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Preparation of core 2 type tetrasaccharide carrying decapeptide by benzyl protection-based solid-phase synthesis strategy

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Abstract—β-D-Gal-(1→4)-β-D-GlcNAc-[β-D-Gal-(1→3)]-α-D-GalNAc-(1→3)-L-Ser/Thr building blocks for solid-phase synthesis of glycopeptide were stereoselectively synthesized in a benzyl-protected form. The key glycosylation reaction to form β-D-GlcNAc linkage was established by the use of protected *N*-trichloroacetyl-D-lactosaminyl fluoride. Usefulness of the building block was demonstrated by solid-phase synthesis of a segment of human leukosialin. Benzyl protecting group was efficiently removed by 'low acidity TfOH' conditions. © 2002 Elsevier Science Ltd. All rights reserved.

It has been recognized that *N*- and *O*-linked glycans present in secreted and cell surface glycoproteins contribute to a variety of properties of the glycoprotein such as stability against proteolysis, immunogenicity, signals for cell adhesion, and conformation.[1] However, detailed functions of those glycans have remained to elucidate because of micro-hetrogeneity in oligosaccharide structures and low availability of the homogeneous samples from natural sources. On the other hand, recent advancement in solid-phase technology have made possible rapid synthesis of the glycopeptides bearing structurally defined oligosaccharides. As part of projects on the synthesis of complex glycopeptides directed toward structure–activity relationship studies, the benzyl protection-based solid-phase synthesis of glycopeptides have been extensively investigated within this group.[2] We report here an efficient synthesis of core 2 *O*-linked tetrasaccharide {β-D-Gal-(1→4)-β-D-GlcNAc-[β-D-Gal-(1→3)]-α-D-GalNAc-(1→3)-L-Ser/Thr} building blocks and solid-phase synthesis of a glycopeptide modeled after the leukosialin (CD 43) segment (215–224) of activated T-lymphocytes.[3]

Recently, we have disclosed the first total synthesis of a core 2 disialylated glycohexaosyl threonine building block in a benzyl-protected form. The synthesis was designed so as to condense two trisaccharide segments in a convergent manner.[4] 2-Azido-2-deoxy-D-glucose derivative was employed as the precursor of GlcNAc residue, based on ready conversion of azide to acetamide group and the conditions being compatible with the presence of base-labile 9-fluorenylmethoxycarbonyl, allyl ester, and NeuAc-Gal-(1→4)-lactone moieties. However, the crucial coupling reaction involving trichloroacetimidate protocol afforded a hardly separable mixture of stereoisomeric hexasaccharides (α/β = 1/3), and thus obtained core 2 hexasaccharyl threonine was insufficient in amount to test the feasibility as building block for solid-phase synthesis. We therefore sought to develop an alternative 2-*N*-masking group to preclude the cumbersomeness arising from the stereoisomers.

Although *N*-phthaloyl group has been widely used in order to synthesize 1,2-*trans* glycoside of D-glucosamine,[5] numerous other *N*-protecting groups such as *N*-tetrachlorophthaloyl,[6,7] *N*-dichlorophthaloyl,[8] *N*-trichloroethoxycarbonyl,[9] *N*,*N*-dithiasuccinoyl,[10] *N*-allyloxycarbonyl,[11] *N*-trichloroacetyl,[12] *N*-pentenoyl,[13] *N*-dimethylmaleoyl,[14] 2,5-dimethylpyrrole,[15] *N*,*N*-diacetyl,[16] *N*-thioglycoloyl,[17] *N*-[1,3-dimethyl-2,4,6(1*H*,3*H*,5*H*)-trioxopyrimidin-5-ylidene]methyl,[18] have been also applied to the stereocontrol. Amongst them *N*-trichloroacetyl group meets our requirements since it was reported to be convertible into acetyl group without basic conditions. In contrast, most of other *N*-protecting groups essentially require base treatment for their cleavage before transformation into acetamide.

