Palladium-catalysed ^{3}H-labelling of Neu5AcOMe and spacered 3′-sialyllactose

Valerii P. Shevchenko,[a] Igor Yu. Nagaev,[a] Nikolai F. Myasoedov,[a] Aleksandr B. Tuzikov[b] and Nicolai V. Bovin*[b]

[a] *Institute of Molecular Genetics, Russian Academy of Sciences, 123182 Moscow, Russian Federation*
[b] *M. M. Shemyakin–Yu. A. Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, 117997 Moscow, Russian Federation. Fax: +7 095 330 5592; e-mail: bovin@carb.siobc.ras.ru*

10.1070/MC2002v012n06ABEH001654

Randomly ^{3}H-substituted Neu5AcαOMe and Neu5Acα2-3Galβ1-4Glc-NHCOCH$_2$NH$_2$ were synthesised from 'cold' components using catalytic solid-phase exchange under the following conditions: tritium gas, Pd/CaCO$_3$, 140–160 °C.

A common method of tritium insertion into sialoglycoconjugates involves periodate oxidation of the glycerol site of sialic acid and further reduction with sodium borotritide.[1] The method is simple, easy to use, and reproducible; however, periodate oxidation results in truncating sialic acid into C8 and C7 derivatives. Undoubtedly, the method of tritium insertion is useful for studying well-characterised sialidase by labelled sialosides, whereupon it is known that the oligosaccharide with the truncated sialic acid remains an enzyme substrate. However, when studying a new enzyme with unknown specificity and for which the significance of interaction with the C8–C9 fragment of the sialic acid residue is still unclear, an alternative non-destructive labelling method proves necessary. We faced this problem upon research into the donor and acceptor specificity of human *trans*-sialidase.[2] Aiming to synthesise full-length (C9) sialosides we used the catalytic solid-phase exchange, when tritium replaces nonspecifically the C-bound hydrogen atoms under the action of a metal catalyst at elevated temperatures.[3] The approach was successfully used for labelling peptides, nucleic acids and proteins.[4]

Neu5Ac α-methyl glycoside sodium salt was labelled as described below. A tube containing 8 mg of the glycoside dissolved in 0.2 ml of water and 200 mg of Pd/BaSO$_4$ (or Pd/CaCO$_3$) was frozen using liquid nitrogen, and water was removed in a vacuum. The tube was filled with tritium gas, which was then sealed and kept for 5–40 min at different temperatures ranging from 140 to 220 °C (Table 1). At 140 °C and a reaction time of 40 min, the level of H→T isotope exchange constituted 16%, the isolated glycoside yield was 95%, while at 200 °C and a reaction time of 5 min the yield was 25% (in this case, 86% substitution was achieved). Using acid hydrolysis, free labelled Neu5Ac can be obtained from methyl sialoside for further studies or transformations into more complex derivatives.

For the preparative labelling of spacered trisaccharide Neu5Acα2-3Galβ1-4Glc-NHCOCH$_2$NH$_2$ (3′SL-NHGly),[5] milder reaction conditions were used, compared to a monosaccharide synthesis, because the substance rapidly degraded at a temperature over 160 °C. The tube was filled with 3′SL-NHGly[6] (4 mg) applied to 5% Pd/CaCO$_3$ as described above and evacuated up to 0.1 Pa. It was then filled with tritium gas under a pressure of 0.33×10^5 Pa and kept at 160 °C for 20 min. The excess of tritium was removed by evacuation; the cooled reaction products were dissolved in water (6 ml), the catalyst was filtered off, and the filtrate was evaporated several times with water to remove the exchangeable tritium. The residue was purified by thin-layer chromatography on silica gel plates (Merck 572) with a methanol–acetonitrile-water (3:2:2) eluent. The spot containing trisaccharide (R_f 0.47) was cut out, and the substance was eluted from silica gel with 5 ml of aqueous methanol (9:1). The final purification was performed by HPLC on a Separon NH$_2$ column (7 μm, 3×150 mm) using an acetonitrile–water–trifluoroacetic acid (80:20:0.08) eluent. The yield of trisaccharide (with radiochemical purity > 95%) was 10–15%, its specific radioactivity was equal to 8–9 Ci mmol^{-1}.

After the dilution of 3′SL-NHGly with cold trisaccharide, it was coupled to polyacrylamide or to biotin-labelled polyacrylamide according to a previously published procedure,[6] which resulted in 3′SL-PAA or 3′SL-PAA-biot with 20 mol% saccharide and 5 mol% biotin. The latter preparation was quantitatively conjugated to streptavidine-Sepharose and then used in a solid-phase assay of human *trans*-sialidase; 3′SL-PAA and a free trisaccharide were also employed as glycoside donors for studying the specificity of the given enzyme.

Table 1 Influence of temperature and the nature of the catalyst on the yield and relative specific radioactivity of Neu5Ac α-methyl glycoside sodium salt.

Catalyst	*T*/°C	Reaction time/min	Yield (%)	*A*[a] (%)
5% Pd/BaSO$_4$	140	40	92	13
	180	5	75	39
5% Pd/CaCO$_3$	140	40	95	16
	160	25	91	22
	180	5	74	46
	200	5	25	86
	220	5	8	100

[a]Relative specific radioactivity (maximum value at 220 °C in this series is taken as 100%).

Thus, the catalytic solid-phase exchange of tritium proves suitable to obtain labelled sialylated saccharides with a high specific radioactivity.

References

1 R. W. Veh, A. P. Corfield, M. Sander and R. Schauer, *Biochem. Biophys. Acta*, 1977, **486**, 145.
2 V. V. Tertov, V. V. Kaplun, I. A. Sobenin, E. Yu. Boytsova, N. V. Bovin and A. N. Orekhov, *Atherosclerosis*, 2001, **159**, 103.
3 N. F. Myasoedov, *J. Label. Comp. Radiopharm.*, 1993, **33**, 391.
4 V. P. Shevchenko, I. Yu. Nagaev and N. F. Myasoedov, *Usp. Khim.*, 1999, **68**, 944 (*Russ. Chem. Rev.*, 1999, **68**, 859).
5 A. B. Tuzikov, A. S. Gambaryan, L. R. Juneja and N. V. Bovin, *J. Carbohydr. Chem.*, 2000, **19**, 1191.
6 N. V. Bovin, E. Yu. Korchagina, T. V. Zemlyanukhina, N. E. Byramova, O. E. Galanina, A. E. Zemlyakov, A. E. Ivanov, V. P. Zubov and L. V. Mochalova, *Glycoconjugate J.*, 1993, **10**, 142.

Received: 31st July 2002; Com. 02/1981