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SYNTHESIS OF TYPE 2 HUMAN BLOOD-GROUP ANTIGENIC DETERMINANTS. THE H, X, AND Y HAPTENS AND VARIATIONS OF THE H TYPE 2 DETERMINANT AS PROBES FOR THE COMBINING SITE OF THE LECTIN I OF *Ulex europaeus**

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ABSTRACT

Chemical syntheses of the human blood-group antigenic determinants derived from *N*-acetyllactosamine are described; namely, the H type 2 [αLFuc(1→2)βDGal-(1→4)βDGlcNAc], X {βDGal(1→4)[αLFuc(1→3)]βDGlcNAc}, and Y {αLFuc-(1→2)βDGal(1→4)[αLFuc(1→3)]βDGlcNAc} determinants as glycosides of 8-carboxymethyloctanol. In order to study the binding of the H type 2 determinant with the lectin I of *Ulex europaeus*, structures designed to specifically alter the hydrophilic and hydrophobic portions of the H type 2 determinant were also prepared; namely, the 6-deoxy derivative, the 4′-epimer, and the 5″-*nor*-homolog. The use of these structures, together with the H type 1 hapten and the *N*-deacetylated forms of both the H type 1 and H type 2 determinants, as inhibitors of the agglutination of O red cells by the lectin allowed the conclusion that the binding of the H type 2 determinant is hydrophobic; the binding involves a wedge-like portion of the determinant that is basically hydrophobic, except for the 5-hydroxymethyl group, which is at the tip of the wedge and forms an intramolecular hydrogen-bond with O-5 for acceptance by a hydrophobic cleft at the surface of the lectin. Blocking procedures involving alkoxymethyl groups and new experiences involving glycosylation reactions are described.

INTRODUCTION

Glycoproteins and glycosphingolipids, as constituents of cell membranes, are now appreciated to be involved, through their complex oligosaccharide ligands, in important intercellular recognition-processes and in the binding of regulatory mole-

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cules vital to the control of cellular life and reproduction[1,2]. This paper is concerned with the chemical synthesis of a number of oligosaccharides designed to assist in elucidating the mode of binding that occurs between lectin 1 of *Ulex europaeus* and the H type 2 human blood-group determinant [αLFuc(1→2)βDGal(1→4)βDGlcNAc].

Previous papers[3,4] have described our approach, based on synthesis and ^{1}H-n.m.r. studies, for the elucidation of the conformational properties of oligosaccharide structures. An empirical method, termed HSEA calculations[5], was developed for the production of molecular models that are in good accord with the demands of the ^{1}H-n.m.r. spectral data. Furthermore, it was recognized[6] that the important driving force for recognition of the combining site of a lectin or antibody is in all probability a hydrophobic effect wherein a hydrophobic cleft in the protein structure is brought into contact with a region of an oligosaccharide's overall topography that is compatible with dehydration. This, in principle, was expected[7] to involve mainly non-hydroxyl regions but could involve one or more hydroxyl groups, especially when these are suitably oriented, relative to a proton-accepting atom, to be energetically well disposed for intramolecular hydrogen-bonding. The bonding of a hydroxyl group with a proton acceptor within the combining site is not precluded. However, although such an interaction can, in principle, be expected to contribute importantly to the specificity of the reaction, for reasons previously presented[7], it seems unlikely that the exchanges in hydrogen bonds between solvent and protein in the overall process would make an important contribution to the driving force for the binding reaction[8].

Much evidence has accumulated that hydrophobicity is an important factor in carbohydrate–protein interactions[9]. Compelling evidence in this regard was recently obtained in a collaboration concerned with the binding of myeloma monoclonal antibody termed anti-I Ma with an "ambiphilic"[6] topography of the trisaccharide[10] βDGal(1→4)βDGlcNAc(1→6)DGal. This conclusion was drawn from the improved inhibition provided by the synthetic 6-*C*-methyl derivative of the trisaccharide which possessed (^{1}H-n.m.r.) a well-defined conformational preference[11]. A hydrogen bond between OH-3′ and O-5″ was proposed in order to render that portion of the molecule more compatible with a hydrophobic surface.

Fig. 1A shows a computer-drawn CPK molecular model for the H type 2 human blood-group determinant. The model is presented in adjoining halves in order to display both sides of a wedge-like portion possessing a surface that can be expected to be compatible with a hydrophobic cleft in the surface of the lectin 1 of *Ulex europaeus* or, indeed, anti-H type 2 antibodies. Thus, the frozen[8] water molecules at the involved hydrophobic areas of the agglutinin would be released and provide the main driving force for the binding reaction. It may be seen that OH-6 (*a*) is presented as hydrogen bonded to O-5. This is the reason why we chose to synthesize the 6-deoxy-H type 2 hapten (**29**), which proved to be a more potent inhibitor of the agglutination of O cells by the lectin 1 of *Ulex europaeus* than was the H type 2 hapten (**22**). Examination of the model presented in Fig. 1A shows that the methyl group at C-5″ of the fucosyl residue occupies space within the region of the structure

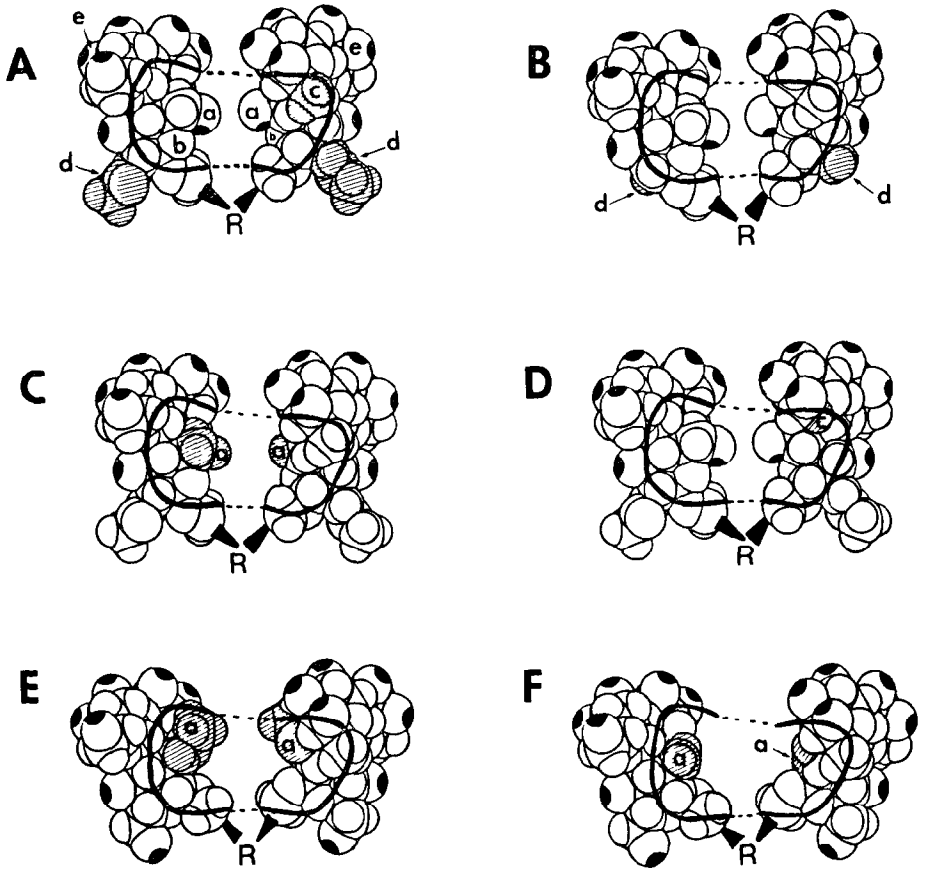


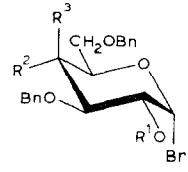
Fig. 1. Computer-drawn (Pluto program) projections of CPK molecular models based on HSEA calculations[4,5] to display the topographies of wedge-like portions of oligosaccharides indicated within the enclosures shown. The enclosures include surfaces on both halves of the molecules and, consequently, the projections are presented for both of these sides with the tip (edge) of the wedge at the center. The molecular models shown in A and E are in accord with ^{1}H-n.m.r. parameters[5] and are expected to be in accord with the favored conformation. The other structures are assumed to have the same favored conformations. For models A, C, D and E, R is $(CH_2)_8CO_2Me$; and for models B and F, R is $(CH_2)_8CO_2H$. A. The H type 2 hapten (**22**) with **a** representing OH-6 hydrogen-bonded to O-5 (**b**). These atoms, which have surfaces on both sides of the wedge, and the *C*-methyl group (**c**) of the αLFuc residue (one side only) are seen to reside within the enclosure that describes both sides of the wedge. The acetamido and the OH-4′ (βDGal residue) groups are indicated by **d** and **e**, respectively, and are situated outside the enclosure. B. The model for *N*-deacetyl H type 2 (**23**), displaying the location of the amino group (**d**). C. The model for 6-deoxy H type 2 (**29**) with **a**, displaying the location of the methyl group at C-5 of the 6-deoxy-βDGlcNAc residue at the cutting edge of the wedge-like portion of the molecule. D. The model for 5″-*nor*-H type 2 (**33**), indicating the location (**c**) of the equatorial H-5″ of the βDAra residue. E. The model for the H type 1 (or Lewis d) hapten[4], showing the location of the acetamido group (**a**). F. The model for *N*-deacetyl-H type 1 hapten, indicating the location of the amine group (**a**). The similarities in the topographies within the enclosure with that displayed for the 6-deoxy-H type 2 hapten (C) are to be noted.

expected to be involved in the binding reaction. For this reason, the 5″-*nor*-H type 2 structure (**33**) was synthesized. It proved to be a relatively poor inhibitor. On the other hand, it may be seen that both the acetamido group and the 4′-hydroxyl group of the H type 2 determinant reside outside the hydrophobic–ambiphilic surface. It is for this reason that the 4′-*epi*-(**47**) and *N*-deacetylated forms of the H type 2 hapten (**23**) were prepared. These changes caused little change in inhibitory power. The relative abilities of these compounds, together with the H type 1 hapten and its *N*-deacetylated form, to inhibit the agglutination established beyond reasonable doubt that the binding involves the hydrophobic–ambiphilic portion of the H type 2 determinant indicated in Fig. 1A, and that the interaction is strictly hydrophobic.

