Tetrahedron Letters, Vol.31, No.8, pp 1109-1112, 1990
Printed in Great Britain

0040-4039/90 $3.00 + .00
Pergamon Press plc

GLYCOSYL-INOSITOL DERIVATIVES II. SYNTHESIS OF 2-AMINO-2-DEOXY-D-GALACTOSYL-α-1,3-D-CHIRO-INOSITOL

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ABSTRACT: The "azide method" has been applied to the preparation of a 2-amino-2-deoxy-α-D-galactosyl-D-chiro-inositol disaccharide, using silver perchlorate and silver carbonate as the coupling reaction catalysts.

A family of glycosyl phosphatidyl inositols (GPI) has been recognized recently as versatile anchor systems for various cell-surface proteins, including enzymes, receptors, antigens and immunological factors.[1] The generic structure of GPI may be depicted as:

Phosphatidyl inositol-hexosamine-hexoses-mannose-6-phosphate--protein

Upon activation, the anchored protein is cleaved by specific endogenous phospholipases and proteases to release the biologically active protein and an inositol glycan frgament. It has also been suggested that, following the interaction of insulin with its cell surface receptor, a similar mechanism releases one or more phosphorylated inosityl-glycans as putative insulin mediators (PIM) which can mimic some biochemical activities of insulin in vitro.[2]

The structure elucidatin of GPI and the similar glycan moiety in PIM[3] has received considerable attention.[4] Recently, the structure determination[4c] and synthesis[5] of the glycan portion of the T. brucei GPI were reported. We have previously found that a PIM preparation from rat liver contains an unusual combination of D-chiro-inositol and D-galactosamine.[3a] In this communication we wish to report the synthesis of an α-D-galactosamine-D-chiro-inositol disaccharide. Due, in part, to the structure of the T. brucei glycan, we have pursued the α-1,3 linkage sequence, which would correspond to a possible PIM structure with the 4-O-glycosyl-inositol-1-phosphate substitution pattern.

Silver triflate promoted condensation of the bromo sugar 1[6] with the protected monobenzyl D-chiro-inositol 2[7] gave the coupling product 3 in 30% yield, as an α--β mixture in the ratio of 1:4. Alternatively, the azido-bromo sugar 4[8] was condensed with the diisopropylidene-D-chiro-inositol 5[7]

using silver percholorate and silver carbonate as promoters, to yield the α-1,3-disaccharide 6 in 30% yield, after 3 days at 25° C.

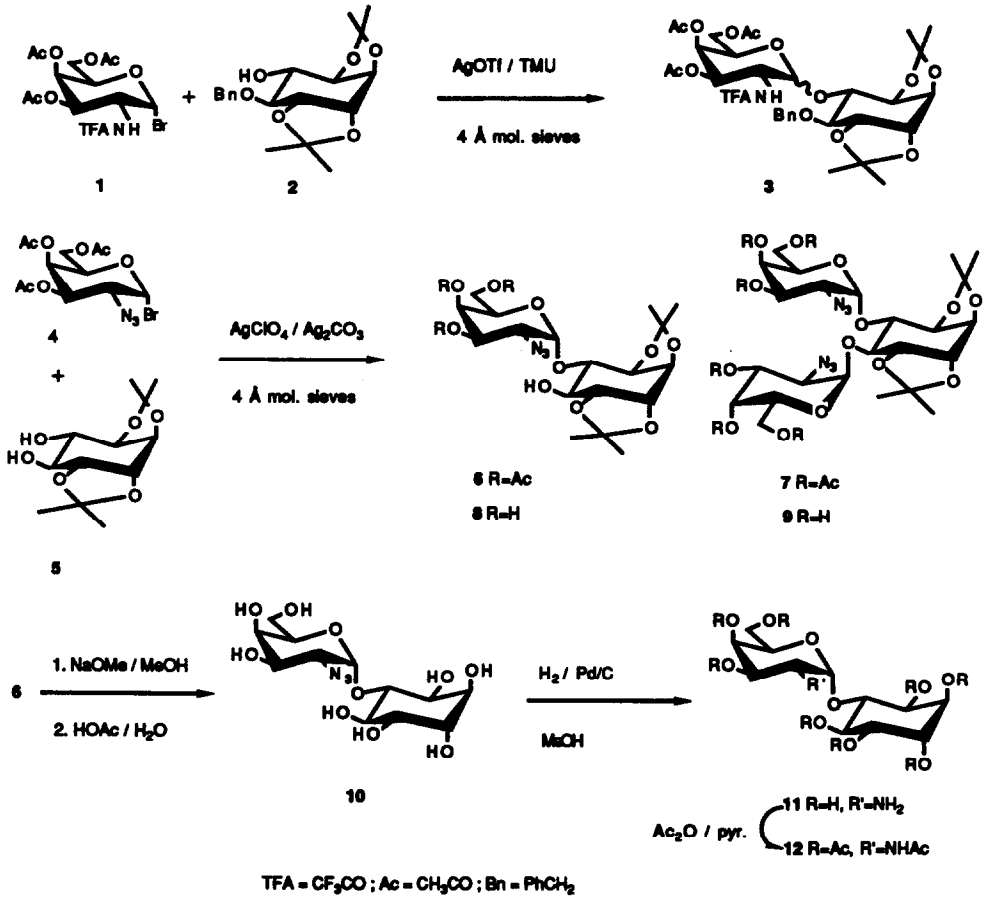
Treatment of the chiro-inositol 5 with 2 eq of the azido-bromide 4 gave, after 3 days at 25° C, a nearly equimolar mixture of the desired disaccharide 6 along with the product of disubstitution 7. These two glycosides, having identical chromatographic mobilities, were conviently separated after Zemplén deacetylation, giving 8 and 9, respectively. The symetrical trisaccharide 9 was easily charactarized by ^{1}H NMR. The ^{1}H C-methyl region of the diisopropylidene chiro-inositol gave only the expected two, 6 H, singlets, and the ring protons for the galactose residue integrated to 2 H relative to the inositol-ring-proton signals. The branched product was not further investigated.