We attempted a new synthesis of core 2 tetrasaccharide by using *N*-trichloroacetyl group.[19] Since suitably protected Gal-β-(1→3)-GalN$_3$-Ser/Thr **3** and **4**, the key

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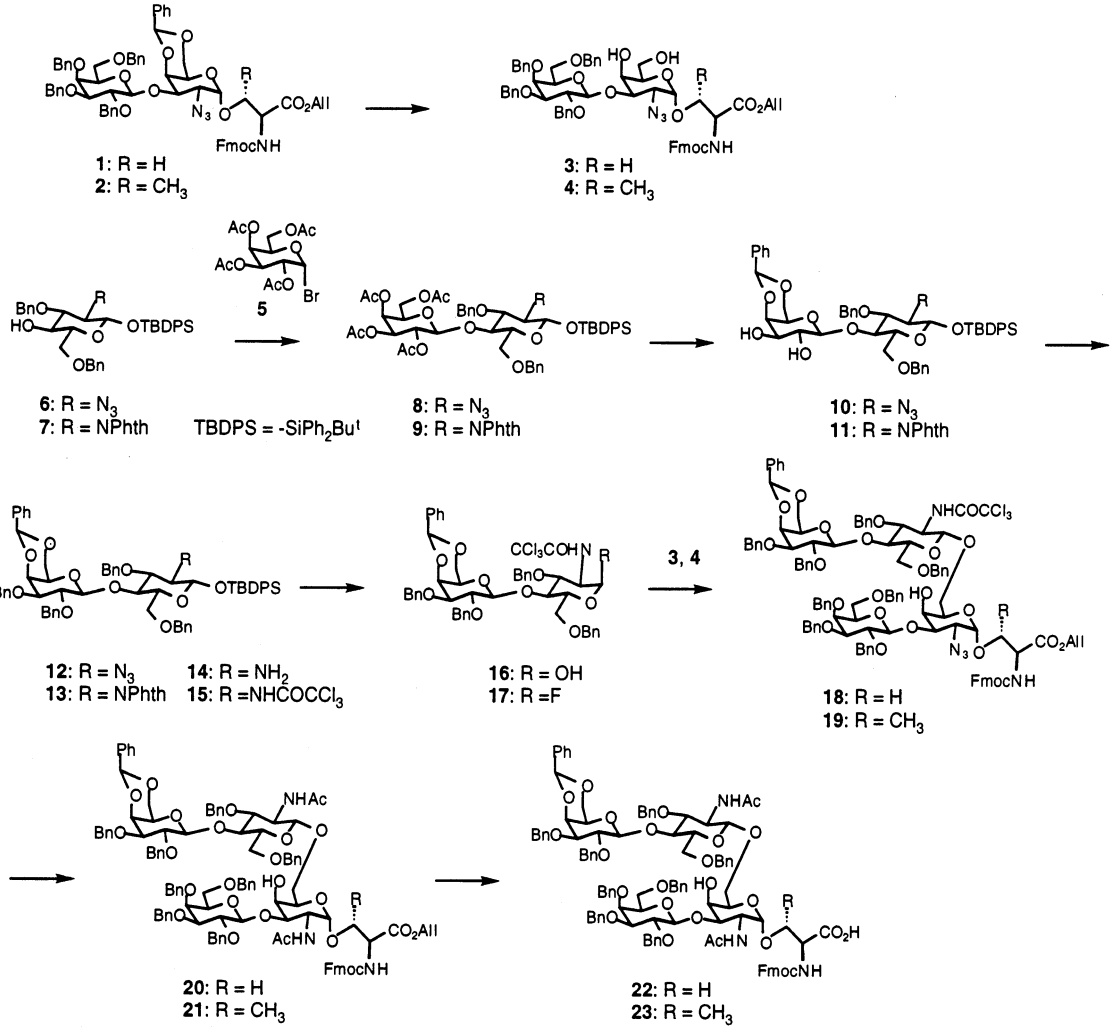
disaccharide components, were readily available by hydrolysis of the benzylidene group of known **1**[2a] and **2**,[2f] respectively, the lactosamine counterpart was synthesized as follows (Scheme 1). AgOTf-mediated glycosylation of *t*-butyldiphenylsilyl 2-azido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranoside **6**[4b] with 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide **5** afforded disaccharide **8** in 80% yield. Deacetylation (NaOMe/MeOH) was followed by benzylidene acetal formation (dimethoxytoluene, *p*-TsOH, CH_3CN) to give diol **10**, which was benzylated (NaH, BnBr, DMF) to produce **12** in 79% yield in three steps. Azide reduction with powdered Zn and AcOH in CH_2Cl_2 proceeded to exclusively give 2-amino derivative **14**. Synthesis of **14** was alternatively performed starting from *t*-butyldiphenylsilyl 3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside **7**[20] through glycosylation with **5** (**9**: 82%), deacetylation (100%), benzylidenation (**11**: 97%), benzylation (**13**: 66%), and dephthaloylation with ethylenediamine in *n*-BuOH (98%). Amine **14** was then treated with trichloroacetyl chloride in pyridine to afford trichloroacetamide **15** in 87% yield. Cleavage of the silyl ether with *n*-Bu_4NF and AcOH in THF gave hemiacetal **16**, which was converted into glycosyl fluoride **17** by treatment with Et_2NSF_3 in THF (84% in two steps). Highly stereoselective generation of the α-fluoride was evidenced by characteristic signal of the anomeric proton.[21]

