Structure–Activity Relationships of Aminoalkyl and -aryl Glycosides Having Insulin-like Activity[1]

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A number of alkyl, aryl, and aralkyl glycosides (mono- and disaccharides) substituted in the aglycon with a primary amino group have been found to exert insulin-like activity on rat adipocytes in vitro. Systematic variations in the saccharide configuration, glycosidic linkage, aglycon moiety, and sugar substitution pattern were investigated to delineate structure–activity relationships. A high degree of structural specificity was observed. Maximal insulin mimicking activity was obtained with the 6-aminohexyl 1-thio-D-mannopyranosides; the β anomer was more active than the α anomer. Modification of the sugar hydroxyl groups resulted, in most cases, in partial or complete loss of biological activity at the levels tested; however, in a few instances, sugar-modified derivatives did show enhanced insulin-like effects. Specific structural types evaluated are discussed in greater detail. 6-Aminohexyl 1-thio-β-D-mannopyranoside also exhibited in vivo insulin-like effects on both diaphragm muscle and omental adipose tissues. The specificities for the sugar as well as the aglycon portions of these carbohydrate derivatives suggest that both parts of the molecule are involved in the expression of the full biological activity observed; their respective roles in the mechanism of the insulin-like activity are discussed.

As part of a research program concerned with the synthesis and biological evaluation of novel saccharide derivatives designed to selectively affect cell surface membranes, we have investigated the interaction of carbohydrates with rat adipocytes in assays that reflect effects on the surface membrane insulin receptor as well as on subsequent intracellular events.

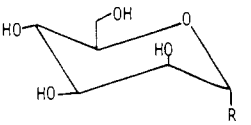
It has been suggested that endogenous saccharide residues on the cell surface may play a role in the binding to insulin.[2-5] Such an involvement of carbohydrates in the function of the insulin receptor has been inferred from studies on the insulin-like properties of plant lectins[2,5] and on the effects of a few *exo*-glycosidases on insulin binding and biological activity.[4] Concanavalin A, wheat germ agglutinin, and several other plant lectins have been shown to mimic some of insulin's biological activities and binding properties.[2,5,6] Lectins are plant proteins that can bind to specific carbohydrate determinants on the surface of mammalian cells. Treatment of fat cells with neuraminidase and β-galactosidase has suggested that galactose residues on the insulin receptor may contribute to the binding with insulin and that sialic acid residues may be involved at some stage in the action of insulin subsequent to binding.[4] Additional evidence for the importance of sugar residues has come from the finding that certain monosaccharides and their methyl glycosides can affect both the binding of insulin–Sepharose to its receptors on fat cells and glucose oxidation by these cells.[7] The possibility that low-molecular-weight, substituted saccharides might perturb the insulin receptor and give rise to insulin-like or insulin-antagonistic activity was therefore considered.

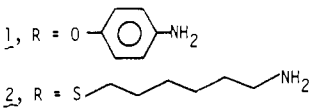
In our laboratories, the interaction between the saccharide binding sites on Con A and the lectin receptor on intact isolated adipocytes has been studied employing the affinity buoyant density method.[8] Simple saccharides were found to block and reverse the binding of Con A–Sepharose to the intact cells, and it was subsequently discovered that these same saccharides also inhibited the binding of insulin–Sepharose to its receptor and that the saccharide inhibitory specificity closely paralleled Con A's saccharide binding affinity.[7] Illustratively, para-substituted phenyl α-D-mannopyranosides exhibited a more potent inhibition of the binding of insulin–Sepharose to fat cells than did the analogous phenyl α-D-glucopyranosides.[7]

Of pertinence at this stage was to ascertain whether, in addition to the simple glycopyranosides,[7] structurally more complex inhibitors of insulin–Sepharose binding to fat cells could also affect the biological activity of insulin. Initially, therefore, inhibitors found in the insulin receptor binding assay were evaluated in the fat cell in vitro insulin bioassay for stimulation or inhibition of glucose utilization (conversion of [^{14}C]glucose to $^{14}CO_2$).

Results and Discussion

On the basis of the inhibitory effects seen in the binding assay, it was expected that most of the compounds examined would antagonize insulin's action. However, from testing in the fat cell insulin bioassay, we found that a certain number of these derivatives expressed insulin-like effects (i.e., stimulated glucose oxidation in the absence of insulin) but at much lower concentrations than the simple glycopyranosides. Thus, *p*-aminophenyl α-D-mannopyranoside (**1**) at 3×10^{-3} M significantly stimulated





glucose oxidation in the isolated fat cells (see Table I, section 4). 6-Aminohexyl 1-thio-α-D-mannopyranoside (**2**) (hereafter referred to as α-AHTM), having an aliphatic stem linking the terminal amino group to the sugar residue, was approximately ten times more potent than the aminoaryl derivative **1** (see Table I, section 4). α-AHTM (**2**) was subsequently found also to mimic insulin in vitro in its ability to stimulate fat cell lipogenesis from [^{14}C]-glucose and to inhibit epinephrine and cholera toxin stimulated lipolysis in rat adipocytes.[1b,9]

