Pergamon

Tetrahedron Letters, Vol. 36, No. 34, pp. 6137-6140, 1995
Elsevier Science Ltd
Printed in Great Britain
0040-4039/95 $9.50+0.00

0040-4039(95)01162-5

Synthesis of a Tetrasaccharide Fragment of the Circulating Anodic Antigen of *Schistosoma mansoni*

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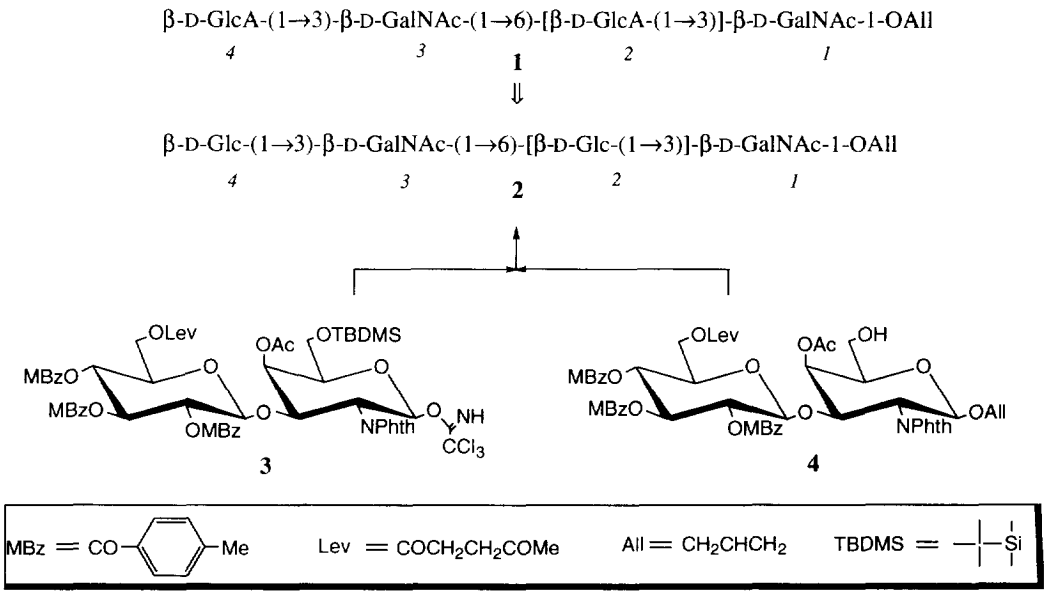
Abstract: A stereocontrolled synthesis of the allyl glycoside of a tetrasaccharide fragment of the O-linked polysaccharide chain of the circulating anodic antigen of *Schistosoma mansoni*, β-D-Glc*p*A-(1→3)-β-D-Gal*p*NAc-(1→6)-[β-D-Glc*p*A-(1→3)]-β-D-Gal*p*NAc, is presented.