With Cp_2ZrCl_2–$AgClO_4$ employed as promoter,[22] coupling reaction of glycosyl donor **17** and acceptors **3**/**4** (1.1 equiv.) in CH_2Cl_2 at −15°C proceeded smoothly (<2 h) to give tetrasaccharides **18** and **19** as the sole product in 72 and 70% yield, respectively. As anticipated, both coupling reactions exclusively afforded β-glycoside, whose anomeric configuration was assignable from the $^1J_{CH}$ coupling constant.[21] The promoter $Sn(OTf)_2$[23] was also effective for this coupling reaction.

Then trichloroacetamide group was transformed into acetamide under reductive conditions. It is to be noted that tributyltin hydride reduction recommended for this transformation[12] is inapplicable to these particular compounds **18** and **19** due to the presence of labile allyl ester group. Dechlorination was effected by treatment with excess (>50 equiv.) powdered Zn and AcOH in CH_2Cl_2 at room temperature though rather slow to complete (3 d). By this treatment, reduction of azide to amine was simultaneously promoted. The products were acetylated with Ac_2O in MeOH to afford **20** and



Scheme 1.

21 in 91 and 96% yield, respectively. Deallylation with $Pd(PPh_3)_4$ and dimedone in THF gave rise to the suitably protected building blocks **22** and **23**.

In order to demonstrate usefulness of the core 2 building block for solid-phase synthesis, a decapeptide segment (215–224) **24** of human T-lymphocyte glycoprotein leukosialin, which carried a single glycan linked to one of the putatively glycosylated threonine residues, was chosen as a model. It is noteworthy that synthesis of the *N*-terminal heptadecapeptide possessing core 1 trisaccharide [α-D-Neu5Ac-(2→3)-β-D-Gal-(1→3)-α-D-GalNAc-(1→3)-L-Thr] was reported recently.[24]

Solid-phase synthesis was commenced with commercial Fmoc Sieber amide resin (0.25 mmol/g). According to Fmoc protocol, the eight amino acids were manually condensed using Fmoc amino acid (4 equiv.), DCC, HOBt, and DIEA in NMP for 1 h (Scheme 2). Removal of Fmoc group was performed with 20% piperidine/NMP. Capping procedure with Ac_2O was employed after each coupling reaction. *t*-Bu (Ser/Thr) and Tr (Asn) groups were employed for side-chain protection. Then octapeptide-bound resin **25** was allowed to react with **23** (2 equiv.) in the presence of the same coupling reagents overnight. Finally Fmoc proline was condensed by double coupling procedure to complete the target sequence.

