks. Successful application of reactivity-based one-pot synthesis relies on a careful selection of protecting groups and design of building blocks. The Fuc-GM₁ hexasaccharide was retrosynthetically disconnected into three saccharide building blocks: fucosyl building block 3, disaccharide building block 4, and sialylated trisaccharide building block 5 (Fig. 2). Implementation of reactivity-based one-pot oligosaccharide synthesis requires a descending order of reactivity for these three building blocks. Based on the reactivity database (13), the perbenzylated fucosyl thioglycoside 3 with a RRV of 7.2 × 10⁴ was selected as the first donor. The reducing end component is the sialylated trisaccharide building block 5, which is prepared from a lactoside acceptor 13 and sialic acid donor 14. Because 5 is an O-glycoside, a zero RRV can be assumed. The design of the disaccharide building block 4 demanded special concern. Because 4 is used as an acceptor and donor, the presence of a C₂-free hydroxyl group on the galactosyl unit as well as a thiotoluene functionality at the anomeric center of the galactosaminyl unit is required. In theory, its RRV should fall between 7.2 × 10⁴ and 0. This criterion could be easily addressed by using appropriate protecting groups.

For an efficient reactivity-based one-pot synthesis, not only is the reactivity value of the thioglycoside building block important, careful selection of the glycosylation promoter is also imperative. Although much success has been observed in our previous one-pot syntheses (14–16), some difficulties occur when either a very reactive donor and/or a poor nucleophilic acceptor is used in glycosylations promoted by NIS/TfOH, mainly because of the competitive formation of an undesired succinimide byproduct (8, 17). To address this problem, a combination of NIS/Tf₂O and DMTST was used initially to establish the one-pot synthetic route for the target glycoside. Subsequent efforts were focused on the application of a recently developed thioglycoside promoter system, the BSP/Tf₂O, to our one-pot synthesis to improve the efficiency of the process (18).

Syntheses of Building Blocks. With the synthetic plan in hand, we started the reactivity-based one-pot synthesis of Fuc-GM₁ by preparing the three saccharide building blocks 3, 4, and 5.
Perbenzylated fucosyl thioglycoside 3 was prepared by a literature procedure (8). The disaccharide building block 4 can be accessed by glycosylation between a galactosyl donor and a galactosaminyl acceptor. The galactosaminyl acceptor should possess a C3-hydroxyl handle, a RRV smaller than 4,000, and a C2-participating group to dictate the β-selective glycosidic bond formation. To address these requirements, we took the advantage of our knowledge on the properties of various protecting groups to design the galactosaminyl thioglycoside 8.

Scheme 1. Reagents and conditions for NIS/Tf2O- and DMTST-promoted one-pot reaction: route a (i) NIS, TfOH, CH2Cl2, −70 °C, 36%; (ii) DMTST, 0 °C, 36%.

Implementation of Reactivity-Based One-Pot Synthesis of Fuc-GM1 Glycoside. With the desired carbohydrate building blocks, 3, 4, and 5, and their RRV determined, the stage was set for the
The reactivity-based one-pot synthesis of the Fuc-GM1 glycoside (Scheme 3, route a). Perbenzylated fucosyl-GM1 building block 3 (RRV = 7.2 × 10^4) was coupled with the less reactive disaccharide building block 4 (RRV = 2,839) in the presence of NIS/TIOH (22). After 2 h at −70°C, trisaccharide acceptor 5 was added, followed by the addition of DMTST (23). The second glycosylation required higher reaction temperatures (0°C) and a longer reaction time (1 day). The slow reaction rate was partially attributed to the glycosylation between two large sugar fragments and this had previously been observed during the synthesis of an N-acetyllactosamine octamer (16). In addition, the Ca-axial hydroxyl is a weak nucleophile, which further lowered the reaction rate. Nevertheless, the fully protected Fuc-GM1 2 was obtained in 36% isolated yield directly from the building blocks without protecting group manipulation and reaction intermediate isolation. This finding corresponds to an average yield of 60% per glycosylation step.