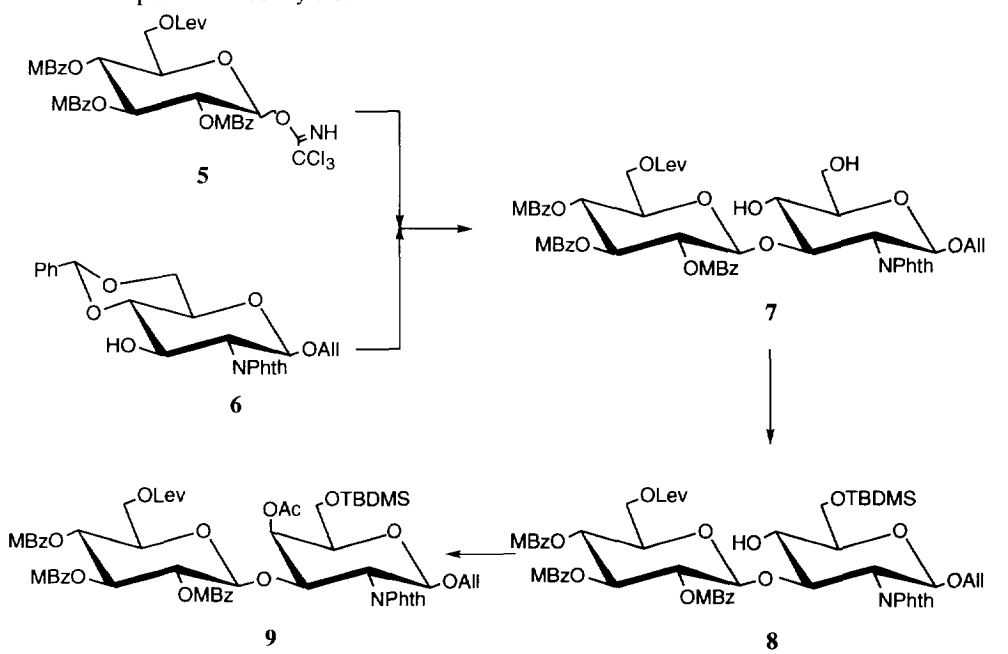
The circulating anodic antigen (CAA), secreted by the parasite *Schistosoma mansoni*, is a glycoprotein in which the major threonine-linked carbohydrate part is built up of disaccharide repeating units, namely {→6)-[β-D-Glc*p*A-(1→3)]-β-D-Gal*p*NAc-(1→}$_n$, probably connected to the protein via an as yet unknown core saccharide with GlcNAc at the reducing end[1]. Schistosomiasis is one of the most widespread parasitic diseases of man[2], and heavily infected individuals may develop severe pathology. Infection occurs by contact with water[3,4], infested with the larvae of the parasite. Because for cure of the infection chemotherapy[5-7] is available, diagnosis is important. Currently, the diagnosis of schistosomiasis in developing countries is based on the parasitological examination of faeces and urine for the presence of *Schistosoma* eggs. In addition, for diagnosis, serological methods are increasingly used, aiming both at detection of specific antibodies and of specific antigens. As CAA is both an important circulating antigen and strongly immunogenic, it would be an interesting target antigen for antibody assays. However, this approach is limited by the availability of CAA from biological sources in sufficient amounts. In order to replace isolated CAA in diagnostic methods, a synthetic program for the preparation of a wide range of medium-sized oligosaccharide fragments of CAA was initiated, to determine the optimal epitope of CAA that can act as an immunologic determinant.

This report describes the stereoselective synthesis of CAA fragment **1**. Oxidation of the primary hydroxyl groups was carried out in the final stage of the synthesis, because D-glucuronic acid has a low reactivity towards glycosidation[10]. To this end, tetrasaccharide **2** was designed, and protected **2** was synthesized from glycosyl donor **3** and glycosyl acceptor **4**. Both synthons could be prepared from the same precursor **9**, which in turn was obtained by coupling of glycosyl donor **5** with glycosyl acceptor **6**. The monosaccharide units **5** and **6** were synthesized as described earlier[11-13].

