Synthesis of a Branched Pentasaccharide: One of the Core Oligosaccharides of Human Blood-group Substances

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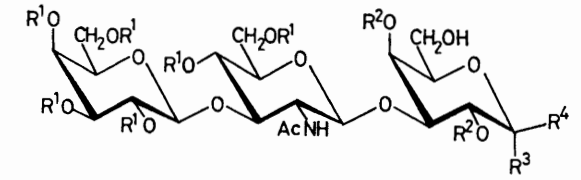
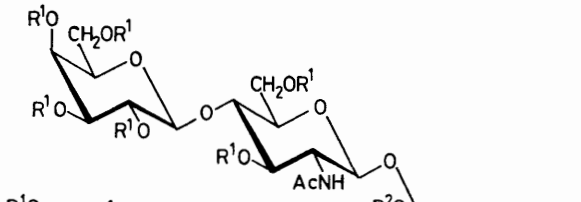
Summary Condensation of the lactosamine-derived per-*O*-acetyl oxazoline (**6**) with the trisaccharide derivative (**1**) followed by removal of the protecting groups, gave the branched pentasaccharide, β-D-Galp(1→3)-β-D-GlcpNAc-(1→3)-[β-D-Galp(1 → 4)-β-D-GlcpNAc(1→6)]-D-Gal (**4**); the reduced form of this compound has been previously reported as present among the degradation products of soluble Le^a-active glycoprotein from ovarian cysts.

BLOOD-GROUP substances from erythrocytes membranes are glycolipids which carry the A-,B-, H-, and Le-determinants on the outer parts of their oligosaccharide chains. Because of their scarcity, most chemical investigations started from the so-called 'soluble blood-group substances'. These are glycoproteins which may be found in the secretions of an individual and carry the same immunochemical specificities as his red cells.[1] Both types of substances appear similar in their chain endings, but it is now established that they differ in their core sequences underlying the blood-group determinants. Glycolipids contain structures with two β-D-*N*-acetyl-lactosamine units ('type 2 chains') branched on the 3- and 6-positions of D-galactose.[2] On the other hand, the pentasaccharide (**4**) was isolated (in the reduced form) from among the degradation products of a Le^a-active glycoprotein from ovarian cysts.[3] This fact indicated branching at one galactose residue, with one β-D-*N*-acetyl-lactosamine unit at the 6-position and a so-called 'type 1 chain', β-D-Galp(1→3)-β-D-GlcpNAc at the 3-position. As such core oligosaccharides are very difficult to isolate from natural sources, we have undertaken their syntheses so that they should be readily available for chemical, enzymic, and immunochemical studies. Our general scheme starts from benzyl 2,4-di-*O*-benzyl-D-galactopyranoside derivatives with allyl ether protecting groups[4] on the 3- or 6-position.[5,6] Condensation of per-*O*-acetylated mono- and di-saccharide oxazolines onto these units should lead to all possible natural structures near the branch point. In the synthesis of 'lacto-*N*-triose I' (**2**), we found[6] that one useful intermediate was the partially protected trisaccharide derivative (**1**) which has now been obtained in the pure state {anhydrous foam, $[\alpha]_D^{20}$ + 7·9° (*c* 1 in chloroform)} by chromatography† (chloroform–acetone, 4 : 1).

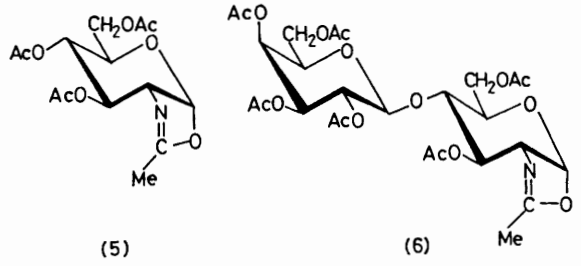
N-Acetyl-lactosamine[7] was converted into the per-*O*-acetylated α-chloride and then into the oxazoline (**6**)[8,9] by a common-ion method already used[10] in the preparation of the oxazoline (**5**). The oxazoline (**6**) (2 equiv.) was added portionwise over 48 h, to a solution of the alcohol (**1**) (0·93 g) in 1 : 1 toluene–nitromethane kept at 60 °C under nitrogen in the presence of traces of toluene-*p*-sulphonic acid. Two consecutive chromatographic purifications, one with ethyl acetate, and the other with 98 : 2 chloroform–ethanol, gave, in 55% yield, the protected pentasaccharide (**3**) {monohydrate, foam, m.p. 125—129 °C after treatment with ether, $[\alpha]_D^{20}$ + 1·9° (*c* 1 in chloroform)}. The removal of protecting groups from compound (**3**) by treatment with 0·05 M sodium methoxide in methanol at room temperature overnight followed by catalytic de-*O*-benzylation (shaking for 48 h at room temperature and atmospheric pressure in acetic acid solution in the presence of hydrogen and 10% palladium on charcoal gave the free pentasaccharide (**4**) purified by chromatography (isopropyl alcohol–ethyl acetate–water; 3 : 2 : 2) (0·20 g; 60%) {m.p. 206—210 °C decomp.; $[\alpha]_D^{20}$ − 6° → + 1·5° (3 h) (*c* 1·6 in H_2O); paper chromatography (p.c.) (n-butanol–pyridine–water, 6 : 4 : 3; 88 h, descending) $R_{lactose}$ 0·1}.



