On the Emerging Role of Chemistry in the Fashioning of Biologics:
Synthesis of a Bidomainal Fucosyl GM1-Based Vaccine for the Treatment of Small Cell Lung Cancer

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The synthesis of the novel small cell lung cancer (SCLC) fucosyl GM1-based vaccine construct, featuring insertion of the HLA-DR binding 15 amino acid sequence derived from Plasmodium falciparum, is described. The resultant glycopeptide has been synthesized in an efficient manner. Finally, successful conjugation of the glycopeptide to the keyhole limpet hemocyanin (KLH) carrier protein completed the preparation of the vaccine.

Introduction

Among the large number of emerging anticancer strategies, the prospect of mobilizing the immune system against the disease is especially attractive. One can imagine employing a vaccine-based therapeutic approach against a number of different primary tumors, as well as against metastatic cells, in an adjuvant mode.1 Along these lines, we are pursuing the idea of targeting as immune system markers complex carbohydrate epitopes, which are overexpressed on cancer cell surfaces. Though this concept has occurred to others, our group has made a particularly strong commitment to accessing these structures by total synthesis.2 A typical carbohydrate-based anticancer vaccine would consist of a complex carbohydrate hapten, overexpressed on the cancer cell, a carrier protein, and a linker attaching the carbohydrate to the protein (Figure 1). Beside being a potent immunogen, the carrier protein is known to provide the MHC-II binding peptides bound to the epitope, thus helping to present the carbohydrate to the T-cells for T-cell activation and initiation of the cellular response.3,4 Consequently, one could imagine that the immunogenicity of a vaccine might well be enhanced by providing MHC-II binding peptides in the environs of the epitope, thereby serving to increase the number of epitopes presented to the CD4+ T cell. In a sense, this rationale is related to the idea of conjugating epitopes to carrier protein to create vaccines. However, this approach of placing an MHC-II binding sequence in a fixed relation to the antigen has been pursued mostly for vaccines unconjugated to carrier protein.5 We hope to explore the possibility that introduction of an

MHC-II binding sequence could also improve the immunogenicity of vaccines incorporating standard carriers such as keyhole limpet hemocyanin (KLH).

To test the notion of upgrading the immunogenicity of a candidate carbohydrate-based vaccine in this way, we pursued the synthesis of the construct illustrated in Figure 1. Fucosyl GM1 is a carbohydrate epitope that is expressed on the surface of small-cell lung cancer (SCLC) cells. A 15 amino acid peptide sequence derived from \textit{Plasmodium falciparum} and illustrated in Figure 1 was chosen as the T-cell epitope. This sequence has been shown to be general for binding up to nine different genetic variants of human HLA-DR with binding capacity prevalently in the nanomolar range.

The appendage of the fucosyl GM1 epitope to the peptide portion could be accomplished using the norleucine linker developed by our group. The long aliphatic chain of this linker would be optimal in preventing potentially adverse interactions between the epitope and the peptide backbone. The amino acid functionality makes this linker a powerful handle for conjugation.

The synthesis of glycoprotein conjugates still presents a challenge for numerous reasons. One of the issues is compatibility and “collegiality” of the protecting groups required for peptidic and carbohydrate assemblies. Thus, a complex glycan is often unstable under the highly acidic conditions that are required to remove peptide protecting groups. Correspondingly, peptides may be unstable under the basic conditions that are required for the deprotection and retrieval of the oligosaccharide ensembles. However, since the protecting groups of the selected peptide would be limited to tert-butylcarboxy for lysine and tert-butyl for tyrosines and threonine, there is, hopefully, no requirement for a comprehensive review on the subject, refer to: Herzner, H.; Reipen, T.; Schulz, M.; Kunz, H. \textit{Chem. Rev.} \textbf{2000}, \textit{100}, 4495–4537.

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**FIGURE 1.** Next-generation bidomainal fucosyl GM1-based vaccine for the treatment of SCLC.

