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Tetrahedron Letters 47 (2006) 2475–2478
Tetrahedron Letters

Synthesis of 2-azidoethyl α-D-mannopyranoside orthogonally protected and selective deprotections

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Received 24 January 2006; revised 7 February 2006; accepted 13 February 2006
Available online 28 February 2006

Abstract—We present the synthesis of a fully orthogonally protected mannosyl glycoside **1** and the corresponding methods for selective deprotections. Mannosyl glycoside **1** contains a functionalized linker at the anomeric position to allow for the attachment of carbohydrate units to scaffolds in order to prepare carbohydrate multivalent systems.

Dendritic cell specific ICAM-3 grabbing non-integrin (DC-SIGN) is a C-type lectin that presents a carbohydrate recognition domain (CRD) at the C-terminus. This lectin is not only capable of interacting with highly glycosylated proteins found at the surface of several pathogens such as viruses, bacteria, fungi and parasites,[1] but also recognizes in a calcium dependent way high mannose structures present in *N*-glycans of pathogen glycoproteins. After the discovery of the role that DC-SIGN plays in the HIV infection process,[2] much effort has been devoted to the synthesis of these mannosylated structures and related neoglycoconjugates in order to obtain multivalent carbohydrate systems that mimic the natural systems.[3–7] The epitope present on high mannose that shows the strongest binding affinity to DC-SIGN is the glycan $Man_9GlcNAc_2$ as represented in Figure 1.[8]

The synthesis of complex oligosaccharides is usually based on a well defined selection of protecting groups in order to achieve efficient protection and deprotection pathways leading to the target compound. Some examples of orthogonal protection–deprotection strategies have been developed for galactose and *N*-acetyl glucosamine both for solution and solid phase synthesis, respectively, with the aim of preparing libraries of saccharides.[9,10] The weak affinity of individual carbohydrate–protein interactions has been overcome in nature by multivalent presentation of the requisite carbohydrates. Consequently, there is a need to prepare glycans functionalized via the anomeric position in such a way as to incorporate the handle necessary to establish the key arrays.

Here, we present a new and effective synthesis of the orthogonally protected mannose derivative **1** functionalized at the anomeric position with a short and functionally versatile spacer moiety. In this synthesis, we have chosen 'permanent' protection for position 4 (*O*-benzyl group) and for one end of the linker (the azido group). These residues will be reduced at the end of the synthetic sequence by hydrogenolysis (palladium on carbon and H_2 at atmospheric pressure). Positions 2, 3 and 6 (corresponding to the branched positions present in high mannose structures, see Fig. 1) have been protected with levulinic ester (Lev), 4-methoxybenzyl (PMB) and *t*-butyldiphenylsilyl (TBDPS) groups, respectively. This selection of orthogonal groups allows for the preparation of different branched oligosaccharides with linkage in 2, 3 or 6 position followed by a simple hydrogenolysis to liberate position 4 and simultaneously provide the amine function at the terminus of the linker. This terminal amine provides a means of incorporating theses mannose derivatives into a multivalent scaffold using classical strategies such as amide,[11–13] urea or thiourea bond formation.[14,15] The synthesis of the mannose derivative **1** begins with commercially available peracetylated α-D-mannose **2**. In three steps, and following the procedure we have published previously based on a modification of earlier work,[11,16] 2-azidoethyl α-D-mannopyranoside (**3**) was prepared in good yield. Glycosylation of **2** with 2-bromoethanol using a large excess of $BF_3{\cdot}Et_2O$ as

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doi:10.1016/j.tetlet.2006.02.063

