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Water-Soluble Sulfo-Fluorous Affinity (SOFA) Tag-Assisted Enzymatic Synthesis of Oligosaccharides

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Abstract: Herein, we report a bifunctional sulfo-fluorous affinity (SOFA) tag-assisted enzymatic synthesis and purification strategy for the facile preparation of bioactive glycans using fluorous solid-phase extraction (FSPE). The incorporation of a sulfonate moiety onto the heavy fluorous tag significantly increases its water solubility, which allows the broad use of the inherently hydrophobic fluorous tag in aqueous buffers. In addition, the SOFA tag contains a photocleavable linker, enabling the easy release of amino-functionalized oligosaccharides by UV irradiation. The SOFA tag was used in the synthesis of both negatively charged and neutral glycans to demonstrate its broad utility as an acceptor toward six different glycosyltransferases, significantly improving the feasibility of the preparation of complex glycans using FSPE. All the reactions were performed in an aqueous buffer, a minimum amount of methanol was used to purify the products, and the SOFA tag was easily recovered after photo-irradiation. Thus, the entire synthetic process is environmentally benign.

Keywords: Enzymatic Synthesis; Glycosyltransferase; Fluorous Tag; Fluorous Solid-phase Extraction; Oligosaccharides; Green Chemistry

1 Introduction

Carbohydrates are one of the most abundant and structurally diverse classes of biomolecules and are present on all cell surfaces, in extracellular matrices as free polysaccharides and as constituents of glycoconjugates. These glycans have been implicated in a wide range of biological processes such as protein folding, fertilization, embryogenesis, bacterial infection, and cell differentiation and mobility.[1–4] As we have become increasingly aware of the importance of these valuable glycans in many biological systems, more and more effort has been devoted to the development of chemical, enzymatic, and chemoenzymatic syntheses of structurally defined complex oligosaccharides.[5–7] Notably, enzymatic synthesis is a more straightforward route for the preparation of human milk oligosaccharides[8] and some glycolipids.[9] The remarkable chemo-, regio-, and stereo-selectivity of enzyme-mediated glycosylations prove their superiority over reactions with chemical catalysts.[10–12]

While the enzyme-catalyzed reactions can effectively produce complex oligosaccharides, purification of the product remains tedious and time-consuming. Due to their high polarity, oligosaccharide purification relies on various techniques either alone or in combination. These techniques include high-performance thin-layer chromatography (HPTLC),[13] high-performance liquid chromatography (HPLC),[14] size-exclusion chromatography (SEC),[15] ion-exchange chromatography,[16] water-soluble polymer-assisted purification[17] and flash silica gel column chromatography[18] which is usually combined with SEC to obtain the desired pure products. To simplify the purification process after enzymatic reactions, Palcic and co-workers first utilized hydrophobic aglycones attached to glycosyl acceptors in which the hydrophobic moiety could be adsorbed on to reversed-phase C18 cartridges by hydrophobic interactions.[19] The product was easily separated from unreacted sugar-nucleotides and by-products by elution with methanol. Inspired by this solid-phase extraction concept, Wang and co-workers

reported the use of sugar acceptors with cleavable hydrophobic tags, a fluorenylmethyloxycarbonyl (Fmoc) group and a carboxybenzyl (Cbz) group, in the enzymatic glycan synthesis to facilitate further application of the synthesized glycans.[20] In general, the combination of SEC and reversed-phase solid-phase extraction gives good separation and yield of the desired pure product.

Fluorous tags (usually perfluoroalkyl chains) show a high affinity for the fluorous-phase surface. This unique interaction has been exploited as a separation technique, fluorous solid-phase extraction (FSPE), for the purification of organic reaction products.[21–22] The fluorinated conjugates can easily be released from the solid phase by elution with a fluorophilic solvent or methanol. In the chemical synthesis of oligosaccharides, fluorous tags have been installed at the anomeric position[23–25] and other positions on the glycosyl acceptors as protecting groups,[26–28] allowing facile purification of the products by FSPE following glycosylation. Alternatively, fluorous tags have been installed as protecting groups on glycosyl donors[29–30] to expedite the purification of the products. Similarly, fluorous tags have been used in enzymatic carbohydrate syntheses ranging from monosaccharide transformations[31] to oligosaccharide assemblies[32–34] as well as *in vitro* oligosaccharide syntheses.[35] In addition, fluorous-tagged lactoside has been immobilized on the surface of a fluorous chip to examine the activities of carbohydrate-active enzymes using nanostructure-initiator mass spectrometry.[36] Recently, Chen and co-workers evaluated the effect of fluorous tag length at the reducing end of a lactose in FSPE purification of oligosaccharides synthesized by enzymatic reactions, and they found that oligo(ethylene glycol)-linked heavy fluorous tags ($-C_8F_{17}$) were optimal for practical glycan syntheses.[34] However, fluorous tags on saccharides can be difficult to remove, which limits the further application of the valuable oligosaccharides synthesized by this method. Moreover, due to the inherent hydrophobicity of the fluorous tags, most examples of their use involve the synthesis of negatively charged glycans, such as the assembly of sialic acid and heparan sulfate;[32–34] this hydrophobicity limits the use of the current fluorous tags in enzymatic syntheses of neutral oligosaccharides. Although the addition of a relatively small amount of DMSO or DMF to the enzymatic reaction may increase the solubility of fluorous-tagged glycans in the reaction buffer, aprotic organic solvents are toxic to enzymes. Obviously, to make enzymatic reactions with fluorous-tagged neutral saccharide acceptors in aqueous solutions more general, substrates with high water solubility are urgently needed. Inspired by the work reported by Siuzdak and co-workers[36] in which the incorporation of a high polar arginine moiety facilitated ionization and improved the water solubility of the perfluorinated acceptor, we installed a sulfonate moiety onto the fluorous tag. The sulfonate group enhanced the water solubility of the conjugated tag better than the use of an arginine unit.[37] As illustrated in Figure 1, the water-soluble sulfo-fluorous affinity (SOFA) tag was applied in the fluorous tag-assisted enzymatic synthesis of both negatively charged and neutral glycans, and the results showed its broad utility as an acceptor toward six different glycosyltransferases. In addition, the introduction of a photocleavable linker between the fluorous tag and glycan enables the release of the amino group at the reducing end of the oligosaccharide by UV irradiation, facilitating subsequent syntheses of corresponding glycoconjugates.

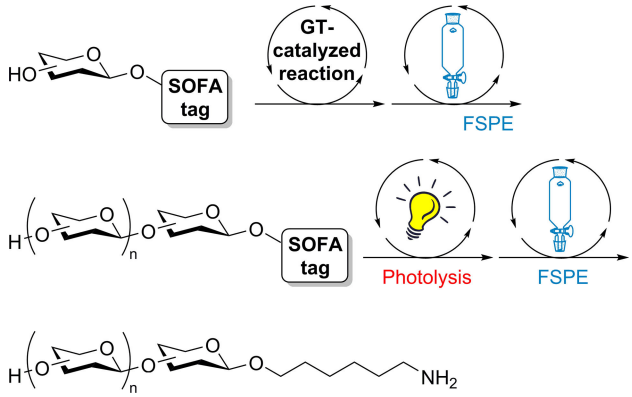


Figure 1. Concept of SOFA tag-assisted enzymatic oligosaccharide synthesis.

2 Results and Discussion

2.1 Synthesis of the Sulfo-fluorous-tagged Acceptors

To examine the applicability of our designed tag, which is tolerated by different glycosyltransferases, acceptors with photocleavable sulfo-fluorous tags at the reducing end, namely, *N*-acetyl glucosamine (GlcNAc **1a** and **1c**) and lactose (Lac **2a**), were synthesized. For comparison, the non-fluorous-tagged acceptors **1b**[38] and **2b**[39] were also prepared (Figure 2).