The glycopeptide thus synthesized was cleaved from resin **27** by treatment with reagent K (aq. TFA, thioanisole, 1,2-ethanedithiol, phenol). The crude product precipitated from ether was debenzylated by using the conditions of low acidity trifluoromethanesulfonic acid (10% TfOH, 30% Me_2S, 8% *m*-cresol, 2% 1,2-ethanedithiol, 50% TFA) at −15 to −5°C.[25] The resulting mixture was analyzed by reversed-phase HPLC with a C18 column to evaluate efficiency of the solid-phase synthesis. Fig. 1 exhibits the chromatogram of the product, in which peaks 1–6 are peptide derivatives. These compounds separated by HPLC were characterized by MALDI TOF MS. Peak 1 corresponds to the unreacted octapeptide, whereas peak 2 represents desired **28**. The accompanying small peaks 3 and 4 are the glycopeptides lacking a Gal residue. Mass spectral data of peak 5 and 6 are coincident with those of the glycopeptide lacking lactosamine moiety and the peptide missing glycothreonine unit, respectively.

Similarly, fully deprotected glycopeptide **24** was released from resin **27** through *N*-deprotection with 20% piperidine followed by treatment with reagent K

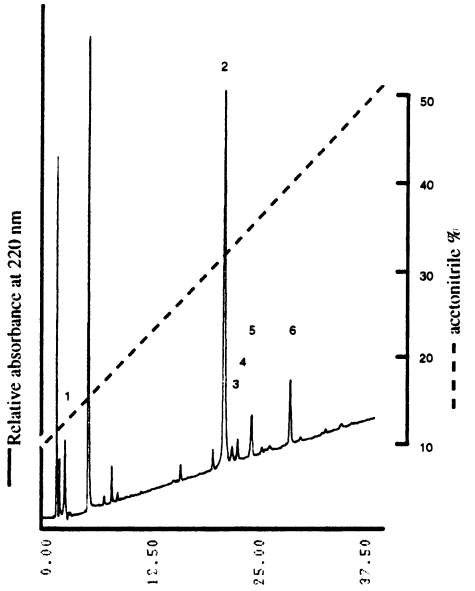
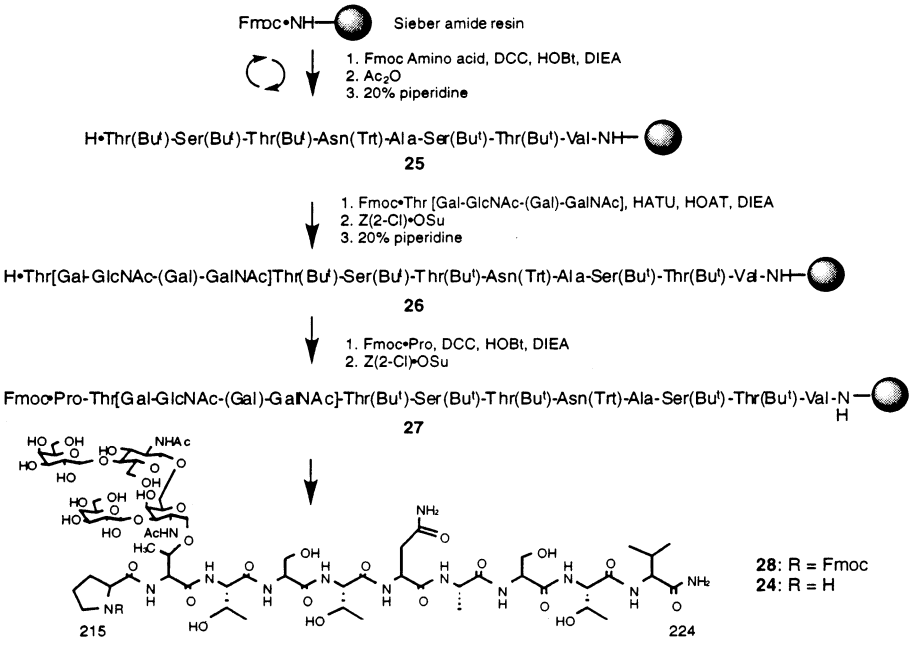


Figure 1.



Scheme 2.

