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Preliminary communication

Enzymic synthesis of *N*-acetyllactosamine on a soluble, light-sensitive polymer

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Inspired by the excellent results of the Merrifield method for polypeptide synthesis and its application to polynucleotide synthesis the potential for polymer-supported synthesis of carbohydrates has gained considerable interest [1–7]. At present, however, this method is not yet competitive with classical glycosylation methods. Frequently, this is attributable to the low access of reagents to polymer-bound substrates. The polymer-supported synthesis provides such advantages as the possible use of a large excess of reagents and the facile separation of polymeric products. Furthermore, saccharide-carrying polymers may serve in affinity chromatography of lectins, enzymes, etc. Herein we describe the synthesis of *N*-acetyllactosamine [**10**, 2-acetamido-2-deoxy-4-*O*-(β-D-galactopyranosyl)-β-D-glucopyranose] on a water-soluble, light-sensitive polyacrylamide, employing UDP-galactose epimerase and galactosyltransferase. The general synthetic route, which was already established in previous contributions [4], was extended by the application of β-galactosidase in the synthesis of (4-carboxymethyl-2-nitrobenzyl) 2-acetamido-2-deoxy-β-D-glucopyranoside (**3a**) (Scheme 1). Methods for the determination of polymer-bound functional groups are briefly reviewed and a new method based on NMR measurements is presented.

The light-sensitive nitrobenzyl glycoside **3a** [mp 185°C; $[\alpha]_D^{20}$ +7.6 (*c* 1.4, $CHCl_3$); ^1H NMR ($CHCl_3$): δ 4.65 (d, $J_{1,2}$ 8.3 Hz, H-1)] is obtained in 35% yield from the Horton chloride [8] (**1**) and methyl 4-hydroxymethyl-3-nitrobenzoate [4] (**2**) in a Koenigs–Knorr procedure with silver perchlorate as a promotor. Deblocking [9] gives **3b** [mp 210°C; $[\alpha]_D^{20}$ +13.5 (*c* 1.1, H_2O); ^1H NMR (D_2O): δ 5.55 (d, $J_{1,2}$ 8.4 Hz, H-1)] in 95% yield. Alternatively **3b** can be synthesized via **3c**

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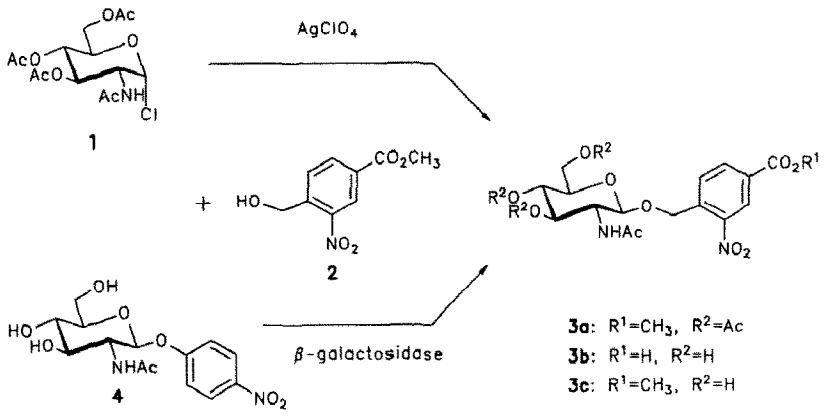
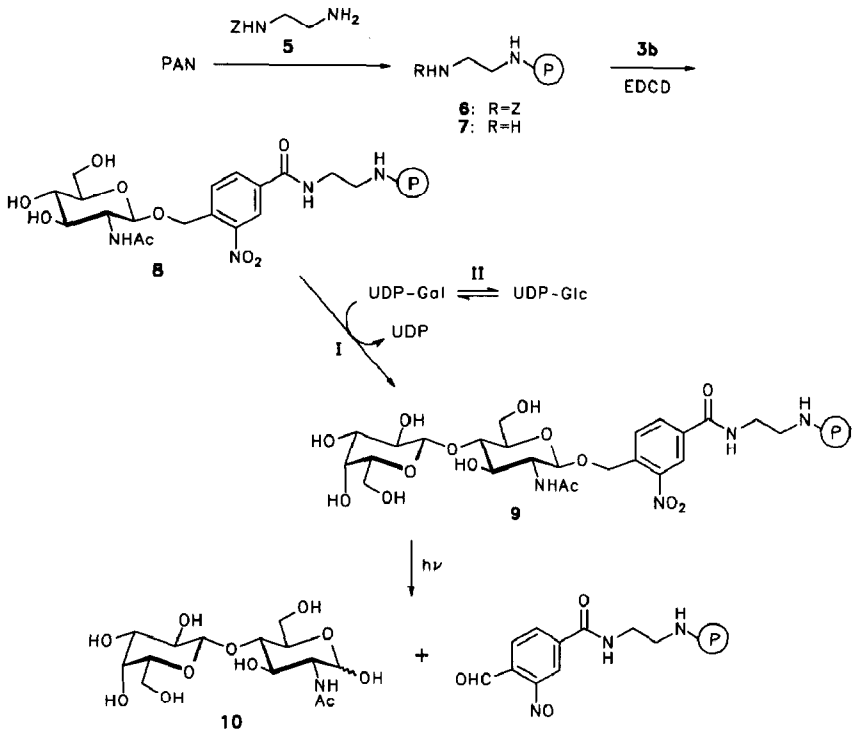