To evaluate the contribution of the sugar moiety to the insulin-like effects observed with α-AHTM (**2**), the biological activities of three aminohexylthio aglycons, i.e., 6-mercapto-*n*-hexylamine (**3**), 6-methylthio-*n*-hexylamine (**4**), and 9,10-dihydroxy-7-thia-*n*-decylamine (**5**), were

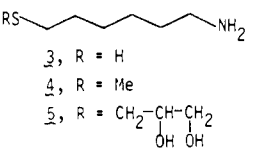


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Table I. Effects of Saccharides on in Vitro Glucose Oxidation by Intact Rat Fat Cells

compd[a]		insulin act., conversion of [^{14}C]glucose to $^{14}CO_2$, % ± SEM[b]
section 1. aglycons		
insulin[e]		100 ± 2.6
2		141.0 ± 3.1
3		15.5 ± 1.7
4		24.8 ± 0.9
5		6.9 ± 0.9
section 2. terminal substituent Z		
insulin[e]		100 ± 5.3
2	CH_2NH_2	40.8 ± 9.7
6	CO_2^- Na^+	0 ± 0.07
7	CH_2CH_3	0 ± 0.01
8	CH_2NHMe	0 ± 0.1
9	CH_2NMe_2	0 ± 0.04
10	CH_2NMe^+ I^-	0 ± 0.09
section 3. length of alkyl chain		
insulin[e]		100 ± 1.8
11	NH_2	0 ± 0.04
12	CH_2NH_2	0 ± 0.1
13	C-2	0 ± 0.1
14	C-4	0 ± 0.1
15	C-5	6.1 ± 1.9
2	C-6	40.9 ± 1.1
16	C-7	14.8 ± 2.1
17	C-8	0 ± 0.3
18		47.1 ± 4.2
section 4. aryl and aralkyl spacer arms		
insulin[e]		100 ± 3.6
2	alkyl	40.9 ± 6.7
19	aryl	0 ± 0.07
20	aralkyl	9.2 ± 0.06
21	aralkyl	0 ± 0.07
22	aralkyl	6.4 ± 0.5
1	aryl	2.9 ± 0.9
1[c]	aryl	23.9 ± 3.5
section 5. anomeric configuration		
insulin[e]		100 ± 8.3
2	α	61.6 ± 2.5
23	β	90.4 ± 0.6
section 6. tautomeric form of saccharide moiety		
insulin[f]		100 ± 8.3
23[d]	pyranoid	128.7 ± 6.2
24[d]	acyclic	0 ± 0.04
25[d]	furanoid	93.6 ± 1.5
section 7. glycosidic linkage (O- vs. S-glycosides)		
insulin[e]		100 ± 1.3
26	β-Glc-*S*-	64.4 ± 2.7
28	β-Glc-*O*-	23.3 ± 2.6
2	α-Man-*S*-	61.6 ± 2.4
27	α-Man-*O*-	23.3 ± 0.9
section 8. glycosidic linkage (amide)		
insulin[e]		100 ± 1.3
23		90.4 ± 0.6
29	C-5	2.6 ± 0.3
30	C-6	20.5 ± 0.7
31	C-7	22.4 ± 11.8
section 9. glycosidic linkage (sulfone)		
insulin[f]		100 ± 9.5
2	-S-	66.7 ± 4.8
32	$-SO_2-$	33.3 ± 2.8
section 10. sugar configuration		
insulin[f]		100 ± 11.3
23	β-Man	95.9 ± 4.8
2	α-Man	64.5 ± 3.2
35	α-Ido	63.7 ± 10.6
36	β-Ido	75.2 ± 6.6
insulin[f]		100 ± 7.3
23[d]	β-Man	197.3 ± 1.5
2[d]	α-Man	132.4 ± 6.3
37[d]	α-Tal	109.9 ± 5.8
insulin[e]		100 ± 5.1
23	β-Man	81.3 ± 5.1
33	β-Gal	68.8 ± 2.0
2	α-Man	58.8 ± 4.4
26	β-Glc	43.8 ± 1.8
34	β-GlcNAc	36.3 ± 3.5
section 11. disaccharide analogues		
insulin[f]		100 ± 10.1
2		60.8 ± 2.2
38	α(1→6)-α	21.6 ± 0.3
39	α(1→4)-α	37.3 ± 2.4
40	α(1→3)-α	15.7 ± 0.3
41	α(1→2)-α	21.6 ± 1.7
42	α(1→2)-β	0 ± 0.09