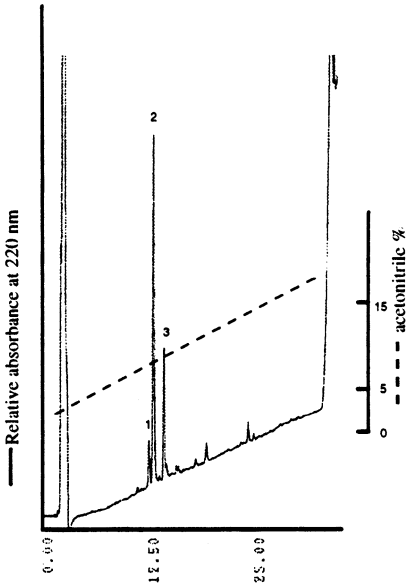


Figure 2.

(82.5% TFA, 5% thioanisole, 5% phenol, 5% H_2O, 2.5% 1,2-ethanedithiol). The resulting partially protected glycopeptide was submitted to debenzylation with the low acidity TfOH mixture. Fig. 2 shows HPLC profile of the product. Glycopeptide **24** was eluted as peak 2, while the unreacted octapeptide and the glycopeptide missing a Gal residue are included in peak 1 and peak 3, respectively. The desired glycopeptide was isolated by HPLC, and characterized by amino acid analysis as well as by mass spectrometry. The overall yield estimated was 27% based on the amino acid analysis.

In conclusion, core 2 *O*-linked tetrasaccharide building blocks **22** and **23** were synthesized via stereocontrolled glycosylation by using *N*-trichloroacetyl-D-lactosaminyl glycosyl donor **17**. The benyl-protected tetrasaccharide was used for the Fmoc-based solid-phase synthesis of glycopeptide **24**, representing leukosialin (215–224). Removal of the benzyl protecting group was efficiently accomplished under the low acidity TfOH conditions. It was observed that the conditions also led to truncation of the glycan chains in part. Further studies aimed at expanding this benzyl-protection approach to the synthesis of glycopeptides bearing complex *N*-linked glycans are currently underway.

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21. Selected physical data. Compound **19**: δ_H 5.77 (dd, 1H, $J=2.5$, 53.7 Hz, H-1a); MALDI-TOF MS calcd for $C_{49}H_{49}C_{l3}FO_{10}$: 958.22 $(M+Na)^+$. Found: 958.03. Compound **20**: δ_C 98.0 ($^1J_{CH}=170.7$ Hz, $GalN_3$-C1), 99.4 ($^1J_{CH}=157.5$ Hz, GlcNTCA-C1), 102.7 ($^1J_{CH}=160.9$ Hz, Gal-C1), 103.9 ($^1J_{CH}=155.9$ Hz, Gal-C1); MALDI-TOF MS calcd for $C_{110}H_{112}C_{l3}N_5O_{24}$: 2014.47 $(M+Na)^+$. Found: 2014.42. Compound **21**: δ_C 99.6 ($^1J_{CH}=172.5$ Hz, $GalN_3$-C1), 99.8 ($^1J_{CH}=159.2$ Hz, GlcNTCA-C1), 102.6 ($^1J_{CH}=160.0$ Hz, Gal-C1), 103.8 ($^1J_{CH}=162.5$ Hz, Gal-C1); MALDI-TOF MS calcd for $C_{111}H_{114}C_{l3}N_5O_{24}$: 2028.68 $(M+Na)^+$. Found: 2028.41. Compound **22**: δ_H 1.81 and 1.56 (2s, 2×3H, AcNH); MALDI-TOF MS calcd for $C_{112}H_{119}N_3O_{25}$: 1928.80 $(M+Na)^+$. Found: 1928.53. Compound **23**: δ_H 1.82 and 1.66 (2s, 2×3H, AcNH); MALDI-TOF MS calcd for $C_{113}H_{121}N_3O_{25}$: 1942.82 $(M+Na)^+$. Found: 1943.17. Compound **28**: MALDI-TOF MS calcd for $C_{82}H_{124}N_{12}O_{39}$: 1951.81 $(M+Na)^+$. Found: 1951.31. Compound **24**: MALDI-TOF MS calcd for $C_{67}H_{114}N_{14}O_{37}$: 1707.76 $(M+H)^+$, 1729.74 $(M+Na)^+$. Found: 1707.89, 1729.65.
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