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A PRACTICAL AND EFFICIENT SYNTHESIS OF COMPLEX-TYPE BIANTENNARY HEPTASACCHARIDE-ASPARAGINE CONJUGATE, A KEY BUILDING BLOCK FOR THE SYNTHESIS OF COMPLEX N-LINKED GLYCOPEPTIDES

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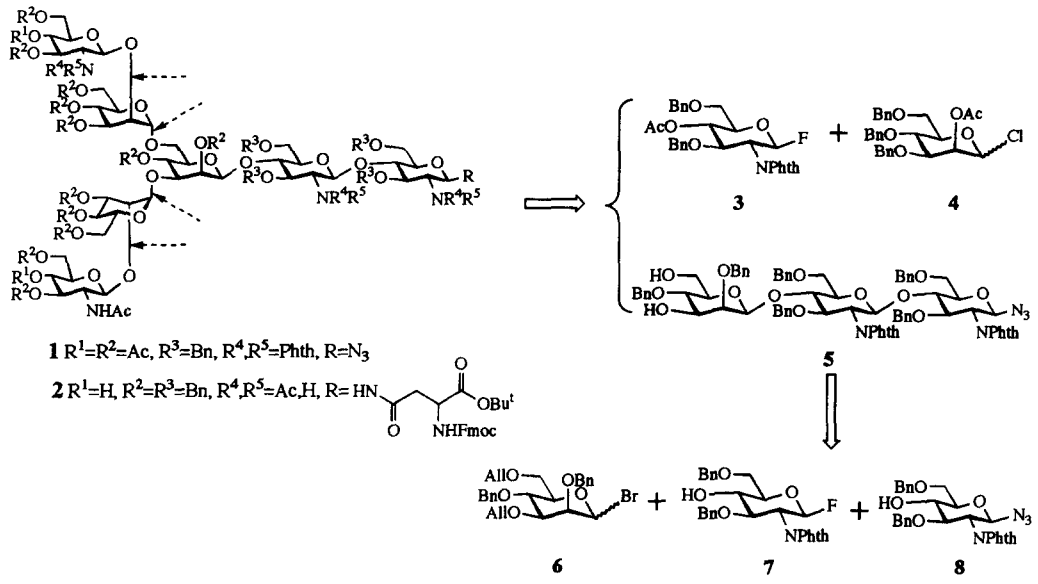
Abstract: The complex-type biantennary heptasaccharide-asparagine conjugate (**2**) of N-linked glycoproteins was synthesized from monosaccharide units **3**, **4**, **6**, **7** and **8** in 7 steps with an overall yield of 18.4%. © 1997 Elsevier Science Ltd.

The oligosaccharide chains of glycoproteins play a significant role in cell recognition and signal transduction during numerous biological processes.[1] Therefore, chemical and chemo-enzymatic synthesis of biologically relevant glycopeptides has become the focus of many research efforts.[2] Nevertheless, the synthesis of complex glycopeptide still remains a challenging problem to current organic chemists, while the problem largely relies on the availability of enough quantity of properly protected complex oligosaccharides. The methodology of enzymatic glycosylation[3] may provide a solution to this problem, but in order to get glycopeptides carrying such as N-linked complex-type biantennary oligosaccharide structures by utilizing glycosyltransferases to elongate glycan chains, one has to first prepare structures containing a heptasaccharide with two N-acetylglucosaminyl residues at both non-reducible ends which is a suitable substrate during enzymatic trans-glycosylations[4,5]. Thus, the heptasaccharide-asparagine conjugate becomes a key building block for such purposes. Unverzagt[6] has recently prepared a partially acetylated and partially benzylated heptasaccharide structure **1** by selective glycosylations in 11 steps (overall yield less than 4%) starting from protected monosaccharides and disaccharides, however, the protective strategy was not compatible to the synthesis of complex glycopeptides. In order to prepare extensively benzylated heptasaccharide-asparagine (Fmoc) conjugate **2** which can fulfil the demand of our recently developed strategy for solid-phase synthesis of glycopeptides employing benzylated oligosaccharide and Fmoc protected amino acids as building blocks,[7] we have developed a practical and efficient route leading to **2**.

