*Carbohydrate Research*, 68 (1979) C17–C20

## Preliminary communication

# Covalent, nitroxide spin-labelling of carbohydrates *via* *s*-triazine residues

MICHAEL J. ADAM* and LAURANCE D. HALL
*Department of Chemistry, The University of British Columbia, Vancouver, B C V6T 1W5 (Canada)*
(Received September 18th, 1978; accepted for publication, October 30th, 1978)

A fundamental prerequisite to all spin-labelling studies of biologically important materials is access to reagents by use of which the label can conveniently be attached, *via* a stable, covalent linkage, to some predetermined locus in the material of interest. Although many reagents suitable for the spin-labelling of proteins and lipids are available[1], there is a general paucity[2] of technology for labelling carbohydrates. In the present report, we draw attention to the use of *s*-triazine trichloride (**1**) as a vehicle[3,4] for attaching either mono- or bi-radicals to monosaccharides *via* *O*-, *S*-, or *N*-linkages in either aqueous or nonaqueous media, and to polysaccharides in aqueous media. This approach has substantial historical precedent, as it has been widely used in the dyestuffs industry[3] since 1956 for dyeing cotton

The spin-labelling reagents** **2** (m p. 108–109°), **3** (m p. 195°, dec.), and **4** (m p. 194–195°) are readily prepared by reaction of **1** with 4-hydroxy-2,2,6,6-tetramethylpiperidin-1-oxyl (**5**) or 4-amino-2,2,6,6-tetramethylpiperidin-1-oxyl (**6**); although the yields are low, the products are isolated directly from the reaction mixture in analytically pure form. Thus, **2** is obtained in 16% overall yield by reaction of **1** (1.8 g, 9.8 mmol) with **5** (2 g, 11.6 mmol), and the biradical **4** in 10% yield from a similar reaction using two equivalents of **5**. The synthesis of the amine spin-label (sl) **3** had already been reported[4], but that now described is far simpler: **1** (0 5 g, 2.7 mmol) and **6** (0.4 g, 2.7 mmol) are stirred in an ice-cold solution of sodium hydrogencarbonate in aqueous acetone for 30 min. The resultant, orange precipitate is filtered off, and washed with water, to give **3** (75% yield) as analytically pure material.

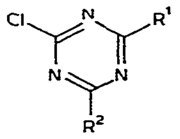
Derivatives **2**, **3**, and **4** are all stable in the crystalline state, and are essentially insoluble in water, but soluble in acetone, benzene, chloroform, and other organic solvents.

The chlorine substituents in **2**, **3**, and **4** can be readily displaced by nucleophiles at low temperature by use of a variety of solvents and base acceptors. The following combinations of solvent and base acceptors were used in this study; aqueous acetone with sodium hydrogencarbonate or sodium hydroxide, acetonitrile with anhydrous, solid sodium carbonate, and benzene with solid sodium hydroxide.

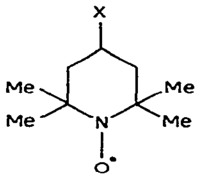
---

*Holder of a National Research Council of Canada Postgraduate Scholarship, 1977–1979.

**All compounds reported here had elemental microanalyses and appropriate spectroscopic parameters in entire accord with the structures assigned