Scheme 1.

employing β-galactosidase from *A. oryzae*, which has low specificity as regards the donor [10]. At this stage, an immobilized enzyme (CNBr-activated Sepharose 4B, Sigma; 60% recovered activity) is used to allow final recovery of the enzyme and also separation from the starch that is always present in the commercial enzyme-preparation. Compound **2** is transferred onto *N*-acetylglucosamine employing 4-nitrophenyl 2-acetamido-2-deoxy-β-D-glucopyranoside (**4**) at pH 5.0 in aqueous DMF or Me_2SO (each 50%) at room temperature for 4 or 7 days, respectively. Compound **3c** is obtained in 23% yield and can be deblocked to give **3b**. Compared to the Koenigs–Knorr procedure, the slightly lower yield is outweighed by avoiding the silver catalyst and the possibility of reusing the immobilized enzyme.

The water-soluble polyacrylamide–poly(*N*-acryloxysuccinimide) (PAN) [11] is derivatized with *N*-benzyloxycarbonyl-1,2-diaminoethane [12] (**5**) to give polymer **6**, which in turn is hydrogenolyzed over Pd–C to yield the functionalized polyacrylamide **7** having 0.24 mequiv NH_2/g. Coupling of **7** with the light-sensitive glycoside **3b** employing 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCD) in water at pH 4.8 for 2 days leads to the *N*-acetylglucosamine-bound polymer **8** (0.13 mequiv GlcNAc/g, 54%); this is then galactosylated using the UDP-glucose epimerase(II)–galactosyltransferase(I) system [13] to give immobilized *N*-acetyllactosamine (**9**). The disaccharide **10** and nongalactosylated *N*-acetylglucosamine are released by photolysis (< 350 nm). Thus far compound **10** has been obtained in only 2% yield with respect to the amount of immobilized *N*-acetylglucosamine after separation by gel-permeation chromatography. Previously, in another experiment, but under comparable conditions, yields above 30% could be achieved, and thus the present reaction will have to be further elaborated and optimized (Scheme 2).

There are several methods to determine the amount of covalently linked functional groups in every step of the synthetic route. The content of active ester groups in PAN may be determined by hydrolysis and photometric determination of the released succinimide [11] (cf. Scheme 3).



Scheme 2.