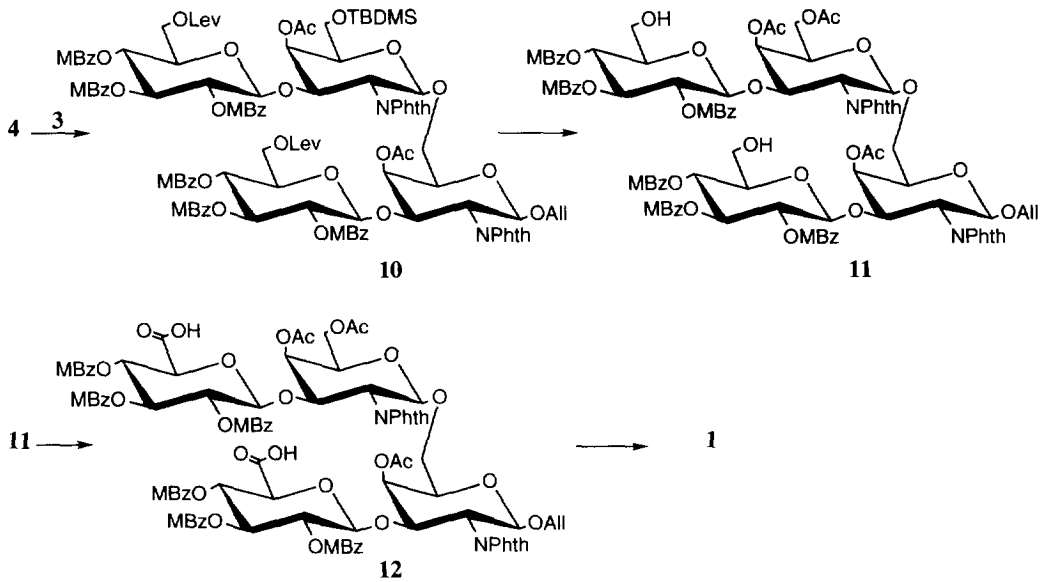
Stereocontrolled glycosylation of **6** with 1.5 equivalents of **5** in CH_2Cl_2 in the presence of TMSOTf (0.05 equivalents) and molecular sieves 4A at 25°C gave, after acid hydrolysis with TFA in CH_2Cl_2 and H_2O,



7[14] in 89% yield. Selective silylation of the primary hydroxyl group with TBDMS-Cl in CH_2Cl_2/py was achieved in 80% yield (→**8**[14]). The glucosamine moiety of **8** was converted in two steps into a galactosamine unit, yielding **9**[14] (*i* Tf_2O in CH_2Cl_2/py, *ii* TBAA in DMF, 78% overall yield)[15,16]. Disaccharide donor **3**[14] was obtained from **9** (*i* $(PPh_3)_4RhCl$ in ethanol[17] followed by NIS in CH_2Cl_2 and H_2O[18], *ii* CCl_3CN, DBU in CH_2Cl_2[19]) in an overall yield of 63%, while desilylation (*p*-TsOH in 9:1 CH_3CN-H_2O) of **9** afforded disaccharide acceptor **4**[14] in 86% yield.



TMSOTf-MS4A promoted glycosylation of **4** with 2 equivalents of disaccharide donor **3** in CH_2Cl_2 at 0°C gave tetrasaccharide derivative **10**[14] in 73% yield. Then, compound **10** was converted in three steps into **11**[14] (*i* *p*-TsOH in 9:1 CH_3CN-H_2O, *ii* Ac_2O-DMAP in py, *iii* NH_2NH_2.OAc in 2:1 ethanol-toluene[20,21], 80% overall yield). The two primary hydroxyl functions of **11** were successfully oxidized to yield **12**[14], using PDC[22] in CH_2Cl_2 in the presence of powdered molecular sieves 4A[23], in 60% yield. Final deprotection of **12** into **1**[14] was achieved in two steps (*i* 33% $MeNH_2$ in ethanol[24], *ii* Ac_2O in methanol, 76% overall yield).



In summary, for the first time a stereocontrolled synthesis of tetrasaccharide **1** was achieved by using key disaccharide **9**. Synthesis of larger oligosaccharides and conjugation to suitable carriers are in progress.

Acknowledgment

The research of Dr. T.M. Slaghek has been made possible by a fellowship of the Royal Netherlands Academy of Art and Sciences.