The syntheses of photocleavable and sulfo-fluorous-tagged sugar acceptors for enzymatic glycosylation are shown in Scheme 1. Photocleavable compound **3** was synthesized according to the reported procedure[40] with minor modifications, and then it was coupled with *N*-Boc-1,3-propanediamne to give **4** in 77% yield. Then, 4-nitrophenyl chloroformate (NPC) was used as a cross-linker between compound **4** and the sugars. The hydroxyl group of **4** was reacted with NPC to form the carbamate, which was subsequently coupled with the amine groups of sugars **5**, **6**, and **7** to afford **8**, **9**, and **10** in overall yields of 81%, 71%, and

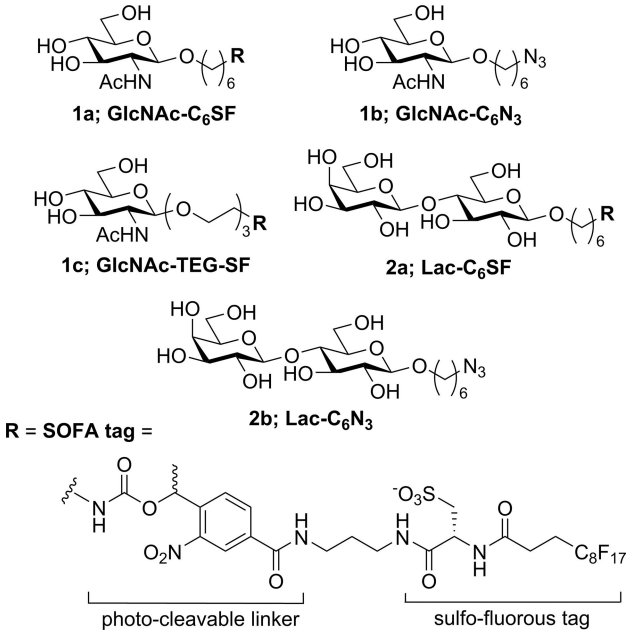
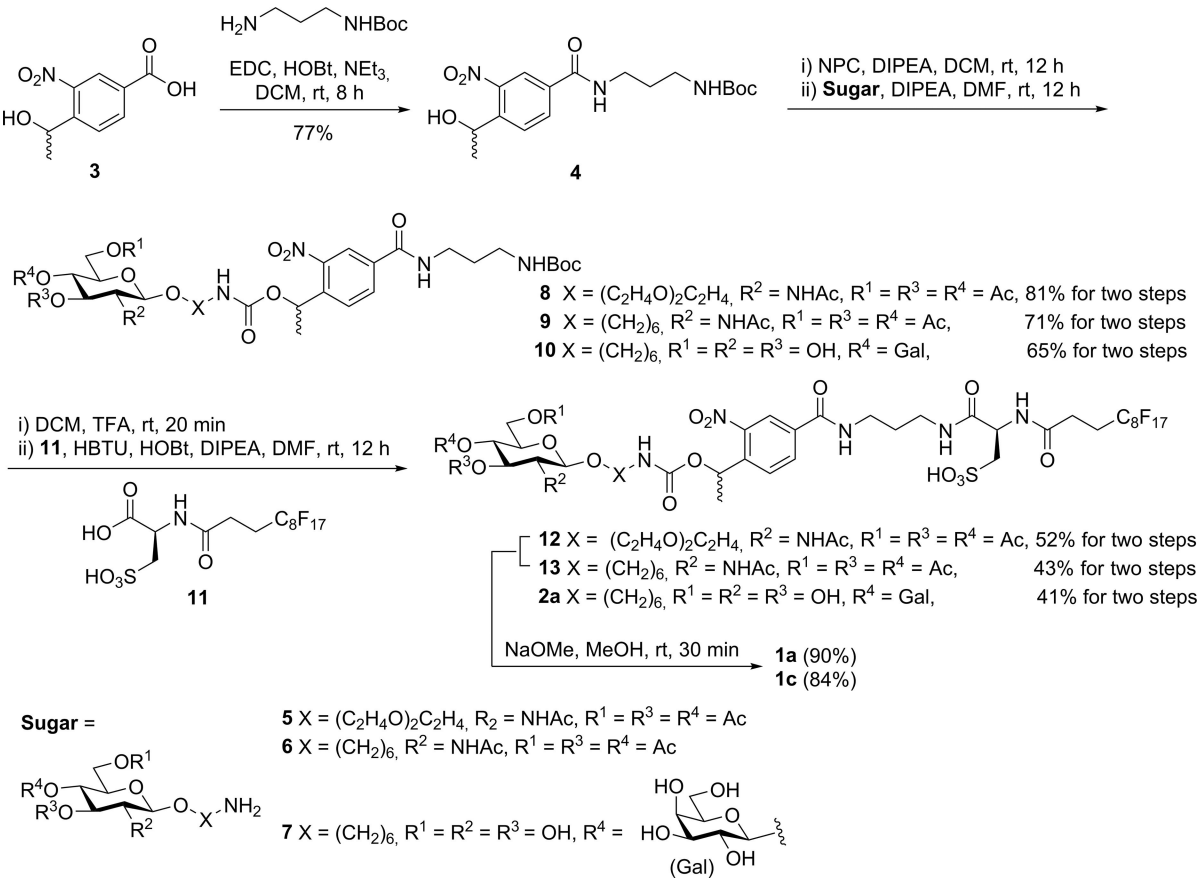


Figure 2. The sulfo-fluorous-tagged acceptors for glycosyltransferase-catalyzed reactions.

65%, respectively, over two steps. The *tert*-butyloxycarbonyl (*N*-Boc) protecting groups on the terminal amines of **8**, **9**, and **10** were removed by a trifluoroacetic acid cocktail followed by coupling with sulfofluorous tag **11**[37] under basic conditions to furnish **12**, **13**, and **2a** in 52%, 43%, and 41% overall yields, respectively, for the two steps. The fluorous-tagged compounds were purified by FSPE, allowing the byproducts such as non-fluorous reagents and base to be easily removed. Finally, removal of the acetyl groups on **12** and **13** was performed by using sodium methoxide in methanol to yield sulfo-fluorous-tagged GlcNAc **1a** and **1c** in 90% and 84% yields, respectively. The water solubilities of GlcNAc **1a** and **1b** were further examined, and **1a** (241 mg/mL, 195 mM) was as hydrophilic as **1b** (72 mg/mL, 208 mM), indicating the importance of the sulfonate group in improving the water solubility.

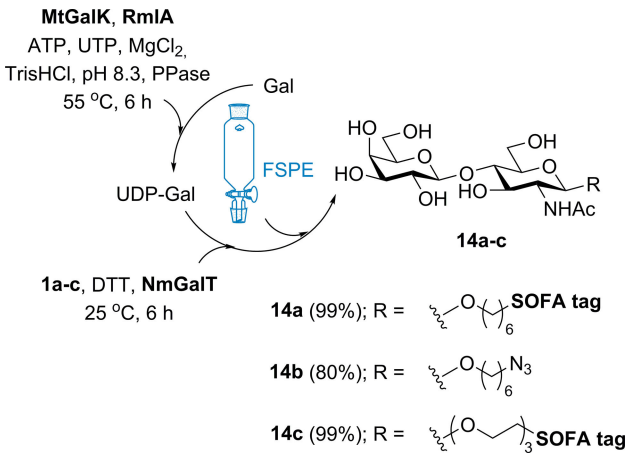
2.2 Synthesis of LacNAc using a SOFA Tag Strategy

In the proof-of-concept experiment, we examined two SOFA-tagged acceptors (**1a** and **1c**) and one conventional azidohexyl-tagged acceptor (**1b**). SOFA-tagged LacNAc **14a** was prepared from GlcNAc **1a** using β-



Scheme 1. Synthesis of photocleavable and sulfo-fluorous-tagged sugar acceptors **1a**, **1c**, and **2a**

1,4-galactosyltransferase from *Neisseria meningitidis* (NmGalT) in the presence of uridine diphosphate galactose (UDP-Gal), which was generated by a sequential one-pot enzymatic reaction using galactokinase from *Meiothermus taiwanensis* (MtGalK)[41] and glucose-1-phosphate thymidylyltransferase from *Aneurinibacillus thermoaerophilus* (RmlA)[42] as reported previously with minor modifications (Scheme 2). After incubation at 25 °C for 6 h, the reaction mixture was transferred to an FSPE column and eluted with water followed by methanol. The pure LacNAc **14a** was eluted by methanol to afford the product in 99% yield (67 mg). Similarly, as described in the synthesis of **14a**, LacNAcs **14b** and **14c** were prepared by using NmGalT and UDP-Gal from **1b** and **1c** to give 80% and 99% yields, respectively. Notably, the presence of the SOFA tag enhances the reaction rate (yields of **14a** and **14c** are higher than that of **14b**) under the same reaction conditions. In addition, one cycle of FSPE purification requires only 30 min to obtain a relatively pure product.



Scheme 2. Enzymatic synthesis of LacNAc **14** using a SOFA tag strategy.

