Communications to the Editor

Combined Use of *trans*-Sialidase and Sialyltransferase for Enzymatic Synthesis of αNeuAc2→3βGal-OR

Yukishige Ito† and James C. Paulson*

CYTEL Corporation and Department of Chemistry
The Scripps Research Institute
3525 John Hopkins Court
San Diego, California 92121

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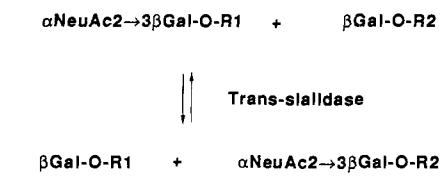
In the application of enzymes to the field of synthetic carbohydrate chemistry,[1] the use of sialyltransferase[2] for enzymatic sialylation is recognized to offer advantages over chemical methods[3] due to its virtually complete stereoselectivity and linkage specificity.[4] However, a major drawback of enzymatic sialylation in general is the rather strict acceptor substrate specificity of these enzymes, which allows the synthesis of a limited number of sialoside sequences. We report here a novel enzymatic method for introducing sialic acid (NeuAc) that addresses this limitation and is widely applicable to the synthesis of glycan chains containing the terminal NeuAcα2→3Gal sequence.

This method takes advantage of a newly described *trans*-sialidase enzyme from *Trypanosoma cruzi*,[5] which has the unique property of catalyzing the reversible transfer of NeuAc from a donor substrate of the sequence NeuAcα2→3βGal-O-R1 to virtually any galactoside acceptor βGal-O-R2 to yield a new product (NeuAcα2→3Gal-O-R2) (eq 1).[6] The primary limitation



in the use of this enzyme for synthetic purposes is that the desired product is produced at the expense of another sialoside used as the donor substrate. In addition, since the transfer of NeuAc is a reversible process, it is difficult to drive the equilibrium in favor of the desired sialoside (NeuAcα2→3βGal-O-R2). To overcome these limitations, we have devised a catalytic *in situ* generation of a sialoside donor substrate (e.g., **1**) using an α-2,3-sialyltransferase to transfer free NeuAc to its precursor galactoside acceptor (e.g., **2**) via the catalytic *in situ* regeneration of CMP-NeuAc according to Ichikawa et al.[2f] The stoichiometric substrates of this enzymatic system are free NeuAc, phosphoenolpyruvate (PEP), and most any galactoside acceptor substrate (e.g., **3**) utilized by the *trans*-sialidase (see Scheme I).

As an example of this reaction, we have prepared the simple sialoside **4**, which represents the oligosaccharide moiety of ganglioside GM4. This simple target compound was chosen to illustrate the utility of this system for two reasons. First, the corresponding galactoside **3**[7] is a poor substrate of all known α-2,3-sialyltransferases and cannot be efficiently sialylated using these enzymes. Second, after protection of hydroxy and carboxylate groups and selective cleavage of 2-(trimethylsilyl)ethyl glycoside, the sialoside **4** is readily converted to a disaccharide glycosyl donor that can be used in the block chemical synthesis of ganglioside GM4[8] and more complex sialoside,[9] as reported by others.

The reaction was performed as follows. A mixture of compound **3** (10.5 μmol), NeuAc (10.5 μmol), lacto-*N*-tetraose[10] (**1a**; 1.0 μmol), CMP (0.9 μmol), ATP (0.09 μmol), phosphoenolpyruvate trisodium salt (46 μmol), $MgCl_2$ (10.5 μmol), $MnCl_2$ (3 μmol), KCl (10.5 μmol), BSA (5%; 5 μL), mercaptoethanol (0.03 μL), myokinase (15 units), pyruvate kinase (25 units), inorganic pyrophosphatase (1.6 units), βGal1,3/4GlcNAc α-2,3-sialyltransferase[11] (16 munits), *T. cruzi trans*-sialidase (7 munits[12]), and CMP-NeuAc synthetase[13] (80 munits) in 200 mM HEPES buffer (pH 7.4; 1.0 mL) was incubated at room temperature for 4 days. The mixture was passed through Sep-Pack C_{18} cartridge (Waters). The cartridge was washed with 0.1 M NH_4HCO_3 and eluted with 50% MeOH. Fractions containing the product were collected and purified by a column of Bio-Gel P2 (0.1 M NH_4HCO_3) to afford compound **4**[14] (3.9 mg, 65%). The same transformation could be achieved in a comparable efficiency by using a different sialyltransferase (βGal1,3GalNAc α-2,3-sialyltransferase[15]) and its prefered galactoside acceptor (**1b**).

The results demonstrate the synthetic potential of the *T. cruzi trans*-sialidase. The multienzyme system can be viewed as an extension of the acceptor substrate specificity of sialyltransferases. Due to the broad specificity of the *trans*-sialidase, many naturally occurring αNeuAc2→3Gal-OR sequences can be synthesized by

* Address correspondence to this author at CYTEL Corporation.
† On leave from The Institute of Physical and Chemical Research (RIKEN), Japan.

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(14) ^1H NMR (D_2O, 300 MHz): δ 4.44 (d, J = 7 Hz, H-1*Gal*), 4.04 (dd, J = 10 and 3 Hz, H-3*Gal*), 3.91 (d, $J \approx 3$ Hz, H-4*Gal*), 3.48 (dd, J = 10 and 7 Hz, H-2*Gal*), 2.72 (dd, J = 12 and 4 Hz, H-3eq*NeuAc*), 2.00 (s, NAc), 1.77 (t, J = 12 Hz, H-3ax*NeuAc*). ^{13}C NMR (D_2O, 75 MHz): δ 175.4, 174.3, 102.3 (C-1*Gal*), 100.3 (C-2*NeuAc*), 76.4 (C-3*Gal*), 75.3, 73.3, 72.2, 69.5, 68.8, 68.7, 68.5, 67.9, 63.0, 61.3, 52.1 (C-5*NeuAc*), 40.1, 22.5 ($CO\mathit{CH_3}$), 18.0 ($\mathit{CH_2}SiMe_3$), −2.1 ($SiMe_3$).

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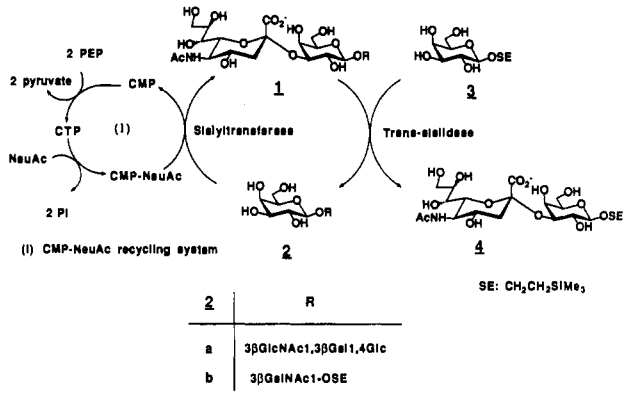
Scheme I



2	R
a	3βGlcNAc1,3βGal1,4Glc
b	3βGalNAc1-OSE

substituting different galactoside acceptor substrates. The other advantage of this multienzyme system is that the equilibrium of the *trans*-sialidase is shifted toward product formation by the sialyltransferase cycle. Since large-scale preparation of both sialyltransferase and CMP-NeuAc synthetase are now possible,[16] further improvement of efficiency should be possible once larger-scale preparation of a recombinant *trans*-sialidase is established.

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Supplementary Material Available: 300-MHz ^{1}H NMR and 75-MHz ^{13}C NMR spectra of **4** (3 pages). Ordering information is given on any current masthead page.

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