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Preliminary ^{1}H NMR Investigation of Sialic Acid Transfer by the *trans*-Sialidase from *Trypanosoma cruzi*

Jennifer C. Wilson,[a,b] Milton J. Kiefel,[a,b] Samia Albouz-Abo[b] and Mark von Itzstein[a,b,*]

[a]*Centre for Biomolecular Science and Drug Discovery, Griffith University (Gold Coast Campus), PMB 50 Gold Coast Mail Centre, Queensland 9726, Australia*

[b]*Department of Medicinal Chemistry, Monash University (Parkville Campus), 381 Royal Parade, Parkville, Victoria 3052, Australia*

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Abstract—^{1}H NMR spectroscopy has been used to investigate the transfer of sialic acid from sialic acid donor molecules to acceptor molecules using the *trans*-sialidase from *Typanosoma cruzi*. It is clearly demonstrated that NMR spectroscopy is an efficient and powerful means of monitoring the *trans*-sialidase promoted transfer of sialic acid from donor to acceptor.

The causative agent of Chagas disease, an incurable, debilitating disease that affects millions of people in Latin America, is the protozoal parasite *Trypanosoma cruzi*.[1] As a part of the infection process the parasite appears to require sialic acid.[2] To satisfy this requirement the parasite scavenges the acidic carbohydrate from exogenous cell surface glycoconjugates and subsequently transfers it to mucin-like acceptor molecules on the trypanosome surface.[3–5] To effect this transfer the parasite up-regulates the expression of the enzyme *trans*-sialidase in the highly infectious trypomastigote stage of its life cycle.[6] Both *trans*-sialidase and sialidase activities have been detected on the surface membrane of trypomastigotes of *T. cruzi* and have been implicated in the parasitic invasion of host cells.[2,7] However, it is considered that the transferase activity is more important than the sialidase activity of this enzyme.[4,7] Structurally, it has been proposed that the enzyme consists of three domains prior to a long C-terminal repeat sequence.[8] Whilst the three N-terminal domains are required for complete transferase activity, the first N-terminal domain by itself retains some transferase activity.[9]

The *trans*-sialidase from *T. cruzi* specifically catalyzes the transfer of α(2,3)-linked sialic acids.[4,5] Interestingly, whilst functionally resembling an α-2,3-sialyltransferase enzyme, the *trans*-sialidase from *T. cruzi* does not require nucleotide-bound sialic acid as the sialyl donor,[10] but rather an α-linked glycoside of sialic acid (Fig. 1). The sialyl acceptor for *T. cruzi trans*-sialidase must be a terminal β-galactoside unit.[4,5,11]

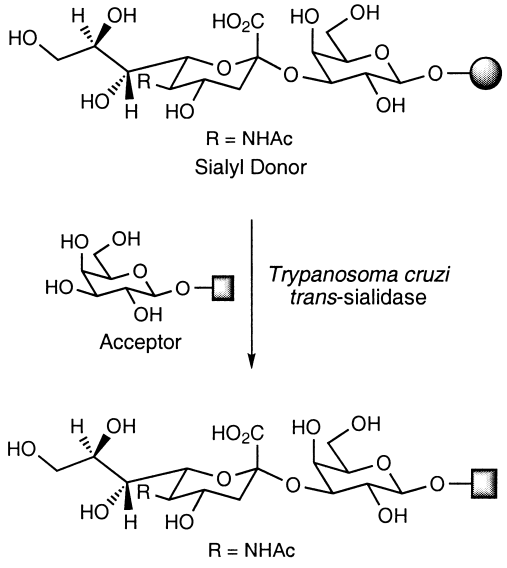

Figure 1. *Trypanosoma cruzi trans*-sialidase catalyzed sialic acid transfer.

*Corresponding author. Tel.: +61-7-5594-8051; fax: +61-7-5594-8098; e-mail: m.vonitzstein@mailbox.gu.edu.au

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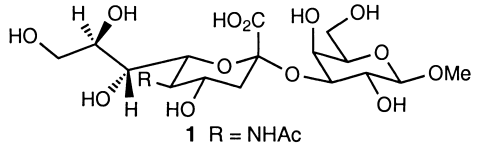
A recent investigation into the reason why the *trans*-sialidase from *T. cruzi* is more efficient in transferring rather than hydrolyzing terminal sialic acid residues centred on the crystal structure of the closely related sialidase from *T. rangeli*.[12] The two enzymes have over 70% sequence identity and comparison of the X-ray crystal structure of *T. rangeli* and a homology model of *T. cruzi* revealed similar three-dimensional structures in the catalytic and lectin domains.[12] Significant differences were however, noted between the active site of *T. rangeli* sialidase and that of *T. cruzi trans*-sialidase.