1 R¹ = R² = Cl
2 R¹ = Cl ; R² = OSl
3 R¹ = Cl , R² = NHSl
4 R¹ = R² = OSl



5 X = OH (OSl)
6 X = $NH_2$ (NHSl)

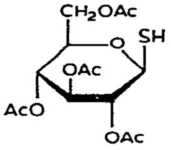
OSl = O spin-label, NHSl = N spin-label

In general, there is no difficulty in effecting monosubstitution, and invariably, the oxygen-linked derivative **2** reacts more rapidly than its NH counterpart **3**, reflecting the fact that the oxygen substituent does not have the same deactivating effect as the amine substituent. For example, reaction of the protected 2-amino-2-deoxy-β-D-glucoside **15** with **2** in acetonitrile and sodium carbonate overnight at room temperature affords **12**, m.p. 90°, $[\alpha]_D^{22}$ −39° (*c* 1, $CHCl_3$) in 53% yield, whereas all attempts to synthesize an analogous compound by use of the nitrogen-linked derivative **3** failed. Synthesis of the two hexylamine derivatives **16** and **17** also reflects this difference; thus, compound **16** (a syrup) was prepared in 70% yield by reaction of **2** with hexylamine in aqueous acetone containing sodium hydrogencarbonate during 20 min at room temperature, whereas the synthesis (80% yield) of compound **17** (m.p. 160°) required 20 min at 45° in the same mixture. As the thio sugar derivatives **9** and **11**, and **8** and **10**, were prepared in different solvents and base acceptors, the relative reactivity of reagents **2** and **3** in these cases cannot be directly compared

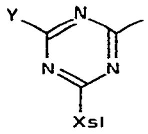
Reaction of the 1-thio sugar **7** with **2** in acetonitrile and sodium carbonate overnight at room temperature afforded **8** (m.p. 85°), $[\alpha]_D^{22}$ −0.5° (*c* 6, $CHCl_3$), in 57% yield, whereas the thio derivative **9** (m.p. 105°), $[\alpha]_D^{22}$ +3.4° (*c* 5, $CHCl_3$), was prepared in 72% yield in aqueous acetone and sodium carbonate during 20 min at room temperature. The bis derivative **10** {m.p. 110°, $[\alpha]_D^{22}$ +5.5° (*c* 1.8, $CHCl_3$)} and **11** {m.p. 108°, $[\alpha]_D^{22}$ +6.6° (*c* 5, $CHCl_3$) were respectively obtained in 78 and 85% yield after reaction for ~18 h in the same solvents and at the same temperatures as their monosubstituted counterparts.

Although reactions with most of the simple, alkylamine nucleophiles occur very readily (*cf.*, the preparation of **3**), the reactivity can vary over wide limits, and, unfortunately, the amino groups of amino sugars often have very low reactivity. For example, although 2-amino-2-deoxy-α,β-D-glucopyranose reacts[6] readily with **1** to give **13**, it gives no detectable product with **3**.

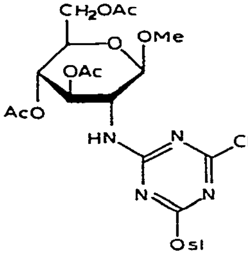
The triazine moiety can serve as a "trivalent locus" for joining together a sugar, a spin label, and an alkyl group, and this is exemplified by the preparation of **14** {m.p. 170°, $[\alpha]_D^{22}$ −5.50° (*c* 1.4, $CHCl_3$)} in 40 %overall yield by reaction of the *O*-linked derivative



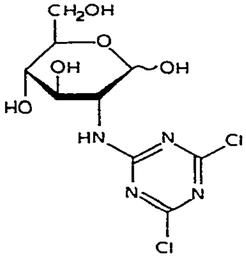
7 (= SGlc)



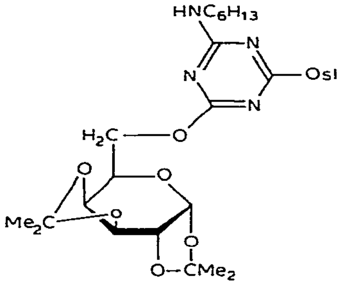
8 X = O ; Y = Cl
9 X = N , Y = Cl
10 X = O  Y = SGlc
11 X = NH , Y = SGlc



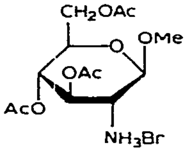
12



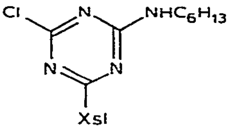
13



14



15



16 X = O
17 X = NH

with 1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose, followed, without isolation, by reaction with hexylamine.