2.3 SOFA Tag-assisted Enzymatic Synthesis of Sialyl Lewis X

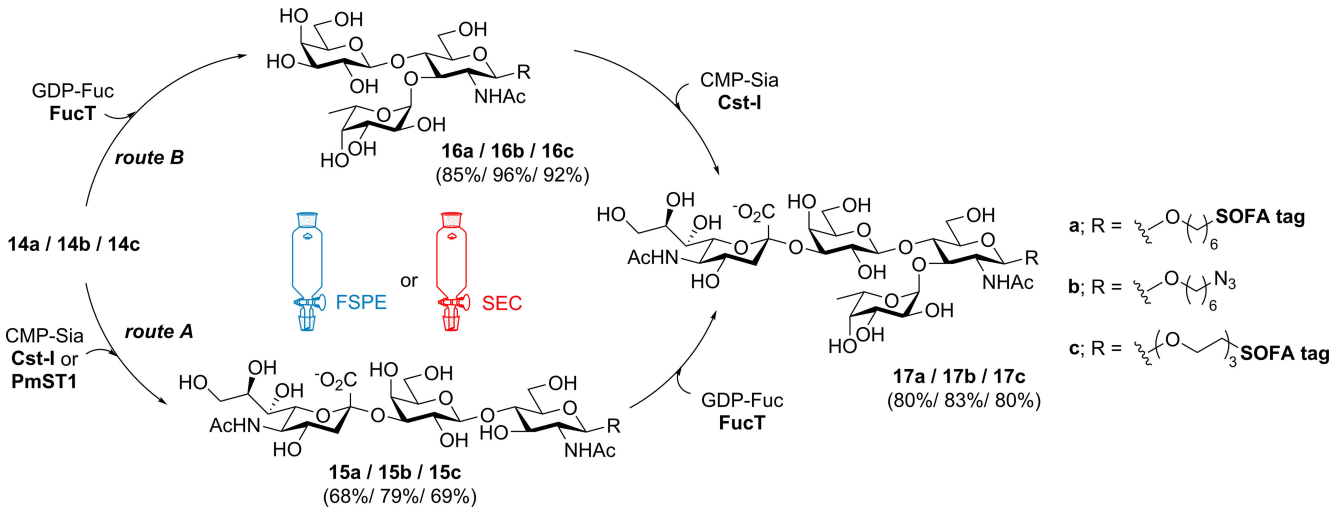
To demonstrate the feasibility of the SOFA tag strategy in the enzymatic synthesis of oligosaccharides, the negatively charged sialyl Lewis X antigen was chosen as the target oligosaccharide. As illustrated in Scheme 3, the glycosylated products with azidohexyl linkers were purified by SEC, while FSPE was used for SOFA-tagged compounds. In route A, the sialylations of LacNAcs **14a** and **14b** were performed by α-2,3-sialyltransferase PmST1 from *Pasteurella multocida* with CMP-sialic acid as the donor substrate to afford α-2,3-sialyl LacNAcs **15a** and **15b** in 68% (15 mg) and 79% (22 mg) yields, respectively. Similarly, the α-2,3-sialyltransferase Cst-I from *Campylobacter jejuni* was able to sialylate LacNAc **14c** to prepare α-2,3-sialyl LacNAc **15c** in 69% yield (15 mg). Both sialyltransferases tolerate the SOFA tag, but the reaction yields were moderate. The reduced yields may be due to the repulsion between the negative charges of the sulfonate of SOFA and the carboxyl group of sialic acid. Subsequent fucosylation of **15a-c** by α-1,3-fucosyltransferase from *Helicobacter pylori* (FucT) in the presence of GDP-fucose produced sialyl Lewis X (SLe^X) **17a**, **17b**, and **17c** in 80% (19 mg), 83% (13 mg), and 80% (13 mg) yields, respectively. In route B, fucosylated LacNAc (Lewis X, Le^X) derivatives were prepared by FucT with GDP-fucose in 85% (**16a**, 33 mg), 96% (**16b**, 18 mg) and 92% (**16c**, 9 mg) yields, indicating that the SOFA tag can serve as water-soluble tag for the synthesis of neutral saccharides. To prepare SLe^X by sialyation of Le^X, **16c** was used as the starting acceptor and two α-2,3-sialyltransferases were tested. PmST1 (wild-type) did not efficiently convert Le^X to SLe^X, which agreed with the reported literature,[43] while Cst-I was able to directly sialylate Le^X **16c** to produce SLe^X **17c** in 65% yield (4.5 mg).

2.4 Enzymatic Synthesis of Globotriose using a SOFA Tag Strategy

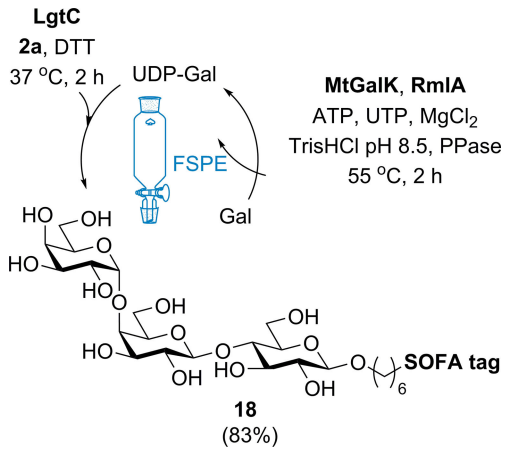
In addition to the synthesis of a negatively charged SLe^X tetrasaccharide, enzymatic syntheses of neutral oligosaccharides, including globotriose trisaccharide (Gb3, also referred to as the P^k antigen), di-LacNAc tetrasaccharide, P1 antigen pentasaccharide, and dimeric Lewis X (hexasaccharide), were also achieved using this SOFA tag strategy. The installation of the sulfonate group dramatically improved the water solubility of the fluorous tag, and the use of either aliphatic or ethylene glycol linkers showed similar results in the enzymatic synthesis. Therefore, we used aliphatic SOFA-tagged substrates (compound number with **a**) for further neutral oligosaccharide syntheses. As shown in Scheme 4, lactoside **2a** was galactosylated by LgtC, an α-1,4-galactosyltransferase from *N. meningitidis*,[44] in the presence of UDP-Gal with 2 h of incubation at 37 °C by a sequential one-pot enzymatic synthesis procedure[41–42] to give Gb3 **18** in 83% yield (19 mg). The efficiency of LgtC catalysis toward SOFA-tagged lactose **2a** was similar to that of azidohexyl lactose **2b** that we reported previously (91%).[41]

2.5 Enzymatic Synthesis of P1 Antigen using a SOFA Tag Strategy

By using a combination of three glycosyltransferases in a sequential one-pot enzymatic synthesis of UDP-



Scheme 3. Synthetic routes to sialyl Lewis X using a SOFA tag strategy. The synthetic yields of **17** indicated are from compound **15**. The yield of **17c** from **16c** is 65%.

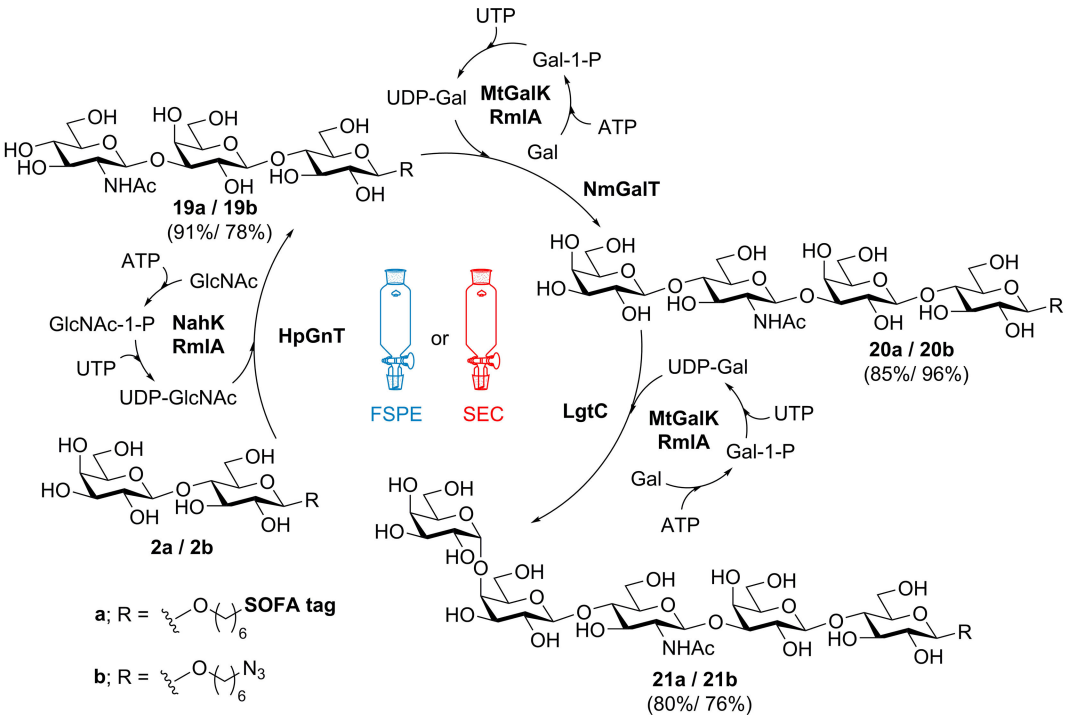


Scheme 4. Enzymatic synthesis of Gb3 **18** using a SOFA tag strategy.SOFA tag strategy.