The present communication describes the chemical synthesis of the H type 2 hapten and the "fraudulent" analogs just indicated. The occasion is also used to describe the syntheses of structures known[12] as the X (**41**) and Y (**35**) determinants which, like the H type 2 determinant, are derivatives of *N*-acetyllactosamine. Jacquinet and Sinaÿ[13,14] have published syntheses of the H type 2 and Y determinants (then believed to be a candidate for the Lewis d determinant) as the free saccharides by routes similar to those used herein. During the course of our synthetic studies, other syntheses of the H type 2 trisaccharide have appeared[15,16], as well as a synthesis of the X structure[17]. The X determinant (**41**) was of interest because of the widespread occurrence of the so-called X enzyme (an αLFuc transferase) in human plasma and secretions[18].

SYNTHESIS OF HAPTENS

Following our general procedure for the preparation of haptens[19,20], the human blood-group antigenic determinants, which have been termed H type 2 (**22**), X (**41**), Y (**35**), as well as variations in the H type 2 structure; namely, 6-deoxy-H type 2 (**29**), 5″-*nor*-H type 2 (**33**), and 4′-epi-H type 2 (**47**), were all synthesized as their 8-methoxycarbonyloctyl glycosides. These syntheses all started from the previously reported 8-methoxycarbonyloctyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranoside (**4**), which had served as starting material for the preparation of the Le^a (ref. 19), Le^b, and Le^d (H type 1) determinants[21]. In order to have available the 2′-hydroxyl group of appropriately protected intermediates for glycosylation reactions, as previously done for the syntheses of the Le^b and Le^d determinants[21], the glycosyl bromides **1–3** were prepared.

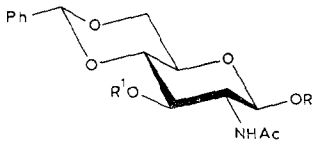


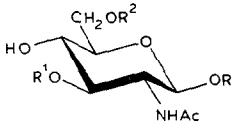
1 R^1 = pNO_2Bz, R^2 = H, R^3 = OBn

2 R^1 = Bz, R^2 = H, R^3 = OBn

3 R^1 = Ac, R^2 = OBn, R^3 = H

Compounds **1–3** were prepared from 3,4,6-tri-*O*-benzyl-1,2-orthoesters[22]. Hydrolysis of 3,4,6-tri-*O*-benzyl-1,2-*O*-(1-ethoxyethylidene)-α-D-galactopyranose to 3,4,6-tri-*O*-benzyl-D-galactose, followed by *p*-nitrobenzoylation and treatment of the 1,2-di-*p*-nitrobenzoate with hydrogen bromide provided[21] compound **1**. Controlled hydrolysis of the orthoester followed by benzoylation yielded 1-*O*-acetyl-2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-α-D-galactopyranose which, on treatment with hydrogen bromide, gave **2**. The glucosyl bromide (**3**) was prepared directly from 3,4,6-tri-*O*-benzyl-1,2-*O*-(1-ethoxyethylidene)-α-D-glucopyranose[23] by treatment with acetyl bromide in the presence of tetraethylammonium bromide. The β anomer of **3** was the first product of the reaction (^{1}H-n.m.r.) and the bromide salt was added to hasten its conversion into the more stable α anomer (**3**).





R = $(CH_2)_8CO_2Me$*

4 R^1 = H

5 R^1 = CH_2OBn

6 R^1 = All

7 R^1 = CH_2OBn, R^2 = H

8 R^1 = R^2 = CH_2OBn

9 R^1 = All, R^2 = H

10 R^1 = All, R^2 = CH_2OBn

11 R^1 = All, R^2 = $CH_2OCH_2CCl_3$

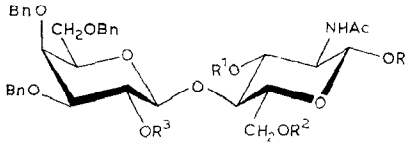
*R denotes this aglycon throughout this discussion of results

In order to make available the 4-hydroxyl group of β-D-GlcNAcO$(CH_2)_8CO_2Me$ for selective glycosylation reactions, the derivatives **7–11** were prepared starting from **4**. The first stage was to treat **4** either with benzyloxymethyl chloride in the presence of 2,6-lutidine to form **5** or with allyl bromide under standard conditions to afford **6**. It should be noted at this point that, in a separate investigation[23a], it became apparent that benzyloxymethyl chloride reacts even more rapidly with 2,6-lutidine than with methanol in dichloromethane, and that the resulting quaternary ammonium salt reacts very slowly with an alcohol as compared with the benzyloxymethyl chloride. Indeed, formation of the benzyloxymethyl ether appeared to be dependent on regeneration of the benzyloxymethyl chloride from the quaternary ammonium salt. Benzyloxymethyl bromide was a very poor alkylating agent in the presence of 2,6-lutidine, and this behavior appeared to be related to the high stability of the quaternary salt. However, when N,N,N′,N′-tetramethylurea was used as buffer, the reaction of the bromide was much faster than that with chloride. This was as expected, as neither of these benzyloxymethyl halides reacted with tetramethylurea and, consequently, were fully available for reaction. Tetramethylurea is now the base of choice in this laboratory for alkylations using alkoxymethyl halides. For strongly hindered hydroxyl groups, molar ratios of alcohol, alkoxymethyl bromide,

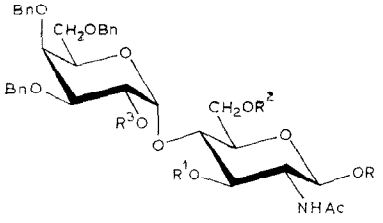
and tetramethylurea of 1 : 2 : 4 are recommended. These insights were not established at the beginning of the present investigations and were gained because of the high resistance to alkylation displayed by certain hindered alcohols when 2,6-lutidine was used as buffer.

The 3-*O*-benzyloxymethyl group of **5** proved sufficiently stable to acidic hydrolysis to allow removal of the 4,6-*O*-benzylidene group by 50% aqueous acetic acid at 80°. The product (**7**, 80% yield) was then preferentially alkylated at the primary position by using benzyloxymethyl chloride in the presence of 2,6-lutidine in *N*,*N*-dimethylformamide. The total yield of crystalline product (**8**) was 70%. This compound was used to prepare the protected disaccharides **14** and **42**, which served as intermediates for the syntheses of the H type 2 (**22**) and epi-H type 2 (**47**) haptens.

Acid hydrolysis of the 4,6-*O*-benzylidene group of the allyl ether **6** to form **9** proceeded without difficulty (80% yield). This compound was then preferentially alkylated at the primary position by using the tetramethylurea as base and with benzyloxymethyl bromide (to form **10**) and with 2,2,2-trichloroethoxymethyl bromide[24,25] to form **11**. The yields were 58 and 66%, respectively. Compound **10** was used as an intermediate in the preparation of the 6-deoxy-H type 2 hapten (**29**) and of the Y hapten (**35**). Compound **11** was prepared to offer a route to the X hapten (**41**).



12 R^1 = R^2 = CH_2OBn, R^3 = $pNO_2C_6H_4CO$
14 R^1 = R^2 = CH_2OBn, R^3 = H
16 R^1 = All, R^2 = CH_2OBn, R^3 = Bz
17 R^1 = All, R^2 = CH_2OBn, R^3 = H

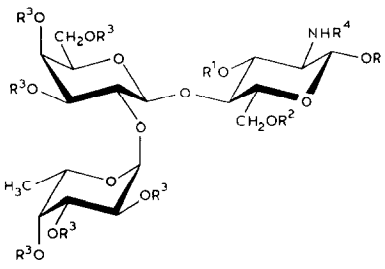


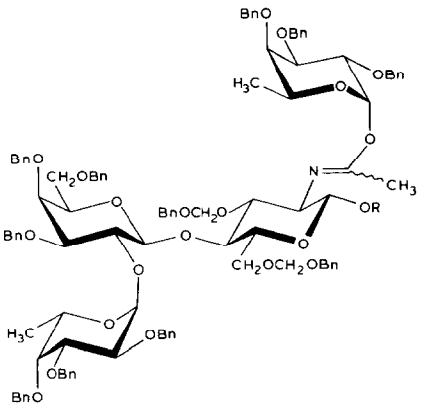
13 R^1 = R^2 = CH_2OBn, R^3 = $pNO_2C_6H_4CO$
15 R^1 = R^2 = CH_2OBn, R^3 = H

Attempted condensation of the *p*-nitrobenzoate–bromide **1** with alcohol **8**, under Helferich conditions[26] using mercuric cyanide, failed to provide the protected lactosamine derivative **12**. However, when the condensation was conducted under the conditions described by Hanessian and Banoub[27], with silver triflate as promoter and tetramethylurea as proton acceptor, an ~60% yield of a mixture of disaccharide derivatives was obtained. Chromatographic separation showed the mixture to be an ~3 : 2 mixture of the β- (**12**) and α- (**13**) linked isomers. Treatment of the mixture with sodium methoxide in methanol to remove the *p*-nitrobenzoyl group provided a mixture of the alcohols **14** and **15**, which were readily separated by chromatography on silica gel.

As will be seen later in connection with the synthesis of the 3-*O*-allyl analog (**16**) of **12**, and as previously commented upon by Sinaÿ[28], Helferich condensations at the 4-position of a protected β-glucoside are strongly influenced by the neighboring 3-substituent. In fact, on changing the 3-*O*-benzyloxymethyl group of **8** to an allyl group to form **10**, the yield of the β-linked disaccharide derivative was increased

from 45 to 76%. Although the yield of **14** from the alcohol **8** was low (25% isolated) and the procedure is not recommended, an adequate amount of the compound was obtained to allow the preparation of the H type 2 hapten. Its α anomer (**15**) was not further investigated. The lack of specificity displayed in the glycosylation reaction is noteworthy, as this method has been claimed[27] to be highly stereoselective for the β configuration.





20

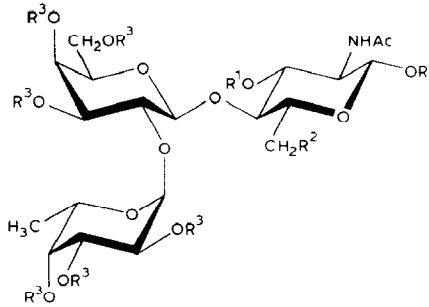
18 R^1 = R^2 = CH_2OBn, R^3 = Bn, R^4 = Ac
19 R^1 = R^2 = CH_2OBn, R^3 = Bn, R^4 = H
21 R^1 = All, R^2 = CH_2OBn, R^3 = Bn, R^4 = Ac
22 R^1 = R^2 = R^3 = H, R^4 = Ac, H type 2 hapten
23 R^1 = R^2 = R^3 = R^4 = H, R = $(CH_2)_8CO_2H$

Compound **14** was subjected to standard bromide ion-catalyzed α-fucosylation[29] by tri-*O*-benzyl-α-L-fucopyranosyl bromide. The conditions involved both Hünig's base and 4A molecular sieve to buffer the mixture. Chromatographic separation of the products provided a main fraction indicated by ^{1}H-n.m.r. to be an ~2:1 mixture of two trisaccharide derivatives. These products were separated by different chromatographic conditions and the major fraction proved to be the desired compound (**18**) (44% yield). The minor component (**19**) of the mixture had ^{1}H-n.m.r. parameters very similar to those of **18** except that there was no *N*-acetyl group present. Acetylation of **19** provided **18** in quantitative yield.

