Sialidase-catalyzed transsialylation using polymer-supported solution-phase techniques†‡

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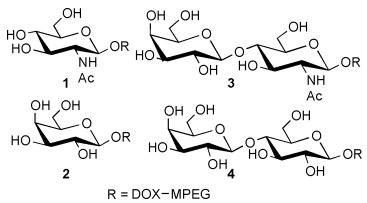
Received (in Cambridge, UK) 17th May 2000, Accepted 22nd August 2000
First published as an Advance Article on the web 18th September 2000

By combination of glycosidase-catalyzed transglycosylation with polyethylene glycol ω-monomethyl ether (MPEG; mw 5000) based solution phase synthesis, various terminally sialylated di- and trisaccharides were synthesized.

Sialyl oligosaccharides are involved in many diseases and function as the receptor determinants for viruses, lectins, toxins and certain tumor-specific antibodies.[1,2] To further investigate the biological functions of these components efficient methodologies for their synthesis are needed.

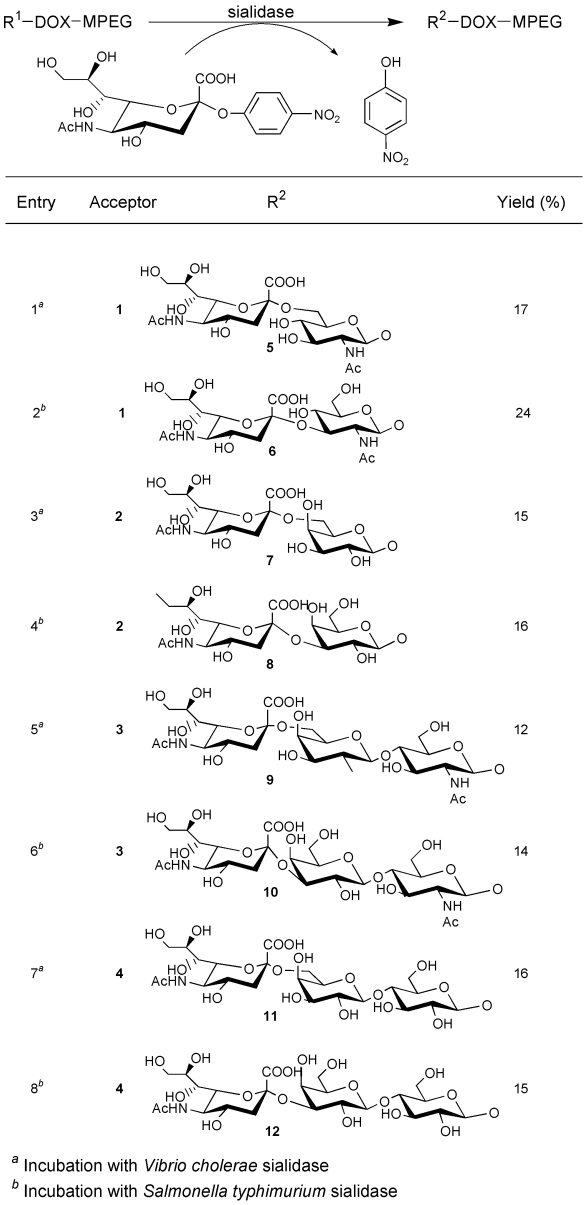
It has been demonstrated that sialidases are effective sialylation tools[3–5] supplying the stereoselective efficiency of a sialyltransferase while avoiding its complex donor-regeneration cycles and the need for intermediate protection and deprotection sequences of classical chemical procedures. Limitations in efficiency of classical chemical and enzymatic glycosylation methods resulting from time-consuming work-up and purification procedures after each glycosylation step can be overcome by application of polymer-supported solution-phase techniques which previously have been shown to be powerful tools in classical chemical synthesis.[6] Whereas solid-phase techniques suffer from various problems due to the heterogenous nature of reactions on the insoluble resin, soluble polymer supports enable standard solution-based reaction conditions to be used while preserving the possibility of exploiting the macromolecular properties of the resin in the purification steps. Combining the glycosidase-catalyzed transglycosylation technique with solid-phase supported work-up procedures, the terminally α(2-3)- and α(2-6)-sialylated oligosaccharides **5**–**12** (Scheme 1) were synthesized from the precursors **1**–**4** employing MPEG as polymeric support using a procedure based on the solubility of the polymer under reaction conditions and its insolubility during work-up in various ethers.[7]† In order to facilitate final cleavage, an α,α′-dioxyxylyl linker (DOX) is introduced.[8] Thus, glycosylation can be performed in the aqueous solution phase whereas work-up is performed in the solid phase.