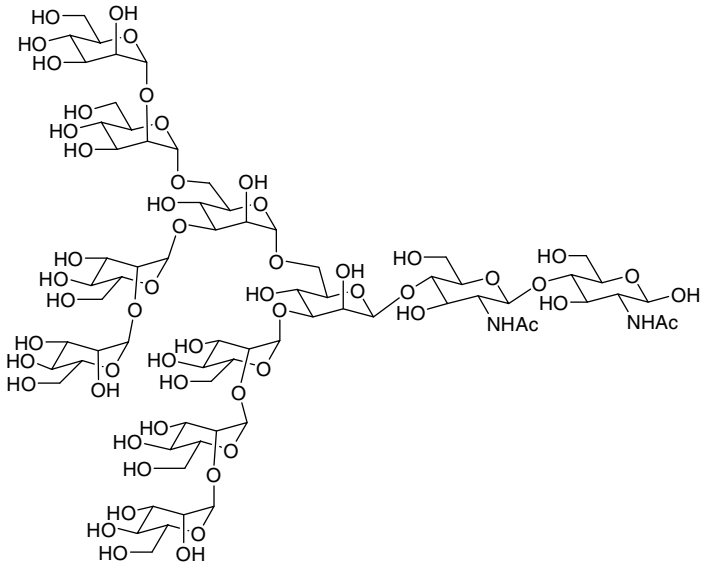
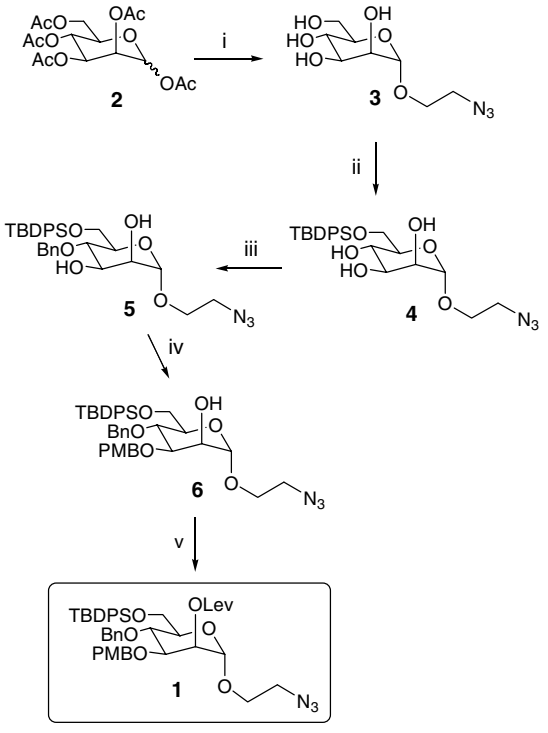


Figure 1. Chemical structure of $Man_9GlcNAc_2$.

promoter, followed by azide displacement and acetate deprotection using Zempler conditions with NaOMe gave glycoside **3** in 63% yield over three steps (Scheme 1).

The primary hydroxyl group in mannose **3** was protected with a silyl group by reaction with *t*-butyldiphenylsilyl chloride using the reaction conditions described in the literature.[17] This compound was purified by flash chromatography using as eluent CH_2Cl_2–MeOH (9.5:0.5) to give silyl ether **4** in 79% yield. ^1H NMR in $CDCl_3$ of **4** showed typical signals corresponding to the aromatic protons at 7.72–7.68 ppm (m, 4H) and 7.48–7.38 ppm (m, 6H) and a singlet at 1.01 ppm for the *t*-Bu group. The next step in the synthesis was the protection of position 4 with a benzyl group as a permanent protecting group. To achieve this required transient protection of positions 2 and 3, which was accomplished by formation of an acetonide with 2,2′-dimethoxy propane and *p*-TsOH in acetone. Subsequently, benzylation of the hydroxyl in position 4 was achieved by treatment with benzyl chloride and NaH in dimethylformamide with high yield. Final cleavage of the acetonide using trifluoroacetic acid in THF at room temperature and chromatographic purification on silica gel using as eluent toluene–AcOEt (9:1) gave mannose **5** in 63% yield over three steps. ^1H NMR in $CDCl_3$ of **5** showed a more complex aromatic region and a clear AB system for the methylene of the benzyl group centered at 4.69 ppm. The H_3 proton deshielded at low field 0.15 ppm by the ring effect and H_4 moved to high field. To selectively protect the position 3 we chose a strategy based on use of a stannylene acetal between hydroxyl groups in 2 and 3.[18] Reaction of **5** with Bu_2SnO followed by *p*-methoxybenzyl chloride (PMBCl) and Bu_4NI in toluene under reflux gave the mannose derivative **6** protected exclusively in position 3 with the *p*-methoxybenzyl group. The final intermediate **6** was purified by chromatography on silica gel using as eluent toluene–MeOH (94:6) and was obtained in 76% overall yield and as a single regioisomer as confirmed by NMR spectroscopy. ^1H NMR in $CDCl_3$ of **6** showed H_2 deshielded at low field and H_3 moved to high field with new singlets for two methylene protons at 4.63 ppm and for three methoxy protons at 3.83 ppm.



