Synthesis of Mucin Glycans from the Protozoon Parasite *Trypanosoma cruzi*

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Dedicated to Professor Sir Jack Baldwin FRS, in honour of his many and varied contributions to organic chemistry

Abstract: A short, blockwise, 2+2-glycosylation approach to the synthesis of a tetrasaccharide component of *Trypanosoma cruzi* mucin is reported. Despite the use of a 1,2-linked disaccharide donor, high yield (79%) and good stereocontrol (>10:1, β:α) were achieved in the key glycosylation step. Preliminary studies indicate that this branched tetrasaccharide can serve as a substrate for enzymatic sialylation by the parasite cell surface *trans*-sialidase.

Key words: oligosaccharide, synthesis, parasite, mucin, *trans*-sialidase, drug target

The South American protozoon parasite *Trypanosoma cruzi* is the causative agent of Chagas' disease, one of the most serious parasitic diseases in tropical regions. Approximately 18 million people are infected with *T. cruzi* and a further 100 million are at risk of infection; 2–3 million chronic cases are current, with around 45000 deaths each year.[1] The repertoire of current drugs for treating Chagas' disease is poor: new leads are needed. As with many pathogenic microorganisms, the nature and extent of cell-surface glycosylation impacts directly on their infectivity.[2] In the case of *T. cruzi*, the cell surface is decorated with a complex glycocalyx composed of very heavily glycosylated mucins (ca. 60% carbohydrate by weight).[3] The mucin glycans are the major substrate for a parasite *trans*-sialidase (TcTS)[4] which, like the mucins themselves, is attached to the parasite surface via a GPI anchor.[5] *trans*-Sialidase catalyses the transfer of sialic acid from host glycoproteins onto the parasite surface as part of its effort to evade the host immune response. *Trypanosoma cruzi* is unable to synthesise sialic acid de novo so the *trans*-sialidase represents a potential drug target. Numerous studies have aimed to understand TcTS structure and action[6] and to exploit it in enzymatic synthesis.[7] In our work,[8] this enzyme has proved invaluable for the preparation of isotopically enriched glycans for NMR structural studies, in particular.[7f,9] However, despite its broad similarity to microbial neuraminidases, for which several potent inhibitors are known (and which form the basis of current drugs, such as tamiflu),[10] this potential parasite drug target remains rather enigmatic. Despite numerous efforts, only very limited progress has been made with inhibitor development.[11] There is therefore a need to better understand how TcTS binds and performs chemistry on its natural substrates, the parasite-cell-surface mucin glycoproteins.[3] Given the heterogeneity of *T. cruzi* mucins, and the issues associated with culturing quantities of human pathogen, we set out to synthesise *T. cruzi* mucin glycopeptides. The de Lederkremer lab, in particular, have made substantial efforts in the chemical synthesis and assessment of the mucin glycans from *T. cruzi* G strain.[12] We have recently reported on the action of TcTS on synthetic glycopeptide fragments of mucins from *T. cruzi* Y strain.[13] Herein we report approaches to the synthesis of a more complex *T. cruzi* Y strain mucin tetrasaccharide, in a form suitable for elaboration into glycoamino acid building blocks for glycopeptide production and for direct assessment as a substrate for TcTS.

In developing a strategy for the synthesis of the tetrasaccharide component of the Y strain mucin glycan, a key consideration was the compatibility of the synthesis with subsequent use of the glycan product for glycopeptide synthesis (Figure 1). The ability to control glycosylation stereochemistry is the key. The challenge with the structure shown is that the acetamido group at C-2 of the core α-GlcNAc has the potential to participate in glycosylation, directing the reaction to give the undesired β-linked product. We have recently reported the direct synthesis of α-linked GlcNAc-threonine from a 2-acetamido donor,[13] although modest yields mean that the method is only practical for a cheap GlcNAc donor but not for complex synthetic oligosaccharides (for a recent review of hexosamine glycoside syntheses, see ref.[15]). This led us to consider a reducing terminus based on 2-azido-2-deoxy-glucose, in the form of a thioglycoside, since we have already shown

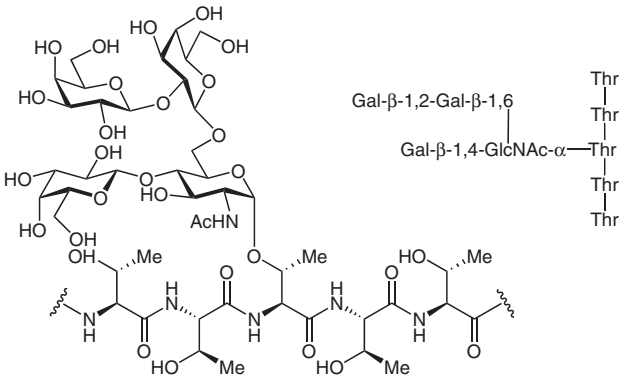

Figure 1 Fragment of *Trypanosoma cruzi* Y strain mucin structure[14]