Essentially, all reactions proceed following the same sequence: TRANSSIALYLATION → CODISTILLATION (with toluene) → REDISSOLUTION (in dichloromethane) → PRECIPITATION (with *tert*-butyl methyl ether) → FILTRATION → RECRYSTALLIZATION (from ethanol).

Employing incubation conditions broadly identical to standard procedures for transsialylation the oligosaccharides **5**–**12** were obtained in yields corresponding to non-polymer-supported acceptor molecules.[5]

As sialidases can display a variety of regioselectivities depending on their biological source, extreme care has to be taken in order to achieve product formation of a single regioisomer. Among a variety of sialidases that not only display the necessary regioselectivity but are also commercially available in sufficient quantitity and purity the sialidases from *Vibrio cholerae* and *Salmonella typhimurium* proved to be the most suitable transsialylation catalysts.§ While the sialidase



Scheme 1 Chemoenzymatic transsialylation of **1**–**4** using *Vibrio cholerae* and *Salmonella typhimurium* sialidase.

† Electronic supplementary information (ESI) available: Further experimental data. See http://www.rsc.org/suppdata/cc/b0/b003943h/

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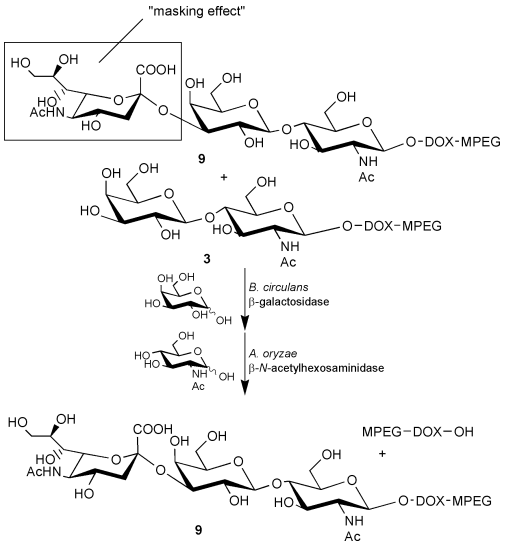
from *V. cholerae* preferentially catalyzes transsialylation in the α(2-6)-position the sialidase from *S. typhimurium* displays a pronounced regioselectivity for the α(2-3)-directed sialylation.

Sialylation of the monosaccharide acceptors **1** and **2** afforded slightly higher yields than the respective disaccharide conjugates **3** and **4**. The relatively high yield of 24% for **6** was achieved by twofold repetition of the transsialylation after intermediate work-up, giving **6** in 13% after the first and 21% yield after the second cycle. Unfortunately, only in this one case could the yield be raised considerably. Increases in yields of 2–3% after renewed incubation did not justify repeated transsialylation cycles for the other acceptor molecules. Instead it proved to be more profitable to run the same reaction in several small batches. The reactions could be scaled up to 100 μmol of product oligosaccharide without great loss in yields. In all instances described, a completely regiospecific transfer of *N*-acetylneuraminic acid could be achieved by careful monitoring of the reaction course by ^{1}H NMR spectroscopy. Regioisomeric transfer ratios were determined on-resin by integration of the axial and equatorial H-3 protons of neuraminic acid, applying the structural-reporter group concept.[9] The shifts of the *N*-acetylneuraminic acid H-3 protons are not only located outside the bulk signal region but also contain characteristic information about the type and linkage position of the glycosidically coupled saccharide. In some cases incubations had to be interrupted before maximum overall product formation was achieved in order to obtain a single regioisomer.

Even with an acceptor turnover rate of 10–25%, 75–90% of unreacted polymer-bound acceptor is still present. Cleavage from the resin at this stage would require an additional chromatographic step. This can be avoided by exploiting the capability of *exo*-glycosidases to stereoselectively hydrolyze specific terminal glycosyl units while leaving internal ones intact. Treatment of **9** with β-galactosidase from *Bacillus circulans* and β-*N*-acetylhexosaminidase from *Aspergillus oryzae* using standard incubation protocols yields MPEG-bound sialyllactosamine while free galactose and *N*-acetylglucosamine are removed as above by precipitation and filtration (Scheme 2). Owing to similar incubation conditions, the reaction in this case can be carried out as a one-pot reaction.