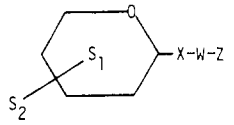
[a] For convenience, concentrations of saccharides are listed as μg/mL, since all compounds were tested on a weight rather than on a molar basis. However, since all of the saccharides tested have similar molecular weights, the molar concentrations are also similar to each other. Unless otherwise designated, all saccharides were tested at 100 μg/mL. [b] Values represent the average of three experiments normalized relative to the effect of insulin. Stimulation of $^{14}CO_2$ production from [^{14}C]-glucose ranged from +500 to +1100%. This range of biological response is due to the variable sensitivities inherent between different preparations of cells and animals. For other details, see the Experimental Section. [c] At 750 μg/mL (2.8×10^{-3} M). [d] At 50 μg/mL. [e] 25 μU/mL. [f] 20 μU/mL.

measured in the fat cell assay. The data obtained for these compounds and for α-AHTM (**2**) are shown in Table I, section 1. The very weak responses observed for the three aglycons (**3**–**5**), as compared to that elicited when a sugar residue is attached, as in α-AHTM (**2**), clearly demonstrated the significant contribution of the carbohydrate moiety to the expression of full biological activity by α-AHTM. The large differences in activity observed with compounds having the same aglycon but chemically modified carbohydrate residues (vide infra) also provided evidence for the functional role of the carbohydrate moiety in the total measured biological effect.

Following these comparisons, a semiempirical, systematic chemical modification of the lead structure, α-AHTM (**2**), was carried out to further enhance biological potency. Several routes were pursued, which included modification of (a) the sugar and anomeric configurations, as well as the tautomeric form; (b) the glycosidic linkage X between the saccharide and aglycon portions of the molecule; (c) the aglycon spacer arm linking the terminal substituent Z with the saccharide through X; (d) the terminal substituent Z; and (e) the hydroxyl groups on the monosaccharide, S_1, including disaccharide analogues by combination of S_2 with S_1 (see Chart I). These manipulations were performed either individually or in combination. Highly specific structure–activity relationships were uncovered and are discussed below.

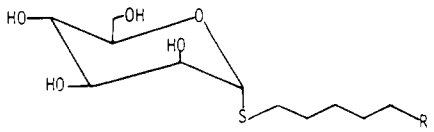
Structure–Activity Relationships. a. Terminal Substituent Z. The first structural parameter to be investigated was the terminal functional group on the aliphatic stem of α-AHTM (**2**). Negatively charged, neutral, and other positively charged (secondary, tertiary,

Chart I



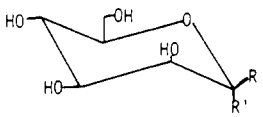
S_1 = substituted or unsubstituted monosaccharide
S_2 = non-reducing moiety of disaccharide
X = glycosidic linkage
W = aglycon spacer arm
Z = terminal functional substituent

and quaternary ammonium) groups were introduced into the molecule in place of the primary amino group. Examination of the biological data given in Table I, section 2, clearly reveals that the primary amino group is a requisite for stimulation of glucose oxidation. Thus, whereas α-AHTM (**2**) expressed significant activity at a concentration of 100 μg/mL, all other terminal substituents abolished the biological activity. This absolute requirement for a primary amino group is readily understandable in light of the mechanism of action discussed below.

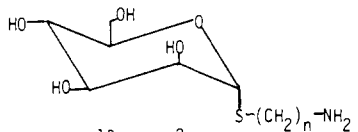


6, R = $CO_2^-Na^+$
7, R = CH_2CH_3
8, R = CH_2NHMe
9, R = CH_2NMe_2
10, R = $CH_2NMe_3^+I^-$
18, R = $CH_2\overset{H}{N}\overset{O}{C}(CH_2)_6NH_2$

b. Length of Alkyl Chain. In order to examine the effect of varying the length of the aliphatic stem linking the terminal substituent to the sugar residue, the following compounds were evaluated in the insulin bioassay: β-D-mannopyranosylamine (**11**), having the amino group



11, R = NH_2; R' = H
12, R = H; R' = CH_2NH_2

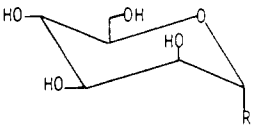


13, n = 2
14, n = 4
15, n = 5
16, n = 7
17, n = 8

directly attached to the anomeric carbon of the pyranoid sugar; 1-amino-2,6-anhydro-1-deoxy-D-*glycero*-D-talo-heptitol (**12**), with a single methylene group bridging the amine function to the sugar; and five additional ω-aminoalkyl 1-thio-α-D-mannopyranosides with n = 2, 4, 5, 7, and 8 (**13**–**17**, respectively). The length of the aglycon spacer arm was observed to have a remarkable effect on the biological activity (see Table I, section 3).