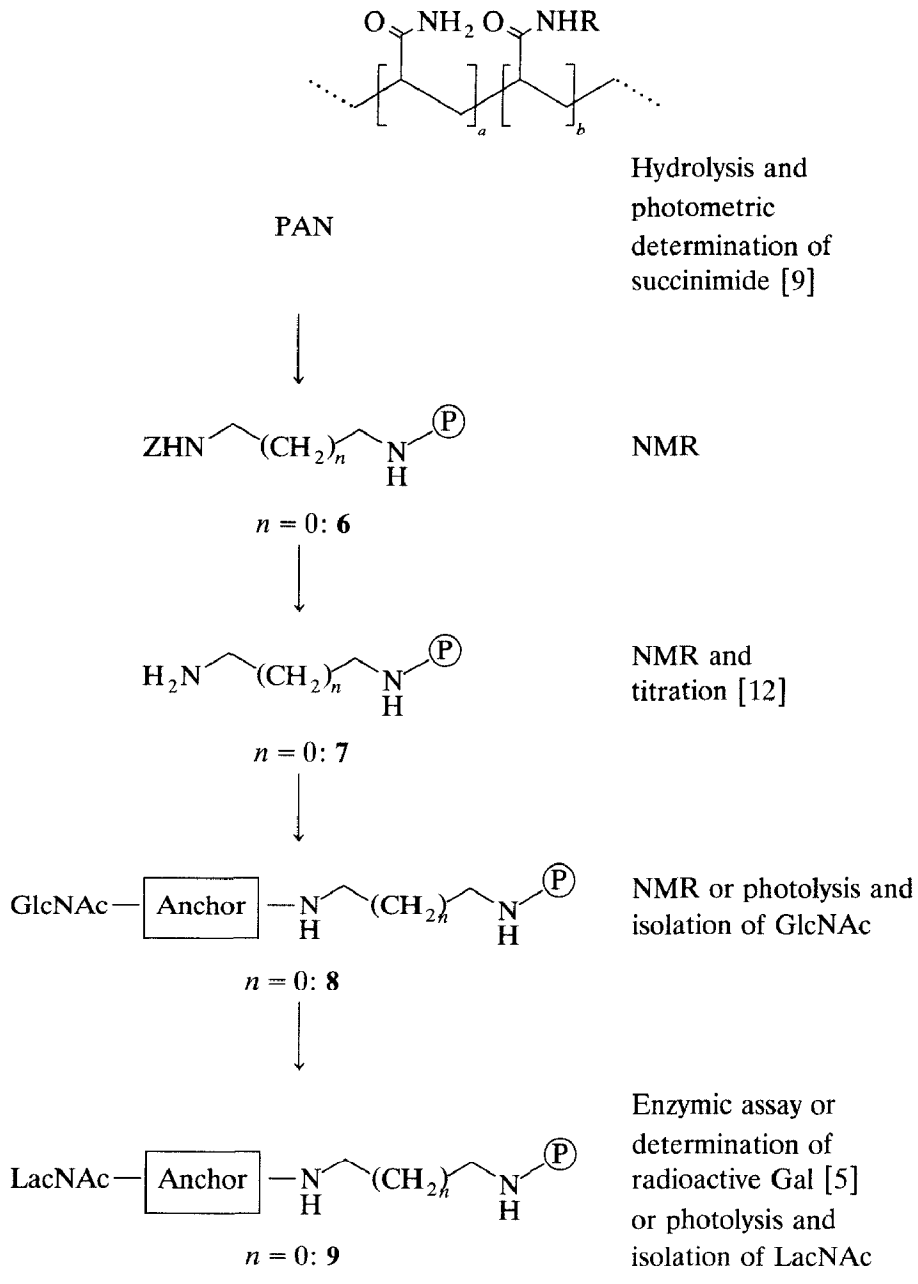
The benzyloxycarbonyl-protected amino groups in polymer **6** are determined by comparing the integrals of the NMR signals of the phenyl group [Int(Ph), $\delta = 7.50$ ppm] and the alkyl chain [Int(G), $\delta = 1.20$–2.60 ppm]. Generally, the density of functional groups D is given by:

$$D = \frac{b}{\mathrm{MW}}\left[\frac{\mathrm{mol}}{\mathrm{g}}\right] \tag{1}$$

were b is the number of functionalized acrylamide units and MW the molecular weight of the polymer. With a being the number of acrylamide units and MW_a and MW_b the molecular weights of units a and b, MW can be substituted by $\mathrm{MW} = a \cdot \mathrm{MW}_a + b \cdot \mathrm{MW}_b$ and it follows from eq. *1* that:

$$D = \frac{1}{\frac{a}{b}\mathrm{MW}_a + MW_b}\left[\frac{\mathrm{mol}}{\mathrm{g}}\right] \tag{2}$$