Prior to the cleavage of the product from the polymer, the carboxy function of the sialyl unit is protected by a methyl ester using standard procedures. Final cleavage of the product is achieved by application of a recently published method using scandium(III) trifluoromethanesulfonate and acetic anhydride,[10] which results in cleavage of the C–O bond between the dioxyxylyl unit and the terminal oxygen of the MPEG. Furthermore, all free hydroxy groups of the oligosaccharide are acetylated, which facilitates chromatographic purification of the product by flash chromatography.



Scheme 2 Down-trimming of the polymer.

In conclusion, we have demonstrated for the first time a combination of glycosidase-catalyzed transglycosylation methodology with polymer-supported solution-phase techniques and have been able to synthesize various terminally α(2-3)- and α(2-6)-sialylated di- and trisaccharides, thus, providing a simple and useful method for the synthesis of heterooligosaccharides.

Notes and references

‡ *Transsialylation assay using V. cholerae sialidase.* Solutions of *p*Np-Neu5Ac (21 mg, 50 μmol) and acceptor (120–160 mg, 25 μmol, donor–acceptor ratio 2 : 1) in degassed incubation buffer (150 μl, 0.1 M NaOAc, 0.5 mM $CaCl_2$, pH5.5) are incubated with *V. cholerae* sialidase (1 U) at 30 °C. Compounds **5** (24 mg, 17%), **7** (21 mg, 15 %), **9** (17 mg, 12 %) and **11** (22 mg, 16%) were obtained as white powders. The amounts refer to the calculated absolute amounts of polymer-bound product, whereas the percentages are relative to polymer-bound acceptor units.

Transsialylation assay using S. typhimurium sialidase. Solutions of *p*Np-Neu5Ac (21 mg, 50 μmol) and acceptor (110–150 mg, 20 μmol, donor–acceptor ratio 2.5 : 1) in degassed incubation buffer (150 μl, 0.1 M NaOAc, pH 5.1) are incubated with *S. typhimurium* sialidase (1 U) at 30 °C. Compounds **6** (34 mg, 24%), **8** (23 mg, 16%), **10** (21 mg, 14%) and **12** (23 mg, 15%) were obtained as amorphous solids.

General procedure. The course of the reaction is followed photometrically at 400 nm and by TLC (EtOH–1 M ammonium acetate (pH 7.4) 5 : 1) using resorcinol spraying reagent for *N*-acetylneuraminic acid detection. The incubation is terminated by addition of 0.1 M Na_2CO_3 (1 ml) followed by ultrasonification for 3 min. The mixture is acidified to pH 6.8 with Dowex 50WX8 H^+ cation exchanger and centrifuged. The supernatant is decanted and codistilled twice with toluene (10 ml). The residue is taken up in dry dichloromethane (10 ml) and precipitated with *tert*-butyl methyl ether (200 ml). The precipitate is filtered, rinsed with *tert*-butyl methyl ether (50 ml) and recrystallized from EtOH.

Cleavage of the product from the resin exemplified for compound **3**. 160 mg of polymer-bound trisaccharide **11** (0.032 mmol) is dissolved in dry dichloromethane (1.5 ml). Acetic anhydride (1.5 ml) and scandium(III) trifluoromethanesulfonate [$Sc(OTf)_3$, 10 mg, 0.02 mmol] are added sequentially. After stirring under argon for 6 h the mixture is cooled to 0 °C in an ice bath and *tert*-butyl methyl ether (150 ml) is added to precipitate the cleaved polymer. The filtrate is concentrated to dryness. The residue is suspended in dry MeOH (10 ml) and 100 mg cation exchange resin (DOWEX 50W-X8 H^+) is added. After stirring for 6 h at rt the resin is filtered off. The filtrate is concentrated and purified by flash chromatography (petrol ether–ethyl acetate 3 : 1 to 1 : 1) to yield *p*-*O*-acetyl-[DOX]yl [methyl(5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosyl)onate]-(2-3)-*O*-(2,3,4-tri-*O*-acetyl-β-D-galactopyranosyl)-(1-4)-*O*-2,3,6-tri-*O*-acetyl-*β*-D-glucopyranoside (1.8 mg, 14% relative to polymer bound **4**, 88% relative to **11**).

§ Sialidase (E.C. 3.2.1.18) from *V. cholerae* was a kind gift from Chiron Behring GmbH, Germany. Recombinant sialidase (E.C.3.2.1.18) from *S. typhimurium* was purchased from New England Biolabs GmbH, Germany. 2-*O*-(*p*-Nitrophenyl)-5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-nonulopyranusonic acid (*p*Np-Neu5Ac) was prepared by the method of Rothermel *et al.*[11] Acceptor **1** was prepared by a procedure developed by Hodosi *et al.*[12]

For further experimental detail see ESI†.

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