At a concentration of 100 μg/mL, the mannosylamine **11** and aminoheptitol **12** gave no response. The insulin-mimicking activity in the ω-aminoalkyl series peaked at n = 6 and fell sharply with either an increase or decrease in the chain length. Interestingly, however, 6-(7-aminoheptanamido)hexyl 1-thio-α-D-mannopyranoside (**18**), a derivative in which the amino group occupies a more distant position from the sugar than in the n-octyl analogue **17**, gave a biological response in the range of α-AHTM itself (see Table I, section 3).

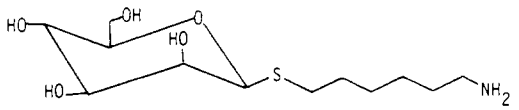
c. Other Aglycon Spacer Arms. To test the effect of aryl and aralkyl spacer arms, the following four derivatives were examined in the fat cell insulin bioassay: p-aminobenzyl and p-(aminomethyl)benzyl 1-thio-α-D-mannopyranoside (**19** and **20**, respectively); o- and p-(α-



19, R = SCH_2–C$_6$H$_4$–NH_2
20, R = SCH_2–C$_6$H$_4$–CH_2NH_2
21, R = O–C$_6$H$_4$–CH_2NH_2
22, R = O–C$_6$H$_4$–CH_2NH_2

D-mannopyranosyloxy)benzylamine (**21** and **22**, respectively). With all four compounds, replacement of the aliphatic stem with an aryl or aralkyl arm resulted in severe loss of biological activity relative to α-AHTM (**2**) (see Table I, section 4).

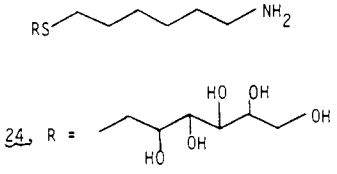
d. Anomeric Configuration. 6-Aminohexyl 1-thio-β-D-mannopyranoside (β-AHTM, **23**), the isomer of α-



23

AHTM having the aminohexylthio group in an equatorial orientation in the favored $^4C_1$ conformation, was found to be considerably more active in the insulin bioassay than α-AHTM (axial aminohexylthio group in the preferred conformation). Thus, this chemical modification of the lead structure afforded significant enhancement of insulin-like biological potency. β-AHTM (**23**) at 10^{-4} M exhibited approximately the same response as 20 μU/mL of the insulin standard control ($\sim 10^{-10}$ M) (see Table I, sections 5 and 6). For reference, the physiological concentration of insulin ranges from about 25 to 1000 μU/mL in the serum.

e. Tautomeric Form of Saccharide. In view of the structural requirement for the saccharide moiety, it was of interest to compare compounds in the pyranoid ring form with the corresponding furanoid and acyclic analogues. Whereas 1-*S*-(6-aminohexyl)-1-thio-D-mannitol (**24**)



24, R = HO OH / OH / HO OH

25, R = D-mannofuranosyl

showed no activity at 50 μg/mL, 6-aminohexyl 1-thio-D-mannofuranoside (**25**), obtained as an anomeric mixture, was significantly active at this concentration. However, the observed stimulation was somewhat weaker than that for β-AHTM (**23**) (see Table I, section 6).

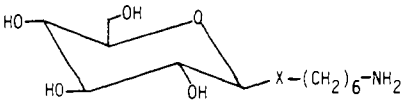
f. Glycosidic Linkage. Thioglycosides were initially studied as lead structures (e.g., **2** and **23**) since it was

Table II. Effect of Sugar Configuration on Insulin-like Activity of Aminohexyl Thioglycopyranosides in Vitro

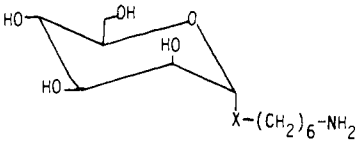
compd[a]	stimulation of glucose oxidation,[b] % effect ± SEM			av effect normalized to insulin's effect[c] ± SEM
	expt 1	expt 2	expt 3	
insulin[d]	727 ± 9	805 ± 21	886 ± 23	100 (n = 6)
β-AHTM (23)	663 ± 5	630 ± 20	634 ± 19	80 ± 6 (n = 3)
β-AHTGal (33)	498 ± 27	554 ± 14		69 ± 0.1 (n = 2)
β-AHTGlc (26)	476 ± 29	475 ± 18	290 ± 22	48 ± 6 (n = 6)
α-AHTM (2)[e]	467 ± 18	472 ± 21		65 ± 7 (n = 5)

[a] Saccharides measured at 100 μg/mL. [b] Percent stimulations represent percent incremental effect of compound above the basal level of glucose oxidation. All values listed under each experiment represent results obtained within the same experiment run "side by side" at the same time on the same batch of cells and with the same reagents used for each other compound in the designated experiment. [c] "Average percent of insulin's effect" is the average of "n" experiments included within the same series of experiments used to calculate each of the other effects in this table; this value represents the effects normalized relative to the effect of insulin at 25 μU/mL, which is assigned a value of 100. [d] 25 μU/mL. [e] α-AHTM (2) was included as a control run within the same series of experiments as for the other saccharides.

