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Total Synthesis of the 2,6-Sialylated Immunoglobulin G Glycopeptide Fragment in Homogeneous Form

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Carbohydrate domains mediate the stability, folding, and biological activity of glycoproteins.[1] Quality investigations into the impact of specific glycosylation patterns are complicated by formation of horrific mixtures of difficult-to-separate glycoforms. A major program underway in our laboratory seeks to address the challenge of obtaining single glycoforms of biologics through the de novo chemical synthesis of homogeneous glycopeptides and glycoproteins of established therapeutic value.[2]

Accordingly, we became interested in monomeric immunoglobulin G (IgG) purified from intravenous immunoglobulin (IVIG). This therapeutically valuable anti-inflammatory agent is commonly employed in the treatment of autoimmune disorders, such as rheumatoid arthritis and immune thrombocytopenia. The biological activity of IVIG derives from its Fc fragment, which presents a biantennary tridecasaccharide attached through an N-linkage to the Asn^{297} residue. Recently, Ravetch and coworkers discovered the critical role of the terminal 2,6-linked sialic acids of the glycan in mediating the anti-inflammatory activity of IVIG.[3] Although the IVIG glycan is biosynthesized as a heterogeneous mixture containing both 2,3- and 2,6-sialylated carbohydrate domains, only the Fc fragment possessing the 2,6-sialylated glycoform demonstrates appreciable anti-inflammatory activity in arthritic mice. Moreover, an IVIG Fc fragment possessing exclusively 2,6-sialic acid linkages was shown to be 10-fold more active in suppressing inflammation in mice than was an Fc fragment isolated from IVIG (containing both 2,6- and 2,3-linkages). These results suggest that an Fc peptide fragment presenting a homogeneous 2,6-sialylated glycan domain (e.g., **1**) could be more potent and effective than the heterogeneous mixture currently employed in clinical settings.

We describe herein the chemical synthesis of the homogeneous tridecasaccharide **1** possessing terminal 2,6-sialic acid linkages. We further describe the aspartylation of **1** with a peptide domain, thereby demonstrating the ability to convert it into a glycopolypeptide (see **28**). Accordingly, glycopeptidic constructs corresponding to the carbohydrate domain of IgG can now be synthesized and screened.

The power of prioritized strategic bond disconnection as a means of guiding synthetic analysis, formalized by the Corey school,[4] is well-appreciated by students of chemical synthesis of complex target systems. Less well appreciated is a type of pattern recognition analysis that can be very helpful in devising total synthesis programs directed toward biologic-level oligosaccharides.[5] System **1** constitutes a significant challenge to chemical synthesis. In particular, the 13-mer contains an often troublesome L-α-fucosyl ring (ring 5) branching from the "reducing-end" GlcNAc of the terminal chitobiose (rings 1 and 2). It also presents the complex β-mannose linkage joining a β-mannose (ring 3) to the chitobiose core system. The β-mannoside (ring 3) in turn is linked in a biantennary fashion from its C_3 and C_6 hydroxyls to two α-linked mannosides (see rings 6 and 7). The C_2 hydroxyls of these mannosides are linked in a β-fashion to two lactosamines, joined in 2,6-linkages to sialic acids (see rings 8−13). The β-linked mannose (ring 3) is also joined at its equatorial C_4 oxygen in a β-linkage to a GlcNAc residue (see ring 4).

On the basis of earlier literature in this general area,[6] it seemed likely that the most demanding phase of the synthesis would involve the attachment of the two trisaccharide ensembles, rings 8−10 and rings 11−13, to their respective axial hydroxyl acceptor sites in ring 6 and ring 7. Looming particularly difficult was the prospect of introducing additional appendages at the interior C_2 axial hydroxyl of ring 7. Indeed, it was our hope for conciseness to conduct simultaneous azaglycosylations at both of these two acceptor sites to introduce β-glucosamine donor residues (see rings 8 and 11). These rings would in turn be joined at their C_4 equatorial hydroxyl groups via β-linkages to C_6-sialylated galactosyl donors. Ring 7 emanates from the particularly hindered C_3 hydroxyl site of ring 3 nestled between the glucosamine moiety at C_4, the axial hydroxyl at C_2, and the complex core substructure (rings 1 and 2). In fact, previous attempts to use the more exposed C_6 hydroxyl of ring 3 as a viable acceptor site met with only the slightest of success. No glycosylation at all had been achieved at the much more hindered secondary hydroxyl acceptor site at C_3 of ring 3. Indeed, the key breakthrough involved the use of a phenylsulfamido function on the future ring 4 (see the * in compound **4**) to allow for introduction of rings 5, 6, and 7 (see below).