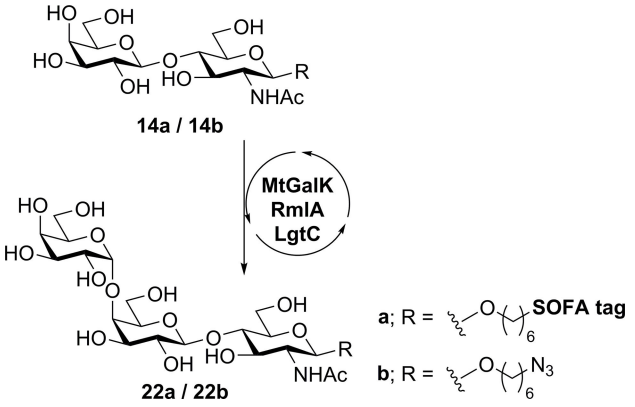
sugars, P1 antigen derivatives **21a** and **21b** were synthesized (Scheme 5). Lactosides **2a** and **2b** were used as substrates for β-1,3-*N*-acetyl-glucosaminyl-transferase HpGnT from *Helicobacter pylori* and reacted with UDP-GlcNAc for 2 days at 25 °C to afford LNTri II **19a** and **19b** in 91% (27 mg) and 78% (26 mg), respectively. The resulting products were further treated with UDP-Gal under the NmGalT catalysis for 2 h at 25 °C to obtain lacto-*N*-neotetraoses (LNnTs) **20a** and **20b** in 85% (25 mg) and 96% (36 mg), respectively. Finally, LgtC was used to generate the α-1,4-galactosidic bond to yield the P1 antigen pentasaccharide **21** in a similar manner to what was described for the synthesis of LNnT. Interestingly, LgtC converted SOFA-tagged acceptor LNnT **20a** to P1 antigen **21a** in 2 h at 37 °C in 80% yield (18 mg), while the enzymatic reaction of non-fluorous-tagged acceptor LNnT **20b** required a much longer reaction time (2 days) to reach 76% yield of **21b** (26 mg) by using the same weight ratio amount of LgtC. The slow reaction rate under LgtC catalysis with acceptor substrate, LNnT, containing an alkyl azide at the reducing end is consistent with the results reported by Cao and co-workers[45] in which the optimal conversion yield of P1 antigen from azidopropyl LNnT was 55% (52 mg). The enhancement of the rate of the LgtC-catalyzed reaction due to the presence of the SOFA tag was further confirmed by using **14a** and **14b** as acceptors (Scheme 6) to examine sulfo-fluorous and non-sulfo-fluorous-tagged substrates for the synthesis of non-reducing end P1 trisaccharides (Galα-1,4-Galβ-1,4-GlcNAc) **22a** and **22b**, and the progress of reactions was monitored by TLC. Under the same LgtC-catalyzed reaction conditions, SOFA-tagged LacNAc **14a** was completely consumed within 2 h; however, non-fluorous-tagged LacNAc **14b** was a poor substrate for LgtC, and the reaction required 16 h to reach completion. Although, in the synthesis of P1 pentasaccharide, the enzymatic reaction yields of different reducing end-tagged acceptors were similar (under the optimized reaction time), the purification of the SOFA-tagged products was very efficient, requiring only 30 min for each separation, while the azidohexyl-tagged compounds required more time for purification (8–36 h).

2.6 SOFA Tag-assisted Enzymatic Synthesis of Dimeric Lewis X

Moreover, the SOFA tag strategy was also applicable to the synthesis of GlcNAc-LacNAc **23** and LacNAc dimer **24** (Scheme 7). Following the procedure for the synthesis of oligoLacNAc reported previously,[38] the



Scheme 5. Synthetic route of P1 antigen using a SOFA tag strategy.



Scheme 6. One-pot synthesis of P1 non-reducing end trisaccharide **22** using sequential MtGalK, RmlA, and LgtC catalysis.

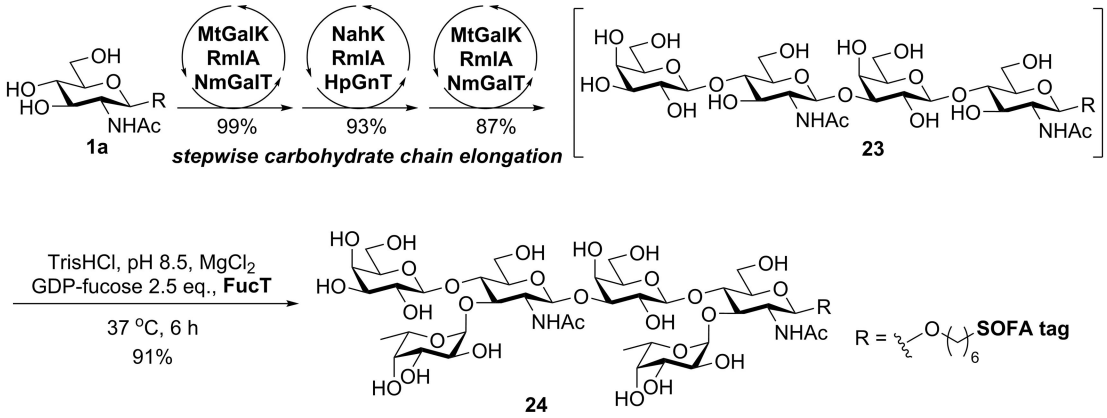
stepwise introduction of Gal, GlcNAc, and Gal onto GlcNAc **1a** gave excellent yields of the corresponding products (99%, 93%, and 87%, respectively). Notably, the octasaccharide (four LacNAc repeating units) shows poor water solubility according to our previous observations.[38] However, with the SOFA tag, which significantly increases the water solubility of the tagged compound, LacNAc derivatives can be enzymatically prepared up to the decasaccharide (data not shown). Furthermore, the LacNAc dimer (intermediate **23**) was subsequently fucosylated by FucT in the presence of 2.5 equivalence of GDP-fucose at 37 °C for 6 h to give dimeric Lewis X **24** in 91% yield (22 mg).

2.7 Photolysis of SOFA-tagged Gb3 (18)

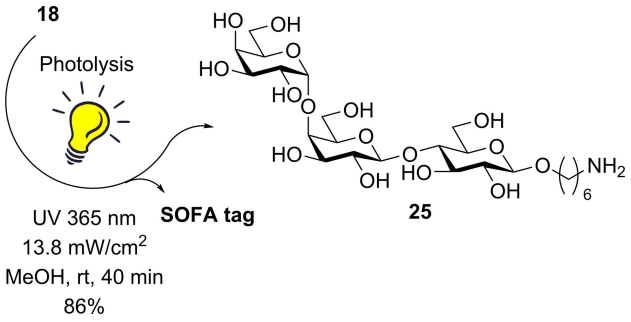
The photolysis of **18** was performed under UV irradiation at 365 nm in methanol for 40 min to yield Gb3-C_6-NH_2 (**25**) with a cleavage efficiency of 86% (Scheme 8). The resulting SOFA tags were trapped by FSPE, allowing the convenient elution of desired Gb3-C_6-NH_2 (**25**) with water and facilitating the release of the reaction product for further applications.

3 Conclusion

In summary, we developed a bifunctional sulfofluorous affinity (SOFA) tag-assisted strategy for the enzymatic syntheses of oligosaccharides, allowing the rapid purification of complex glycans. The incorporation of a sulfonate moiety onto the heavy fluorous tag significantly increased the water solubility of the compounds, which avoids the challenges typically associated with the use of inherently hydrophobic fluorous tags in aqueous buffers. In addition, the photocleavable linker enables the easy release of the amino-functionalized oligosaccharides by UV irradiation. The benefits of the SOFA tag strategy in enzymatic glycosylations have been demonstrated by using several glycosyltransferases including HpGnT,



Scheme 7. A sequential, one-pot, enzymatic synthesis of dimeric Lewis X **24** from GlcNAc **1a**. The fucosylation was carried out with FucT in the presence of 2.5 eq. of GDP-Fuc.



Scheme 8. Photolysis of SOFA-tagged Gb3 (**18**) by UV irradiation.

NmGalT, LgtC, FucT, PmST, and Cst-I. Facile syntheses of both negatively charged and neutral oligosaccharides including sialyl Lewis X, Lewis X, P1 pentasaccharide, globotriose, and Lewis X dimer were achieved. Notably, Cst-I can directly sialylate Le^X to produce SLe^X, which furnishes an alternative approach to the preparation of SLe^X. However, the yields of sialylation are moderate, which may be due to repulsion between the charges on the carboxylate of the sialic acid and the sulfonate group of the SOFA tag. By contrast, sulfo-fluorous-tagged acceptors substantially improve the rate of the LgtC-catalyzed reaction. In general, fluorous tag is applied to solid phase extraction for separation of tagged compounds. Our previous studies indicated that the fluorous-fluorous interaction is stable in buffer solution.[37] Thus, the SOFA tag could be used for immobilization of primer sugar on the perfluorinated alkyl group coated particles and should be feasible in conjunction with enzymatic solid phase synthesis.[46] In addition, the use of SOFA tag is environmentally benign and is more "green" than the corresponding chemical syntheses of oligosaccharides.