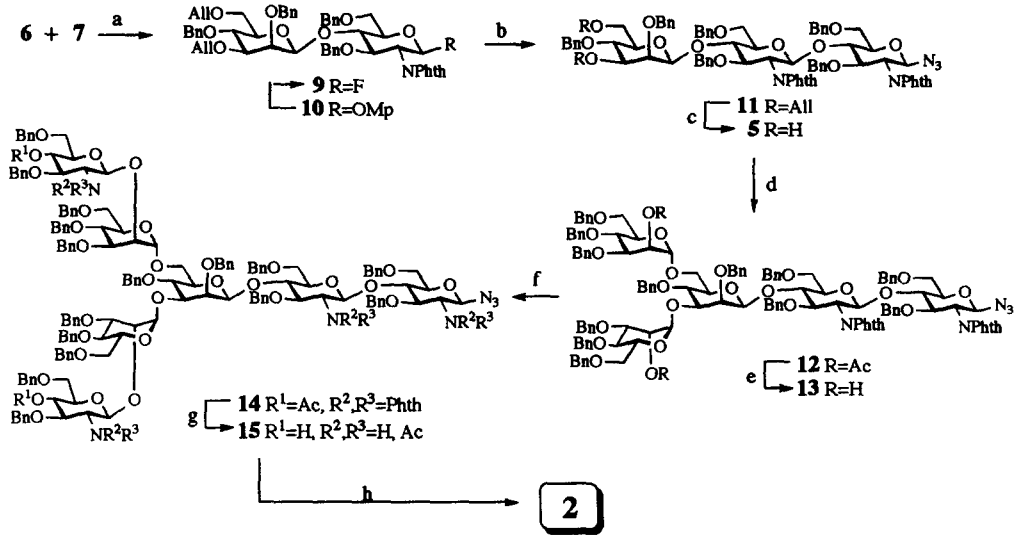
Based on the idea of orthogonal glycosylation strategy, we designed monosaccharide units **6**[9], **7**[8] and **8**[10] (Scheme 1), whose anomeric centers can be activated or transformed under different conditions, for the preparation of a key trisaccharide intermediate **5** (Scheme 2). Thus, reaction of **6** and **7**, promoted by silver alumina-silicate[11], afforded **9** and its α-isomer (1.4 : 1.0) in 91% yield, without influencing the anomeric fluoride. Compound **9** was easily isolated from the reaction mixture by column chromatography and was then directly employed as the glycosyl donor for the next glycosylation. Compared to the procedure to produce **9** we

Scheme 1.



recently reported[10], the new one saved two steps: first, oxidative deprotection of anomeric *para*-methoxyphenyl group in **10**, in which step purification of the resulting hemiacetals from the brown contaminants was proved to be difficult and the yield was rather low (70%), and then, fluorination of the resulting hemiacetals. Reaction between **9** and **8** in the presence of hafnocene dichloride-silver perchlorate (1:2)[12] proceeded smoothly to give trisaccharide **11** (76%) which was deallylated by an iridium-catalyzed process[13] to afford **5** (86%). Glycosylation of compound **5**, using **4** as glycosyl donor and silver trifluoromethanesulfonate as promoter, gave pentasaccharide **12** in excellent yield (97%). Thus far, the core pentasaccharide structure **12** was synthesized on a multi-gram scale. Treatment of **12** with 1M NaOMe in ethanol removed both acetyl groups on terminal mannosyl residues to result in **13** (90%) which served as a glycosyl acceptor to react with fluoride **3** in the presence of hafnocene complex again giving heptasaccharide **14** (84%). Dephthaloylation of **14** with ethylenediamine in 1-butanol[14] was proved to be very slow (5 days for completion) and all four possible intermediates were observed by TLC during the course of the reaction. It was also observed that selective acetylation of the amino groups in resulting product using acetic anhydride in methanol was unsuccessful because the acetylation of amino groups was not completed even after prolonged reaction time at room temperature and therefore, a two step procedure, i.e. peracetylation using acetic anhydride in pyridine followed by selective removal of O-acetyl groups using $LiOH/H_2O_2$, was applied to pursue this purpose. Thus, dephthaloylation of **14**, peracetylation of the resulting product and selective removal of O-acetyl groups were carried out in a one-pot-three-step manner to produce **15** in 82% yield. During all above transformations, anomeric azide which served as a protected amino group was unaffected. Finally, reduction of the azide group in **15** with Lindlar catalyst under hydrogen atmosphere followed by coupling reaction of the resulting amino sugar with aspartic acid moiety activated by 1-hydroxybenzotriazole (HOBt) / dicyclohexylcarbodiimide (DCC)[15] gave the desired product **2** (77%)[16].

Scheme 2.



a. silver alumina silicate, MS 4A, CH_2Cl_2, 0°C to r.t., 4h, 91%(α:β=1.0:1.4). b. MS 4A, Cp_2HfCl_2, $AgClO_4$, CH_2Cl_2, **8**, -20°C to r.t., overnight, 76%. c. $[Ir(COD)(PMePh_2)_2]PF_6$, THF, r.t. 1h; then HgO/$HgCl_2$, acetone, r.t., 1h, 86%. d. AgOTf, MS 4A, **4**, CH_2Cl_2, -40°C to r.t., overnight, 97%. e. 0.5N NaOMe, MeOH, r.t., 1.5 h, 90%. f. MS 4A, Cp_2HfCl_2, $AgClO_4$, **3**, CH_2Cl_2, -20°C to r.t., overnight, 84%. g. i) $NH_2CH_2CH_2NH_2$, n-BuOH, 90°C, 5 d; ii) Ac_2O, Pyridine, r.t. 6 h, iii) 1M LiOH, THF, H_2O_2, r.t., 4h, 82%. h. i) Lindlar catalyst, H_2, MeOH, r.t., 5h; ii) Fmoc-Asn-Bu^t/HOBt/DCC, CH_2Cl_2, r.t., overnight, 77%.