**SCHEME 1**

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"Key: (a) TBAF, AcOH, THF; (b) NaOMe, MeOH; (c) NaOH, THF; (d) Na, NH₃, THF; (e) Ac₂O, DMAP, Py; (f) DMAP, MeOH; (g) Ac₂O, DMAP, Py; 56% (7 steps); (h) A, B, CH₂Cl₂, rt; (i) H₂, Pt/C, MeOH–H₂O; 49%, two steps.
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for prolonged treatment with acid in the deprotection phase. Thus, the glycoside linkages of peracetylated fucose and sialic acid moieties could be stable under the deprotection conditions. Of course, the cleavage of a methyl ester and 17 acetate groups could prove to be a challenging task that might well require careful selection of hydrolysis conditions.

Results and Discussion

The construction of the vaccine began from the known Fuc-GM1 hexasaccharide, obtained through a previously disclosed sequence. After deprotection of the trisopropylsilyl groups with TBAF/acetic acid and subsequent cleavage of acetates, carbonate, and sialic acid methyl ester, the resultant product was debenzylated under Birch conditions. The obtained acid was exhaustively peracetylated to provide a corresponding lactone that was subsequently opened with methanol and DMAP. Acylation afforded (56%, seven steps). Glycoside was treated with Fmoc-L-allylglycine benzyl ester and Hoveyda-Grubbs catalyst under the previously developed conditions, and the resultant olefin cross-metathesis product was subjected to catalytic hydrogenation. The side-chain olefinic linkage was reduced with concomitant selective removal of

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the benzyl protecting group in the presence of Fmoc-protected amine to afford the cassette 5 (49%, 2 steps) ready for coupling (Scheme 1).

The synthesis of peptide 7 was accomplished by standard Fmoc solid-phase peptide synthesis (SPPS), starting from the protected tyrosine, 6, preloaded on NovaSyn TGT resin. Peptide 7 was obtained in 95% yield after cleavage from the resin, in more than 95% purity, as judged by LC/MS and 1H NMR analysis. The elaboration of 7 to peptide 8 was executed by first conjugating 7 to the linker C14 using the standard EDCI/HOBt protocol.10c The Fmoc protecting group was next removed by treatment with piperidine, thereby providing glycopeptide 8 in 71% yield (two steps). The attachment of 8 to carbohydrate epitope 5 proceeded in 81% yield, thereby providing glycopeptide 9 (see Scheme 2).

Compound 9 was treated sequentially with piperidine/DMF and Ac2O/Py. The allylcarboxy protecting group was exchanged to the acetate-protected 2-sulfhydrylacetate linker by reduction with Pd(PPh3)4/PhSiH3 followed by acylation with SAMAPbf.10c Finally, three tert-butylcarboxy and one tert-butylcarboxy groups were removed by treating 10 with TFA/PhOH/H2O/TIPS providing the corresponding deprotected product in 71% yield (five steps) from 9 following purification by HPLC. This compound was treated with a degassed solution of NaOH in MeOH/H2O (pH = 10.5), providing the desired deprotected product 11 in 19% yield following HPLC purification.15 Minor amounts of a dehydration side product (ca. 5% yield after HPLC) were also isolated.

Next, the conjugation of construct 11 to maleimide-activated KLH 12 was examined (Scheme 3). Thus, 11 was pretreated with TCEP gel for 2 h and then treated with freshly prepared 12 at pH = 7.2. The efficiency of the coupling was estimated by a combination of Bradford protein assay16 and neuraminic acid determination according to Svennerholm17 to be 210 epitopes per molecule of KLH (MW = 8 MDa).