Experimental Section

GlcNAc-TEG-SF-tag (1c). Compound **12** (74 mg, 0.05 mmol) was dissolved in MeOH (2 mL) and stirred in the presence of NaOMe (2.6 mg, 0.048 mmol) at 4 °C. After being stirred for 30 min at room temperature, the reaction mixture was neutralized with Amberlite IR-120 (H^+) resin, filtered, and concentrated *in vacuo* to give the crude product. The mixture was purified by column chromatography using silica gel (DCM/MeOH, 3:1) to give compound **1c** (61 mg, 84%) as a light-yellow syrup. R_f=0.25 (DCM/MeOH=3:1); ^{1}H NMR (400 MHz, CD_3OD) δ 8.43 (s, 1H), 8.19 (d, J= 8.2 Hz, 1H), 7.84 (d, J=8.2 Hz, 1H), 6.30–6.00 (m, 1H), 4.73 (dd, J=7.6, 4.6 Hz, 1H), 4.59 (br, 1H), 4.46 (dd, J=8.4, 1.4 Hz, 1H), 3.94 (dd, J=9.9, 5.1 Hz, 1H), 3.87 (d, J= 11.7 Hz, 1H), 3.75–3.64 (m, 4H), 3.64–3.53 (m, 7H), 3.52–3.41 (m, 5H), 3.38–3.33 (m, 2H), 3.30–3.25 (m, 2H), 3.22 (dd, J= 8.4, 5.5 Hz, 2H), 2.74–2.42 (m, 4H), 1.97 (s, 3H), 1.87–1.75 (m, 2H), 1.62 (d, J=6.5 Hz, 3H). ^{13}C NMR (100 MHz, CD_3OD) δ 173.9, 172.7, 167.3, 157.8, 149.1, 148.6, 142.8, 136.0, 133.2, 128.8, 124.6, 102.9, 78.0, 76.1, 72.1, 71.5, 71.4, 71.3, 71.0, 69.9, 69.5, 62.7, 57.3, 52.7, 52.5, 41.6, 38.1, 37.6, 29.8, 27.6 (t, $^2J_{CF}$=21 Hz, CH_2CF_2), 27.5, 23.1, 22.3. HRMS (ESI-TOF) m/z calcd for $C_{42}H_{51}F_{17}N_5O_{18}S$ $[M-H]^-$: 1268.2678, found: 1268.2671.

GlcNAc-TEG-tag (8). To a stirred solution of compound **4** (456 mg, 1.25 mmol) and DIPEA (310 μL, 2 mmol) in DCM (12 mL) was added 4-nitrophenyl chloroformate (383 mg, 1.9 mmol) at 0 °C under nitrogen. The reaction mixture was stirred at room temperature for 12 h and then quenched by $NaHCO_3$ (1 mL). The mixture was extracted with EtOAc, and the combined organic layer was washed with brine, dried over $MgSO_4$, and concentrated. The residue was purified by column chromatography using silica gel (hexane/EtOAc 1:1) to afford the pure residue. Compound **5** (478 mg, 1 mmol) and DIPEA (465 μL, 3 mmol) were added to a solution of above residue in DMF (10 mL) at room temperature. After being stirred for 12 h, the reaction mixture was concentrated and purified by column chromatography using silica gel (hexane/EtOAc 1:1, with 20% MeOH) to give compound **8** (705 mg, 81%) as light yellow syrup. R_f=0.36 (hexane/EtOAc=1:1, with 20% MeOH); ^{1}H NMR (400 MHz,

CD_3OD) δ 8.43 (s, 1H), 8.16 (d, J = 8.3 Hz, 1H), 7.84 (d, J = 7.9 Hz, 1H), 6.15 (q, J = 6.4 Hz, 1H), 5.21 (ddd, J = 10.4, 9.4, 5.3 Hz, 1H), 5.02–4.93 (m, 1H), 4.71 (dd, J = 8.4, 3.7 Hz, 1H), 4.61 (s, 1H), 4.28 (dd, J = 12.5, 4.4 Hz, 1H), 4.13 (d, J = 12.5 Hz, 1H), 3.89 (t, J = 9.4 Hz, 2H), 3.80 (d, J = 7.5 Hz, 1H), 3.76–3.65 (m, 1H), 3.65–3.52 (m, 6H), 3.49 (t, J = 5.3 Hz, 2H), 3.44 (t, J = 6.8 Hz, 3H), 3.22 (dd, J = 11.8, 5.5 Hz, 2H), 3.13 (t, J = 6.7 Hz, 2H), 2.05 (s, 3H), 2.00 (s, 3H), 1.97 (s, 3H), 1.91 (s, 3H), 1.85–1.72 (m, 2H), 1.63 (d, J = 6.5 Hz, 3H), 1.43 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.1, 170.7 (×2), 169.3, 164.6, 157.1, 155.5, 147.5, 141.4, 134.4, 131.9, 127.5, 123.2, 101.6, 79.7, 73.1, 71.7, 71.0, 70.3, 70.2, 69.8, 68.7, 68.5, 68.4, 62.1, 54.1, 53.4, 40.5, 37.0, 36.2, 29.8, 28.3(x3), 22.9, 22.0, 20.7, 20.6. HRMS (ESI-TOF) m/z calcd for $C_{38}H_{57}N_5O_{18}Na$ $[M+Na]^+$: 894.3596, found: 894.3594.

GlcNAc-TEG-SF-tag (12). A solution of TFA (1 mL) in 3 mL of DCM was added to compound **8** (250 mg, 0.28 mmol) at 4 °C. The reaction was stirred at room temperature for 20 min and then concentrated *in vacuo*. The reaction mixture in MeOH (10 mL) was neutralized with Dowex resin (OH^-), filtered, and then concentrated *in vacuo* to give the crude compound as a yellow oil. The resulting amine was used in the next reaction without further purification. Compound **11** (186 mg, 0.28 mmol), HBTU (159 mg, 0.42 mmol), HOBt (57 mg, 0.42 mmol) and DIPEA (90 µL, 0.56 mmol) were dissolved in dry DMF (1 mL) and stirred for 30 min at room temperature under a nitrogen atmosphere. To a stirred solution of the activated ester in dry DMF (1 mL) was added the above residue and DIPEA (45 µL, 0.28 mmol). After being stirred for 12 h at room temperature, the crude mixture was purified by flash column chromatography on fluorous silica gel (eluted with 80% aq. MeOH) to afford product **12** (204 mg, 52%) as a light-yellow syrup. R_f = 0.10 (hexane/EtOAc = 1:1, with 20% MeOH); ^{1}H NMR (400 MHz, CD_3OD) δ 8.43 (s, 1H), 8.18 (d, J = 6.9 Hz, 1H), 7.83 (dd, J = 8.2, 2.3 Hz, 1H), 6.15 (q, J = 6.1 Hz, 1H), 5.37–5.11 (m, 1H), 4.99 (t, J = 9.7 Hz, 1H), 4.77 (dd, J = 7.8, 4.6 Hz, 1H), 4.71 (dd, J = 8.4, 4.0 Hz, 1H), 4.28 (dd, J = 12.3, 4.5 Hz, 1H), 4.12 (dd, J = 12.3, 2.1 Hz, 1H), 3.94–3.85 (m, 2H), 3.85–3.77 (m, 1H), 3.76–3.64 (m, 1H), 3.64–3.52 (m, 7H), 3.52–3.41 (m, 4H), 3.39–3.33 (m, 4H), 3.26–3.15 (m, 3H), 2.75–2.44 (m, 4H), 2.04 (s, 3H), 2.00 (s, 3H), 1.97 (s, 3H), 1.91 (s, 3H), 1.86–1.76 (m, 2H), 1.62 (d, J = 6.5 Hz, 3H). ^{13}C NMR (100 MHz, CD_3OD) δ 173.5, 172.9, 172.5, 172.4, 171.8, 171.3, 167.1, 157.7, 148.9, 142.8, 135.9, 133.1, 128.8, 124.4, 102.4, 74.2, 72.8, 71.4, 71.3, 71.0, 70.2, 70.1, 69.4, 63.3, 55.3, 52.6, 52.5, 41.6, 38.1, 37.7, 29.8, 27.5 (t, $^2J_{CF}$ = 21 Hz, CH_2CF_2), 22.9, 22.4, 22.3, 20.7, 20.6 (×2). HRMS (ESI-TOF) m/z calcd for $C_{47}H_{56}F_{17}N_6O_{21}S$ $[M-H]^-$: 1395.2948, found: 1395.2936.