Although we have no direct evidence for the formation of the fucosyl acetamidate **20** in the course of the bromide ion-catalyzed condensation, the circumstantial evidence appears convincing. Pougny and Sinaÿ[30] observed the formation of *O*-glycosyl imidates as by-products in Koenigs–Knorr-type condensations, and these observations then formed the base for the imidate procedure for the formation of α-linked glycosides[31]. The glycosyl imidates are extremely sensitive to hydrolysis and do not survive chromatography on silica gel[32]. Hydrolysis of imidates may be controlled to produce either free amine or to regenerate the amide[33]. Therefore, the imidate **20** could lead to the amine **19** in the course of the isolation procedure. The production of acetimidates was also observed in other halide-ion glycosylations, and

it seemed likely that their formation was related to the presence of the Hünig's base in the mixture. As it was known[34] that the halide ion-catalyzed glycosylation reactions do not require base catalysts, the preparation of **21** was conducted using only the 4A molecular sieve as buffer. The isolated yield was 94% and the product did not contain a detectable amount of the amine. We therefore no longer recommend the use of hindered organic bases as buffers in halide ion-catalyzed glycosylation reactions unless the reactants contain an extremely acid-sensitive grouping. As previously reported[34], 4A molecular sieve (BDH) absorbs hydrogen bromide sufficiently well to prevent the reaction of liberated hydrogen bromide with an *O*-trityl group.

As control experiments had shown that benzyloxymethyl ethers are readily cleaved by hydrogenolysis in the presence of palladium to generate the alcohol, the H type 2 hapten (**22**) was readily obtained (85% yield) on hydrogenolysis of **18.**

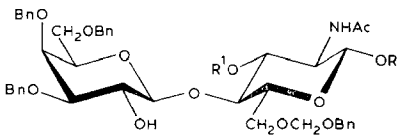


21 R^1 = All, R^2 = OCH_2OBn, R^3 = Bn
24 R^1 = H, R^2 = OCH_2OBn, R^3 = Bn
25 R^1 = Ac, R^2 = OCH_2OBn, R^3 = Bn
26 R^1 = Ac, R^2 = OH, R^3 = Bn
27 R^1 = Ac, R^2 = OMs, R^3 = Bn
28 R^1 = Ac, R^2 = I, R^3 = Bn
29 R^1 = R^2 = R^3 = H, 6-deoxy-H type 2 hapten

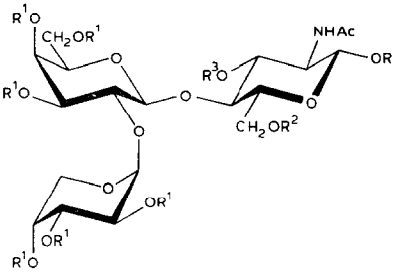
The choice of the allyl group[35] for protection of the 3-position[17] in the synthesis of **16** was to protect this group with a substituent that could be preferentially removed in order to convert **21** into **24**. The procedure developed by Corey[36] employing *tris*(triphenylphosphine)rhodium(I) chloride as catalyst for isomerization of the allyl group to 1-propenyl, followed by hydrolysis in the presence of mercuric chloride, was used.

Acetylation of **24** gave **25**, from which the 6-*O*-benzyloxymethyl protecting group was selectively removed to provide the alcohol **26** by treatment for 5 min in the cold with a solution of hydrogen bromide in dichloromethane–acetic acid. Mesylation of **26** provided **27** and replacement of the mesyloxy group by iodine to form **28**, followed by hydrogenolysis and transesterification, then furnished the desired 6-deoxy-H type 2 hapten (**29**). The overall yield from **25** to **29** was very low (7.5%) and there can be no doubt that the overall procedure could be improved.

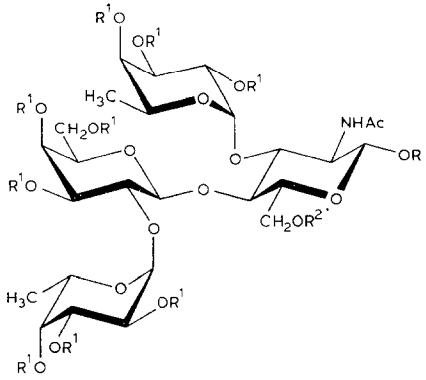
Nevertheless, sufficient material (7 mg) was obtained for proper characterization by ^{1}H-n.m.r. at 400 MHz.



17 R^1 = All 30 R^1 = H



31 R^1 = Bn, R^2 = CH_2OBn, R^3 = All
32 R^1 = Bn, R^2 = CH_2OBn, R^3 = H
33 R^1 = R^2 = R^3 = H, 5″-nor-H type 2 hapten

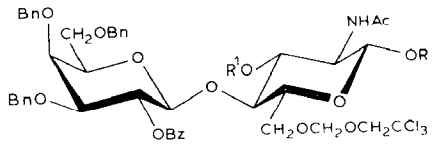


34 R^1 = Bn, R^2 = CH_2OBn
35 R^1 = R^2 = H, Y hapten

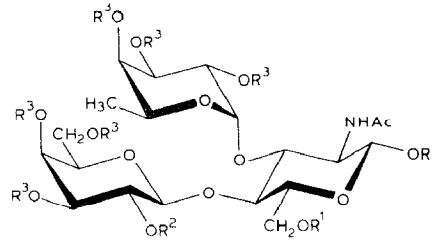
Compound **17** was used to prepare βDAra(1→2)βDGal(1→4)βDGlcNAcO-$(CH_2)_8CO_2Me$ (**33**), which is referred to as the 5″-*nor*-H type 2 hapten. The chloride ion-catalyzed condensation between 2,3,4-tri-*O*-benzyl-β-D-arabinopyranosyl chloride (prepared by treatment of 2,3,4-tri-*O*-benzyl-D-arabinose[37] with the Vilsmeier reagent[38] and the alcohol **17** was slow at room temperature. By using 1,2-dichloroethane as solvent, the reaction temperature was raised to 65°. After 4 days, the desired product **31** was obtained in 52% yield, by chromatographic separation, in admixture with a small amount of an unidentified compound. This crude product was then de-allylated conventionally to give **32** as a colorless chromatographically pure syrup having n.m.r. parameters consistent with expectations. Hydrogenolysis of **32** to remove the benzyl and benzyloxymethyl groups proceeded in high yield (80%) to produce the desired 5″-*nor*-H type 2 hapten (**33**).

The alcohol **17** was also used to prepare the Y hapten (**35**). The first step was to remove the allyl group (90% yield) to form the diol **30**. This product was then difucosylated using the bromide ion-catalyzed reaction. The isolated yield of the

desired product (**34**) was 75%. Hydrogenolysis of **34** in the presence of palladium-on-charcoal then generated the Y hapten (**35**) in 87% yield.



36 R^1 = All
37 R^1 = H



38 $R^1 = CH_2OCH_2CCl_3$, R^2 = Bz, R^3 = Bn
39 R^1 = H, R^2 = Bz, R^3 = Bn
40 $R^1 = R^3$ = H, R^2 = Bz
41 $R^1 = R^2 = R^3$ = H, X hapten

Although we presume that the X hapten (**41**) could have been prepared starting from **16**, the trichloroethoxymethyl analog of **16**, namely **36**, was prepared for this purpose. Trichloroethoxymethyl chloride was introduced as a reagent by Jacobson and Clader[25]. We prepared the corresponding bromide for the preparation of **36** in order to achieve more-rapid introduction of the trichloroethoxymethyl group. The intention was to also prepare the 6-deoxy-X hapten, but this objective was not met in this investigation. The derivative of *N*-acetyllactosamine **36** was prepared by condensation of the glycosyl bromide **2** with the alcohol **11**. In this case, 10:9[39,40] silver triflate–*sym*-collidine was used to promote the condensation. The isolated yield of the crystalline β-disaccharide derivative **36** was only 29%. Removal of the allyl group provided **37**, which was then fucosylated under bromide ion-catalyzed conditions using the 4A molecular sieve to absorb the liberated hydrogen bromide, and *N*,*N*-dimethylformamide in dichloromethane as solvent in order to speed up the reaction. The yield of **38** was 80%.

Attempts to remove the trichloroethoxymethyl group of **38** by reduction[25] with zinc dust encountered difficulties. The yields were variable depending on the source of the zinc dust, and were generally unacceptable, primarily because the reaction did not go to completion. The situation was not substantially improved by using Zn-Cu or Zn-Pt couples. However, the use of a Zn-Ag couple proved most effective, regardless of the source of the zinc dust, and allowed the preparation of **39** in acceptable yield (66%). This product was then subjected to hydrogenolysis to form **40**, which was subjected directly to transesterification to give the desired X hapten (**41**) in 83% yield from **39**. The ^{1}H-n.m.r. spectrum of **41** bears a relationship to that of the H type 2 (**22**) and Y haptens (**35**), which is very similar to that between the Le^a hapten and the Le^d (H type 1) and Le^b haptens[4]. These relationships are readily appreciated from the 400-MHz ^{1}H-n.m.r. spectra (see Table I) which are reproduced in Fig. 2. The specific deshielding of H-5″ (fucosyl residue) by O-3 and O-5′, as previously noted for the Le^a hapten[4], is also seen to be present for the X hapten.