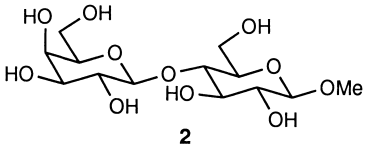
Another study has used NMR spectroscopy to investigate the sialidase activity of recombinant *T. cruzi trans*-sialidase.[13] The stereoselectivity of sialoside hydrolysis of two substrates, namely 4-methyl-umbelliferyl *N*-acetyl-neuraminoside and α(2,3)-sialyl-lactose, was investigated. The initial product of release in the case of both substrates was found to be the thermodynamically less stable α-anomer of sialic acid (Neu5Ac) that subsequently mutarotated to the β-anomer.[13] This is consistent with previous studies on other sialidases,[14–19] which have all been identified as retaining enzymes.

Since the sialic acid transfer functionality of *T. cruzi trans*-sialidase is believed to play the more significant role in the pathogenesis of this microorganism,[4,7] we decided to investigate the sialic acid transfer function of this enzyme. In line with our previous studies of other sialidases[17–19] and with the studies on the sialidase activity of *T. cruzi trans*-sialidase,[13] we chose to use ^{1}H NMR spectroscopy to investigate the transferase activity of this enzyme. To the best of our knowledge this is the first time that a *trans*-sialidase catalyzed sialic acid transfer reaction has been probed using NMR spectroscopy.

As shown in Figure 1, *T. cruzi trans*-sialidase utilizes a Neu5Acα(2,3)-galactoside based donor and an asialogalactoside as an acceptor. For the successful ^{1}H NMR analysis of the *T. cruzi trans*-sialidase catalyzed transfer of Neu5Ac from one galactoside to another, we needed to choose two galactosides that would appear different by ^{1}H NMR spectroscopy when they were linked in an α(2,3) manner to Neu5Ac. From a related synthetic project, we felt that the use of Neu5Acα(2,3)Galβ1Me (**1**)[20] as donor and methyl β-D-lactoside (**2**) as acceptor would be suitable for this investigation. However, it was first necessary to ascertain if the product of Neu5Ac transfer from **1** to **2**, namely Neu5Acα(2,3)Lacβ1Me (**3**), could be differentiated from **1** and **2** by ^{1}H NMR spectroscopy.



1 R = NHAc



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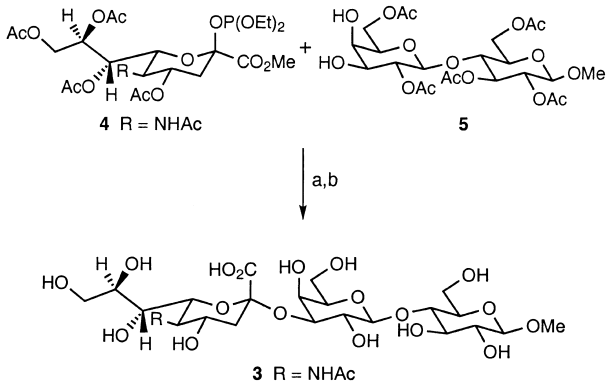
The target sialoside Neu5Acα(2,3)Lacβ1Me (**3**) was readily prepared by a similar method to that previously described for **1**.[20] Thus, TMSOTf-mediated coupling[21,22] between the sialosyl phosphite **4** and the lactoside **5**, gave the desired sialyllactoside **3** after deprotection (Scheme 1).



4 R = NHAc **5**

3 R = NHAc

Scheme 1. Reagents and Conditions: (a) **5** (2.5 equiv), THF, TMSOTf (0.2 equiv), 4 Å sieves, −40 to 0 °C, 3 h, 56%; (b) NaOMe then NaOH (1 M), 77%.

Each of the three possible components of the proposed *T. cruzi trans*-sialidase reaction, i.e. compounds **1**, **2** and **3**, were dissolved separately in deuterated phosphate buffered saline (PBS) (pH 7.4) and the ^{1}H NMR spectra recorded at 600 MHz and 25 °C. This spectroscopic examination revealed that each of the compounds could be readily identified from their respective anomeric proton resonances. This is displayed in Figure 2 which shows the anomeric regions (δ 4.2–4.7 ppm) of the ^{1}H NMR spectra of the sialyl donor **1** (Fig. 2a), the sialyl acceptor **2** (Fig. 2b) and the anticipated product **3** of Neu5Ac transfer (Fig. 2c). As is clearly shown, the presence of each of the three components of the transfer reaction can be easily monitored by examining the anomeric region of the ^{1}H NMR spectrum of a *trans*-sialidase catalyzed transfer reaction as it occurs.

With the appropriate sialyl donor and acceptor molecules in hand, our attention turned to the *trans*-sialidase from *T. cruzi*. Partially purified enzyme was obtained from the culture supernatants utilizing a sialic acid-based affinity chromatographic medium[23] as previously described.[24]

The ^{1}H NMR monitored *trans*-sialidase catalyzed transfer reactions were recorded in deuterated phosphate buffered saline (PBS) (pH 7.4) at 600 MHz and 25 °C. In a typical experiment, a mixture of the sialyl donor **1** and acceptor **2** (molar ratio 1:2) were dissolved in deuterated PBS and the ^{1}H NMR spectrum recorded ($t = 0$ min). *T. cruzi trans*-sialidase was then added to the mixture of donor and acceptor and ^{1}H NMR spectra recorded at regular intervals over a period of 2 days. As shown in Figure 2d, after 46 h it is evident that the transfer reaction is being successfully catalyzed by

T. cruzi trans-sialidase, albeit slowly. The appearance of an additional signal at δ 4.55 in the ^{1}H NMR spectrum of the reaction mixture is indicative of the formation of the product **3**. Additionally, the spectrum of the transfer reaction mixture (Fig. 2d) reveals a new peak at δ 4.34, which corresponds to the anomeric proton of methyl β-D-galactoside released from the sialyl donor.

