

## Preliminary communication

### Synthesis of luminescent probe—sugar conjugates of either protected or unprotected sugars

LAURANCE D. HALL\* and MANSUR YALPANI

*Department of Chemistry, The University of British Columbia, Vancouver, B.C. V6T 1W5 (Canada)*

(Received October 1st, 1979; accepted for publication, October 20th, 1979)

Fluorescent carbohydrate conjugates have recently received considerable attention in a variety of biological fields ranging from cell-surface topography<sup>1</sup>, membrane transport<sup>2</sup>, polysaccharide mobility<sup>3</sup>, and gelling mechanism<sup>4</sup>, to the analysis of glycoproteins<sup>5</sup>. Concurrently, the need has arisen for a broader spectrum of labelling methods<sup>6</sup>, and, in this report, we focus attention on reactions whereby an amine-containing luminescent probe (L-NH<sub>2</sub>) can be covalently attached to a partially protected or unprotected sugar, and also to polysaccharides.

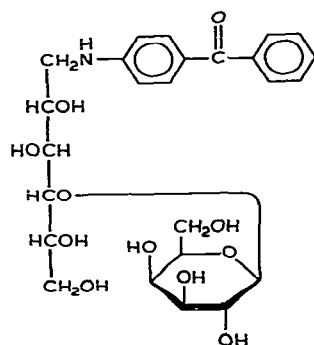
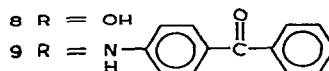
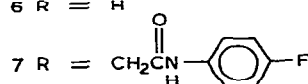
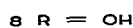
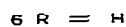
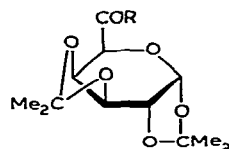
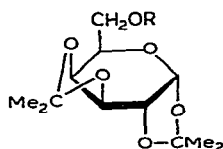
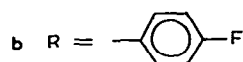
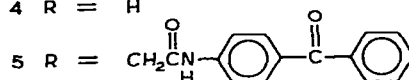
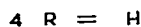
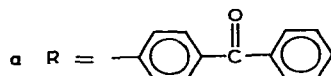
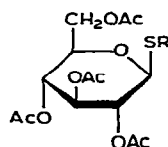
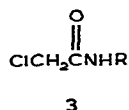
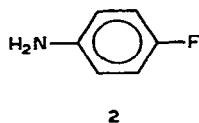
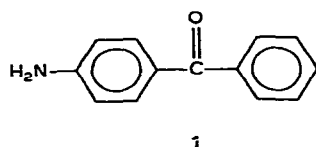
Reaction of the amines<sup>7,8</sup> **1** and **2** with ClCH<sub>2</sub>COCl in ethyl acetate or ether under reflux (~2 h), followed by concentration by use of a stream of dry N<sub>2</sub> at room temperature, affords the pure chloroacetamide derivative<sup>†</sup>, in high yield, directly from the reaction mixture: **3a**, m.p. 133.5° (from toluene) and **3b**, m.p. 131.0–131.5° (dec., from toluene). Such derivatives, which constitute a novel, and versatile, type of luminescent reagent can be used to alkylate thiol, amino, or hydroxyl functionalities under appropriate reaction-conditions; being less reactive than the equivalent iodoacetamides<sup>9</sup>, they offer the advantage of greater selectivity. For example, **3a** reacts with the thio sugar tetraacetate **4** in CHCl<sub>3</sub> in the presence of solid NaHCO<sub>3</sub> during 18 h to yield **5** in 67% yield, m.p. 130° (from ethanol–H<sub>2</sub>O), [α]<sub>D</sub><sup>22</sup> –30° (c 1.6, CHCl<sub>3</sub>); in contrast, no reaction between **3a** and the D-galacto derivative **6** was observed under similar conditions. However, with NaH–HCONMe<sub>2</sub><sup>10</sup>, the ether **7** was produced in 39% yield, m.p. 128° (from isopropyl alcohol), [α]<sub>D</sub><sup>22</sup> –12° (c 1.1, C<sub>6</sub>H<sub>6</sub>).

Luminescent amide derivatives may be obtained *via* carbodiimide-mediated coupling; for example, the D-galacturonic acid derivative **8** reacts with **1** and dicyclohexylcarbodiimide<sup>11</sup> in CH<sub>2</sub>Cl<sub>2</sub> solution during 35 h at room temperature to afford, after chromatography on a column of silica gel, compound **9** in 48% yield, m.p. 117° (dec., from isopropyl alcohol), [α]<sub>D</sub><sup>22</sup> –122.5° (c 0.4, CHCl<sub>3</sub>).

Complementary to the foregoing procedures are methods for selectively labelling, in polar media, unprotected sugars. Reductive amination<sup>12</sup> of oligosaccharides using sodium cyanoborohydride in methanol under reflux proceeds in high yields<sup>13</sup>. For

\*To whom correspondence should be addressed.

†All derivatives reported here have n.m.r. spectra, elemental analyses, and/or high-resolution, mass spectra in accord with the structures assigned.



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example, **1** and lactose reacted during 23 h to afford the 1-deoxylactitol-1-yl derivative **10**, in 75% yield after chromatography on a column of silica gel, m.p. 157–159° (from ethanol),  $[\alpha]_D^{22} -15.8^\circ$  (*c* 1.1, H<sub>2</sub>O). This compound was further characterised as the nona-acetate, m.p. 61–63° (from EtOH–H<sub>2</sub>O),  $[\alpha]_D^{22} +25.9^\circ$  (*c* 1.1, CHCl<sub>3</sub>).

The same, reductive-amination procedure can be equally well applied to polysaccharides. Thus, sodium alginate that had been periodate-oxidized<sup>14</sup> (26%) to the dialdehyde derivative reacted with *p*-fluoroaniline and sodium cyanoborohydride to yield,

after *extensive* dialysis and lyophilization, the luminescent alginate derivative. Conversely, chitosan reacted<sup>15</sup> with 9-anthraldehyde in 1:49 aqueous acetic acid–MeOH to form a stiff gel which was repeatedly washed (MeOH and ether) to afford the luminescent Schiff-base derivative. This derivative could be stabilized against hydrolysis by cyanoborohydride reduction, either simultaneous with its formation or following its isolation.

Insofar as the yields reported here are typical of those we have obtained for other similar reactions, we consider that these three methods will find widespread application in covalent labelling (luminescent, e.s.r., and n.m.r.) of many sugar-containing systems. In the case of the polysaccharide reactions where conventional, chemical characterization is not easy, we draw attention to the advantage of using as models the reactions of analogous, spin-labelled compounds, which can be accurately assayed by e.s.r. spectroscopy<sup>16</sup>.

#### ACKNOWLEDGMENTS

This work was supported by operating grants from the National Research Council of Canada (A 1905 to L.D.H.).

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