Conclusion

In summary, a new kind of fucosyl GM1 epitope-based vaccine has been efficiently prepared through the coupling of the fucosyl GM1 cassette with a promiscuous HLA-DR binding peptide. The resultant construct was further functionalized and deprotected to provide the glycopeptide, which was next conjugated to the carrier protein (KLH). The results of immunological evaluations of the vaccine will be forthcoming. This synthetic accomplishment is in keeping with an important theme in our laboratory, to the effect that there have emerged exciting opportunities for chemistry in the design of carbohydrate structure types previously perceived as strictly “biologies”. It goes without saying that chemistry provides vast opportunities (and challenges) in de novo design, thus offering far greater flexibility than is available though strictly biology-driven routes to biologics.

Experimental Section

Synthesis of Acetylated Glycoside 4 (Steps a–c, Scheme 1). To a solution of the hexaasaccharide 2 (142 mg, 0.056 mmol) in THF (6.0 mL) were added glacial Ac2O (0.12 mL) and TBAF (1.0 M in THF, 1.3 mL). The reaction mixture was stirred at rt for 2 days, poured into ice—water (25 mL), and extracted with EtOAc. The organic extracts were dried over MgSO4, and concentrated. The resulting triol was dissolved in anhydrous MeOH (6 mL), and sodium methoxide was added (25% solution in MeOH, 0.6 mL). The contents were stirred at rt for 3 days, and then water (6.0 mL) and THF (6.0 mL) were added. Stirring at rt for an additional 2 days was followed by neutralization with Dowex-H+ filtration with MeOH washing, and concentration. The crude material was allowed to dry under high vacuum for 1 day.

Synthesis of Acetylated Glycoside 4 (Steps d–g, Scheme 1). To a blue solution of sodium (160 mg) in liquid NH3 (50 mL) was added a solution of the white solid from above in THF (5.0 mL), and the resulting mixture was stirred at −78 °C for 2 h. The reaction was quenched by the addition of anhydrous MeOH (20 mL), warmed to rt, and concentrated with a stream of dry argon. The residue was diluted with MeOH (70 mL) and treated with Dowex 50WX8-400 until pH was nearly 5–6. The mixture was filtered and concentrated to provide a solid. This solid was dissolved in a mixture of pyridine (12.0 mL) and Ac2O (6.0 mL) at rt. To the solution of tetrasaccharide was