TABLE 1

^{1}H- AND ^{13}C-N.M.R. CHEMICAL SHIFTS FOR THE H TYPE 2 (**22**), Y (**35**), AND X (**41**) HAPTENS

Residue:	*βDGlcNAc*							*βDGal*						
Position:	1	2	3	4	5	6	6′	1	2	3	4	5	6	6′
^{1}H-n.m.r. data[a]														
H type 2	4.79	4.02	3.95	4.06	3.75	4.27	4.09	4.83	3.96	4.17	4.19	3.99	4.08	4.02
Y	4.79	4.13	(4.12)	4.21	3.74	4.31	4.12	4.79	3.94	4.14	4.16	3.89	4.04	4.00
X	4.82	4.15	(4.18)	4.23	3.86	4.29	4.15	4.75	3.79	3.95	4.19	3.89	4.04	4.00
^{13}C-n.m.r. data[b]														
H type 2	101.89	56.25	73.12	77.26	76.12	61.13		101.28	77.36	74.43	69.98	76.02	61.86	
Y	101.76	56.93	76.48	74.16	75.72	60.83		101.10	77.38	74.42	69.58	75.72	62.29	
X	101.96	56.93	76.41	73.61	75.98	60.91		102.89	71.55	74.53	69.41	75.98	62.47	
Residue:	*αLFuc(1→2)*							*αLFuc(1→3)*						
Position:	1	2	3	4	5	6		1	2	3	4	5	6	
^{1}H-n.m.r. data[a]														
H type 2	5.58	4.11	(4.09)	4.10	4.52	1.54								
Y	5.55	4.10	(4.12)	(4.12)	4.54	1.58		5.37	3.99	4.21	4.08	5.15	1.55	
X								5.39	3.98	4.20	4.09	5.13	1.49	
^{13}C-n.m.r. data[b]														
H type 2	100.28	69.14	70.55	72.56	67.73	16.11								
Y	100.38	68.63	70.65	72.63	67.69	16.35		99.41	69.19	70.07	72.80	67.69	16.35	
X								99.61	68.81	70.32	73.00	67.72	16.34	

[a]Measured at 400 MHz and 300K using 0.02M solutions in D_2O with acetone, set at 2.48 p.p.m., as the internal standard. The assignments were made conventionally by Dr. H. Thøgersen[4]. The chemical shifts in parentheses are ±0.04 p.p.m. [b]Measured with 0.06M solutions containing 1,4-dioxane set at 67.40 p.p.m. as internal standard. The signals for **22** were assigned as previously described[4] and those for **35** and **41** were assigned by inspection with reference to the assignments for **22** and those previously assigned for the Lewis a, b, and d haptens[4].

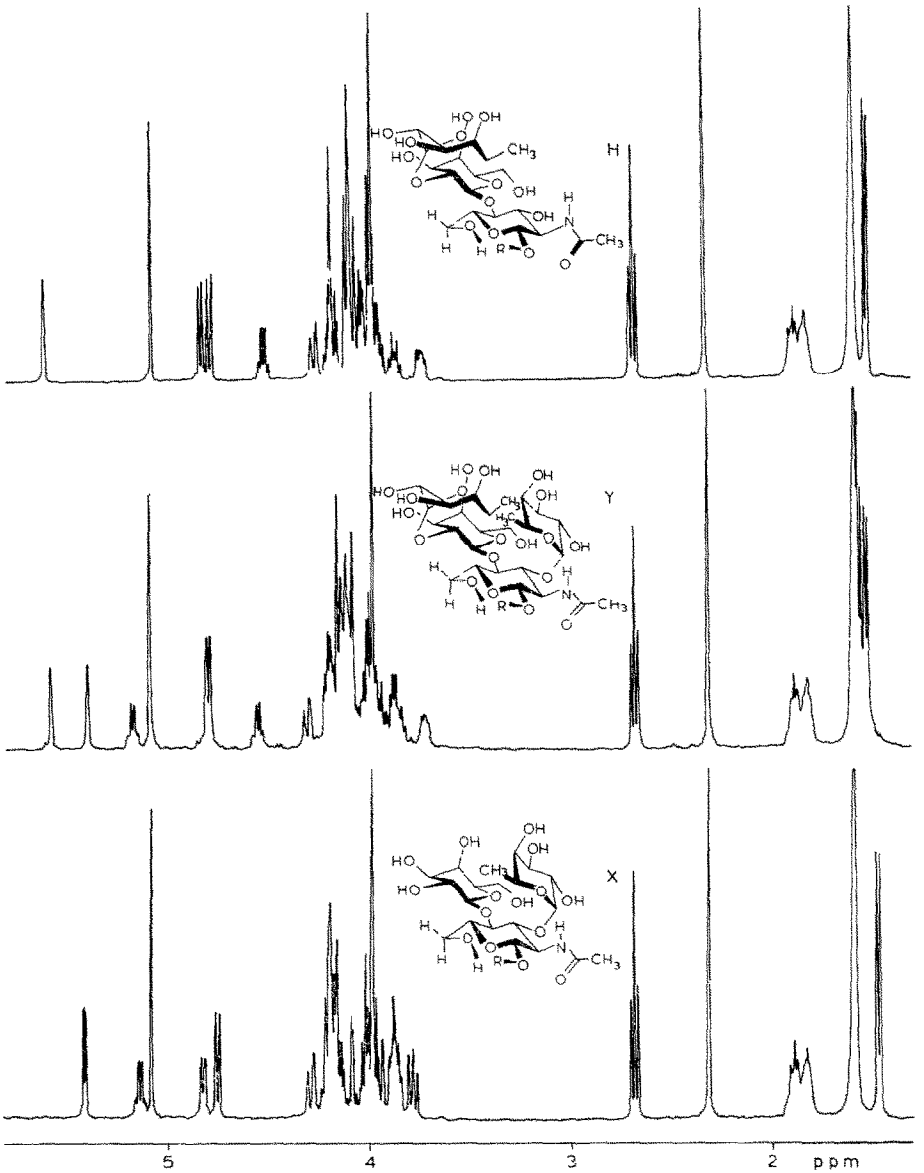
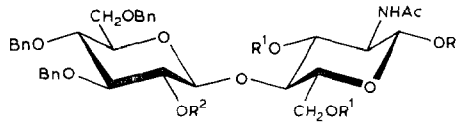
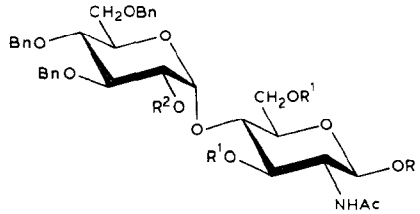


Fig. 2. 400-MHz ^{1}H-n.m.r. spectra in D_2O to display the resolution achieved and the correspondence of signals in the spectra of the H type 2 (H, **22**), and X (**41**) haptens to features in the spectrum of the Y hapten (**35**). The ^{1}H- and ^{13}C-n.m.r. parameters for these structures are recorded in Table I.

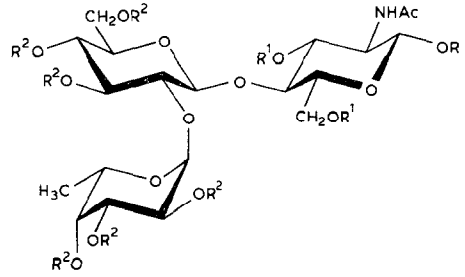
However, in this case, the marked deshielding of H-5″ is expected (HSEA calculations[5]) to arise because of its close proximity to both O-4 (2.50A) and O-5′ (2.57A). The conformational properties of the H type 2, X, and Y antigenic determinants will be considered in detail in a separate communication[6].



42 R^1 = CH_2OBn, R^2 = Ac
44 R^1 = CH_2OBn, R^2 = H



43 R^1 = CH_2OBn, R^2 = Ac
45 R^1 = CH_2OBn, R^2 = H



46 R^1 = CH_2OBn, R^2 = Bn
47 R^1 = R^2 = H ; 4′-epi-H type 2 hapten

In order to prepare the 4′-*epi*-H type 2 hapten (**47**), as in the preparation of **12**, the Hanessian–Banoub conditions[27] were used to condense 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-glucopyranosyl bromide (**3**) with the 3,6-di-*O*-benzyloxymethyl derivative of βDGlcNAc-O$(CH_2)_8CO_2Me$ (**8**). Again, the condensation provided a mixture of the β- and α-linked disaccharides, in this case **42** and **43**. The compounds were isolated as a mixture by chromatography. Deacetylation by transesterification yielded a mixture of the alcohols **44** and **45**, which could now be readily separated by chromatography (31 and 11% overall yields, respectively). Fucosylation of the β,β′-anomer **44** under bromide ion-catalyzed conditions using both Hünig's base and 4A molecular sieve as buffers provided the desired trisaccharide derivative **46** in 57% yield. This yield could likely have been improved by the omission of the Hünig's base, but this insight was not available at the time this preparation was made. Hydrogenolysis to remove both the benzyloxymethyl and benzyl groups then provided the 4′-*epi*-H type 2 hapten (**47**) in 85% yield.

Although the lectin 1 of *Ulex europaeus* was known to not bind the H type 1 (Lewis d) hapten, it was considered[41] possible that removal of the acetyl group to form the *N*-deacetyl H type 1 hapten (**48**) would provide a suitable substrate. The conditions described by Lindberg and co-workers[42] were explored for this purpose and it was observed, in preliminary experiments, that although the *N*-deacetylation of the H type 2 hapten (**22**) to form **23** proceeded readily at 100°, this was not the case for the H type 1 hapten. In the latter instance, a reaction time of 65 h at 120° was required for completion. These conditions were used for preparation of the amino acid **23** and the amino acids **48**, **49**, and **50** from the H type 1, Lewis a, and Lewis b haptens, respectively. Several laboratories[42-44] have commented on the influence of a neighboring substituent on the ease of *N*-deacetylation of *N*-acetyl hexosamine derivatives.

TABLE II

INHIBITION OF THE AGGLUTINATION OF OLe^{a+b-} HUMAN RED CELLS BY LECTIN I OF *Ulex europaeus*

Hapten[a]	*Structure*	*Minimum amount (μg)*	*Relative potency*
H type 2 (**22**)	αLFuc(1→2)βDGal(1→4)βDGlcNAc	0.25	1
de-*N*Ac-H type 2 (**23**)	αLFuc(1→2)βDGal(1→4)βDGlcNH$_2$	0.25	1
4′-*epi*-H type 2 (**47**)	αLFuc(1→2)βDGlc(1→4)βDGlcNAc	0.5	2
6-deoxy-H type 2 (**29**)	αLFuc(1→2)βDGal(1→4) 6-deoxy-βDGlcNAc	0.03	0.1
5″-*nor*-H type 2 (**33**)	βDAra(1→2)βDGal(1→4)βDGlcNAc	8.0	32
Methyl α-L-fucopyranoside	αLFuc	8.0	32
H-disaccharide	αLFuc(1→2)βDGal	8.0	32
N-Acetyllactosamine	βDGal(1→4)βDGlcNAc αLFuc ↓ 1,3	>64	>250
X (**41**)	βDGal(1→4)βDGlcNAc αLFuc ↓ 1,3	>64	>250
Y (**35**)	αLFuc(1→2)βDGal(1→4)βDGlcNAc	1	4
H type 1 (Le^d)	αLFuc(1→2)βDGal(1→3)βDGlcNAc	>64	>250
de-*N*-Ac-H type I (**48**)	αLFuc(1→2)βDGal(1→3)βDGlcNH$_2$ αLFuc ↓ 1,4	2	8
Lewis a	βDGal(1→3)βDGlcNAc αLFuc ↓ 1,4	~64	~250
de-*N*-Ac-Lewis a (**49**)	βDGal(1→3)βDGlcNH$_2$ αLFuc ↓ 1,4	16	64
Lewis b	αLFuc(1→2)βDGal(1→3)βDGlcNAc αLFuc ↓ 1,4	>64	>250
de-*N*-Ac-Lewis b (**50**)	αLFuc(1→2)βDGal(1→3)βDGlcNH$_2$	8	64

[a]Except for methyl α-L-fucopyranoside, these structures are glycosidically attached to a linking arm.