Scheme 1. Reagents and conditions: (i) see Ref. 11; (ii) TBDSCl, Im, DMF, rt, 79%; (iii) (a) 2,2′-dimethoxypropane, PPTS, acetone; (b) BnBr, NaH, DMF; (c) TFA, DCM, 63% in three steps; (iv) (a) Bu_2SnO, toluene, 110 °C; (b) PMBCl, Bu_4NI, 76%; (v) levulinic acid, DCC, DMAP, DCM, 95%.

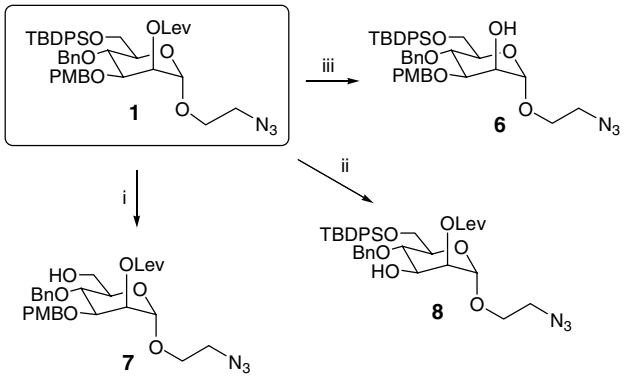
More significant was the ^{13}C NMR spectrum where C_3 moved from 72 to 81 ppm and the position of C_2 did not change.

The protection of the axial hydroxyl group in position 2 represents the final step in the synthesis of the orthogonally protected mannose derivative **1**, for which a levulinic ester was selected. Although levulinate is not one of the most common protecting groups used in carbohydrate chemistry, the selective deprotection of this unit in the presence of several functional groups using hydrazine acetate makes this group very attractive. Reaction of **6** with levulinic acid in the presence of 1,3-dicyclohexylcarbodiimide (DCC) and 4-dimethylaminopyridine (DMAP) using the conditions previously described in our group provided the fully protected mannose derivative **1** in 95% isolated yield.[19] Compound **1** was completely characterized using mass spectrometry, as well as mono- and two-dimensional NMR experiments.[20] The most indicative data were the deshielding of H_2 signal from 4.06 to 5.34 ppm and the presence of new signals corresponding to the methylene groups at 2.83–2.61 (m, 4H) and to the methyl group at 2.51 ppm of the levulinic ester.

Having synthesized the mannose derivative **1** with the array of orthogonal protecting groups shown, we have performed selective deprotections to demonstrate the orthogonal behavior of the protecting groups used; these steps are critical for the potential applications of this mannose in complex and versatile synthesis of oligosaccharides. Specific conditions were chosen to achieve a complete and selective removal of each protecting group and these are illustrated in Scheme 2.

The cleavage of the silyl group at position 6 in the mannose derivative **1** was easily achieved cleanly and highly efficiently using tetrabutylammonium fluoride (TBAF) in THF at room temperature[21] or with the HF–Py complex in HOAc–THF as solvent.[19] The resulting mannose derivative **7** was purified by flash chromatography with hexane–AcOEt (1:1.5) as eluent. In the NMR spectrum the absence of the *t*-butyl signal at 1.56 ppm and the simplification of the aromatic region with the loss of two phenyl groups were indicative.

Mannose derivative **8** with a free hydroxyl group at position 3 was obtained in quantitative yield by treatment of glycoside **1** with trifluoroacetic acid in dichloromethane at −20 °C.[19] The loss of the *p*-methoxybenzyl group was observed in the NMR spectrum by the disappearance of the AB system of the benzyl methylene at 4.53 ppm and the singlet corresponding to the methoxy group at 3.83 ppm. Also the ^1H NMR in $CDCl_3$ showed that the H_3 moved to low field from 3.80 to 4.22 ppm and ^{13}C NMR showed the shielding of C_3 from 55.7 to 70.2 ppm.