As the coupled product 6 obtained via the azide method was easier to prepare than 3, the synthesis of the hexosamine-disaccharide 11 was pursued from this derivative. Removal of the acetyl gropus by Zemplén deacetylation followed by acidic hydrolysis of the acetal groups gave the crystalline-azido disaccharide 10, mp 188-190°C, (from acetone). The sample gave $[\alpha]_D$ 78.9° (c 0.66 water), and showed the expected azide band at 2118 cm^{-} in the infrared spectrum. Reduction of the azido function was effected with either Pd/C or $Pd(OH)_2$, and low pressures of hydrogen, to give the D-galactosamine-D-chiro-inositol disaccharide 11. The amino-sugar derivative 11 could be easily purified by ion-exchange chromatography on Bio-Rad AG 50W-X8 resin (H^+ form). The adsorbed product was eluted cleanly with 1.0 M aqueous ammonia. The sample, which appeared very pure by ^{1}H NMR, did not give satisfactory elemental analysis, and was not readilly crystallized as the free base or the hydrochloride salt. The H'-2 signal shifted from 3.49 ppm in 10 to 3.01 ppm in 11, and the hydrochloride salt of 11 showed H'-2 at 3.10 ppm in the ^{1}H NMR spectrum. The values for $[\alpha]_D$ of 11 and its hydrochloride were measured as 122.6°, (c 0.88 water), and 90.4° (c 0.35 water), respectively. In all of the disaccharide derivatives the α linkage was confirmed by H-1 at about 5.3 ppm, with $J_{1,2}$=3.7 Hz.

In order to fully charactarize the amino disaccharide 11, the peracetate 12 was prepared in 72% yield from a sample of 11, with pyridine and acetic anhydride. The sample was decolorized by passage over silica gel and further purified by crystallization from ether. The peracetate 12 had mp 122-124° C, and $[\alpha]_D$ 68.5° (c 0.9, $CHCl_3$). The peracetate 12 gave satisfactory elemental analysis, as the mono-hydrate, and gave, in the mass spectrum, M+1 at 721 (CI, using isobutane). The ^{1}H NMR spectrum of 12 showed the expected 11 signals for acetyl methyls, and the 13 ring protons.

The purification and structure elucidation of PIMs from biological media have not yet been completed. The minimal structural requirement for producing insulin-like activities is totally unknown. To facilitate further progress in this field, the conversion of derivatives of disaccharide 11 and its protected precursors into phosphorylated and glycosylated derivatives as research probes is currently underway.

SCHEMES



The assignments for the ring protons of the disaccharides 6 through 12 are given in Table I.

TABLE I

Chemical shifts are given in ppm, relative to the solvent line. Coupling constants for $J_{a,b}$ are given in parenthesies, and are in Hz.

Cmpd.	H-1	H-2	H-3	H-4	H-5	H-6	H'-1	H'-2	H'-3	H'-4	H'-5	H'-6a	H'-6b
	δ												
6	4.07	4.37	3.44	3.64	4.37	4.07	5.21	3.88	5.37	5.44	4.48	4.11-4.18	
$CDCl_3$	-	-	dd	dd	-	-	d	dd	dd	-	t	m	
	-	-	(8.1)	(9.9)	-	-	(3.4)	(9.9)	(3.1)	-	(7.1)	-	
7	4.34	4.15	3.42	3.64	4.15	4.34	5.14	3.79	4.04	4.08	4.10	3.93	3.85
$CDCl_3$	-	-	dd	dd	-	-	d	dd	dd	-	-	dd	dd
	-	-	(1.1)	(7.8)	-	-	(3.7)	(10)	(3.1)	-	-	-	-
10	3.88	-	3.62--3.78		-	3.88	5.41	3.49	4.02	3.78	4.27	3.62--3.78	
MeOH	-		m			-	d	dd	dd	-	t	m	
d-4	-						(3.8)	(10.7)	(3.2)	-	(6.1)		
11	-	3.94--3.92/3.83--3.84			-	5.22	3.01	-	-	4.19		-	
MeOH		-	3.63--3.78		-		d	dd	-	-	t		-
d-4			-				(3.8)	(9.6)	-	-	(6.1)		-
12	5.40	5.20	4.22	5.49	5.17	5.32	5.15	4.59	5.05	5.36	4.30	4.17	4.00
$CDCl_3$	t	dd	t	t	dd	t	d	ddd	dd	t	t	dd	dd
	(3.7)	(7.0)	(9.3)	(9.8)	(3.2)	(4.0)	(3.8)	(12)	(3.1)	(0.7)	(6.6)	(11)	-

Acknowledgement: We wish to thank Professor L. Anderson for the generous gift of diisopropylidene-D-pinitol. This work was supported by NIH grant #DK40137-02.

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(Received in USA 27 September 1989)