(1) R^1 = Ac; R^2 = $PhCH_2$; R^3 = CH_2Ph; R^4 = H
(2) R^1 = R^2 = H, R^3, R^4 = H, OH



(3) R^1 = Ac, R^2 = $PhCH_2$, R^3 = OCH_2Ph, R^4 = H
(4) R^1 = R^2 = H, R^3,R^4 = H, OH



(5) (6)

† All chromatographic separations were performed on silica gel columns.

TABLE. ^{1}H N.m.r. spectral data[a] for compounds (**4**) and (**7**) and related oligosaccharides.

Compounds		Anomeric protons of component monosaccharide units					NAc	Ref.
		D-Gal	β-D-GlcpNAc(1→3)	β-D-Galp(1→3)	β-D-GlcpNAc(1→6)	β-D-Galp(1→4)		
(**9**)	α	5·28	4·76 (8·4)				2·06	5
	β	4·58	4·77 (8·4)					
(**8**)[b]	α					4·49 (7·5)	2·06	
	β							
(**10**)	α	5·26	4·77 (8·0)		4·62 (8·0)		2·08	5[c]
	β	4·58 (7·8)	4·78 (8·0)					
(**2**)	α	5·29	4·87	4·52 (7·3)			2·09	6
	β	4·63 (7·75)						
(**7**)	α	5·22 (3·4)	4·77	4·45 (7·5)	4·58 (8·2)		2·07; 2·09	
	β	4·55 (7·0)						
(**4**)	α	5·21 (3·5)	4·86	4·47 (7·5)	4·58	4·44 (7·0)	2·03; 2·05	
	β	4·55 (8·0)						

[a] Spectra were recorded at 250 MHz with a Cameca Model STN 250 spectrometer with Fourier transform unit, for solution in D_2O at 80 °C. Chemical shifts in p.p.m. (δ) relative to Me_4Si (0·2% solution in $CDCl_3$ as external reference) are followed by ^3J coupling constants in Hz in parentheses. [b] Protons of reducing unit: α isomer, δ 5·22 (2 Hz); β anomer, δ 4·74 (broad). [c] See also S. David and A. Veyrières, *Carbohydrate Res.*, 1975, **40**, 23.

The tetrasaccharide (**7**), which is useful for comparison of ^{1}H n.m.r. chemical shifts, was obtained in the same way from the protected trisaccharide (**1**) and the oxazoline (**5**), in 50% overall yield {trihydrate, m.p. 186—188 °C, $[\alpha]_D^{20}$ + 2·5° (*c* 1 in water) no mutarotation; p.c.: (ethyl acetate–pyridine–water, 2:1:2, upper phase), $R_{lactose}$ 0·19; (butanol–pyridine–water, 5:3:2), $R_{lactose}$ 0·22}.

The ^{1}H n.m.r. signals in the spectrum of (**4**) were attributed to its five anomeric protons by comparison with the spectra of oligosaccharides (**2**), (**7**), (**8**), (**9**), and (**10**), which contain parts of the sequence. We assume that the chemical shifts of the inter-glycosidic anomeric protons of each component residue would not change much between these compounds, and the Table shows that this leads to a fully consistent interpretation.

β-D-GlcpNAc (1→6) β-D-Galp(1→4)-D-GlcpNAc (**8**)

β-D-Galp(1→3)-β-D-GlcpNAc(1→3)-D-Gal (**7**)

β-D-GlcpNAc(1→3)-D-Gal (**9**)

β-D-GlcpNAc(1→3)-D-Gal (**10**)

Borohydride reduction of (**4**) gave a compound with $R_{lactose}$ 0·17 when examined by p.c. in the same system as the parent pentasaccharide. The same figure has been recorded[3] for the reduced pentasaccharide obtained from the Le^a-active glycoprotein of ovarian cysts.

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