As access to the one-pot synthesis of the Fuc-GM1 glycoside was accomplished, we turned our attention to improving the efficiency of the established one-pot synthetic protocol. Drawbacks of the present NIS/TIOH and DMTST system were the slow reaction rate (1 day) and moderate yield (36%). To solve this problem, we sought a better promoter. Such a promoter should enable the activation of either a reactive or nonreactive thioglycoside donor without the formation of undesired competitive byproducts. BSP/Tf2O, a recently developed thiophilic reagent by Crich and Smith (18), is compatible to our reactivity-based one-pot glycosylation protocol and acts by in situ conversion of thioglycosides to glycosyl trifluoromethanesulfonates, which then couple with the nucleophilic acceptors efficiently.

To investigate the compatibility of BSP/Tf2O with the present one-pot protocol, we first carried out a reactivity-based glycosylation reaction with a model system (Fig. 3), which incorporated a very active donor and a poor acceptor. This combination was known to be problematic, using the NIS/TIOH promoter system as succinamide byproducts are observed. Thioglycoside acceptor 15 (8) with a RRV of 9.4 was glycosylated with a fucosyl donor 3 (RRV = 7.2 × 10^4) in the presence of BSP/Tf2O. Despite the hindered acceptor site of 15, disaccharide product 16 was obtained in 95% isolated yield. Rather interestingly we have also determined that using a substoichiometric amount of BSP/Tf2O provides efficient disaccharide formation. One possible rationalization may be that the reaction pathway produces sulfonium byproducts, which can further promote glycosylations, as shown in Fig. 3. This possibility is supported by the fact that slightly more than half an equivalent of BSP/Tf2O provided a 95% yield of disaccharide product 16. Although no direct evidence for these sulfonium byproducts has been observed, detailed mechanistic studies should shed some light into the reaction pathway. It is also important to note that the reaction also proceeds sufficiently with one equivalent of BSP/Tf2O (90%), but the reaction must be quenched at low temperatures (−60°C) with triethylamine for optimal yields. Being encouraged by these results, we further investigated the application of the BSP/Tf2O promoter system to the one-pot synthesis of the Fuc-GM1 (Scheme 3, route b). For the BSP/Tf2O promoted one-pot operation, the first stage glycosylation between fucosyl donor 3 and disaccharide acceptor 4 was completed in 1 h. For the second stage glycosylation between two trisaccharide fragments, the expected longer reaction time (3 h) was needed. The isolated yield of this BSP/Tf2O promoted one-pot reaction was 47%, corresponding to an average of 67% per glycosylation step.

In terms of the reaction rate and product yield, the BSP/Tf2O system was substantially better than the NIS/TIOH and DMTST combination in promoting the one-pot glycosylation.

With the development of an improved reactivity-based one-pot synthetic operation, we proceeded to the global deprotection of Fuc-GM1. This process began with the removal of a Troc protecting group and reacetylation of the exposed amino functionality by reduction with zinc dust in acetic anhydride. The O-acetyl groups were removed, followed by alkaline hydrolysis of the methyl ester on the sialic acid residue. Unfortunately, the final hydrogenolysis of benzyl and Cbz protecting groups proved problematic by using either the Birch reduction conditions (24) or palladium-catalyzed hydrogenolysis in methanol. After a series of experiments, a successful deprotection was finally accomplished by catalytic transfer hydrogenation using palladium black in methanol with 10% formic acid under hydrogen (1 atm) at room temperature (25). Fully deprotected Fuc-GM1 glycoside 1 was obtained in 44% yield from 2. The target glycoside 1 was characterized with standard NMR spectroscopic methods and high-resolution MS, which were in agreement with the literature reported data (9).

Conclusion. The total synthesis of a cancer related and antigenic carbohydrate epitope, the Fuc-GM1 glycoside, was accomplished by an improved reactivity-based one-pot synthetic strategy. Through selection and careful design of various carbohydrate building blocks and application of the BSP/Tf2O promoter system, an efficient reactivity-based one-pot synthetic protocol was successfully developed. Not only does the established one-pot protocol simplify the synthesis of the
Fuc-GM1 glycoside, application of the BSP/Tf₂O promoter system improves the efficiency of the synthesis by shortening the reaction time and increasing the glycosylation yield. The building blocks and their reactivity developed in this study should find use in the one-pot synthesis of other oligosaccharides. It is reasonable to assume that the incorporation of the BSP/Tf₂O promoter system in our reactivity-based one-pot strategy can apply to the synthesis of more challenging targets.

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