The principal use for these triazine reagents is in the spin-labelling of polysaccharides, readily effected for either **2**, **3**, or **4** by using aqueous sodium hydroxide as the reaction medium. The products from insoluble polysaccharides are subsequently washed with acetone and water, to remove the excess of reagent and adsorbed spin-label, whereas those from the soluble ones, with the exception of starch, are repeatedly precipitated with acetone, filtered off, and redissolved in water. The starch sample was purified by passing it through a column of Sephadex LH-20. By using this procedure, we have labelled the following substances; e.p.r. integration provides a facile measure of the degree of substitution, and the data are given in parentheses as the number of sugar units per spin-label residue: Cellulose (Whatman CF-11 powder, from W. & R. Balston, Ltd., U.K.), reagent 2 (8,800), reagent 3 (11,200), reagent 4 (20,600); Sephadex G50 coarse (from Pharmacia Fine

Chemicals, AB, Uppsala, Sweden), reagent 2 (2700); reagent 3 (4200), reagent 4 (2100), xanthan gum (from Kelco, 8355 Aero Drive, San Diego, California 92123), reagent 2 (1900); agarose [(SKA-ME 11335) Marine Colloids, Rockland, Maine 04841], reagent 2 (4800); guar gum (Kelco), reagent 2 (900).

In summary, we consider that reagents 2, 3, and 4 reported here have substantial potential for the spin-labelling of carbohydrates ranging from mono- to poly-saccharides. Additionally, we draw attention to the novel use of the triazinyl residue as a trivalent locus for the general derivatization of sugars. It is also worth noting that this reactivity extends to many other substrates; for example, we have successfully labelled aluminum oxide (Brockman activity 1) *via* 2 at a level of 1.3 × $10^{19}$ spins/g, and bovine serum albumin (B.S.A.) *via* 2 in a level of 12 spins per B.S.A. molecule. The chemical insight obtained here extends to the attachment of other types of labels and substituents to many naturally occurring carbohydrates; noteworthy in this regard is the low, overall cost of the reagents and the simplicity of the operations.

## ACKNOWLEDGMENTS

This work was supported by the National Research Council of Canada *via* operation grants (A1905, to L.D.H.) and a Postgraduate Scholarship (to M.A.).

## REFERENCES

1 E. G. Rosantzev, *Free Nitroxyl Radicals*, H Ulrich (translator and Ed.), Plenum, London, 1970; B J. Gaffney, in L. J. Berliner, Jr. (Ed.), *Spin Labelling, Theory and Applications*, Academic Press, New York, 1976, p. 184.

2 D. Gagnaire and L Odier, *Bull. Soc. Chim Fr.*, (1974) 2325–2328; J. M J. Tronchet, J. Ohja-Poncet, E. Winter-Mihaly, and B. Kohler, *Helv. Chim. Acta*, 60 (1977) 888–891, J. D. Aplin and L. D Hall, *J. Am Chem. Soc.*, 99 (1977) 4162–4163; *Carbohydr. Res.*, 59 (1977) C20–C24; *J. Am. Chem Soc.*, 100 (1978) 1934–1936.

3 R. L. M. Allen, *Colour Chemistry*, Appleton–Century–Crofts, New York, 1971.

4 G. I. Likhtenshtein and P. K. H. Bobodzhanov, *Biofizika*, 14 (1969) 783–785, G. I. Likhtenshtein, *Mol. Biol. (Moscow)*, 2 (1968) 193–198, G. I Likhtenshtein, Yu. B Grebenshchikov, P. K. H. Bobodzhanov, and Yu V. Kokhonov, *ibid*, 4 (1968) 550–558, R. M. Marupov, P. Ky. Bobjanov, N. V. Kostina, and A. B. Shapiro, *Biofizika*, 21 (1976) 848–851.

5 C T. Bishop and A. S. Chaudhari, *Can. J. Chem*, 50 (1972) 1987–1991.