THE COMBINING SITE OF THE LECTIN

Table II lists the minimum amounts of blood-group related haptens which, under the standard conditions described in the Experimental section, caused complete inhibition of the agglutination of OLe^{a+b-} human red cells by the lectin 1 of *Ulex europaeus*. It may be seen that hydrolysis of the H type 2 hapten (**22**) to form the H type 2 amino acid (**23**) had no influence on the inhibition potency. This result is in accord with the observation by Pereira and coworkers[45], who found that 2′-*O*-fucosyllactose was as good an inhibitor of the agglutination of O cells by the lectin as was 2′-*O*-fucosyl-*N*-acetyllactosamine. The conclusion that the acetamido group is not bound by the lectin was therefore confirmed. As the amino acid **23** was fully effective as an inhibitor, we further concluded that the presence of an 8-carboxyoctyl aglycon rather than an 8-methoxycarbonyloctyl aglycon has no appreciable influence on the binding of the structure with the lectin.

In view of the foregoing results, it was anticipated that the H type 2 determinant is bound within a hydrophobic cleft in the surface of the lectin that has complimentarity in structure to the region described in the CPK model projection shown for the H type 2 determinant in Fig. 1A. In fact, the H type 2 trisaccharide may be seen to possess a topographical feature that is wedge-like and, except for the 6-hydroxyl group, could be expected to be compatible with dehydration for hydrophobic bonding within a hydrophobic cleft. The event would leave the acetamido group and the remaining seven hydroxyl groups of the H type 2 trisaccharide solvated by water. As may be seen from the projection in Fig. 1B, removal of the acetamido group would alter a region of the hapten outside this ambiphilic region and therefore remain compatible with the lectin's combining site. Also, on this basis, a change in the orientation of a hydroxyl group in the strongly hydrophilic region of the hapten (outside the region enclosed in Fig. 1A) should have little influence, if any, on the strength of the binding. Indeed, the 4′-*epi*-H type 2 hapten (**47**) was virtually as good an inhibitor, and this result requires that the hydroxylic portion of the βDGal unit not be involved in the binding.

As already mentioned and as might be appreciated from Fig. 1A, the 6-hydroxyl group of the βDGlcNAc residue would be at the edge of the ambiphilic wedge that is proposed to enter a hydrophobic cleft. The OH-6 group of glucopyranosides is known[46] to be well disposed for intramolecular hydrogen-bonding with the neighboring O-5 atom. Lemieux and co-workers[7] achieved circumstantial evidence that intramolecular hydrogen-bonding may render a hydroxyl group compatible with hydrophobic bonding, and this appears established for the I Ma antigenic determinant[10]. Therefore, it was expected that the H type 2 hapten may be bound by the lectin with OH-6 intramolecularly hydrogen-bonded either in the *syn–trans* orientation displayed in the CPK projection presented in Fig. 1A or in the likely less favorable *syn–syn* orientation. The descent of the ambiphilic region of the H type 2 determinant into a hydrophobic cleft would displace several water molecules at the hydrophobic surface of the protein while providing, through complementarity of structure, im-

portant multipoint Van der Waals' forces of attraction[47]. Therefore, it was expected that the removal of OH-6 to form the 6-deoxy H type 2 hapten (**29**) would provide a structure that would still be bound by the lectin unless this hydroxyl group becomes intermolecularly hydrogen-bonded to the surface of the combining site. In fact, **29** proved to be nearly 10 times *more* potent as an inhibitor than the H type 2 hapten. This observation is considered to prove beyond reasonable doubt that OH-6 is intramolecularly hydrogen-bonded when the lectin enters the combining site and that the basic driving force for the reaction is hydrophobic.

In order to further confirm the hypothesis that the region outlined in Fig. 1 well represents the topography of the H type 2 determinant that is bound by the lectin, it was decided to synthesize the 5″-*nor*-H type 2 hapten (**33**). As may be seen in Fig. 1A, the methyl group of the αLFuc residue occupies a position in the proposed ambiphilic region that is on one side of the wedge. Replacement of this methyl group by hydrogen would be expected not only to reduce importantly the hydrophobicity of the topography but, also, to influence adversely the complementarity of atoms in the strengthening of the bonding by way of Van der Waals' forces of attraction after the hapten has entered the combining site (Compare Figs. 1A and 1D). Therefore, it was to be expected that the 5″-*nor*-H type 2 hapten would be a relatively poor inhibitor. Indeed, as may be seen from Table II, this fraudulent hapten was about 32 times less potent an inhibitor of the agglutination.

The lectin I of *Ulex europaeus* is well known to bind methyl α-L-fucopyranoside weakly[45]. Table II shows that, under the conditions of our inhibition studies, it is 32 times less potent than the H type 2 hapten. Examination of Fig. 1A shows that the binding site is expected to bind the βDGal residue only about the CH-1′, CH-3′, and CH-5′ atoms. The important binding is indicated to be with portions of the βDGlcNAc and αLFuc residues. Removal of either of these residues to form the αLFuc(1→2)-βDGal or βDGal(1→4)βDGlcNAc haptens listed in Table II should lead to poor inhibitors. In fact, the latter structure was very poor, whereas the αLFuc(1→2)βDGal hapten was not superior as an inhibitor to methyl α-L-fucopyranoside. The fact that this disaccharide was not superior supports the important contribution to the binding that is attributed to the βDGlcNAc residue of the H type 2 hapten.

In view of the foregoing discussion, it was to be expected that the X hapten (**41**) would be ineffective as an inhibitor, whereas the H type 2-related Y hapten (**35**) would be a good inhibitor. As may be seen in Table III, the Y hapten was four times less potent. That this structure was not quite so effective is undoubtedly related to the fact that the αLFuc(1→3) component occupies a region in space close to the αLFuc-(1→2′) residue[3,4].

The fact that the Lewis a, Lewis b, and Lewis d (or H type 1) structures do not react appreciably with the lectin I of *Ulex europaeus* has already been mentioned[41]. As may be appreciated from Fig. 1E, the acetamido group of the H type 1 determinant is expected to be at the surface of an ambiphilic region somewhat similar in size and shape to that proposed for the H type 2 determinant. This hapten, however, is not well bound by the lectin. Replacement of the *N*-acetyl group of the H type 1 hapten to

form the H type 1 amine (**48**) was expected to provide an ambiphilic region (see Fig. 1F) that is more compatible. Comparison of this topography with that presented by the 6-deoxy-H type 2 hapten (**29**) (Fig. 1C) is particularly impressive in this regard. Therefore, it was not surprising that the H type 1 amino acid (**48**) at pH 7.2 was a good inhibitor — only 8 times less potent than the H type 2 hapten (**22**). It is noteworthy in this regard that the only similarities between the ambiphilic wedges of **29** and **48** are topography and hydrophobicity — the two basic requirements for effective hydrophobic bonding. Thus, the potency of **48** as an inhibitor provides strong confirmation of our tenet that the driving force for binding of the lectin with the H type 2 determinant is hydrophobic coupled with a complementarity of structure that establishes multipoint Van der Waals' forces of attraction for the further stabilization of the complex and which are at the source of the specificity of the reaction.

In conclusion, it is noted that the Lewis a hapten was ineffective as an inhibitor, although it contains an αLFuc residue. Our interpretation of the ^{1}H-n.m.r. spectrum for this hapten, which conforms with the model obtained by HSEA calculation, places the required topography of the αLFuc residue in a hydrophilic environment and, therefore, energetically less available for binding than is this topography in the simple methyl α-L-fucopyranoside. As may be seen in Table II, *N*-deacetylation of the Lewis a hapten to form the amino acid (**49**) provided a still very weak but superior inhibitor. This behavior may arise because of an increase in the freedom of rotation about the O-3–C-3 bond of the βDGlcNH$_2$ residue of **49** as compared to when the 2-substituent is the bulky acetamido group. Only a small decrease in the H-3–C-1′ ψ torsion angle from 15° (ref. 4) to 0° (which brings the acetamido group closer to OH-2) would substantially relieve the fucosyl residue from the neighboring hydrophilic hydroxyl groups of the βDGal residue. We consider these observations to lend support to our working hypothesis[6] that lectins and antibodies, perhaps in contrast to enzymes, are designed to accept oligosaccharides in their energetically favorable conformations.

The observation (Table II) that the Lewis b tetrasaccharide hapten is not bound appreciably by the lectin is attributed to the reasons already outlined, which caused neither the Lewis a nor the Lewis d (H type 1) determinant to be effective as inhibitors. The fact that the Lewis b amino acid (**50**) showed some inhibition could be assigned to the same reason that the H type 1 (Lewis d) amino acid (**48**) was a good inhibitor. That the Lewis b amino acid was a poorer inhibitor may be assigned to the same reason that was already discussed to rationalize the weaker potency of the Y (**35**) as compared to the H type 2 hapten (**22**).

EXPERIMENTAL

General methods. — All solvents and reagents were purified and dried according to standard procedures[48]. The molecular sieve (British Drug House, 4A) was dried for 24 h at 180° just prior to use. Solution transfers were conducted under dry nitrogen by standard syringe techniques[49]. The thin-layer chromatograms were performed

on precoated plates of silica gel (60-F254, E. Merck, Darmstadt) and spots made visible by quenching of fluorescence and/or by charring after spraying with 5% sulphuric acid in ethanol. For column chromatography, the loading was in the range 1:50 to 1:100 on silica gel (H type 60, E. Merck, Darmstadt) and distilled solvents were used to develop the chromatograms. The melting points are uncorrected. All samples of 2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl bromide were prepared just prior to use from 2,3,4-tri-*O*-benzyl-1-*O*-*p*-nitrobenzoyl-β-L-fucopyranose[50]. Except where otherwise stated, the chemical shifts are given relative to internal tetramethylsilane. The specific rotations were measured at room temperature (22 ± 1°).