In Scheme 1, by focusing on key building blocks, we recapitulate the strategic analysis that eventually achieved success. Thus, as shown, we envisioned reaching tetrasaccharide **4** through coupling of trisaccharide **5** with sulfonamide donor **6**.[7] As noted, the presence of the sulfonamide functionality on diol **4** would prove to be key to the efficient installation of the "wing" mannose and fucose units, furnishing core heptasaccharide **3**.[8] Elongation of the mannose units with a β-GlcNAc donor would yield the nonasaccharide, **2**, which would finally be subjected to diglycosylation with disaccharide **10**, affording the target compound, **1**. Happily, in this synthetic analysis, we were able to revisit the chemistry developed in connection with our longstanding glycal assembly program.[9]

Our synthesis of key intermediate **2** commenced with the known monosaccharides **11**[10] and **12** (Scheme 2). Glycosylation of **12** with donor **11** through exposure to NIS/TMSOTf[11] under sonication conditions proceeded rapidly to afford the disaccharide in 82% yield.[12] Subsequent saponification furnished acceptor **13**. The latter was then glycosylated with donor **14**,[13] which gave trisaccharide **15** with good selectivity (β/α = 7:1). The β-isomer was isolated

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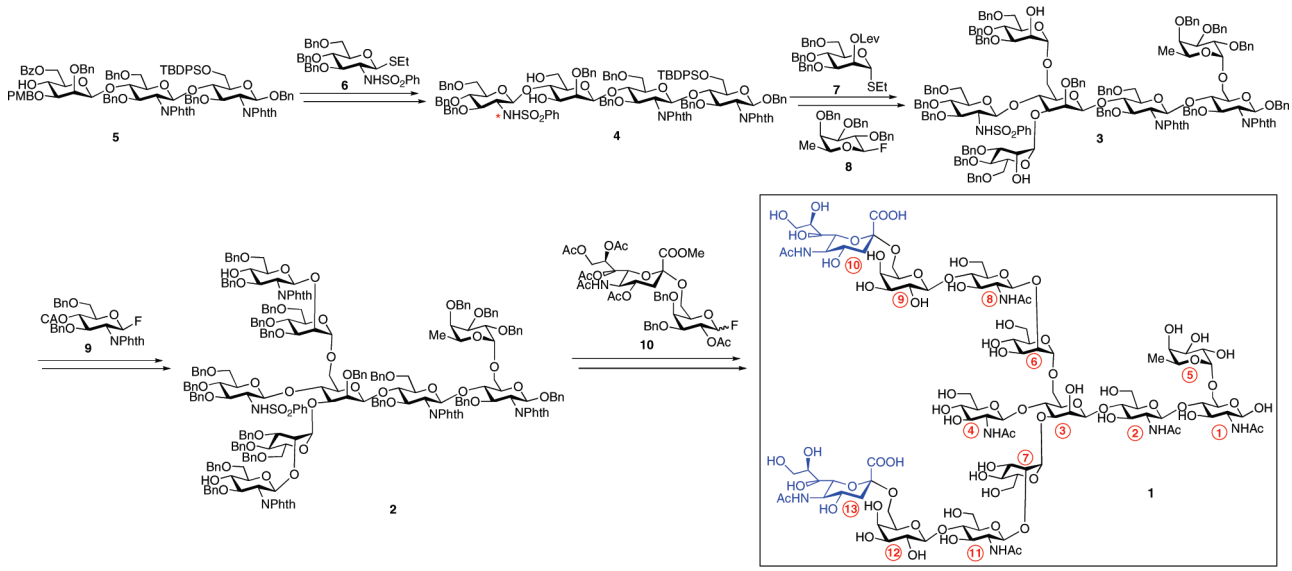
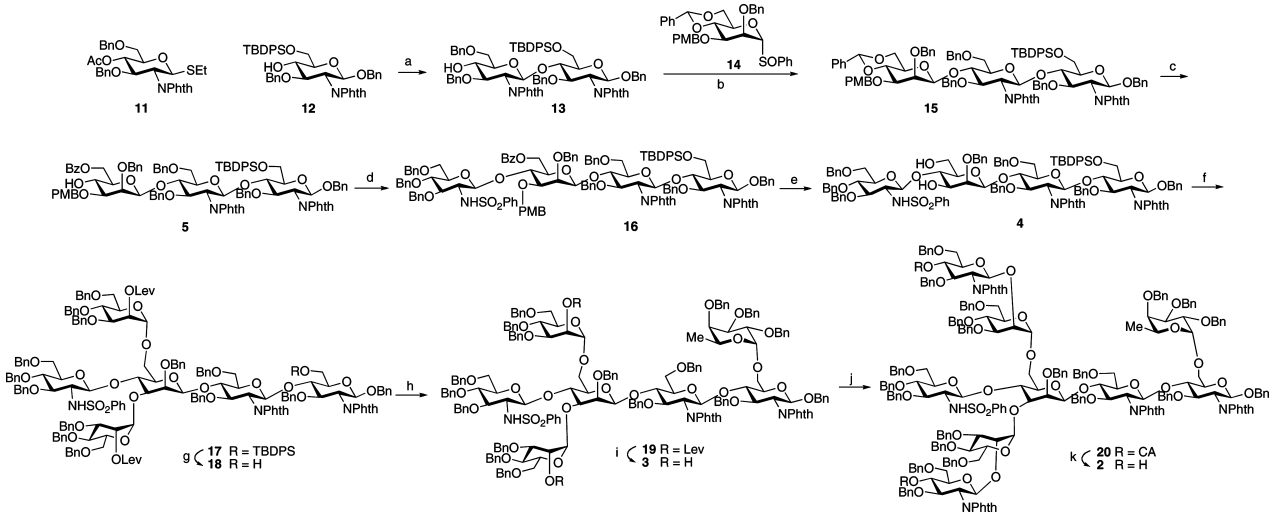
Scheme 1. Synthetic Strategy Toward Tridecasaccharide **1**