added DMAP (10 mg), and the mixture was stirred for an additional 2 days. The reaction mixture was cooled to 0 °C and treated with MeOH (24 mL). To this solution was added DMAP (15 mg), and the resultant mixture was stirred at rt for an additional 4 days. The reaction mixture was then concentrated and coevaporated with toluene (4 × 100 mL). The residue was dissolved in pyridine (5.0 mL) and Ac2O (10.0 mL) at rt. The mixture was stirred for 1 day and then concentrated. The resultant oil was dissolved in MeOAc (10.0 mL) and MeOH (0.2 mL). To the solution was added cesium carbonate (33 mg), and the mixture was stirred for 1 h and then diluted with methyl acetate (250 mL). The organic phase was washed with brine/NH4Cl (1:1, 100 mL), NaHCO3 (100 mL), and brine (100 mL) and dried over MgSO4. Concentration followed by flash chromatography (silica, 5% methanol/dichloromethane) provided the acetylated glycoside 4 (142 mg, 56% from 2): [α]D = −40.4 (c 1.00, CHCl3); IR (film CHCl3) 2969, 1746, 1689, 1530, 1371, 1231, 1131, 1058 em−1; 1H NMR (CDCl3, 600 MHz) δ 7.22 (d, J = 5.9 Hz, 1H), 5.76–5.69 (m, 1H), 5.61–5.58 (m, 1H), 5.43 (d, J = 3.5 Hz, 1H), 5.34–5.30 (m, 4H), 5.21–5.10 (m, 4H), 5.02–4.97 (m, 4H), 4.82 (t, J = 8.8 Hz, 1H), 4.74 (t, J = 11.4, 3.6 Hz, 1H), 4.65 (d, J = 7.7 Hz, 1H), 4.52 (d, J = 7.8 Hz, 1H), 4.49–4.45 (m, 1H), 4.39–4.33 (m, 3H), 4.21–4.17 (m, 2H), 4.14–4.02 (m, 6H), 4.00–3.89 (m, 3H), 3.85–3.70 (m, 9H), 3.58–3.16 (m, 2H), 3.45–3.40 (m, 2H), 3.01 (dt, J = 12.7, 5.6 Hz, 1H), 2.81 (dd, J = 12.9, 4.1 Hz, 1H), 2.19 (s, 3H), 2.12 (s, 3H), 2.10 (s, 3H), 2.09 (s, 3H), 2.03–1.97 (m, 33H), 1.95 (s, 3H), 1.94 (s, 3H), 1.93 (s, 3H), 1.80 (s, 3H), 1.70 (t, J = 12.8 Hz, 1H), 1.64–1.57 (m, 2H), 1.22–1.20 (m, 2H), 1.11 (d, J = 6.3 Hz, 3H), 11C NMR (CDCl3, 150 MHz) δ 173.6, 171.0, 170.9, 170.4, 170.4, 170.3, 170.3, 170.3, 170.2, 170.2, 170.1, 169.7, 169.6, 169.5, 169.4, 169.2, 168.2, 137.7, 114.9, 102.0, 100.4, 98.7, 97.3, 94.4, 75.6, 73.5, 73.4, 73.3, 72.8, 72.5, 72.0, 71.8, 71.7, 71.3, 70.9, 70.3, 70.2, 69.8, 69.4, 69.2, 69.1, 68.7, 68.1, 67.7, 67.4, 67.2, 67.0, 65.0, 63.3, 62.4, 62.4, 62.4, 60.6, 60.3, 55.5, 53.7, 52.5, 49.3, 37.1, 31.6, 29.7, 29.2, 28.5, 23.5, 23.0, 21.3, 20.9, 20.8, 20.7, 20.7, 20.7, 20.6, 20.6, 20.5, 20.4, 20.4, 15.9, 14.1; ESI/MS exact mass calced for C43H42N2O10 [M + Na]+ 1975.6, found 1963.9, 1977.0.