3,4,6-Tri-O-*benzyl-2*-O-p-*nitrobenzoyl*-α-D-*galactopyranosyl bromide*[21] (**1**). — 3,4,6-Tri-*O*-benzyl-1,2-*O*-(1-ethoxyethylidene)-α-D-galactopyranose[51] was hydrolyzed by sequential treatment with 95% acetic acid at room temperature for 25 min and, after evaporation of the solvent, 2:8:1 triethylamine–methanol–water for 12 h at room temperature. Di-*O*-*p*-nitrobenzoylation of the resultant product provided 3,4,6-tri-*O*-benzyl-1,2-di-*O*-*p*-nitrobenzoyl-β-D-galactopyranose, m.p. 132°, which was isolated by crystallization from ethyl acetate–pentane[21]. Conventional treatment of this material with hydrogen bromide provided a syrupy product (**1**) that was used without further purification. The ^{1}H-n.m.r. spectrum was in accord with the structural assignment.

2-O-*Benzoyl-3,4,6-tri*-O-*benzyl*-α-D-*galactopyranosyl bromide* (**2**). — 3,4,6-Tri-*O*-benzyl-1,2-*O*-(1-ethoxyethylidene)-α-D-galactopyranose (15 g) was dissolved in 95% aqueous acetic acid (100 mL) and kept for 20 min at room temperature. The solution was then evaporated and acetic acid in the syrupy residue removed by evaporation of toluene from it (twice). The ^{1}H-n.m.r. spectrum indicated the product to be essentially pure 1-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-galactopyranose. Without further purification, this crude product was dissolved in dry dichloromethane (100 mL) and the solution cooled to 0° in the presence of 4A molecular sieve (~5 g), and then a solution of benzoyl chloride (6.0 mL, 51 mmol) and pyridine (4.03 mL, 50 mmol) in dichloromethane (50 mL) was added. After stirring overnight at 0°, the product was isolated conventionally and chromatographed on a column of silica gel (600 g) with 17:3 toluene–ethyl acetate as eluent. The main fraction readily crystallized and was recrystallized from diethyl ether–*n*-hexane (68% yield), m.p. 104–105°, $[\alpha]_D$ +104° (*c* 0.5, chloroform). The structure was evident from the ^{1}H-n.m.r. spectrum and the material was used directly, without further characterization, for preparation of the title compound (**2**). The material (5.67 g, 9.52 mmol) was dissolved in dry dichloromethane (45 mL) at 0° and 35% hydrogen bromide in acetic acid (4.5 mL) was added. The solution was stirred for 30 min at room temperature and the product isolated conventionally. The material did not crystallize but appeared to be essentially pure (t.l.c. and ^{1}H-n.m.r.). The ^{1}H-n.m.r. spectrum left no doubt as to the identity of **2**; three *O*-benzyl groups and one *O*-benzoyl group were present and the signal for H-1 (δ 6.87, d, 4 Hz), was in the region expected for an α-glycosyl bromide. Also, the signal for H-2, δ 5.56 (dd, 4 and 9.5 Hz), was in the region expected for a 2-benzoate.

The compound was unstable on storage and was used directly without further purification.

2-O-Acetyl-3,4,6-tri-O-benzyl-α-D-glucopyranosyl bromide[23] (**3**). — A mixture of 3,4,6-tri-*O*-benzyl-1,2-*O*-(1-ethoxyethylidene)-α-D-glucopyranose[23] (2.21 g, 4.25 mmol), tetraethylammonium bromide (0.42 g, 2.01 mmol), molecular sieve (3.3 g), and dichloromethane (16 mL) was stirred for 2 h at room temperature and then acetyl bromide (0.60 mL, 8.2 mmol) was added. After 1 h, t.l.c. (4:1 benzene–ethyl acetate) showed almost exclusive conversion into a more-mobile product. The mixture was then poured into a vigorously stirring mixture of saturated sodium hydrogencarbonate, ice, and dichloromethane and filtered to remove the sieves. The organic phase was washed with cold, aqueous sodium hydrogencarbonate, water, and dried before evaporation to a clear syrup. The n.m.r. spectra of this product showed it to consist of >90% of **3**: ^{1}H-n.m.r. ($CDCl_3$): δ 7.40–7.10 (m, 15 H), 6.63 (d, $J_{1,2}$ 4.0 Hz, $\geq$ 0.9H, H-1), 4.90–4.37 (m, 7 H, H-2 and benzylic), 4.16–3.55 (m, 5 H), and 2.00 (s, $\geq$ 2.8H, $COCH_3$); ^{13}C-n.m.r. ($CDCl_3$): δ 169.87 (C=O), 138.34, 137.97, 137.90, 128.48, 127.89, 127.64, 89.53 (C-1), 80.40, 76.37, 75.53 (2C), 75.29, 73.52, 73.40, 67.61, and 20.69 ($COCH_3$).

8-Methoxycarbonyloctyl 2-acetamido-4,6-O-benzylidene-3-O-benzyloxymethyl-2-deoxy-β-D-glucopyranoside (**5**). — A solution of 8-methoxycarbonyloctyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranoside[19] (**4**) (18.6 g, 38.4 mmol), 2,6-lutidine (8.9 mL, 77 mmol), and benzyloxymethyl chloride (52) (8.0 mL, 58 mmol) in dry acetonitrile (140 mL) was kept for 12 h at 80°. Methanol (10 mL) was then added and, after 1 h, the solution was taken to dryness. The residue was dissolved in dichloromethane (400 mL) and washed with cold 2% hydrochloric acid, saturated sodium hydrogencarbonate, and water before drying and evaporation. The residual, dark syrup was then dissolved in 2:1 dichloromethane–ethyl acetate and decolorized with alumina.

Evaporation of the eluate and crystallization from methanol provided the title compound (18.2 g, 79%); m.p. 159–160°, $[\alpha]_D$ +8.4° (*c* 1.0, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 7.49–7.22 (m, 10 H), 5.79 (d, 1H, NH), 5.50 (s, 1H, benzylidene), 5.00–4.77 [m, 3H, H-1 (δ 4.84, $J_{1,2}$ 8.0 Hz) and OCH_2O (AB, δA = 4.93, δB = 4.82, J_{AB} 7.0 Hz)], 4.72–4.44 [2H, benzyl (AB, δA = 4.61, δB = 4.56 J_{AB} 12.0 Hz)], 4.41–4.22 (m, 2H, H-3 and H-6e), 3.99–3.44 (m, 9H, with OCH_3 at δ 3.64), 2.29 (t, 2H, CH_2CO), 1.84 (s, 3H, $NHCOCH_3$), and 1.70–1.20 (m, 12H); ^{13}C-n.m.r. ($CDCl_3$): δ 174.23 ($COOCH_3$), 170.41 ($NHCOCH_3$), 137.89, 137.31, 128.88, 128.30, 128.14, 127.72, 127.48, 126.09, 101.30 (C-1), 81.79, 75.75, 69.93, 68.73, 65.79, 56.48 (C-2), 34.02 (CH_2CO), 29.50, 29.09, 28.99, 25.75, 24.86, and 23.33 ($NHCOCH_3$).

Anal. Calc. for $C_{33}H_{45}NO_9$: C, 66.09; H, 7.56; N, 2.34. Found: C, 66.22; H, 7.60; N, 2.14.

8-Methoxycarbonyloctyl 2-acetamido-3-O-allyl-4,6-O-benzylidene-2-deoxy-β-D-glucopyranoside (**6**). — Barium oxide (17 g), barium hydroxide octahydrate (5 g), and allyl bromide (4.2 mL, 48.2 mmol) were added to a solution of 8-methoxycarbonyloctyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranoside (**4**, 12 g, 25.0

mmol) in dry *N,N*-dimethylformamide (170 mL). The mixture was shaken for 3 h at room temperature and then partitioned between dichloromethane and ice-cold 2M hydrochloric acid. The organic layer was washed with hydrochloric acid and aqueous sodium hydrogencarbonate and then evaporated. The residue was taken up in methanol. Crude **6** precipitated (8.3 g) on keeping the solution at 4°. Chromatography of the mother liquor on a column of silica gel (400 g) using 1:1 toluene–ethyl acetate as eluent provided more material (1.4 g) for a total crude yield of 74%. Recrystallization from methanol gave the analytical sample, m.p. 204–205°, $[\alpha]_D$ −17°. The ^{1}H-n.m.r. spectrum was consistent with the structural assignment.

Anal. Calc. for $C_{28}H_{41}O_8N$: C, 64.7; H, 7.89; N, 2.69. Found: C, 64.7; H, 7.94; N, 2.56.

*8-Methoxycarbonyloctyl 2-acetamido-3-*O*-benzyloxymethyl-2-deoxy-β-*D*-glucopyranoside* (**7**). — Warm 50% aqueous acetic acid (80°, 200 mL) was added to a solution of compound **5** (16.2 g, 27.0 mmol) in 1,4-dioxane (50 mL) and the resulting solution was kept for 75 min at 80°. The solvent was then evaporated and traces of acetic acid were removed by the addition and evaporation of 3 portions of 1,4-dioxane (150 mL). The residue was purified by column chromatography with 10:5:5:2 dichloromethane–*n*-hexane–ethyl acetate–ethanol as eluent. The major component was precipitated as an amorphous, white powder from acetone–*n*-hexane (11.1 g, 80%), $[\alpha]_D$ +4.2° (*c* 1.2, chloroform); ^{1}H-n.m.r. (acetone-d_6, D_2O exchanged): δ 7.40–7.10 (m, 5H), 5.01–4.74 (AB, δA = 4.96, δB = 4.79, J_{AB} 6.7 Hz, 2H, OCH_2O), 4.63–4.51 (3H, H-1 and benzyl), 3.89–3.16 (m, 11H, with OCH_3 at δ 3.56), 2.24 (t, 2H, CH_2CO), 1.78 (s, 3H, $NHCOCH_3$), and 1.70–1.20 (m, 12H); ^{13}C-n.m.r. (CD_3OD): δ 175.82 ($COOCH_3$), 173.17 ($NHCOCH_3$), 139.21, 129.31, 128.70, 128.58, 102.52 (C-1), 96.91 (OCH_2O), 83.07, 77.65, 71.97, 70.58, 70.51 (OCH_3), 34.72 (CH_2CO), 30.56, 30.29, 30.22, 30.05, 26.97, 25.94, and 23.12 ($NHCOCH_3$).

Evaporation of the trailing fractions from the column provided 8-methoxycarbonyloctyl 2-acetamido-2-deoxy-β-D-glucopyranoside (1.28 g, 12%), identified by comparison with an authentic sample[19].