The observation that the transfer reaction from the donor **1** to the acceptor **2** proceeds relatively slowly may well be a reflection of the production of methyl β-D-galactoside during the course of the transfer reaction. Methyl β-D-galactoside has been reported[11] to inhibit *T. cruzi trans*-sialidase. Alternatively, the slow transfer may be due to the preference of *T. cruzi trans*-sialidase for a donor that is larger than a simple disaccharide such as **1**. In an attempt to explore this issue further, we thought it of value to employ a different donor/acceptor system. Accordingly, we chose to use α(2,3)-sialyllactose (**6**)[25] as the sialyl donor and *N*-acetyllactosamine (**7**)[26] as the acceptor, since their respective ^{1}H NMR spectra show distinct resonances for the anomeric protons.

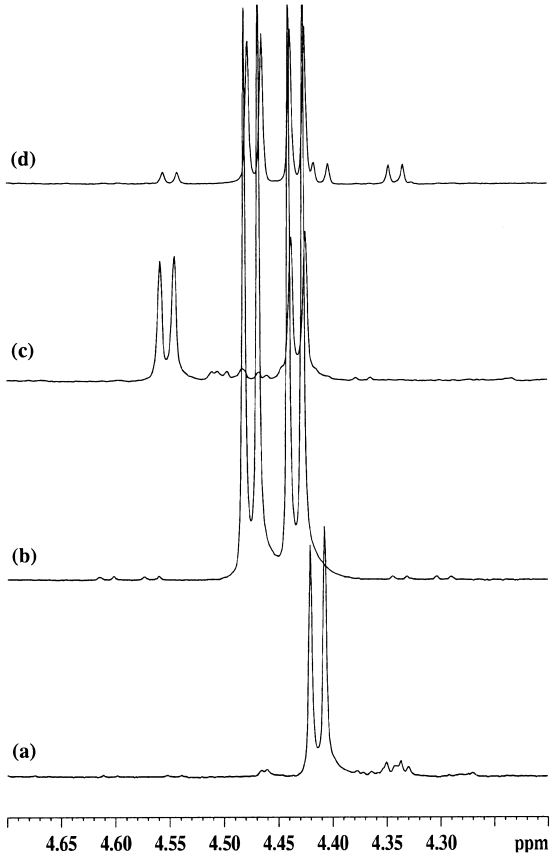
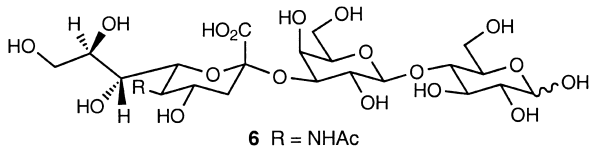
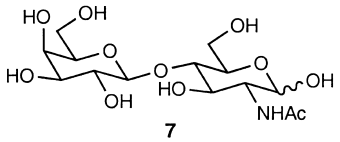


Figure 2. Anomeric region of the 600 MHz ^{1}H NMR spectra of: (a) Neu5Acα(2,3)GalβMe (**1**) (donor); (b) methyl β-D-lactoside (**2**) (acceptor); (c) Neu5Acα(2,3)LacβMe (**3**) (product); and (d) *trans*-sialidase catalyzed transfer reaction mixture after 46 h.



6 R = NHAc



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Thus, in an analogous manner to that described above, a mixture of α(2,3)-sialyllactose (**6**) as donor and *N*-acetyllactosamine (**7**) as acceptor (molar ratio of 1:2) was exposed to *T. cruzi trans*-sialidase, and the progress of the reaction monitored by ^{1}H NMR spectroscopy. New peaks in the spectrum appeared at δ 4.45 and δ 4.55, being entirely consistent with the formation of δ(2,3)-sialyllactosamine. Interestingly, as with the previous donor/acceptor system employed, this transfer reaction was also relatively slow.

The work described herein has clearly demonstrated the effectiveness of using ^{1}H NMR spectroscopy to examine in situ the *trans*-sialidase catalyzed transfer of Neu5Ac from donor to acceptor molecules. Although at this stage it remains to be established what the optimum sialyl donor and acceptor molecules are for such experiments, there is a clear indication that this technique can provide extremely useful information regarding *trans*-sialidase catalyzed sialic acid transfer reactions. We are presently exploring some of these possibilities.

Acknowledgements

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26. *N*-Acetyllactosamine was purchased from Fluka (#01240) and used without further purification.