LacNAc-TEG-SF-tag (14c). A buffered (50 mM Tris-HCl, pH 8.3) solution (5 mL) of galactose (17 mg, 0.094 mmol), 40 mM $MgCl_2$, 20 mM ATP (52 mg, 0.094 mmol), 20 mM UTP (50 mg, 0.094 mmol) and inorganic pyrophosphatase (10 U) was incubated at 55 °C in the presence of MtGalK (10 µg/mL) and RmlA (1 mg/mL) for 6 h. The reaction solution was monitored by RP-HPLC coupled with a UV detector (the formation of UDP-Gal was measured). The solution was centrifuged (10,000 × g, 10 min) to remove insoluble precipitates, and the supernatant was added to compound **1c** (60 mg, 0.047 mmol), 0.2 mM DTT and NmGalT (50 µg/mL). The resulting solution was incubated at 25 °C for 6 h. When the reaction was completed by TLC analysis, the reaction solution was centrifuged (10,000 × g, 10 min), and the supernatant was purified by flash column chromatography on fluorous silica gel (eluted with 70% aq MeOH, followed by 100% MeOH). Product-containing fractions were identified by TLC and were combined and lyophilized to give compound **14c** (67 mg, 99%). R_f = 0.35 (DCM/MeOH = 2:1); ^{1}H NMR (400 MHz, CD_3OD) δ 8.43 (s, 1H), 8.19 (d, J = 7.7 Hz, 1H), 7.84 (d, J = 8.3 Hz, 1H), 6.15 (q, J = 6.1 Hz, 1H), 4.71 (t, J = 7.5 Hz, 1H), 4.60 (s, 1H), 4.49 (dd, J = 8.4, 3.3 Hz, 1H), 4.38 (d, J = 6.9 Hz, 1H), 3.89 (ddd, J = 26.5, 11.5, 5.3 Hz, 4H), 3.79–3.72 (m, 2H), 3.72–3.68 (m, 1H), 3.68–3.53 (m, 14H), 3.53–3.37 (m, 6H), 3.21 (dt, J = 13.7, 6.9 Hz, 2H), 2.72–2.43 (m, 4H), 1.96 (s, 3H), 1.87–1.75 (m, 2H), 1.62 (d, J = 6.5 Hz, 3H). 13C NMR (100 MHz, D_2O) δ 175.3, 173.2, 172.5, 167.1, 157.8, 148.2, 142.3, 135.0 133.2, 128.7, 124.1, 104.0, 102.1, 79.4, 76.4, 75.8, 73.6, 73.6, 72.1, 70.7, 70.6, 70.3, 70.0, 69.7, 62.5, 62.1, 61.1, 60.5, 56.2, 52.2, 52.0, 41.1, 41.1, 38.0, 37.7, 29.2, 27.2 (t, $^2J_{CF}$ = 21 Hz, CH_2CF_2), 23.3, 21.5; HRMS (ESI-TOF) m/z calcd for $C_{48}H_{61}F_{17}N_5O_{23}S$ $[M-H]^-$: 1430.3207, found: 1430.3205.

Sialyl LacNAc-TEG-SF-tag (15c). The reaction was carried out in a 15-mL centrifuge tube with 2 mL of Tris-HCl buffer (20 mM, pH 7.5) containing CMP-sialic acid (20 mg, 31 µmol), LacNAc **14c** (30 mg, 21 µmol), $MgCl_2$ (20 mM), alkaline phosphatase (1 U), and Cst-I (75 µg/mL). The reaction mixture was incubated at 37 °C for 6 h with shaking (200 rpm), and the reaction was monitored by TLC. When the reaction was completed by TLC, the reaction solution was centrifuged (10,000 × g, 10 min), and the supernatant was purified by flash column chromatography on fluorous silica gel (eluted in 50% aq MeOH, followed by 100% MeOH). Product-containing fractions were identified by TLC and were combined and lyophilized to give compound **15c** (25 mg, 69%; conversion appeared quantitative by TLC). R_f = 0.6 (EtOAc/MeOH/H_2O/AcOH = 4:2:1:0.5); ^{1}H NMR (400 MHz, CD_3OD) δ 8.43 (s, 1H), 8.18 (d, J = 8.4 Hz, 1H), 7.84 (d, J = 8.3 Hz, 1H), 6.15 (dd, J = 12.7, 6.1 Hz, 1H), 4.73 (dd, J = 8.0, 4.6 Hz, 2H), 4.61 (s, 4H), 4.46 (d, J = 7.9 Hz, 2H), 4.06 (dd, J = 9.7, 2.7 Hz, 1H), 3.98–3.80 (m, 7H), 3.80–3.68 (m, 5H), 3.68–3.52 (m, 18H), 3.52–3.36 (m, 7H), 3.24–3.15 (m, 3H), 2.86 (dd, J = 11.9, 2.4 Hz, 1H), 2.72–2.47 (m, 4H), 2.01 (s, 3H), 1.97 (s, 3H), 1.85–1.75 (m, 2H), 1.62 (d, J = 6.6 Hz, 3H). ^{13}C NMR (150 MHz, CD_3OD) δ 175.5, 174.0, 173.6, 172.9, 172.7, 167.3, 157.8, 149.0, 142.8, 136.1, 133.1, 128.9, 124.4, 105.1, 105.0, 102.9, 81.1, 77.7, 77.1, 76.6, 74.9, 74.3, 73.0, 71.6, 71.5, 71.3, 70.90, 70.86, 70.1, 70.0, 69.5, 69.4, 69.1, 64.6, 62.7, 61.9, 56.6, 56.5, 54.0, 52.7, 52.5, 42.0, 41.6, 38.1, 37.7, 29.8, 27.6 (t, $^2J_{CF}$ = 21 Hz, CH_2CF_2), 23.0, 22.6, 22.3. HRMS (ESI-TOF) m/z calcd for $C_{58}H_{77}F_{17}N_7O_{31}S$ $[M-H]^-$: 1722.4113, found: 1722.4101.

Lewis^x-TEG-SF-tag(16c). Reaction was carried out in a 15 mL centrifuge tube with 0.6 mL Tris-HCl buffer (50 mM, pH 7.5) containing 20 mM $MgCl_2$ and 20 mM $MnSO_4$, 10 mM LacNAc **14c** (9 mg, 6 µmol), 15 mM GDP-fucose (5 mg, 9 µmol), and 60 µg/mL α-1,3-FucT. The resulting mixture was incubated at 37 °C for 2 h. The reaction was monitored by TLC analysis. When the reaction was completed by TLC analysis, the reaction solution was centrifuged (10,000 × g, 10 min) and the supernatant was passed through fluorous

silica gel in a two-stage extraction eluting first with H_2O and then with 100% MeOH. Lyophilized Lewis^X-TEG-SF-tag **16c** (9 mg, 92%). R_f=0.15 (DCM/MeOH=2:1); ^{1}H NMR (400 MHz, CD_3OD) δ 8.43 (s, 1H), 8.20 (d, J=8.5 Hz, 1H), 7.84 (d, J=8.2 Hz, 1H), 6.30–6.04 (m, 1H), 5.04 (d, J=3.8 Hz, 1H), 4.71 (dd, J=7.9, 4.8 Hz, 1H), 4.62 (s, 1H), 4.53 (dd, J=7.6, 4.3 Hz, 1H), 4.49–4.40 (m, 1H), 4.02–3.84 (m, 7H), 3.80 (d, J=3.3 Hz, 2H), 3.78–3.53 (m, 14H), 3.53–3.38 (m, 9H), 3.25–3.15 (m, 3H), 2.73–2.48 (m, 5H), 1.96 (s, 3H), 1.87–1.73 (m, 2H), 1.62 (d, J=6.5 Hz, 3H), 1.18 (d, J=6.6 Hz, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 175.2, 173.3, 172.9, 167.1, 157.8, 149.0, 136.0, 133.1, 128.9, 124.4, 111.4, 103.9, 102.4, 100.2, 77.4, 76.6, 75.2, 74.8, 73.7, 72.8, 71.5, 71.4, 71.3, 71.2, 70.9, 69.94, 69.88, 69.8, 69.5, 67.7, 62.8, 61.7, 61.4, 57.2, 52.7, 52.5, 41.6, 38.1, 37.6, 29.8, 27.6 (t, $^2J_{CF}$=21 Hz, CH_2CF_2), 23.2, 22.3, 16.6; HRMS (ESI-TOF) m/z calcd for $C_{54}H_{71}F_{17}N_5O_{27}S$ $[M-H]^-$: 1576.3786, found: 1576.3777.