Scheme 2. Synthesis of Intermediate **2**[a]

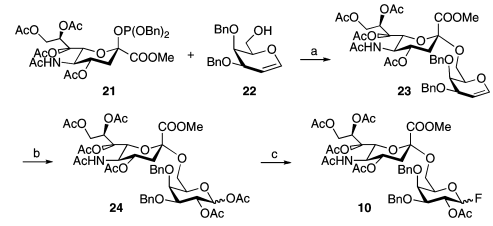


[a] Key: (a) 1. NIS/TMSOTf, CH_2Cl_2, sonication, 82%; 2. NaOMe/MeOH, 92%. (b) **14**, Tf_2O, DTBP, CH_2Cl_2, 65%. (c) 1. $Zn(OTf)_2$/EtSH, CH_2Cl_2, 75%; 2. BzCl, Py, CH_2Cl_2, 93%. (d) **6**, MeOTf, DTBP, CH_2Cl_2, 92% (β/α = 2.7:1). (e) 1. NaOMe/MeOH, 83%; 2. TMSOTf, CH_2Cl_2, 93% (CAN, 66%). (f) **7**, NIS/TMSOTf, CH_2Cl_2, 90%. (g) HF/Py, THF, sonication, 88%. (h) **8**, $Cp_2Zr(OTf)_2$, DTBP, toluene/THF, 93% (α/β = 12:1). (i) $N_2H_4 \cdot H_2O$, Py/AcOH (1:1), CH_2Cl_2, 93%. (j) **9**, toluene, $BF_3 \cdot OEt_2$. (k) K_2CO_3, MeOH, 52%.

in 65% yield. Following removal of the benzylidene acetal[14] and selective benzoylation of the resulting 4,6-diol, compound **5** was in hand. MeOTf-promoted[15] glycosylation with glucosamine building block **6** afforded a 92% yield of **16** (β/α = 2.7:1).

Sequential removal of the benzoate and PMB protecting groups of tetrasaccharide **16** afforded 3,6-diol **4** in 77% yield over two steps. Twofold glycosylation of **4** was accomplished with an excess of donor **7** and NIS/TMSOTf, providing hexasaccharide **17** in 90% yield. The TBDPS group was cleaved upon exposure to HF/Py (88% yield). To the resultant free hydroxyl group was appended fluoride donor **8**[16] under $Cp_2Zr(OTf)_2$ mediation[17] to afford compound **19** in 93% yield and excellent selectivity (α/β = 12:1). Following removal of the levulinoyl protecting group, intermediate **3** was in hand. $BF_3 \cdot OEt_2$-promoted diglycosylation of **3** was achieved through the use of excess donor **9**.[18] Finally, removal of the chloroacetate groups on the glucosamine units provided the target nonasaccharide **2** in 52% yield over two steps.