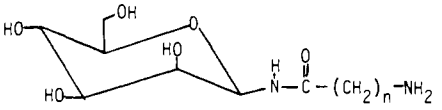
anticipated that a thioglycosidic linkage would be relatively resistant to enzymatic hydrolysis. In both the α-D-manno and β-D-gluco configurations, the aminohexyl thioglycopyranosides (2 and 26, respectively) proved to be more



26, X = S
28, X = O



27, X = O
32, X = SO_2

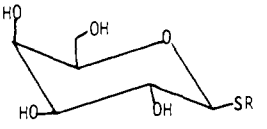


29, n = 5
30, n = 6
31, n = 7

potent insulin-like agents than the corresponding O-linked derivatives (27 and 28, respectively) (see Table I, section 7). Three analogues with an amide linkage, 6-aminohexanoyl-, 7-aminoheptanoyl-, and 8-aminooctanoyl-β-D-mannopyranosylamine (29–31, respectively), exhibited considerably weaker biological activity than β-AHTM (23) (see Table I, section 8). Within this series, however, the biological activities of the aminoheptanoyl (n = 6) and aminooctanoyl (n = 7) compounds (30 and 31, respectively) were about the same and much greater than that of the aminohexanoyl (n = 5) derivative 29.

Oxidation of the sulfide linkage in α-AHTM (2) to the sulfone 32 reduced the activity by half (see Table I, section 9).

g. Sugar Configuration. On the basis of the apparent significant role of the sugar moiety in the biological activity expressed by the aminohexyl thiomannosides (as noted above), a more systematic comparison of monosaccharide analogues with different sugar configurations was carried out. The 6-aminohexyl 1-thioglycopyranosides of five of the eight possible D-hexose configurations, as well as that of *N*-acetyl-D-glucosamine, were evaluated in the bioassay (Table II). Consistent with an important role for the carbohydrate moiety, the β-thiomannoside 23 remained the most active isomer. This was followed in decreasing order by the β-thiogalactoside 33 and the β-thioglucoside



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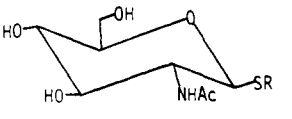

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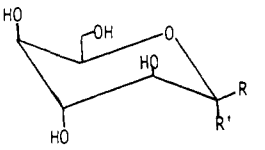
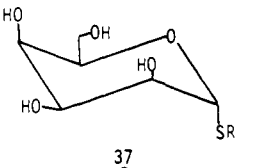
R = $(CH_2)_6NH_2$





35, R = H; R' = S-$(CH_2)_6$-NH_2
36, R = S-$(CH_2)_6$-NH_2; R' = H

37

26. To emphasize the preferential activity of the mannosides, the α-thiomannoside 2, which was previously found to be less potent than the β anomer 23, proved to be no less active than the β-thiogalactoside 33 and, on the average, even more active than the β-thioglucoside 26 (Table II).

The bioactivity of 6-aminohexyl 1-thio-α-D-idopyranoside (35) was in the range of that of α-AHTM, whereas the *N*-acetyl-β-D-thioglucoside and the α-thiotaloside (34 and 37, respectively) were somewhat less active. The β-thioidoside 36 appeared more active than α-AHTM (2) but still (as all other isomers) less active than β-AHTM (23) (see Table I, section 10). Briefly, the above compounds may be listed in the following decreasing order of activity: β-Man > β-Gal ≈ β-Ido > β-Glc > β-GlcNAc; α-Man ≈ α-Ido > α-Tal.