*8-Methoxycarbonyloctyl 2-acetamido-3,6-di-*O*-benzyloxymethyl-2-deoxy-β-*D*-glucopyranoside* (**8**). — A solution of compound **7** (10.5 g, 20.5 mmol), benzyloxymethyl chloride (3.11 mL, 22.5 mmol), and 2,6-lutidine (3.6 mL, 31.0 mmol) in *N,N*-dimethylformamide (35 mL) was kept for 16 h at room temperature. Methanol (2 mL) was then added and, after 1 h, the solution was diluted with dichloromethane (300 mL) and washed with cold 2% hydrochloric acid (300 mL), saturated aqueous sodium hydrogencarbonate (300 mL) and water, each time back-extracting with dichloromethane (100 mL). Drying and evaporation left a white gum (11.6 g) that crystallized from ethanol–water (7.86 g, 61%). Chromatography of the mother liquor provided an additional 1.2 g (9%), m.p. 112–113°, $[\alpha]_D$ −24.4° (*c* 1.4, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 7.43–7.14 (m, 10 H), 5.82 (d, 1 H, NH), 4.94–4.48 (m, 9 H), 4.12–3.06 (m, 12 H with OCH_3 at δ3.62), 2.26 (t, 2 H, CH_2CO), 1.89 (s, 3H, $NHCOCH_3$), and 1.70–1.20 (m, 12 H); ^{13}C-n.m.r. ($CDCl_3$): δ 174.30 (CO_2CH_3),

170.57 ($NHCOCH_3$), 137.83, 136.81, 128.55, 128.39, 127.85, 127.64, 99.95 (C-1), 96.19 and 94.78 (2 OCH_2O), 83.55, 74.82, 70.56, 70.24, 69.54, 69.21, 67.04, 56.76 (C-2), 51.41 (OCH_3), 34.04 (CH_2CO), 29.50, 29.09, 28.98, 25.79, 24.87, and 23.47 ($NHCOCH_3$).

Anal. Calc. for $C_{34}H_{49}NO_{10}$: C, 64.64; H, 7.82; N, 2.22. Found: C, 64.34; H, 7.80; N, 2.16.

8-Methoxycarbonyloctyl 2-acetamido-3-O-allyl-2-deoxy-β-D-glucopyranoside (**9**). — Compound **6** (9.0 g) was kept in 7:3 acetic acid–water (450 mL) for 1 h at 70° and the solution was then evaporated. Several evaporations of 2-propanol from the residue left a semi-solid residue that was crystallized from 2-propanol and ethyl acetate (6.0 g, 80%), m.p. 138–140°, $[\alpha]_D$ −19° (chloroform).

Anal. Calc. for $C_{21}H_{37}O_8N$: C, 58.45; H, 8.59; N, 3.23. Found: C, 58.7; H, 8.71; N, 3.25.

8-Methoxycarbonyloctyl 2-acetamido-3-O-allyl-6-O-benzyloxymethyl-2-deoxy-β-D-glucopyranoside (**10**). — Compound **9** (5.90 g, 13.7 mmol) was dissolved in dry dichloromethane (150 mL) containing tetramethylurea (6.56 mL, 54.8 mmol) and 4A molecular sieve (10 g). Benzyloxymethyl bromide[53] (2.29 mL, 16.4 mmol) was added, dropwise with stirring, while cooling in an ice bath. After 2 h at 0°, more bromide (0.3 mL, 2.15 mmol) was added and the mixture was stirred for an additional h at 0°. Processing of the mixture as described for the preparation of compound **5** gave a crude syrup that was chromatographed on a column of silica gel (500 g). Dichloromethane–ethyl acetate–ethanol (10:10:1) first eluted a compound that, by its ^{13}C-n.m.r. spectrum, appeared to be the 4,6-dialkylated product (0.14 g). The main band eluted was a semi-solid (4.42 g, 58%). Recrystallization from ethyl acetate gave the analytical sample, m.p. 102–103°, $[\alpha]_D$ −13° (chloroform). The ^1H- and ^{13}C-n.m.r. spectra were in accord with the structural assignment.

Anal. Calc. for $C_{29}H_{45}NO_9$: C, 63.14; H, 8.17; N, 2.54. Found: C, 63.3; H, 8.10; N, 2.44.

Further elution of the column with 10:10:3 dichloromethane–ethyl acetate–ethanol provided some starting material (1.06 g, 21%).

8-Methoxycarbonyloctyl 2-acetamido-3-O-allyl-2-deoxy-6-O-(2,2,2-trichloroethoxy)methyl-β-D-glucopyranoside (**11**). — 2,2,2-Trichloroethoxymethyl bromide (2.43 g, 10 mmol) (b.p. 50–70° at 5 torr), freshly prepared from trichloroethanol, paraformaldehyde, and hydrogen bromide according to the procedure of Salomaa and Linnantie[24], was added to a solution of **9** (2.2 g, 5.0 mmol) in 2:1 nitromethane–dichloromethane (60 mL). The mixture was stirred at 35° and after 4 h more bromide (1.21 g, 5 mmol) was added. The stirring was continued for 2 h at 35°. Pyridine (5 mL) was added (to decompose the excess of bromide) and the mixture was left overnight at room temperature. Partitioning of the mixture between dichloromethane and 2M sulfuric acid and washing of the organic layer with aqueous sodium hydrogencarbonate and water gave a colorless solution that was evaporated to dryness. The syrupy residue was chromatographed on a column of silica gel (200 g) with 5:10:2 toluene–ethyl acetate–ethanol as eluent. The chromatographically pure product

(1.96 g, 66%) was a colorless, waxy solid; $[\alpha]_D$ −9° (chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 4.96 (s, 2H, OCH_2O) and 4.22 (s, 2H, OCH_2CCl_3), the remainder of the spectrum was also in accord with the structural assignment; ^{13}C-n.m.r. ($CDCl_3$): δ 135.2 and 117.1 ($CH_2=CH$), 99.8 (C-1), 96.9 (CCl_3), and 96.2 (OCH_2O). The signal for C-6 was at 67.78 p.p.m., as compared with 62.43 p.p.m. for C-6 of the starting material.

*8-Methoxycarbonyloctyl 2-acetamido-3,6-di-*O*-benzyloxymethyl-2-deoxy-4-*O*-(3,4,6-tri-*O*-benzyl-β-*D*-galactopyranosyl)-β-*D*-glucopyranoside* (**14**) *and the α anomer* (**15**). — A solution of 3,4,6-tri-*O*-benzyl-2-*O*-*p*-nitrobenzoyl-α-D-galactopyranosyl bromide[21] (**1**, 5.45 mmol) in dichloromethane (8 mL) was added to a solution of compound **8** (1.90 g, 3.01 mmol), silver trifluoromethanesulfonate (1.36 g, 5.29 mmol), and tetramethylurea (1.4 mL, 11.7 mmol) in dry dichloromethane (13 mL) and the mixture was stirred for 42 h at room temperature, protected from light and moisture. The mixture was then filtered through a pad of Celite and the filtrate made up to 125 mL with dichloromethane, washed once with saturated sodium hydrogencarbonate and twice with water prior to drying and evaporation. The residual brown syrup was chromatographed with 1 : 1 ethyl acetate–*n*-hexane as eluent, to provide two fractions.

The first fraction (R_F 0.55) was *O*-de-*p*-nitrobenzoylated using sodium methoxide in methanol. The main component was isolated by chromatography on a column of silica gel, eluting first with dichloromethane, and then with 1 : 1 dichloromethane–ethyl acetate. This compound proved to be the α anomer (**15**), namely, 8-methoxycarbonyloctyl 2-acetamido-3,6-di-*O*-benzyloxymethyl-2-deoxy-4-*O*-(3,4,6-tri-*O*-benzyl-α-D-galactopyranosyl)-β-D-glucopyranoside, which was obtained as a clear syrup (661 mg, 21% from **8**); ^{1}H-n.m.r. ($CDCl_3$): δ 5.17 (d, $J_{1',2'}$ 3.5 Hz, 1H, H-1′); ^{13}C-n.m.r. ($CDCl_3$): δ 99.96, 99.15 (C-1, C-1′), 95.16, and 94.69 (2 OCH_2O).

Evaporation of the second crude fraction (R_F 0.30) provided slightly impure 8-methoxycarbonyloctyl 2-acetamido-3,6-di-*O*-benzyloxymethyl-2-deoxy-4-*O*-(3,4,6-tri-*O*-benzyl-2-*O*-*p*-nitrobenzoyl-β-D-galactopyranosyl)-β-D-glucopyranoside (**12**, 1.31 g, 36%); ^{1}H-n.m.r. ($CDCl_3$): δ 5.54 (dd, $J_{1',2'}$ 8.0, $J_{2',3'}$ 9.5 Hz, 1H, H-2′); ^{13}C-n.m.r. ($CDCl_3$): δ 100.55, 100.40 (C-1, C-1′), 95.65, and 94.94 (2 OCH_2O). Removal of the *p*-nitrobenzoyl group by transesterification, followed by chromatography as described for the α anomer, provided a main fraction that crystallized from ethanol (787 mg, 25% from **8**), m.p. 108–109°, $[\alpha]_D$ +29.0° (*c* 0.7, chloroform) and to which structure **14** was assigned; ^{1}H-n.m.r. ($CDCl_3$): δ 7.36–7.22 (m, 25H), 5.66 (d, 1H, NH), 4.95–4.29 (m, 16H), 4.01–3.32 (m, 17H with OCH_3 at δ3.64), 2.26 (t, 2H, CH_2CO), and 1.60–1.20 (m, 16H with $NHCOCH_3$ at δ1.70).

Anal. Calc. for $C_{61}H_{77}NO_{15}$: C, 68.84; H, 7.29; N, 1.32. Found: C, 68.65; H, 7.10; N, 1.36.

*8-Methoxycarbonyloctyl 2-acetamido-3-*O*-allyl-4-*O*-(2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-6-*O*-benzyloxymethyl-2-deoxy-β-D-glucopyranoside* (**16**). — *A. Using silver trifluoromethanesulfonate.* A solution of the alcohol **10** (0.90 g, 1.64 mmol) and the bromide **2** (3.27 mmol) in 15 mL of 1 : 1 nitromethane–toluene was stirred and cooled to −35° in the presence of 4A molecular sieve (1 g). A solution of silver trifluoromethanesulfonate (0.90 g, 3.50 mmol) and *sym*-collidine (183 μL,

1.39 mmol) in 1:1 nitromethane–toluene (10 mL) was then added, and the stirring was continued for 15 min at −30°. Pyridine (0.50 mL) was added and the mixture was allowed to reach room temperature. After dilution with 1:1 diethyl ether–ethyl acetate and filtration, the filtrate was processed conventionally. Evaporation left a syrup that was chromatographed on a column of silica gel (120 g). Toluene–ethyl acetate (1:1) eluted pure **16** (1.20 g, 67%) as the main band. The syrup crystallized on standing. Recrystallization from diethyl ether–*n*-hexane provided the analytical sample as needles, m.p. 87–89°, $[\alpha]_D$ −10° (chloroform); ^{13}C-n.m.r. ($CDCl_3$): δ 174.24, 169.99 and 165.97 (3 C=O), 135.0 and 116.43 (CH_2=CH), 100.54 and 99.93 (C-1 and C-1′), and 94.93 (OCH_2O).