Scheme 2. Reagents and conditions: (i) Bu_4NF, THF, rt or HF–Py, HOAc–THF, 95%; (ii) TFA, DCM, −20 °C, 100%; (iii) N_2H_4HOAc, MeOH, rt, 87%.

Mannose derivative **6** deprotected at position 2 was obtained directly as the last intermediate in the synthetic pathway leading to the fully protected mannose derivative **1** (Scheme 1). However, we wanted to demonstrate the selective removal of this protecting group in the presence of the other protecting groups selected for this orthogonally protected mannose. Hydrazine acetate in methanol at room temperature was used to achieve this cleavage[22] and mannose derivative **6**, which was purified by flash chromatography using toluene–MeOH (100:1) as eluent, was obtained in 87% yield.

In conclusion, we have synthesized a functionalized and fully orthogonally protected mannose glycoside **1**. The selective cleavage of protecting groups from **1** was performed in very good yields and with excellent selectivity. Final hydrogenation will provide a terminal primary amine ready to allow the attachment of this carbohydrate to a multivalent scaffold. Glycosylation of mannose deravative **1** with activated mannosyl donors to prepare complex mannosyl structures is underway.

Acknowledgements

We would like to thank FIS (PI030093), for financial support. We also thank Professor Timothy Gallagher, for critical reading of this manuscript.

References and notes

1. van Kooyk, Y.; Geijtenbeek, T. B. H. *Nat. Immunol.* **2003**, *3*, 697–709.
2. Geijtenbeek, T. B. H.; Kwon, D. S.; Torensma, R.; van Vliet, S. J.; van Duijnhoven, G. C. F.; Middel, J.; Cornelissen, I. L. M. H. A.; Nottet, H. S. L. M.; KewalRamani, V. N.; Littman, D. R.; Figdor, C. G.; van Kooyk, Y. *Cell* **2000**, *100*, 587–597.
3. Ratner, D. M.; Plante, O. J.; Seeberger, P. H. *Eur. J. Org. Chem.* **2002**, 826–833.
4. Zhang, J.; Ma, Z.; Kong, F. *Carbohydr. Res.* **2003**, *338*, 2039–2046.
5. Lee, H.-K.; Scanlan, C. N.; Huang, C.-Y.; Chang, A. Y.; Calarese, D. A.; Dwek, R. A.; Rudd, P. M.; Burton, D. R.; Wilson, I. A.; Wong, C.-H. *Angew. Chem., Int. Ed.* **2004**, *43*, 1000–1003.
6. Geng, X.; Dudkin, V. Y.; Mandal, M.; Danishefsky, S. J. *Angew. Chem., Int. Ed.* **2004**, *43*, 2562–2565.
7. Schratt, X.; Unverzagt, C. *Tetrahedron Lett.* **2005**, *46*, 691–694.
8. Mitchell, D. A.; Fadden, A. J.; Drickamer, K. *J. Biol. Chem.* **2001**, *276*, 28939–28945.
9. Wong, C.-H.; Ye, X.-S.; Zhang, Z. *J. Am. Chem. Soc.* **1998**, *120*, 7137–7138.