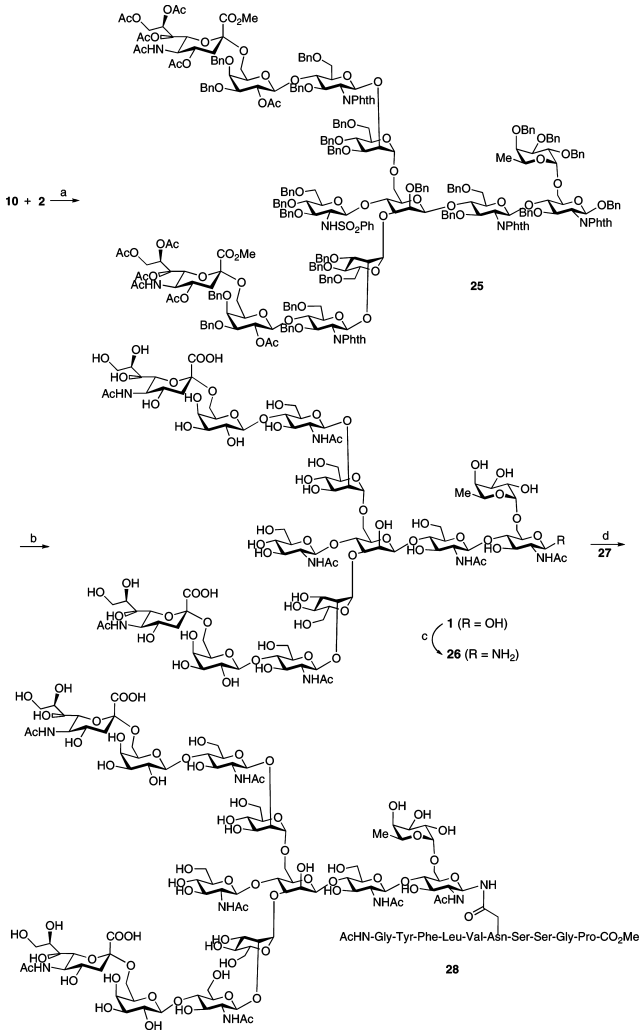
Scheme 3. Synthesis of Disaccharide Donor **10**[a]



[a] Key: (a) TMSOTf, toluene/THF, 81%. (b) 1. $PhI(OAc)_2/BF_3 \cdot OEt_2$, CH_2Cl_2; 2. Py, Ac_2O, 89%. (c) 1. $N_2H_4 \cdot AcOH$, DMF; 2. DAST, CH_2Cl_2, 82%.

Having completed the synthesis of compound **2**, we then turned to the assembly of disaccharide donor **10** (Scheme 3). In the event, coupling of phosphite **21**[19] and galactal **22**[20] proceeded smoothly to afford disaccharide **23** with good selectivity (α/β = 11:1). The predominant α isomer was isolated in 81% yield. Oxidation of **23** with $PhI(OAc)_2$[21] followed by acetylation furnished disaccharide

Scheme 4. Synthesis of Tridecasaccharide **1** and Glycopeptide **28**[a]



[a] Key: (a) $BF_3 \cdot OEt_2$, CH_2Cl_2, 54%. (b) 1. NaOMe/MeOH, THF/MeOH/H_2O; 2. $H_2NCH_2CH_2NH_2$; 3. Ac_2O, Et_3N, MeOH; 4. Na, NH_3(l); 5. Ac_2O, $NaHCO_3$, H_2O, 52%. (c) NH_4HCO_3, H_2O. (d) Peptide **27**, HATU, DIEA, DMSO, 20% yield over two steps. **27**: AcHN-Gly-Tyr-Phe-Leu-Val-Asp-Ser-Ser-Gly-Pro-CO_2Me.

24 in one pot.[22] Finally, deprotection of the anomeric acetate was accomplished through exposure to hydrazine acetate, providing the hemiacetal, which was further reacted with DAST to afford the target fluoride donor **10**.

With the two component pieces in hand, we now turned our attention to the completion of the tridecasaccharide synthesis. As outlined in Scheme 4, $BF_3 \cdot OEt_2$-mediated coupling of excess amounts of donor **10** with **2** provided mainly tridecasaccharide **25** (54% yield) along with a small amount of monocoupled product. The global deprotection of **25** was accomplished in five steps, as shown. First, the methyl ester and acetyl groups were removed using excess NaOMe. Next, the phthalimide groups were cleaved, and the resultant free amines were acetylated. We were pleased to find that the 25 benzyl groups and the sulfonamide group could be efficiently cleaved upon exposure to sodium in liquid ammonia.[23] Selective acetylation was accomplished through treatment with acetic anhydride in saturated $NaHCO_3$, affording **1**.

Finally, we examined the merger of tridecasaccharide **1** with a peptide domain and found that exposure of **1** to Kochetkov conditions[24] provided **26** (Scheme 4). Aspartylation of **26** with **27** provided the target glycopeptide **28**, albeit in modest yield.

In summary, we have synthesized the homogeneous 2,6-sialylated tridecasaccharide of the Fc sector of IVIG and successfully coupled the carbohydrate with a peptide domain to provide a glycopeptide adduct (**28**). The merger of **1** with appropriate Fc-derived peptide fragments and the results of biological evaluations of the glycopeptides will be reported in due course.

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Supporting Information Available: Experimental procedures, characterization, and copies of spectral data. This material is available free of charge via the Internet at http://pubs.acs.org.

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