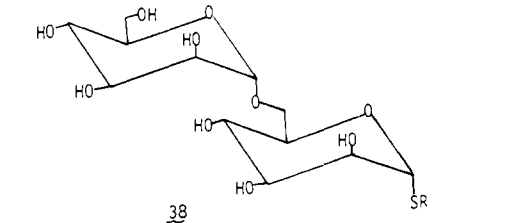
h. Modification of Sugar Hydroxyl Groups. The effect of replacing one of the hydroxyl groups with a second monosaccharide unit was also studied on the assumption that, if the "insulin-like" glycosides function by interacting with a native saccharide receptor site on the fat cell surface membrane,[7] this site would most likely contain an extended carbohydrate binding region. Indeed, this was suggested by Goldstein and co-workers[10] to be the case for the cell surface combining sites of Con A; these authors showed that the trisaccharide *O*-α-D-man-[1→2]-*O*-α-D-man-[1→2]-D-man interacts more strongly with Con A than does the disaccharide *O*-α-D-man-[1→2]-D-man, which, in turn, interacts more strongly than does D-mannose.[10]

Table III. Effect of Sugar Substitution on Insulin-like Activity of 6-Aminohexyl 1-Thio-D-mannopyranosides in Vitro

compd	concn (per mL)	insulin act., conversion of [^{14}C]-glucose to $^{14}CO_2$, % ± SEM[a]
insulin	25 μU	100 ± 4.5
2	100 μg	70.0 ± 8.2
43	100 μg	0 ± 0.08
44	100 μg	37.5 ± 11.3
insulin	20 μU	100 ± 4.7
2	100 μg	52.8 ± 1.6
45	100 μg	64.2 ± 2.1
insulin	20 μU	100 ± 13.7
2	100 μg	85.3 ± 2.4
46	100 μg	102.9 ± 13.5
insulin	20 μU	100 ± 4.8
23	50 μg	109.7 ± 4.8
47	50 μg	129.0 ± 3.4

[a] Values represent the average of three experiments normalized relative to the effect of insulin. Stimulation of $^{14}CO_2$ production from [^{14}C]glucose ranged from 300 to 650%. For other details, see Table I.

The biological activities of the 6-aminohexyl 1-thioglycopyranosides of the following mannose disaccharides, which contain the more common naturally occurring linkages, were evaluated in the insulin bioassay: *O*-α-D-man-[1→6]-*O*-α-D-man (**38**), *O*-α-D-man-[1→4]-*O*-α-D-man (**39**), *O*-α-D-man-[1→3]-*O*-α-D-man (**40**), *O*-α-D-man-[1→2]-*O*-α- and β-D-man (**41** and **42**, respectively). Although insulin-like effects were observed with most of these disaccharide analogues, none was more active than the monosaccharide derivatives, α- and β-AHTM (see Table I, section 11).



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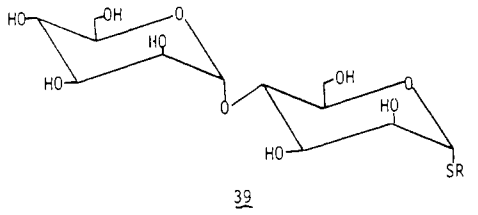

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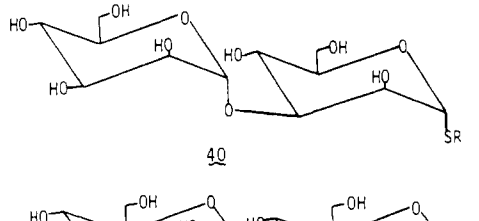

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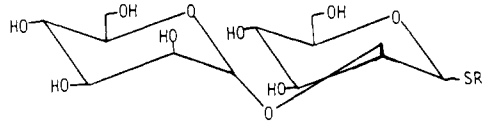
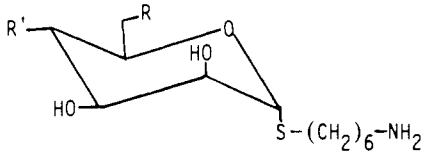


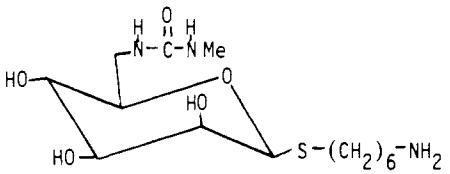
41, α
42, β

R = $(CH_2)_6NH_2$

Derivatives of α- and β-AHTM, in which one or more of the sugar hydroxyls were modified or replaced with another substituent, were also investigated. The results are given in Table III. For the most part, partial or complete loss of activity resulted. As a remarkable illustration of the structural specificity, simple introduction of a deoxy function at the C-6 position of the sugar moiety in α-AHTM (**2**) [affording the derivative, 6-aminohexyl 1-thio-α-D-rhamnopyranoside (**43**)] led to complete loss of



43, R = H; R' = OH
44, R = OMe; R' = OH
45, R = OH; R' = O-C(=O)-NH_2
46, R = O-C(=O)-NH_2; R' = OH



the insulin-like activity at 100 μg/mL. 6-Aminohexyl 6-*O*-methyl-1-thio-α-D-mannopyranoside (**44**) showed intermediate behavior between **43** and α-AHTM (**2**). On the other hand, introduction of a carbamate or ureido function into the molecule did provide derivatives which showed some enhancement of biopotency. Some of the compounds evaluated are shown above and the biological data given in Table III.