In short, the benzylated heptasaccharide-asparagine conjugate **2**, which is a key intermediate for the synthesis of glycopeptides carrying complex-type biantennary N-oligosaccharide chains, was prepared in seven separate steps with an overall yield of 18.4% from monosaccharide units **3**, **4**, **6**, **7** and **8**. The synthesis presented here was proved to be a very short, practical and efficient route, by which it was possible to work on a multi-gram scale. In addition, **14** and **15** have either an easily differentiated acetyl group at each O-4 or a free hydroxyl group at each C-4 of both glucosaminyl residues on non-reducible ends, and it is at these positions that important extensions are mounted in natural structures. Therefore, **14** and **15** are also suitable intermediates for chemical synthesis of more complicated biantennary N-oligosaccharides[17].

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16. **2** $[\alpha]_D$ = +0.6° ($CHCl_3$, c = 1.0). ^1H NMR (600 MHz, for $CDCl_3$ solution with internal TMS as standard): δ 7.75-6.94 (m, 88 H-aromatic), 5.95 (d, 1 H, J = 8.8 Hz, NH-α^{Asn}), 6.61 (d, 1 H, J = 6.8 Hz, NH^5), 5.33 (b, 1 H, NH), 5.14 (d, 1 H, J = 6.8 Hz, $NH^{5'}$), 5.05 (s, 1 H, H-1^4), 4.92, 4.91, 4.89 (3 d, 3 H, J = 12.2, 12.7, 12.2 Hz, 3 H-Bn), 4.87 (d, 1 H, J = 8.0 Hz, H-1^5), 4.84 (d, 1 H, J = 12.7 Hz, H-Bn), 4.77 (s, 1 H, H-$1^{4'}$), 4.74 (d, 1 H, J = 8.8 Hz, H-1^1), 4.72 (d, 1 H, J = 12.2 Hz, H-Bn), 4.71-4.63 (m, 5 H-Bn), 4.62 (s, 1 H, H-1^3), 4.60 (d, 1 H, J = 12.0 Hz, H-Bn), 4.47 (d, 1 H, J = 8.8 Hz, H-$1^{5'}$), 4.45 (m, 1 H, H-α^{Asn}), 4.55-4.40 (m, 21 H-Bn), 4.38 (m, 1H, H-β^{Fmoc}), 4.36 (d, 1 H, J = 8.0 Hz, H-1^2), 4.31 (dd, 1 H, H-3^5), 4.21 (dd, 1 H, J = 6.3, 17.0 Hz, H-β'^{Fmoc}), 4.20 (dd, 1 H, J = 6.3, 7.8 Hz, H-α^{Fmoc}), 4.08(bd, 1 H, J = 1.0 Hz, H-2^4), 4.06 (dd, 1 H, J = J = 6.8 Hz, H-4^1), 3.98 (s, 1 H, H-2^4), 3.94 (bdd, 1 H, H-2^1), 3.87 (bd, 1 H, H-3^4), 3.82 (bs, 1 H, H-2^3), 3.81-3.51 (m, 27 H of sugar rings), 3.48 (dd, 1 H, J = 10.2 Hz, H-$4^{5'}$), 3.40 (dd, 1 H, J = 7.8, 9.3 Hz, H-3^1), 3.26 (m, 1 H, H-5), 3.22 (m, 1 H, H-5^5), (ddd, 1 H, J = 9.3, 8.8, 8.8 Hz, H-$2^{5'}$), 3.09 (bs, 1H, OH-4^5), 3.01 (m, 1 H, H-5^5), 2.95 (m, 1 H, H-2^5), 2.79 (dd, 1 H, J = 4.9, 16.6 Hz, H-β^{Asn}), 2.72 (m, 1 H, H-$5^{5'}$), 2.60 (dd, 1 H, J = 3.9, 16.6 Hz, H-β'^{Asn}), 2.55 (bs, 1 H, OH-$4^{5'}$), 1.77, 1.74, 1.66, 1.65 (4 s, 4 x 3 H, 4 Ac), 1.41 (s, 9 H, t-Bu). ^{13}C NMR: δ 189.00, 172.06, 171.69, 170.87, 170.69, 169.85, 156.15 (7 C=O), 101.55 (C-1^4), 99.61 (C-1^3), 99.25 (C-1^2), 98.41 (C-$1^{4'}$), 97.96 (C-1^5), 97.55 (C-$1^{5'}$), 81.97 (-$OCMe_3$), 79.50 (C-1^1), 58.00, 56.81, 55.49, 52.29 (4 C-2 of GlcN), 50.94 (C-α^{Asn}), 47.12 (C-α^{Fmoc}), 37.72 (C-β^{Asn}), 27.87 (-$OCMe_3$), 23.52, 23.44, 23.39, 22.93 (4 -NHCOMe).
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