10. Zhu, T.; Boons, G.-J. *Tetrahedron Asymmetry* **2000**, *11*, 199–205.
11. Arce, E.; Nieto, P. M.; Díaz, V.; García Catro, R.; Bernad, A.; Rojo, J. *Bioconjug. Chem.* **2003**, *14*, 817–823.
12. Kleinert, M.; Rockendorf, N.; Lindhorst, T. K. *Eur. J. Org. Chem.* **2004**, 3931–3940.
13. Wu, X.; Bundle, D. R. *J. Org. Chem.* **2005**, *70*, 7381–7388.
14. Roy, R.; Pagé, D.; Figueroa Perez, S.; Verez Bencomo, V. *Glycoconj. J.* **1998**, *15*, 251–263.
15. Corbell, J. B.; Lundquist, J. J.; Toone, E. J. *Tetrahedron Asymmetry* **2000**, *11*, 95–111.
16. Lindhorst, T. K.; Kötter, S.; Krallmann-Wenzel, U.; Ehlers, S. *J. Chem. Soc. Perkin Trans. 1* **2001**, 823–831.
17. Baeschlin, D. K.; Chaperon, A. R.; Green, L. G.; Hahn, M. G.; Ince, S. J.; Ley, S. V. *Chem. Eur. J.* **2000**, *6*, 172–186.
18. Cherif, S.; Clavel, J.-M.; Monneret, C. *J. Carbohydr. Chem.* **1998**, *17*, 1203–1218.
19. Reichardt, N.-C.; Martín-Lomas, M. *Angew. Chem., Int. Ed.* **2003**, *42*, 4674–4677.
20. Analytical data for 2-azidoethyl 4-*O*-benzyl-6-*O*-(*t*-butyldiphenylsilyl)-2-*O*-levulinoyl-3-*O*-(4-methoxybenzyl)-α-D-mannopyranoside (**1**): ESI-MS (MeOH): $C_{44}H_{53}N_3O_9Si$ M_r (calcd) 795.4, M_r (found) 818.3 $(M+Na)^+$; $[\alpha]_D$ +2.10 (*c* 0.75, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$): $\delta = 7.76$–7.65 (m, 4H, Ar), 7.46–7.30 (m, 6H, Ar), 7.30–7.22 (m, 5H, Ar), 7.21–7.13 (m, 2H, Ar), 6.83 (d, 2H, $J = 8.7$ Hz, $CH_{2(PMB)}$), 5.39 (dd, 1H, $J = 1.9$ and 2.8 Hz, H_2), 4.89 (d, 1H, $J = 10.6$ Hz, H_{Bn}), 4.87 (s, 1H, H_1), 4.62 (d, 1H, $J = 10.9$ Hz, H_{PMB}), 4.57 (d, 1H, $J = 10.7$ Hz, H_{Bn}), 4.45 (d, 1H, $J = 10.9$ Hz, H_{PMB}), 4.03–3.87 (m, 4H, $2H_6$, H_5 and H_3), 3.87–3.77 (m, 4H, $-OCH_3$ and $-OCH_2-$), 3.74–3.66 (m, 1H, H_4), 3.58 (dt, 1H, $J = 10.6$, 5.1 Hz, $-OCH_2-$), 3.34 (t, 2H, $J = 5.1$ Hz, $-CH_2N_3$), 2.83–2.61 (m, 4H, $-CH_2CH_{2-(lev)}$), 2.51 (s, 3H, $CH_{3-(lev)}$), 1.56 (s, 9H, *t*-Bu); ^{13}C NMR (300 MHz, $CDCl_3$): $\delta = 206.8$ (CO), 172.6 (COO), 159.7 (C_{Ar}), 136.3 (C_{Ar}), 136.0 (C_{Ar}), 134.1 (C_{Ar}), 133.6 (C_{Ar}), 130.5 (C_{Ar}), 130.3 (C_{Ar}), 128.8 (C_{Ar}), 128.3 (C_{Ar}), 128.1 (C_{Ar}), 128.0 (C_{Ar}), 114.2 (C3 and C5), 98.2 (C1), 78.1 (C3), 75.7 (CH_2Bn), 74.3 (C5), 73.3 (C4), 71.8 (CH_2PMB), 69.3 (C2), 66.9 (C7), 63.3 (C6), 55.7 ($-OCH_3$), 50.8 (C8), 38.4 (CH_2lev), 30.3 (CH_3lev), 28.5 (CH_2lev), 27.2 ($CH_{3(t\text{-But})}$), 19.8 ($C_{t\text{-But}}$).
21. Hanessian, S.; Lavallee, P. *Can. J. Chem.* **1975**, *53*, 2975–2977.
22. van Boom, J. H.; Burgers, P. M. J. *Tetrahedron Lett.* **1976**, *17*, 4875–4878.