Synthesis of sialyloligosaccharides using the *trans*-sialidase from *Trypanosoma cruzi*: novel branched and di-sialylated products from digalactoside acceptors

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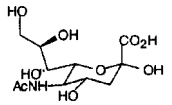
Received (in Cambridge, UK) 22nd March 2000, Accepted 14th April 2000
Published on the Web 22nd May 2000

The *trans*-sialidase from *Trypanosoma cruzi* efficiently catalyses transfer of α-sialyl residues from *p*-nitrophenyl sialic acid (*p*-nitrophenyl *N*-acetylneuraminic acid) to acceptors containing β-galactose units.

The sialic acids are a class of C_9 monosaccharides comprising over thirty five variants.[1] The archetypal member is *N*-acetylneuraminic acid **1**. Colloquially this is referred to as



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'sialic acid' unless another member of the family is indicated. Sialic acids are ubiquitous components of mammalian glycoproteins.[2] They are present in numerous other glycoconjugates[1] and are crucial determinants of their biological properties.[3]

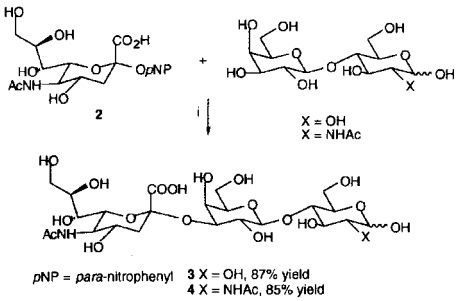
Non-enzymatic syntheses of sialic acid-containing oligosaccharides[4] suffer from the drawbacks of a requirement for multiple protection–deprotection sequences and problems of control of configuration at the anomeric centre. In addition yields may be affected by side reactions involving 2,3-elimination. Accordingly, enzymatic methods have received much attention. Sialyl transferases catalyse sialylation regiospecifically and in high yield.[5] A major drawback to their use is that they require cytidine monophosphate *N*-acetylneuraminic acid (CMP-NeuAc) as sialyl donor. Although non-enzymatic syntheses of CMP-NeuAc have been reported[6] enzymatic methods have been preferred[7] and have been developed to permit multigram production[8] and *in situ* regeneration of the sialyl donor.[9]

Owing to the complexity of the donor and the limited availability of appropriate enzymes, sialylations with sialyl transferases have usually been limited to the preparation of sialylated oligosaccharides on the μmol scale. Sialyl transfers catalysed by sialidases has been demonstrated but proceed in extremely low yields ($< 15\%$).[10]

Against this background, the report of a novel retaining α-*trans*-sialidase in the parasite *Trypanosoma cruzi* was of particular interest.[11] Noteworthy was the finding that sialyl transfer was catalysed with much greater efficiency than hydrolysis.[12] The *trans*-sialidase required sialyl glycosides or oligosaccharides as sialyl donors. It was stated to be selective for transfer to β-galactosyl units; α-galactosides were reported not to be acceptors.[13]

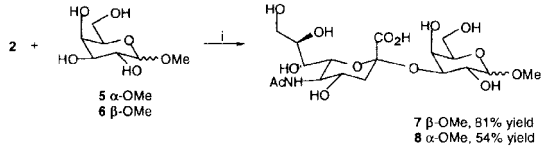
The *trans*-sialidase has been shown to transfer sialyl residues α(2 → 3) on to galactose residues using sialyllactose,[14–17] 4-methylumbelliferyl α-sialoside[18–20] or *p*-nitrophenyl α-sialoside[13,21,22] as donors. However, the full synthetic potential of the enzyme has not been realised. The studies described above were carried out either on an analytical scale or, if preparatively, on a scale of < 10 μmol. We have now developed an approach that permits the synthesis of sialyl galactosides on a mmol or greater scale. The key to the improvement reported here is the use of the irreversible sialyl donor *p*-nitrophenyl α-sialoside **2** made readily available by improvement of a published synthesis[23] and the use of the recombinant form of the *T. cruzi trans*-sialidase referred to above, and which consists of the *N*-terminal catalytic domain. This fragment expresses the full catalytic activity of the wild-type enzyme.[24]

The effectiveness of the *trans*-sialidase is evident in the synthesis, on a mmol scale, of sialyllactose **3** and sialyl-*N*-acetyllactosamine **4** (Scheme 1). Yields of sialylated products are essentially quantitative; the data in Scheme 1 relate to pure, isolated products. It was noteworthy that hydrolysis of the sialyl donor **2** occurred to a significant extent only in the absence of acceptor.

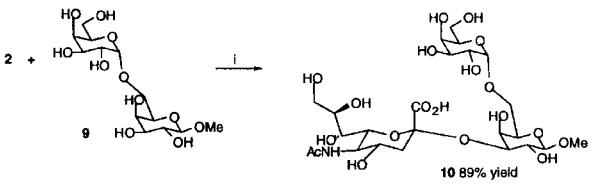


Scheme 1 *Reagent*: i, *trans*-Sialidase from *Trypanosoma cruzi*.