Sialyl Lewis X-C$_6$-TEG-SF-tag (17c). The reaction was carried out in a 15-mL centrifuge tube with 1 mL of Tris-HCl buffer (20 mM, pH 7.5) containing 8.7 mM α-2,3-sialyl LacNAc **15c** (15 mg, 8.7 μmol), 15 mM GDP-fucose (8 mg, 13 μmol), 20 mM $MgCl_2$, 20 mM $MnSO_4$, and 219 μg/mL α-1,3-FucT. The resulting mixture was incubated at 37 °C for 5 h, and the reaction was monitored by TLC. When the reaction was completed by TLC, the mixture was centrifuged (10,000×g, 10 min), and the supernatant was passed through fluorous silica gel in a two-stage extraction eluting first with H_2O and then with 100% MeOH. Product-containing fractions were identified by TLC analysis and were combined and lyophilized to give compound **17c** (13 mg, 80%). R_f=0.5 (EtOAc/MeOH/H_2O/AcOH=4:2:1:0.5); ^{1}H NMR (600 MHz, CD_3OD) δ 8.43 (s, 1H), 8.18 (d, J=8.2 Hz, 1H), 7.84 (d, J=8.2 Hz, 1H), 6.15 (dd, J=12.7, 6.1 Hz, 1H), 5.48 (s, 1H), 5.05 (d, J=3.7 Hz, 1H), 4.75–4.68 (m, 1H), 4.62–4.49 (m, 5H), 4.46 (t, J=7.6 Hz, 1H), 4.09–4.02 (m, 1H), 4.01 (dd, J=11.8, 3.4 Hz, 1H), 3.97–3.81 (m, 9H), 3.80–3.67 (m, 6H), 3.67–3.51 (m, 16H), 3.51–3.40 (m, 8H), 3.26–3.17 (m, 3H), 2.94–2.81 (m, 1H), 2.74–2.49 (m, 4H), 2.01 (s, 3H), 1.96 (s, 3H), 1.88–1.76 (m, 2H), 1.72 (t, J=11.7 Hz, 1H), 1.64 (t, J=13.3 Hz, 3H), 1.16 (dd, J=6.5, 2.1 Hz, 3H). ^{13}C NMR (150 MHz, CD_3OD) δ 175.5, 174.8, 173.9, 172.9, 172.7, 167.3, 157.8, 149.1, 142.8, 136.1, 133.1, 128.9, 124.4, 104.0, 102.5, 100.9, 100.1, 77.9, 77.4, 76.7, 76.4, 75.4, 75.0, 73.7, 73.1, 71.5, 71.4, 71.3, 71.1, 71.0, 70.9, 70.2, 70.0, 69.9, 69.5, 69.4, 68.8, 67.7, 64.7, 63.0, 62.3, 61.3, 57.1, 54.0, 52.7, 52.5, 42.3, 41.6, 38.2, 37.7, 29.8, 27.6 (t, $^2J_{CF}$=21 Hz, CH_2CF_2), 23.14 22.6, 22.3, 16.6. HRMS (ESI-TOF) m/z calcd for $C_{64}H_{87}F_{17}N_7O_{35}S$ $[M-H]^-$: 1868.4692, found: 1868.4671.

Gb3-C$_6$-SF-tag (18). Reaction was carried out in a 15-mL centrifuge tube with 1.57 mL Tris-HCl buffer (50 mM, pH 8.5) containing 20 mM ATP (17 mg, 31 μmol), 20 mM galactose (6 mg, 31 μmol), 40 mM $MgCl_2$, and 5 μg/mL GalK at 55 °C for 2 h. Following addition of 20 mM UTP (16 mg, 31 μmol) and 1 mg/mL RmlA, the resulting mixture was incubated at 55 °C for 2 h. The formation of UDP-GlcNAc was detected and monitored by RP-HPLC coupled with a UV detector. Finally, 10 mM lactose **2a** (21 mg, 15 μmol) and 100 μg/mL LgtC were added. The resulting mixture was incubated at 37 °C for 2 h, and the reaction was monitored by TLC analysis. When the reaction was completed by TLC analysis, the reaction solution was centrifuged (10,000 x g, 10 min), and the supernatant was passed through fluorous silica gel in a two-stage extraction eluting first with H_2O and then with 100% MeOH to give Gb3-C$_6$-SF-tag **18** (19 mg, 83%). R_f=0.42 (EtOAc/MeOH/H_2O/acetic acid=6:2:1:0.5); ^{1}H NMR (600 MHz, CD_3OD) δ 8.43 (s, 1H), 8.18 (d, J=8.3 Hz, 1H), 7.82 (d, J=8.2 Hz, 1H), 6.13 (q, J=6.5 Hz, 1H), 4.95 (d, J=3.7 Hz, 1H), 4.72–4.70 (m, 1H), 4.43 (d, J=7.2 Hz, 1H), 4.29–4.25 (m, 2H), 3.99 (d, J=2.0 Hz, 1H), 3.92 (d, J=2.9 Hz, 1H), 3.88 (d, J=3.2 Hz, 2H), 3.86–3.82 (m, J=9.4, 6.6, 4.1 Hz, 3H), 3.81 (d, J=3.6 Hz, 1H), 3.79–3.77 (m, 1H), 3.74 (dd, J=11.2, 7.0 Hz, 1H), 3.69 (dd, J=11.0, 5.4 Hz, 2H), 3.64 (s, 1H), 3.61–3.37 (m, 10H), 3.33–3.26 (m, 1H), 3.25–3.15 (m, 2H), 3.06–2.96 (m, 2H), 2.71–2.49 (m, 4H), 1.85–1.77 (m, 2H), 1.61 (d, J=6.5 Hz, 3H), 1.47–1.24 (m, 8H). ^{13}C NMR (150 MHz, CD_3OD) δ 172.86, 172.70, 167.31, 157.78, 149.07, 142.98, 136.04, 133.08, 128.73, 124.39, 105.34, 104.20, 102.67, 80.99, 79.76, 76.51, 76.45, 76.40, 74.88, 74.65, 72.83, 72.65, 71.28, 71.06, 70.77, 70.56, 69.31, 62.69, 61.93, 61.46, 52.72, 52.46, 41.54, 38.11, 37.64, 30.70, 30.61, 29.76, 27.56, 27.44, 26.62, 22.21. HRMS (ESI) m/z calcd for $C_{51}H_{67}N_5O_{26}F_{17}S$ $[M-H]^-$: 1520.3524, found: 1520.3597.

Lacto-*N*-triose-C$_6$-SF-tag (19a). The reaction was carried out in a 15-mL centrifuge tube with 1.94 mL of Tris-HCl buffer (100 mM, pH 7.5) containing 20 mM ATP (21 mg, 39 μmol), 20 mM *N*-acetylglucosamine (9 mg, 39 μmol), 40 mM $MgCl_2$, and 0.5 mg/mL NahK at 37 °C for 2 h. Following the addition of 20 mM UTP and 1 mg/mL RmlA, the resulting mixture was incubated at 55 °C for 2 h. The formation of UDP-GlcNAc was detected and monitored by RP-HPLC coupled with a UV detector. Then, 10 mM lactose **2a** (26.4 mg, 19 μmol) and 0.5 mg/mL HpGnT were added. The resulting mixture was incubated at 25 °C for 48 h, and the reaction was monitored by TLC. When the reaction was completed by TLC, the solution was centrifuged (10,000×g, 10 min), and the supernatant was passed through fluorous silica gel in a three-stage extraction eluting first with H_2O, second with 70% aq MeOH, and then with 100% MeOH. Product-containing fractions were combined and lyophilized to give Lacto-*N*-triose-C$_6$-SF-tag **19a** (27 mg, 91%). R_f=0.4(EtOAc/MeOH/H_2O/acetic acid=6:2:1:0.5); ^{1}H NMR (600 MHz, CD_3OD) δ 8.44 (s, 1H), 8.18 (d, J=8.2 Hz, 1H), 7.82 (d, J=8.2 Hz, 1H), 6.13 (q, J=6.4 Hz, 1H), 4.75–4.71 (m, 1H), 4.65 (d, J=8.4 Hz, 1H), 4.39 (d, J=7.0 Hz, 1H), 4.27 (d, J=7.8 Hz, 1H), 4.05 (br, 1H), 3.91–3.81 (m, 4H), 3.78 (dd, J=11.5, 7.6 Hz, 1H), 3.72–3.67 (m, 3H), 3.65–3.41 (m, 10H), 3.41–3.26 (m, 4H), 3.26–3.14 (m, 3H), 3.05–2.96 (m, 2H), 2.71–2.47 (m, 4H), 2.01 (s, 3H), 1.84–1.76 (m, 2H), 1.68–1.50 (m, 5H), 1.49–1.22 (m, 6H). ^{13}C NMR (150 MHz, CD_3OD) δ 174.6, 172.9, 172.7, 167.3, 157.8, 149.0, 143.0, 136.1, 133.1, 128.7, 124.4, 105.0, 104.2 (×2), 83.4, 80.6, 77.9, 76.7, 76.4 (×2), 75.9, 74.8, 71.9, 71.7, 70.8, 70.0, 69.3, 62.5 (×2), 61.9, 57.6, 52.7, 52.5, 41.5, 38.1, 37.6, 30.7, 30.6, 29.8, 27.6, 27.4, 26.6, 23.1, 22.2. HRMS (ESI) m/z calcd for $C_{53}H_{70}N_6O_{26}F_{17}S$ $[M-H]^-$: 1561.3789, found: 1561.3777

Lacto-*N*-neotetraose-C$_6$-SF-tag (20a). The reaction was carried out in a 15-mL centrifuge tube with 1.74 mL of Tris-HCl buffer (50 mM, pH 7.5) containing 20 mM ATP (19 mg, 35 μmol), 20 mM galactose (6 mg, 35 μmol), 40 mM $MgCl_2$, and 5 μg/mL GalK at 55 °C for 2 h. Following the addition of