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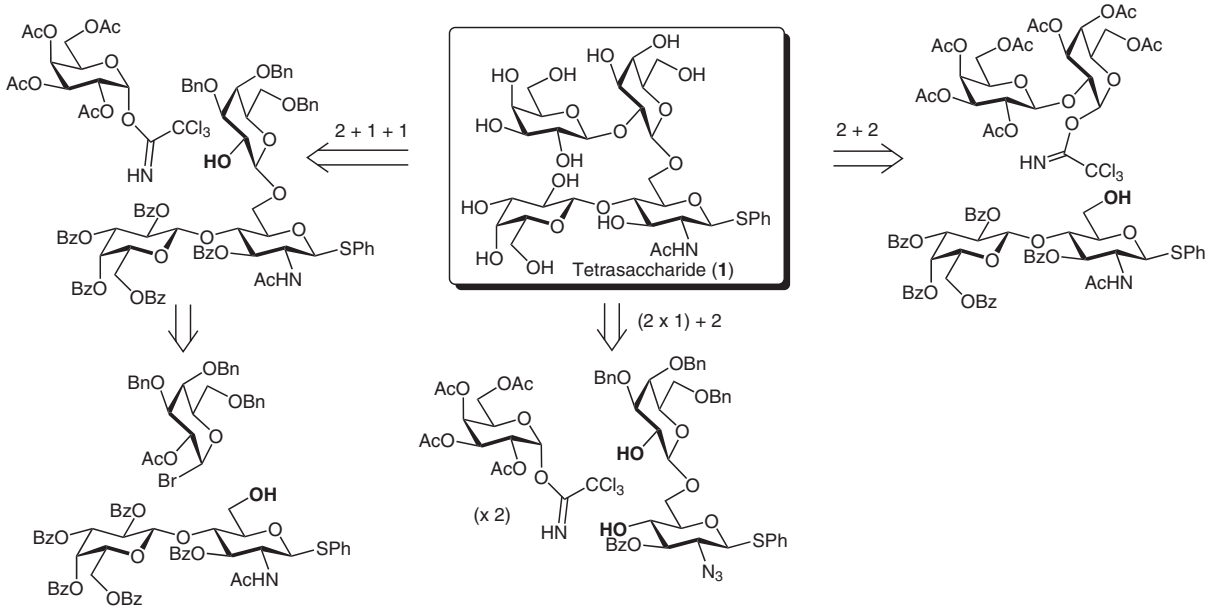


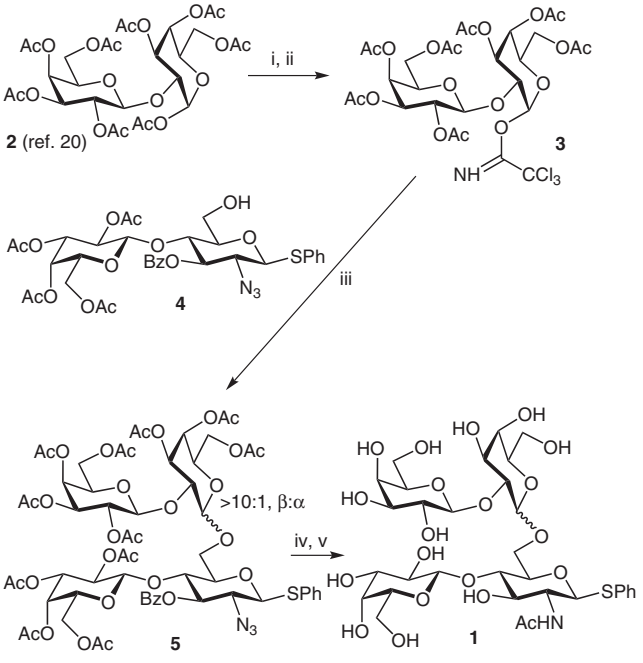
Scheme 1 Outline of potential disconnections of the target tetrasaccharide **1**

that 2-azido-2-deoxy-lactose donors can be convenient for the installation of α-linked LacNAc disaccharide units.[13,16]

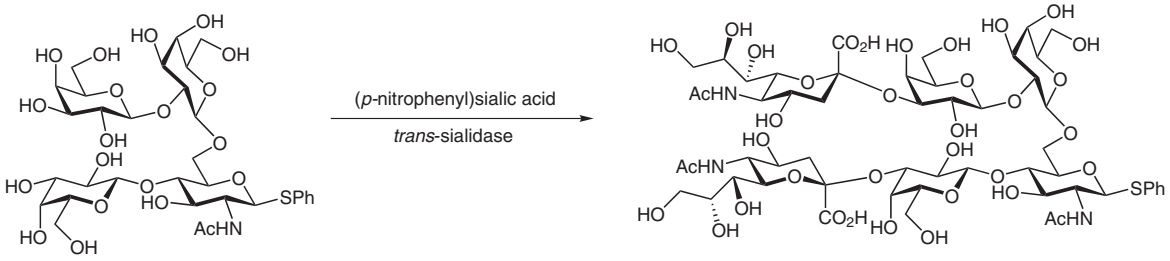
Target tetrasaccharide **1** presents a number of potential disconnections (Scheme 1). In initial efforts, we explored both a 2+1+1 and a (2×1)+2 approach. The former route was unsuccessful due to our inability to selectively de-*O*-acetylate the 2-position of the 1,6-linked galactose in the presence of benzoate protecting groups[17] elsewhere in a trisaccharide intermediate. Attempts to perform double glycosylation of a 1,6-linked diol acceptor in a (2×1)+2 approach yielded only 1,2-glycosylated product, the 4-OH of the azido-glucose unit proving unreactive. Concerns about the difficulty of effecting stereocontrolled glycosylation with a 1,2-linked glycosyl donor[18] initially put us off a blockwise 2+2 approach, but in light of the failure of other approaches this strategy was adopted.