Because it had been reported that the *trans*-sialidase does not catalyse transfer to α-galactosides,[13] the acceptor behaviour of methyl α- and β-galactopyranosides **5** and **6**, respectively, was studied (Scheme 2). The β-anomer was an efficient acceptor giving the sialylgalactoside **7** in 81% yield. Surprisingly, the α-anomer was also sialylated to give glycoside **8** with moderate efficiency (54% yield). Clearly, a small aglycone in the α-anomeric position does not constitute a total barrier to *trans*-sialylation. However, it was considered that the presence of a bulky carbohydrate moiety in place of the much smaller methyl group in glycoside **5** would indeed create problems. Accordingly we studied the acceptor properties of disaccharide glycoside **9**. To our surprise, this was efficiently sialylated but the product was found to be the branched trisaccharide **10** (Scheme 3), formed in 89% yield. Thus the barrier to sialylation of α-galactosides is clearly expressed but the predeliction for sialylation of β-galactosides was not negated by substitution at C-6 in the acceptor.

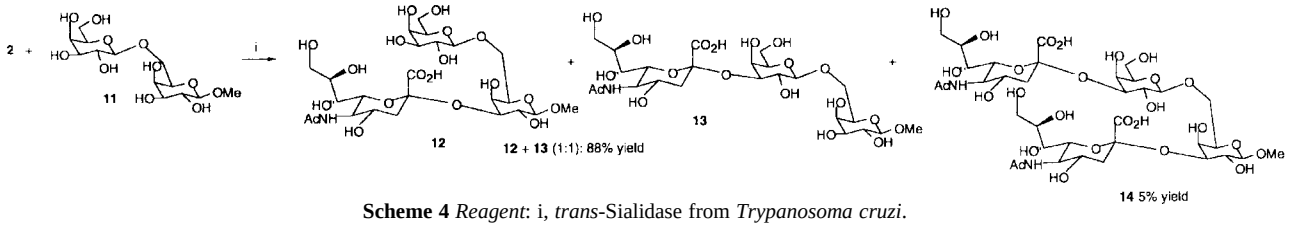


Scheme 2 *Reagent*: i, *trans*-Sialidase from *Trypanosoma cruzi*.



Scheme 3 *Reagent*: i, *trans*-Sialidase from *Trypanosoma cruzi*.

DOI: 10.1039/b002302g



Scheme 4 *Reagent*: i, *trans*-Sialidase from *Trypanosoma cruzi*.

With β-D-Gal*p*-(1 → 6)-β-D-Gal*p*–OMe **11** as acceptor a mixture of three products was obtained (Scheme 4). The major component (88% isolated yield) proved to be a 1 : 1 mixture of the 3- and 3′-monosialylated products **12** and **13** which required HPLC separation. The third, minor product, formed in 5% isolated yield, proved to be the bis-sialylated product **14**. It was established that both of the monosialylated compounds **12** and **13** were converted quantitatively into the tetrasaccharide **14**. The selectivity for β-configured oligosaccharide acceptors and the specificity for α-(2 → 3) transfer were emphasised by the failure of sialyl transfer to α-D-Gal*p*-(1 → 3)-β-D-Gal*p*-(1 → 4)-D-GlcNAc*p*, α-D-Gal*p*-(1 → 3)-β-D-Gal*p*–OMe and α-D-Gal*p*-(1 → 3)-α-D-Gal*p*–OMe.

The studies reported here establish the *T. cruzi trans*-sialidase as a highly efficient catalyst for the sialylation of β-galactosides on a mmol scale. Moreover, they reveal the previously unsuspected ability of the enzyme to catalyse the formation of branched structures. We find that the enzyme is readily immobilised (Eupergit C) and retains its activity undiminished over at least nine catalytic cycles. Clearly, it should now be considered as a viable alternative to CMP-sialic acid-dependent sialyl transferases for α-2,3-sialylation of β-galactose-terminated oligosaccharides and with the added potential for sialylation of C-6-subsituted internal β-galactose units.

Besides its importance for the synthesis of sialyl oligosaccharides, the *T. cruzi trans*-sialidase provides a paradigm for the development, through mutagenesis, of glycosidases that would have high glycosyl transfer but low hydrolytic activity. The *T. cruzi* enzyme is homologous (*ca.* 70% identity for 640 amino acids) with the sialidase from *T. rangeli*, which lacks *trans*-sialidase activity. The latter enzyme has been crystallised. X-Ray structures have been determined at 2.2 Å resolution for the enzyme and at 2.9 Å resolution for the complex between the enzyme and 2,3-didehydro-2-deoxy-*N*-acetylneuraminic acid.[25] Homology modelling combined with mutagenesis studies have revealed several structural features that are crucial for the expression of *trans*-sialidase activity in the *T. cruzi* enzyme.[25,26] The acceptor studies reported here will also contribute to the elucidation of the *trans*-sialidase/sialidase mechanistic dichotomy.

Field and coworkers have also obtained evidence for the formation of branched sialyl galactosides (*cf.* Schemes 3 and 4).[27] We thank Professor Field for a useful exchange of information.

In a typical procedure, the *p*-nitrophenyl glycoside of sialic acid (0.1 g, 0.223 mmol) and *N*-acetyllactosamine (0.5 g, 1.31 mmol) in sodium acetate buffer (50 mM, pH 6.0, 7 cm^3) were incubated at 30 °C with recombinant *trans*-sialidase (1.38 U cm^{-3}, 0.225 cm^3) for 9 h. The reaction was stopped by filtration through a PTFE filter (0.45 μm pore size). The filtrate was applied to a Bio-Gel P-2 column which was eluted with water to give sialyl-*N*-acetyllactosaminelactose [α-Neu5Ac-(2 → 3)-β-D-Gal*p*-(1 → 4)-D-GlcNAc*p*] **4** (Scheme 2), 0.133 g (85%).

We thank Glaxo Wellcome for a gift of sialic acid and the BBSRC for financial support.

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