Other Insulin-like Effects of α-AHTM. In a preliminary communication, the ability of α-AHTM (**2**) to (a) enhance conversion of [^{14}C]glucose to [^{14}C]-glycerol/glycerides, (b) enhance conversion of [^{14}C]glucose to fatty acids (lipogenesis), and (c) inhibit hormone-stimulated lipolysis was demonstrated.[1b] In an extensive biochemical analysis to be published separately, the details of these effects will be described.[9]

In Vivo Biologic Evaluation. Since 6-aminohexyl 1-thio-β-D-mannopyranoside (**23**) was among the most active structures in vitro, it was selected for evaluation of in vivo insulin-like activity in the Rafaelsen bioassay (see the Experimental Section). Stimulation of both lipogenesis in adipose tissue and glycogenesis in muscle tissue of the rat was measured. The results are shown in Table IV. Compared to insulin at a concentration of 200 mU/kg rat weight, β-AHTM (**23**) at a concentration of 500 mg/kg rat weight appeared to exhibit weak insulin-like effects on both lipogenesis and glycogenesis.

Mechanism of Insulin-like Action. In a separate study, biochemical analysis of the time dependence of the insulin-like activity of the aminoalkyl thioglycosides revealed that the initial structure is converted to aldehyde **48** and hydrogen peroxide in a reaction catalyzed by an

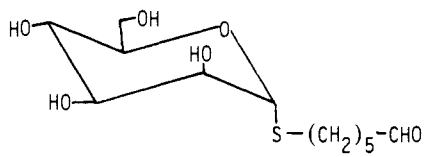


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Table IV. Stimulation of Glycogenesis and Lipogenesis by 6-Aminohexyl 1-Thio-β-D-mannopyranoside (23) in Vivo[a]

	% above control ± SEM[b]		
	[^{14}C]glucose to [^{14}C]glycogen		[^{14}C]glucose to [^{14}C]lipids,
	expt 1	expt 2	expt 3
control	0 ± 21	0 ± 19	0 ± 37
insulin (200 mU/kg)	+3757 ± 347	+3138 ± 159	+1885 ± 393
β-AHTM (23, 500 mg/kg)	+274 ± 51	+74 ± 13	+86 ± 11

[a] Experimental details are given in the Experimental Section. [b] Each number represents the mean of five animals.

amine oxidase present in both the bovine serum albumin used in the in vitro bioassay and on the intact cell membrane.[1b,9] Since catalase, which destroys hydrogen peroxide, and aminoacetonitrile, a known inhibitor of amine oxidase, suppressed the biological activity of the saccharides, and since the effects of hydrogen peroxide are identical with those produced by the saccharides in the same experimental systems, it was concluded that hydrogen peroxide is the mediator of most of the insulin-like action observed with these carbohydrate derivatives.[1b,9] Hydrogen peroxide has been hypothesized[11] to activate the glucose transport system in fat cells by oxidizing a key protein component of the fat cell surface membrane to the disulfide form. However, in view of the antilipolytic action of hydrogen peroxide[1b,9,12] and the saccharides,[1b,9] the activation of a process in addition to glucose transport must be involved. The mechanism of action of the aminoaryl compounds remains to be clarified.

It was subsequently discovered[9] that the surface membrane of the intact rat adipocyte naturally contains amine oxidase activity capable of generating "insulin-like" hydrogen peroxide from the aminohexyl thioglycopyranosides in the complete absence of albumin. This observation could provide an explanation for the in vivo insulin-like activity obtained with β-AHTM (23) shown in Table IV.

Conclusions

The present study has revealed highly specific structural requirements for the expression of insulin-like activity by certain suitably substituted carbohydrate derivatives. The structure–activity relationships described have indicated significant roles for both the sugar and aglycon portions of the glycosides in the stimulation of glucose utilization by isolated rat adipocytes in vitro. Of particular note are the findings that (a) in the aglycon, a primary amino group must be positioned at a specific distance from the sugar residue (aminohexyl thioglycoside) for optimal biological activity; (b) the greatest activity is obtained with the manno-sugar configuration; (c) the β anomer of 6-aminohexyl 1-thiomannopyranoside is more active than the α-anomer; and (d) the nature of the substituents on the sugar residue is also critical for insulin-like activity; several carbamate and ureido derivatives expressed enhanced activity over the unsubstituted precursors.

The specificity for the carbohydrate moiety suggests its participation in the mechanism of the insulin-like activity of these compounds. It may therefore be speculated that the carbohydrate moiety interacts with a "saccharide receptor" site on the cell membrane and that this interaction contributes to the expression of the full biological activity observed.