Synthesis of Amino Acid 5 (Step h, Scheme 1). The first-generation Hoveyda–Grubbs catalyst (B, 9.6 mg, 0.016 mmol) was added to a solution of acetylated glycoside 4 (124 mg, 5160 J. Org. Chem. Vol. 74, No. 15, 2009
0.064 mmol) and allyl glycine A (273 mg, 0.640 mmol) in CH₂Cl₂ (1 mL) at rt. The reaction mixture was stirred for 12 h and exposed to air for 3 h. The mixture was concentrated, and the resultant residue was purified by flash chromatography (100% ethyl acetate) to provide the coupled product.

**Synthesis of Amino Acid 5 (Step i, Scheme 1).** At/C (10% w/w, 15 mg) was added to a solution of the metathesis adduct from above in MeOH (3 mL) and H₂O (0.2 mL), and the hydrogen atmosphere was established. The reaction mixture was stirred for 4 days at rt, filtered through a short pad of silica gel, and concentrated. The residue was purified by flash chromatography (10% MeOH in CH₂Cl₂) to give the amino acid 5 (70 mg, 49% over two steps); [α]D²⁰ = −53.2 (c 1.00, CHCl₃); IR (film CHCl₃) 3470, 2928, 2854, 1746, 1429, 1370, 1232, 1057 cm⁻¹; ¹H NMR (CDCl₃, 600 MHz) 8.16 (d, 1H, J = 6.4 Hz, 7.79) (2H, J = 7.4 Hz), 7.67–7.65 (m, 2H), 7.39–7.37 (m, 2H), 7.30 (br.s, 2H), 5.62–5.60 (m, 1H), 5.48–5.47 (m, 2H), 3.59 (d, 1H, J = 10 Hz), 5.25–5.23 (m, 3H), 5.14–5.05 (m, 3H), 4.97–4.88 (m, 6H), 4.68 (d, 1H, J = 7.5 Hz), 4.64 (d, 1H, J = 7.8 Hz), 4.57 (s, 3H), 4.54–4.53 (m, 2H), 4.47 (d, 1H, J = 11.2 Hz), 4.40–4.32 (m, 1H), 4.29–4.10 (m, 3H), 4.07–3.76 (m, 15H), 3.72–3.65 (m, 3H), 3.57 (s, 1H), 3.44 (m, 1H), 3.25–3.17 (m, 1H), 2.86 (dd, 1H, J = 3.9 and 12.5 Hz), 2.27 (s, 3H), 2.16 (s, 3H), 2.14 (s, 3H), 2.12 (s, 6H), 2.11 (s, 3H), 2.08 (s, 3H), 2.06 (s, 6H), 2.03 (s, 6H), 2.02 (s, 3H), 1.98 (s, 6H), 1.82 (s, 1H), 1.61 (t, 2H, J = 12.5 Hz), 1.52 (br.s, 2H), 1.37–1.25 (m, 7H), 1.18 (d, 3H, J = 6.2 Hz); ¹³C NMR (CDCl₃, 150 MHz) δ 180.2, 175.4, 173.6, 172.4, 172.4, 172.3, 172.2, 172.1, 171.8, 171.7, 171.7, 171.5, 171.4, 171.1, 169.7, 158.2, 145.5, 145.4, 142.6, 128.8, 128.2, 126.3, 121.0, 102.6, 102.3, 101.7, 101.1, 98.7, 97.3, 77.6, 75.2, 75.0, 74.8, 74.4, 74.3, 74.2, 73.8, 73.3, 73.2, 73.0, 72.7, 72.4, 72.2, 71.9, 71.3, 71.0, 70.6, 69.2, 69.2, 68.8, 64.7, 67.7, 66.2, 65.0, 65.8, 63.6, 63.5, 62.7, 57.6, 55.8, 53.8, 50.0, 48.6, 46.8, 38.7, 34.1, 30.6, 30.2, 27.0, 26.8, 24.2, 22.8, 21.7, 21.7, 21.0, 21.0, 20.9, 20.9, 20.8, 20.8, 20.7, 20.6, 20.6, 16.3; ES/MS exact mass calced for C₁₁₀H₁₅₁N₃O₅₄ [M + Na]+ 2275.8, [M + 2Na]⁺ 2275.4, 1149.4, found 2275.5, 1149.3.

**Synthesis of Peptide 7 (Step a, Scheme 2).** Novasyn TGT resin (purchased from NovaBiochem) was chlorinated, esterified with Fmoc-Tyr(Bu)-OH, and treated with a 4-fold excess of HATU and Fmoc amino acids in 1 M DIEA/DMF, and for deblocking, a solution of 2% piperidine/CH₂Cl₂ was used. The amino acids used were, in order of addition: Fmoc-Ala-OH, Fmoc-Leu-OH, Fmoc-Gly-OH, Fmoc-Val-OH, Fmoc-Phe-OH, Fmoc-Lys(Boc)-OH, Fmoc-Tyr(tBu)-OH, Fmoc-Ala-OH, Fmoc-Gly-OH, Fmoc-Pro-OH, Fmoc-Val-OH, Fmoc-Phe-OH, Fmoc-Lys(Boc)-OH, and the appropriate fractions were concentrated (Rf > 0.5: 10% MeOH/CH₂Cl₂) to afford 18 mg of product 7 in 81% yield. This material was found to be >95% pure as judged by reversed-phase LC/ESI MS (C4 column): MS exact mass calced for C₁₁₀H₁₅₁N₃O₅₄ [M + H]+ 2185.2, [M + Na]+ 2207.2, [M + 2H]²⁺ 982.1, found 2184.8, 2206.8, 982.1.