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14. Physical data for new compounds are given below, values for $[\alpha]_D$ and δ_H were measured at 25°C for solutions in $CHCl_3$ and $CDCl_3$ (TMS as internal standard), respectively, unless noted otherwise. Signal assignment such as 1^3 stands for a proton at C-1 of monosaccharide residue *3*. **1**: $[\alpha]_D$ +8° (c 0.3, H_2O); δ_H (D_2O) 2.006 and 2.012 (2s, 2NHAc), 4.154 and 4.182 (2d, 3.25 Hz, 4^1 and 4^3), 4.490 and 4.506 (2d, 7.9 and 8.0 Hz, respectively, 1^2 and 1^4), 4.511 and 4.528 (2d, 8.2 and 8.4 Hz, respectively, 1^1 and 1^3). **3**: $[\alpha]_D$ +19° (c 1.0); δ_H 0.028 and 0.043 (2s, Me_2Si), 0.870 (s, *t*BuSi), 2.209 (s, Ac), 2.233 (s, Lev), 2.259 (s, MeBz), 2.327 (s, 2MeBz), 4.795 (d, 7.8 Hz, 1^2), 5.706 (d, 3.3 Hz, 4^1), 6.334 (d, 8.8 Hz, 1^1). **4**: $[\alpha]_D$ +3° (c 1.0); δ_H 2.203 (s, Ac), 2.226, 2.309 and 2.322 (3s, 3MeBz), 2.296 (s, Lev), 4.871 (d, 7.9 Hz, 1^2), 5.062 (d, 8.6 Hz, 1^1), 5.599 (d, 3.2 Hz, 4^1). **7**: $[\alpha]_D$ +4° (c 1.0); δ_H 2.215 (s, Lev), 2.223, 2.314 and 2.355 (3s, 3MeBz), 4.816 (d, 8.0 Hz, 1^2), 4.997 (d, 8.5 Hz, 1^1). **8**: $[\alpha]_D$ +5° (c 1.0); δ_H 0.081 and 0.090 (2s, Me_2Si), 0.909 (s, *t*BuSi), 2.210 (s, Lev), 2.220, 2.313 and 2.334 (3s, 3MeBz), 4.801 (d, 8.0 Hz, 1^2), 4.940 (d, 8.4 Hz, 1^1). **9**: $[\alpha]_D$ +8° (c 1.0); δ_H 0.064 (s, Me_2Si), 0.893 (s, *t*BuSi), 2.193 (s, Ac), 2.215 (s, Lev), 2.228 (s, MeBz), 2.326 (2s, 2MeBz), 4.779 (d, 7.8 Hz, 1^2), 5.042 (d, 8.6 Hz, 1^1), 5.595 (d, 3.0 Hz, 4^1). **10**: $[\alpha]_D$ -1° (c 1.0); δ_H 0.077 (s, Me_2Si), 0.907 (s, *t*BuSi), 4.682 and 4.752 (2d, 7.8 Hz, 1^2 and 1^4), 4.731 and 5.031 (2d, 8.5 Hz, 1^1 and 1^3), 5.403 and 5.659 (2d, 3.1 and 3.2 Hz, respectively, 4^1 and 4^3). **11**: $[\alpha]_D$ +1° (c 1.0); δ_H 2.108, 2.208 and 2.314 (3s, 3Ac), 2.213, 2.270 and 2.330 (3s, 6MeBz), 4.793 and 4.870 (2d, 7.8 and 7.9 Hz, respectively, 1^2 and 1^4), 4.775 and 5.027 (2d, 8.6 and 8.5 Hz, respectively, 1^1 and 1^3), 5.531 and 5.730 (2d, 3.6 and 3.4 Hz, respectively, 4^1 and 4^3). **12**: $[\alpha]_D$ +37° (c 1.0); δ_H 2.108, 2.208 and 2.315 (3s, 3Ac), 2.218, 2.290 and 2.334 (3s, 6MeBz), 4.774 and 4.868 (2d, 7.8 Hz, 1^2 and 1^4), 4.791 and 5.025 (2d, 8.5 and 8.6 Hz, respectively, 1^1 and 1^3), 5.530 and 5.728 (2d, 3.8 and 3.0 Hz, respectively, 4^1 and 4^3). The corresponding dimethylester was prepared by treatment with CH_2N_2: δ_H 3.675 and 3.707 (2s, 2 $COOCH_3$), 4.743 and 4.841 (2d, 7.5 and 7.7 Hz, respectively, 1^2 and 1^4), 4.765 and 5.006 (2d, 8.5 and 8.6 Hz, respectively, 1^1 and 1^3).
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(*Received in UK* 19 *May* 1995; *accepted* 23 *June* 1995)