A preference for a thioglycoside at the reducing terminus of the target tetrasaccharide **1** dictated the use of glycosylation chemistry that would not result in thioglycoside activation. With this in mind, the acid-activated trichloracetimidate donor system was employed.[19] Hence, known digalactoside **2**[20] was subjected to selective anomeric deprotection and conversion to the corresponding trichloracetimidate **3**. TMS triflate-catalysed glycosylation with imidate **3** in MeCN, as a participating solvent,[21] was used to convert disaccharide acceptor **4**, which was prepared by conventional means (full details will be reported elsewhere), into tetrasaccharide thioglycoside **5**. The tetrasaccharide was obtained in 79% yield as an inseparable mixture of diastereoisomers, with the required β-linked compound dominant (>10:1, β:α), as judged by ^{1}H NMR spectroscopy.[22,23] Subsequent reductive acetylation[13,24] of azide **5** and final de-*O*-acetylation gave the target tetrasaccharide thioglycoside **1** (Scheme 2).

To summarise, we have developed a convenient synthesis of *T. cruzi* Y strain mucin tetrasaccharide in the form of a thioglycoside. Preliminary experiments (Scheme 3) show that this thioglycoside is capable of acting as an acceptor substrate for *Trypanosoma cruzi trans*-sialidase. In contrast to expectations from the biological literature,[14] a doubly sialylated glycan product is evident in such biotransformations; full details will be reported in due course.



Scheme 2 *Reagents and conditions*: (i) $H_2NNH_2{\cdot}OAc$ (1 equiv), DMF, 5 h, quant.; (ii) Cl_3CCN (4 equiv), DBU (0.3 equiv), CH_2Cl_2, 0 °C, 1 h, 88%; (iii) TMSOTf (0.1 equiv), MeCN, 0 °C, 1 h, 79%; (iv) Zn powder (13 equiv), $CuSO_4$, THF–AcOH–Ac_2O (3:1:2), 24 h, 45%; (v) Na, MeOH, 18 h, quant.



Scheme 3 Action of recombinant *T. cruzi trans*-sialidase on synthetic mucin tetrasaccharide **1**.[7d]

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(22) In the parallel series with a mannose at the reducing terminus, the corresponding coupling gave complete β-stereocontrol albeit in lower yield (54%).
(23) **Selected Analytical Data**
Imidate **3**: $[\alpha]_D^{20}$ +41.0 (*c* 1, $CHCl_3$). ^1H NMR (400MHz, $CDCl_3$): δ = 8.65 (s, 1 H, NH), 6.51 (d, 1 H, H1, $J_{1,2}$ = 3.6 Hz), 4.66 (d,1 H, H1′, $J_{1',2'}$ = 7.8 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 160.7 (C=NH), 101.3 (C1′), 94.9 (C1). HRMS: *m/z* calcd for $C_{28}O_{18}NCl_3H_{36}$ [M + NH_4]: 797.1336; found: 797.1339.
Tetrasaccharide **5**: $[\alpha]_D^{20}$ –4.0 (*c* 1 in $CHCl_3$). ^1H NMR (600 MHz, $CDCl_3$): δ = 4.78 (d, 1 H, H1c, $J_{1,2}$ = 8.0 Hz), 4.70 (d, 1 H, H1a, $J_{1,2}$ = 10.0 Hz), 4.60 (d, 1 H, H1b, $J_{1,2}$ = 7.7 Hz), 4.55 (d, 1 H, H1d, $J_{1,2}$ = 7.9 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 101.7 (C1b), 100.9 (C1c), 100.3 (C1d), 85.5 (C1a). MS (ES): *m/z* = 1372.5 [M + $Na]^+$.
Tetrasaccharide **1**: $[\alpha]_D^{20}$ +15.0 (*c* 1 in H_2O). ^1H NMR, (400 MHz, D_2O): δ = 4.87 (d, 1 H, H1a, $J_{1,2}$ = 10.4 Hz), 4.37 (d, 1 H, H1b, $J_{1,2}$ = 6.8 Hz), 4.34 (d, 1 H, H1c, $J_{1,2}$ = 8.0 Hz), 4.31 (d, 1 H, H1d, $J_{1,2}$ = 7.9 Hz). ^{13}C NMR (75 MHz, D_2O): δ = 175.1 (C=ONHAc), 104.2 (C1c), 103.8 (C1d), 102.2 (C1b), 85.9 (C1a). HRMS: *m/z* calcd for $C_{32}H_{49}N_3O_{20}S$ [M + $Na]^+$: 822.2461; found: 822.2487.
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