**Synthesis of Compound 8 (Step c, Scheme 2).** The product from above (26.8 mg, 0.0123 mmol) was dissolved in 1.0 mL of DMF, and to this solution was added piperidine (0.25 mL). After 1 h, LC/MS analysis indicated the completion of the reaction. The mixture was concentrated under reduced pressure and purified via flash chromatography (silica, 10% MeOH/CH₂Cl₂) to afford 18 mg of product 8 in 75% yield. This material was found to be >95% pure as judged by reversed-phase LC/ESI analysis: Rf = 0.5 (C4 column, 40–85% MeCN in H₂O, 30 min); exact mass calced for C₁₁₀H₁₅₁N₃O₅₄ [M + H]+ 1984.9, [M + Na]+ 2006.8, [M + 2H]²⁺ 982.1, found 1962.9, 1984.9, 982.1.
11.2, 6.0 Hz, 2H), 4.05 (dt, J = 9.1, 4.0 Hz, 2H), 3.95 (m, 2H),
3.94–3.81 (m, 8H), 3.83 (s, 3H), 3.80 (m, 3H), 3.75 (m, 1H),
3.71 (m, 1H), 3.68 (m, 1H), 3.64 (m, 3H), 3.58 (m, 1H), 3.50
(s, 1H), 3.42 (m, 1H), 3.15 (dd, J = 13.3, 6.2 Hz, 2H), 3.12 (m, 1H),
3.11–3.03 (m, 4H), 2.97 (t, J = 6.2 Hz, 2H), 2.93–2.83 (m, 4H),
2.80 (dd, J = 12.7, 4.4 Hz, 1H), 2.21 (s, 3H), 2.20–2.13 (m, 2H),
2.11 (s, 3H), 2.09 (s, 3H), 2.06 (s, 9H), 2.01 (s, 3H), 2.00 (s, 3H),
1.99 (s, 3H), 1.97 (s, 6H), 1.96 (s, 6H), 1.94 (s, 3H), 1.92 (s, 3H),
1.91 (s, 1H), 1.89 (s, 1H), 1.89 (s, 1H), 2.13–1.77 (m, 13H), 1.75
(s, 3H), 1.58–1.48 (m, 12H), 1.36 (s, 3H), 1.34–1.24 (m, 8H),
1.23 (s, 18H), 1.21 (s, 1H), 1.15 (s, 6H), 1.15–1.09 (m, 6H),
1.05 (d, J = 6.2 Hz, 3H), 0.91 (d, J = 6.7 Hz, 3H), 0.89 (d, J = 8.6
Hz, 6H), 0.87 (d, J = 7.0 Hz, 6H), 0.85 (d, J = 6.6 Hz, 3H), 0.75
(m, 3H); Rf  = 22.5 (C4 column, 50–95% MeCN in H2O, 30 min);
exact mass calculated for C194H279N21O74S [M + 2Na]+ 2026.4,