Experimental Section

Chemical. The synthesis of the carbohydrate derivatives described in the present study will be reported elsewhere.

Biological. Fat cells were isolated from epididymal fat pads that were excised from male, albino Charles River rats (CD) weighing between 130 and 185 g and fed ad libidum on Purina Chow by the procedure of Rodbell.[13] The fat cell bioassay for insulin-like activity, which measures the stimulation by insulin of the utilization of [^{14}C]glucose and its oxidation to $^{14}CO_2$ by the cells, was performed according to the procedure of Gliemann.[14] About 5×10^4 cells were incubated for 2 h at 37 °C with or without insulin or the designated carbohydrate derivative in 1.0 mL of Krebs–Ringer bicarbonate buffer, pH 7.4, containing 4% (w/v) bovine serum albumin (Fr. V, powder) and 0.75 mM [^{14}C]glucose (0.1 Ci/mol). $^{14}CO_2$ was collected after termination of the reaction and counted in a Packard Tri-Carb Scintillation Counter, Model 3310.

The in vivo assay for the determination of insulin-like activity was conducted essentially according to the procedure described by Rafaelsen and co-workers,[15] except that female rats were used. Rats (140–150 g), fasted overnight, were injected ip with a mixture of insulin (200 mU/kg rat weight), or the designated amount of saccharide neutralized to pH 7.5 with 0.1 N HCl, and [^{14}C]glucose (2 μCi/rat) dissolved in physiological saline solution. Control animals were injected with the same solution, except in the absence of insulin and saccharide. Two hours later the rats were sacrificed, and the diaphragm muscle and omental adipose tissues were removed, weighed, and assayed for ^{14}C radioactivity incorporated per gram of tissue into the glycogen and total lipids extracted from the muscle and adipose tissues, respectively, as described.[15]

Insulin (recrystallized twice) was purchased from Schwarz/Mann Co. All other materials were obtained from the usual commercial sources.

References and Notes

(1) Preliminary reports on the study have appeared: (a) P. L. Durette, R. L. Bugianesi, M. M. Ponpipom, and T. Y. Shen, *Abstr. Pap., Chem. Inst. Can., Am. Chem. Soc., It. Conf., 2nd*, CARB-25 (1977); (b) M. A. Cascieri, R. A. Mumford, and H. M. Katzen, *Fed. Proc., Fed. Am. Soc. Exp. Biol.*, **36**, 915 (1977).

(2) P. Cuatrecasas and G. P. E. Tell, *Proc. Natl. Acad. Sci. U.S.A.*, **70**, 485 (1973); P. Cuatrecasas, *Biochemistry*, **12**, 1312 (1973); *J. Biol. Chem.*, **248**, 3528 (1973).

(3) S. S. Solomon, L. E. King, Jr., and K. Hashimoto, *Horm. Metab. Res.*, **7**, 297 (1975).

(4) P. Cuatrecasas and G. Illiano, *J. Biol. Chem.*, **246**, 4938 (1971); P. Cuatrecasas, *Insulin Action, Proc. Symp., 1971*, 137 (1972).

(5) H. M. Katzen and R. A. Mumford, *Fed. Proc., Fed. Am. Soc. Exp. Biol.*, **35**, 1719 (1976).

(6) M. P. Czech and W. S. Lynn, *Biochim. Biophys. Acta*, **297**, 368 (1973); M. P. Czech, J. C. Lawrence, Jr., and W. S. Lynn, *J. Biol. Chem.*, **249**, 7499 (1974).

(7) H. M. Katzen, unpublished results.

(8) H. M. Katzen and D. D. Soderman, *Biochemistry*, **14**, 2293 (1975).

(9) M. A. Cascieri, R. A. Mumford, and H. M. Katzen, unpublished results.

(10) I. J. Goldstein, C. M. Reichert, and A. Misaki, *Ann. N.Y. Acad. Sci.*, **234**, 283 (1974).

(11) M. P. Czech, J. C. Lawrence, Jr., and W. S. Lynn, *Proc. Natl. Acad. Sci. U.S.A.*, 71, 4173 (1974); M. P. Czech, *Mol. Cell. Biochem.*, **11**, 51 (1976); *J. Cell. Physiol.*, **89**, 661 (1976).

(12) J. N. Livingston, P. A. Gurny, and D. H. Lockwood, *J. Biol. Chem.*, **252**, 560 (1977).

(13) M. Rodbell, *J. Biol. Chem.*, **239**, 375 (1964).

(14) J. Gliemann, *Diabetes*, **14**, 643 (1965).

(15) O. J. Rafaelsen, *Acta Physiol. Scand.*, **61**, 314 (1964); O. J. Rafaelsen, V. Lauris, and A. E. Renold, *Diabetes*, **14**, 19 (1965).