The value of a/b is reflected in the ratio of Int(Ph) and Int(G), which is given for



Scheme 3.

a polymer with an indefinite spacer ($n \geq 0$). The inner methylene groups of the spacer have the same chemical shift as the alkyl-chain protons:

$$\frac{\text{Int(Ph)}}{\text{Int(G)}} = \frac{5b}{3(a+b)+2nb}\left[\frac{\text{mol}}{\text{g}}\right] \qquad (3)$$

Rearrangement of eq. *3* and substitution of a/b in eq. *2* gives:

$$D(\mathbf{6}) = \frac{1}{\frac{1}{3} \cdot \mathrm{MW}_a \left(5 \cdot \frac{\mathrm{Int(G)}}{\mathrm{Int(Ph)}} - 3 - 2n \right) + \mathrm{MW}_b} \left[\frac{\mathrm{mol}}{\mathrm{g}} \right] \quad (4)$$

which allows calculation of D by measuring Int(Ph) and Int(G).

The same method is applied for the deblocked polymer **7**. Taking into account the signals of the protons of the α- and the inner methylene groups [Int(α, $-CH_2$), $\delta = 3.25$ and 3.10, respectively] it follows that:

$$\frac{\mathrm{Int}(\alpha/\omega\text{-}\mathrm{CH_2})}{\mathrm{Int(G)}} = \frac{4b}{3(a+b) + 2nb} \left[\frac{\mathrm{mol}}{\mathrm{g}} \right] \quad (5)$$

and with eq. *2* this gives:

$$D(\mathbf{7}) = \frac{1}{\frac{1}{3} \cdot \mathrm{MW}_a \left(4 \cdot \frac{\mathrm{Int(G)}}{\mathrm{Int}(\alpha/\omega\text{-}\mathrm{CH_2})} - 3 - 2n \right) + \mathrm{MW}_b} \left[\frac{\mathrm{mol}}{\mathrm{g}} \right] \quad (6)$$

D(**7**) may be also determined by titration of the terminal amino groups [14]. However, because of the use of empirical factors, this method does not seem to be very accurate especially in the case of small amounts of attached saccharide units.

The glycosylated polymer **8** is best examined by the integrals of the two vicinal aromatic protons [Int(4_{Ar},5_{Ar}), $\delta = 8.50$ and 8.15, respectively]. They are isolated and therefore easy to integrate. With:

$$\frac{\mathrm{Int}(4_{\mathrm{Ar}},5_{\mathrm{Ar}})}{\mathrm{Int(G)}} = \frac{2b}{3(a+b) + 2nb} \left[\frac{\mathrm{mol}}{\mathrm{g}} \right] \quad (7)$$

it follows from eq. *2* that:

$$D(\mathbf{8}) = \frac{1}{\frac{1}{3} \cdot \mathrm{MW}_a \left(2 \cdot \frac{\mathrm{Int(G)}}{\mathrm{Int}(4_{\mathrm{Ar}},5_{\mathrm{Ar}})} - 3 - 2n \right) + \mathrm{MW}_b} \left[\frac{\mathrm{mol}}{\mathrm{g}} \right] \quad (8)$$

Alternatively, D(**8**) may be determined by measuring the UV absorption of the aromatic moiety or by photolytic cleavage and subsequent gravimetric determination of the sugar. The latter is of course an indirect method, with all its associated disadvantages.

For the galactosylated polymer **9**, easily integratable signals are lacking. When radioactively labled UDP-[^{14}C]Gal is used, the amount of immobilized *N*-acetyllactosamine may be determined by measuring the radioactivity of the polymer [5]. As a convenient indirect method, UDP generated as a by-product during the galactosylation step may be determined by enzymic assay [15].

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