Synthesis of Compound 10 (Step a, Scheme 3).
Compound 9 (10.5 mg, 0.00250 mmol) was dissolved in 1.0 mL of DMF,
and piperidine (0.25 mL) was added. After 1 h, LC/MS analysis
indicated the completion of the reaction: Rf = 15.3 (C4 column,
50–95% MeCN in H2O, 30 min). The reaction mixture was stirred for
40 h before being neutralized with MAC-3 Dowex resin to pH = 5,
filtered, and purified by HPLC. Rf = 14.5 (C18 column, 10–85% MeCN in H2O, 30 min)
to afford 11 (1.0 mg, 19% yield from 10a, 13% from 9). The product was found to be >95% pure as judged by LC/MS and
1H NMR: 1H NMR (500 MHz, D2O) δ 7.46 (d, J = 9.7 Hz, 1H),
7.38 (d, J = 7.4 Hz, 1H), 7.37 (d, J = 7.6 Hz, 2H), 7.32 (t, J =
7.4 Hz, 2H), 7.26 (d, J = 7.0 Hz, 2H), 7.07 (d, J = 8.5 Hz, 2H),
6.87 (d, J = 8.6 Hz, 2H), 6.81 (d, J = 7.4 Hz, 2H), 5.28 (s, 1H),
5.26 (d, J = 3.9 Hz, 1H), 4.70 (dd, J = 5.2, 1.5 Hz, 2H), 4.66 (d, J =
6.9 Hz, 2H), 4.62 (d, J = 8.0 Hz, 1H), 4.60 (d, J = 5.5 Hz, 2H),
4.47 (t, J = 7.5 Hz, 2H), 4.16 (t, J = 6.1 Hz, 2H), 4.10 (at, J = 5.5
Hz, 4H), 4.07 (d, J = 8.5 Hz, 2H), 3.48 (m, 1H), 3.42 (d, J =
10.0, 2.0 Hz, 1H), 3.28 (t, J = 8.9 Hz, 1H), 3.20 (d, J = 13.1, 6.4
Hz, 1H), 3.12 (dd, J = 13.5, 6.8 Hz, 1H), 2.58 (dd, J = 12.1, 4.4
Hz, 1H), 2.31 (m, 1H), 2.24 (s, 3H), 2.05 (s, 3H), 2.04 (s, 3H),
2.01 (s, 3H), 1.87 (t, J = 13.2 Hz, 1H), 1.81 (dd, J = 6.5 Hz, 1H),
1.42 (d, J = 8.5 Hz, 3H), 1.26 (d, J = 6.1 Hz, 3H), 1.24 (d, J =
6.6 Hz, 3H), 1.01 (d, J = 6.8 Hz, 3H), 0.99 (d, J = 6.8 Hz, 3H),
0.94 (d, J = 6.3 Hz, 3H), 0.91 (d, J = 6.7 Hz, 3H), 0.89 (d, J =
6.3 Hz, 3H), 0.87 (d, J = 6.7 Hz, 3H); exact mass calculated for sodium
salt C134H108N21Na2O3S[M + 2H]2+ 1516.7 [M + 3H]3+ 1011.5,
[M + CF3CO2−]− 1572.2, found 1517.7, 1012.3, 1572.9.
Preparation of Conjugate 1 (Step g, Scheme 3). A solution of
sulfamyl SMCC (10 mg/mL, 0.10 mL) in 0.1 M sodium
sulfate, 0.09 M NaCl (pH = 7.2), was added to the reconstituted with
water solution of KLH (Aldrich, H7017, 10 mg/mL, 1.0 mL).
The resultant solution was stirred for 1 h and then
purified over a G-25 Sephadex column using 0.1 M sodium
sulfate, 0.09 M NaCl, 0.1 M EDTA, pH = 7.2 for elution.
The fractions containing KLH were collected and combined,
giving a total volume of 3.0 mL. Compound 11 (2 mg, 0.665 μmol)
in 0.2 mL of the pH = 7.2 buffer was treated with TCEP gel for 2 h,
filtered, combined with the solution of KLH (0.6 mL), and
reacted under argon for 2 h. The resultant solution was purified by repetitive centrifugation over a molecular filter (30 kDa cut
off) resulting in ca. 1 mL of the final solution of the vaccine
conjugate. The degree of the epitope incorporation was estimated
to be 210 epitopes per molecule of KLH using Bradford protein
assay with KLH as a standard and Svennerholm sialic acid assay
to determine the carbohydrate concentration.16,17

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Supporting Information Available: 1H and 13C NMR
spectra for compounds 4 and 5, LC/MS data for compounds
7, 8a, 8b, 9, 10b, 10a, 11a, and 11, and selected intermediates,
and 1H NMR spectra for compounds 7, 8a, 9, 10b, 11a, and 11.
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