20 mM UTP (18 mg, 35 μmol) and 1 mg/mL RmlA, the resulting mixture was incubated at 55 °C for 2 h. The formation of UDP-GlcNAc was detected and monitored by RP-HPLC coupled to a UV detector. Finally, lacto-*N*-triose **19a** (27 mg, 17 μmol) and 120 μg/mL NmGalT were added. The resulting mixture was incubated at 25 °C for 2 h, and the reaction was monitored by TLC. When the reaction was completed by TLC, the solution was centrifuged (10,000 × g, 10 min), and the supernatant was passed through fluorous silica gel in a three-stage extraction eluting first with H_2O, second with 70% aq MeOH, and then with 100% MeOH. Product-containing fractions were combined and lyophilized to give lacto-*N*-neotetraose **20a** (25 mg, 85%). R_f = 0.2(EtOAc/MeOH/H_2O/acetic acid = 6/2/1/0.5); ^{1}H NMR (600 MHz, CD_3OD) δ 8.43 (s, 1H), 8.18 (d, *J* = 8.2 Hz, 1H), 7.82 (d, *J* = 8.2 Hz, 1H), 6.13 (q, *J* = 6.4 Hz, 1H), 4.72–4.70 (m, 1H), 4.67 (d, *J* = 8.4 Hz, 1H), 4.38 (d, *J* = 7.6 Hz, 2H), 4.27 (d, *J* = 7.9 Hz, 1H), 4.04 (br, 1H), 3.91–3.26 (m, 28H), 3.26–3.11 (m, 3H), 3.04–2.99 (m, 2H), 2.71–2.49 (m, 4H), 2.00 (s, 3H), 1.83–1.80 (m, 2H), 1.68–1.49 (m, 5H), 1.46–1.26 (m, 6H). ^{13}C NMR (150 MHz, CD_3OD) δ 174.4, 172.9, 172.7, 167.3, 157.8, 149.1, 143.0, 136.0, 133.1, 128.7, 124.4, 105.1, 105.0, 104.2 (×2), 83.4, 80.6, 80.5, 77.2, 76.7, 76.5, 76.4, 74.83, 74.75, 74.0, 72.6, 71.7, 70.8, 70.4, 70.0, 69.3, 62.6, 62.5, 61.9, 61.7, 56.9, 52.7, 52.5, 41.6, 38.1, 37.6, 30.7, 30.6, 29.8, 27.6, 27.4, 26.6, 23.1, 22.2. HRMS (ESI) m/z calcd for $C_{59}H_{80}N_6O_{31}F_{17}S$ $[M-H]^-$: 1723.4317, found: 1723.4312.

P1-C_6-SF-tag (21a). The reaction was carried out in a 15-mL centrifuge tube with 0.78 mL of Tris-HCl buffer (50 mM, pH 7.5) containing 20 mM ATP (9 mg, 16 μmol), 20 mM galactose (3 mg, 16 μmol), 40 mM $MgCl_2$, and MtGalK (5 μg/mL) at 55 °C for 2 h. Following the addition of 20 mM UTP (8 mg, 16 μmol) and RmlA (1 mg/mL), the reaction was incubated at 55 °C for 2 h. The formation of UDP-Gal was detected and monitored by RP-HPLC coupled to a UV detector. Finally, Lacto-*N*-neotetraose **20a** (13 mg, 8 μmol) and LgtC (300 μg/mL) were added. The resulting mixture was incubated at 37 °C for 2 h, and the reaction was monitored by TLC. The crude reaction product was loaded on an FSPE column and was passed through fluorous silica gel in a three-stage extraction eluting first with H_2O, second with 70% aq MeOH, and then with 100% MeOH. Product-containing fractions were combined and lyophilized to give **21a** (11 mg, 80%). R_f = 0.2 (EtOAc/MeOH/H_2O/acetic acid = 5:2:1:0.5); ^{1}H NMR (600 MHz, CD_3OD) δ 8.43 (s, 1H), 8.18 (d, *J* = 8.2 Hz, 1H), 7.82 (d, *J* = 8.2 Hz, 1H), 6.13 (q, *J* = 6.5 Hz, 1H), 4.72–4.70 (m, *J* = 8.0, 4.7, 1.4 Hz, 1H), 4.68 (d, *J* = 8.4 Hz, 1H), 4.43 (d, *J* = 7.1 Hz, 1H), 4.38 (d, *J* = 7.4 Hz, 1H), 4.30–4.22 (m, 2H), 4.04 (br, 1H), 3.98 (br, 1H), 3.94–3.25 (m, 33H), 3.24–3.11 (m, 3H), 3.06–2.94 (m, 2H), 2.71–2.45 (m, 4H), 2.00 (s, 3H), 1.85–1.74 (m, 2H), 1.69–1.48 (m, 5H), 1.47–1.23 (m, 6H). ^{13}C NMR (150 MHz, CD_3OD) δ 174.5, 172.9, 172.7, 167.3, 157.8, 149.1, 143.0, 136.0, 133.1, 128.7, 124.4, 105.4, 105.0, 104.2, 104.1, 102.6, 83.4, 80.9, 80.6, 79.7, 76.7, 76.6, 76.4, 74.8, 74.7, 74.0, 72.8, 72.7, 71.7, 71.3, 71.1, 70.8, 70.6, 70.0, 69.3, 62.7, 62.5, 61.9, 61.7, 61.5, 57.1, 52.7, 52.5, 41.5, 38.1, 37.6, 30.7, 30.6, 29.8, 27.6, 27.4, 26.6, 23.1, 22.2. HRMS (ESI) m/z calcd for $C_{65}H_{91}N_6O_{36}F_{17}S$ $[M-H]^-$: 1886.4924, found: 1886.4995.

Dimeric Lewis X-C6-SF-tag (24). The elongation of LacNAc on compound **1a** is similar as described in the synthesis of **19a** and **20a**. The yields of the assembly of galactose, *N*-acetylglucosamine and galactose are 99% (66 mg), 93% (150 mg), and 85% (38 mg), respectively, and gave intermediate **23**. The synthesis of compound **24** is similar as described in the synthesis of **16a**. The crude product was passed through fluorous silica gel in a two-stage extraction eluting with H_2O followed by 100% MeOH to give dimeric Lewis X-C_6-SF-tag **24** (22 mg, 91%). R_f = 0.2 (EtOAc/MeOH/H_2O/acetic acid = 4:2:1:0.5); ^{1}H NMR (600 MHz, CD_3OD) δ 8.43 (s, 1H), 8.18 (d, *J* = 4.0 Hz, 1H), 7.82 (d, *J* = 5.9 Hz, 1H), 6.13 (q, *J* = 6.5 Hz, 1H), 5.08 (d, *J* = 3.9 Hz, 1H), 5.02 (d, *J* = 3.4 Hz, 1H), 4.71(d, *J* = 7.9 Hz, 2H), 4.58 (s, 1H), 4.46–4.44 (m, 3H), 4.03–3.24 (m, 37H), 3.24–3.20 (m, 2H), 3.06–2.97 (m, 2H), 2.72–2.42 (m, 4H), 2.01–1.88 (m, 6H), 1.86–1.74 (m, 2H), 1.61 (d, *J* = 6.5 Hz, 3H), 1.52–1.21 (m, 8H), 1.17 (dd, *J* = 17.7, 6.6 Hz, 6H). ^{13}C NMR (150 MHz, CD_3OD) δ 174.60, 173.78, 172.84, 172.70, 167.27, 157.78, 149.08, 142.97, 136.05, 133.08, 128.72, 124.38, 103.88, 103.80, 103.73, 102.34, 100.44, 100.24, 83.97, 77.37, 77.23, 76.72(×2), 76.43, 76.17, 75.18, 74.90 (×2), 73.71, 73.65, 72.79, 71.98, 71.20 (×2), 70.54, 69.99 (×2), 69.46, 69.32, 67.67 (×2), 62.85, 62.72, 61.41, 61.24, 57.67, 52.72, 52.50, 41.54, 38.12, 37.65, 30.77, 30.46, 29.77, 27.56, 26.67, 23.19, 23.10, 22.19, 16.61. HRMS (ESI) m/z calcd for $C_{73}H_{102}N_7O_{39}F_{17}S$ $[M-2H]^{2-}$: 1027.7831, found: 1027.7827.

Gb3-C_6NH_2 (25).[47] To a solution of compound **18** (14 mg, 9.2 μmol) in MeOH (4.6 mL) was added acetic acid (20 μL, 0.3 mmol) at room temperature. The mixture was irradiated by UV lamp (15 W × 16, 365 nm, UV lamp power: 13.8 mW/cm^2) (Panchum, photochemical reactor PR-2000) at 37 °C for 40 min. The mixture was concentrated to dryness *in vacuo*. The crude reaction product was loaded on the fluorous solid-phase extraction cartridge (F-SPE). P^k-C_6-amine was eluted out in 10% MeOH. The product was lypholyzed to give Gb3-C_6NH_2 **25** (4.8 mg) in 86% photo-cleavage yield. R_f = 0.05 (EtOAc/MeOH/H_2O/Acetic acid = 6:2:1:0.5); HRMS (ESI) m/z calcd for $C_{24}H_{45}NNaO_{16}$ $[M+Na]^+$: 626.2636, found: 626.2636. The ^{1}H NMR spectrum of **25** is the same as reported spectrum.

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