Anal. Calc. for $C_{63}H_{77}NO_{15}$: C, 69.53; H, 7.08; N, 1.29. Found: C, 69.5; H, 7.18; N. 1.20.

B. Using mercuric cyanide. The bromide **2** (9.52 mmol) in toluene (10 mL) was added during a period of 1 h to a stirred solution of the alcohol **10** (3.00 g, 5.44 mmol) in 1:1 nitromethane–toluene (75 mL) containing mercuric cyanide (2.50 g, 9.90 mmol) and 4A molecular sieve (1 g). Stirring was continued for 3 h and then the mixture was diluted with ethyl acetate and washed, successively, with aqueous sodium hydrogencarbonate, aqueous potassium bromide, and water. The syrup obtained on evaporation was taken up in diethyl ether–*n*-hexane. Crystals (1.87 g) were deposited after 3 days. The mother liquor was evaporated and chromatographed on a column of silica gel (600 g) with 1:1 toluene–ethyl acetate as eluent. The same compound (2.65 g) was obtained for a total yield of 76%. The compound was identical to that (**16**) already described.

*8-Methoxycarbonyloctyl 2-acetamido-3-*O*-allyl-6-*O*-benzyloxymethyl-2-deoxy-4-*O*-(3,4,6-tri-*O*-benzyl-β-*D*-galactopyranosyl)-β-*D*-glucopyranoside* (**17**). — Compound **16** (4.42 g) was treated with sodium methoxide in methanol until *O*-debenzoylation was complete (t.l.c.). The solution was made neutral with Amberlite IR-120 (H^+) and evaporated to a syrupy residue that was taken up in dichloromethane for passage through a short column of silica gel (200 g) with 2:3 dichloromethane–ethyl acetate as eluent. Evaporation of the major fraction left a solid that was recrystallized from ethyl acetate–*n*-hexane to give colorless crystals (3.31 g, 83%), m.p. 98–101°, $[\alpha]_D$ +7° (chloroform).

Anal. Calc. for $C_{56}H_{73}NO_{14}$: C, 68.34; H, 7.43; N, 1.42. Found: C, 68.4; H, 7.46; N, 1.24.

*8-Methoxycarbonyloctyl 2-acetamido-3,6-di-*O*-benzyloxymethyl-2-deoxy-4-*O*-[3,4,6-tri-*O*-benzyl-2-*O*-(2,3,4-tri-*O*-benzyl-α-*L*-fucopyranosyl)-β-*D*-galactopyranosyl]-β-*D*-glucopyranoside* (**18**) *and its* N*-deacetyl derivative* (**19**). — A solution of 2,3,4-tri-*O*-benzyl-α-*L*-fucopyranosyl bromide [freshly prepared from 2,3,4-tri-*O*-benzyl-1-*O*-*p*-nitrobenzoyl-α,β-*L*-fucopyranose (906 mg, 1.55 mmol)] in dichloromethane (1.0 mL) was added to a mixture of compound **14** (633 mg, 0.59 mmol), tetraethylammonium bromide (185 mg, 0.88 mmol), diisopropylethylamine (0.28 mL, 1.60 mmol), molecular sieve (830 mg), *N*,*N*-dimethylformamide (0.50 mL), and dichloromethane (2.5 mL). After stirring for 26 h at room temperature, the mixture was

diluted with dichloromethane, filtered through Celite, and the filtrate made up to 60 mL with dichloromethane. Washing twice with water, followed by drying and evaporation, left a brown syrup that was purified by chromatography, eluting first with 2:1 *n*-hexane–ethyl acetate and then with 1:1 dichloromethane–ethyl acetate, to provide a major fraction (765 mg). The n.m.r. spectra of this material showed it to be a mixture of two compounds, separated by chromatography (4:1 dichloromethane–ethyl acetate). Evaporation of the second fraction (R_F 0.49) provided compound **18** (385 mg, 44%) as a clear syrup; $[\alpha]_D$ −28.2° (*c* 0.6, chloroform).

The ^{1}H-n.m.r. spectrum ($CDCl_3$) was in accord with the structural assignment and showed the presence of the *N*-acetyl group at 1.74 p.p.m. The complex structure was better characterized by the ^{13}C-n.m.r. spectrum ($CDCl_3$): δ 174.25 (*C*OOCH$_3$), 170.12 (NH*C*OCH$_3$), 139.12, 139.07, 138.85, 138.71, 138.36, 138.22, 138.16, 138.01 and 18 lines in the region 128.96–126.58, 101.73 (C-1), 100.46 (C-1′), 97.73, 95.98 and 95.19 (C-1″ and the two OCH$_2$O), 84.14, 79.78, 78.46, 76.48, 76.42, 76.07, 75.34, 74.99, 74.57, 73.49, 73.05, 72.81, 71.49, 69.86, 69.65, 69.42, 68.65, 67.00, 66.69, 54.77 (C-2), 51.36 (OCH$_3$), 34.14 (*C*H$_2$CO), 29.76, 29.64, 29.21, 29.12, 26.00, 24.98, 23.40 (NHCO*C*H$_3$), and 16.82 (C-6″).

Anal. Calc. for $C_{88}H_{105}NO_{19}$: C, 71.38; H, 7.15; N, 0.95. Found: C, 71.61; H, 7.30; N, 0.95.

Evaporation of the first fraction (R_F 0.58) provided 8-methoxycarbonyloctyl 2-amino-3,6-di-*O*-benzyloxymethyl-2-deoxy-4-*O*-[3,4,6-tri-*O*-benzyl-2-*O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-β-D-galactopyranosyl]-β-D-glucopyranoside (**19**) as a syrup (191 mg, 22%): $[\alpha]_D$ −24.8° (*c* 1.1, chloroform). This assignment was made on the basis that both its ^{1}H-n.m.r. and ^{13}C-n.m.r. spectra were very similar to those obtained for **18** but neither of the spectra showed signals characteristic for an *N*-acetyl group. Furthermore, treatment of **19** with 2:1 methanol–acetic anhydride at room temperature overnight resulted in its quantitative conversion into **18**, as evidenced by t.l.c. and ^{1}H- and ^{13}C-n.m.r. spectra.

*8-Methoxycarbonyloctyl 2-acetamido-3-*O*-allyl-6-*O*-benzyloxymethyl-2-deoxy-4-*O*-[3,4,6-tri-*O*-benzyl-2-*O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-β-D-galactopyranosyl]-β-D-glucopyranoside* (**21**). — A solution of 2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl bromide (3.83 mmol) in dichloromethane (10 mL) was added to the alcohol **17** (1.50 g, 1.53 mmol) dissolved in a mixture of tetraethyl ammonium bromide (0.80 g, 3.83 mmol), 4A molecular sieve (10 g), *N*,*N*-dimethylformamide (2 mL), and dichloromethane (40 mL). After 3 days at room temperature, methanol (2 mL) was added and the mixture was stirred for 6 h. The product was isolated conventionally as a syrup and applied to a column of silica gel (250 g) for chromatography (3:2 toluene–ethyl acetate). The main band provided a syrup (1.96 g, 94% yield) judged (t.l.c. and n.m.r.) to be essentially pure **21**, ^{13}C-n.m.r. ($CDCl_3$): δ 174.12 and 170.14 (2 C=O), 135.18 and 116.65 (CH$_2$=CH), 101.44, 99.62 and 97.54 (C-1, C-1′ and C-1″), 95.00 (OCH$_2$O), and 16.37 (C-6″).

*8-Methoxycarbonyloctyl 2-acetamido-2-deoxy-[4-*O*-(2-*O*-α-L-fucopyranosyl)-β-D-galactopyranosyl]-β-D-glucopyranoside* (**22**). — Compound **18** (323 mg, 0.22 mmol)

was dissolved in 95% ethanol (8 mL) containing 5% palladium-on-carbon (340 mg) and hydrogenated at 140 lb.in^{-2} for 22 h. T.l.c. examination at this point showed the presence of a single spot (R_F 0.54 in 4:1 2-propanol–water). The catalyst was removed by filtration on Celite and washed with several portions of hot ethanol. Solvent removal followed by freeze-drying an aqueous solution of the residue provided the title trisaccharide as a white powder (131 mg, 86%): $[\alpha]_D$ −70.4° (*c* 0.9, H_2O).

The ^{1}H- and ^{13}C-n.m.r. parameters of **22** are reported in Table I (see also Fig. 2). These spectral data are considered to firmly establish the structure and indicate the level of purity to be very high.

*8-Methoxycarbonyloctyl 2-acetamido-6-*O*-benzyloxymethyl-2-deoxy-4-*O*-[3,4,6-tri-*O*-benzyl-2-*O*-(2,3,4-tri-*O*-benzyl-α-*L*-fucopyranosyl)-β-*D*-galactopyranosyl]-β-*D*-glucopyranoside* (**24**). — A solution of compound **21** (1.82 g) in ethanol–toluene–water (30:12:4, 46 mL) was added to tris(triphenylphosphine)rhodium(I) chloride (100 mg) and the solution was boiled under reflux until t.l.c. (ethyl acetate) showed complete disappearance of **21** (3.5 h). The mixture was then diluted with dichloromethane and washed with saturated aqueous potassium chloride and then 2M hydrochloric acid and, finally, aqueous sodium hydrogencarbonate. Evaporation left a syrup that was dissolved in 10 mL of 10:1 acetone–water. Mercuric oxide (0.40 g) was added, followed by the addition of a solution of mercuric chloride (0.40 g) in 10 mL of 10:1 acetone–water. After stirring for 30 min at room temperature, the solids were removed by filtration. The filtrate was diluted with 1:1 diethyl ether–ethyl acetate and washed successively with aqueous potassium iodide and water. Evaporation left a syrup that was applied to a column of silica gel (120 g). Elution with 1:1 toluene–ethyl acetate removed some impurities. Continued elution with 3:7 toluene–ethyl acetate gave **24** (1.23 g, 70%) as a syrup, $[\alpha]_D$ −39° (*c*, 0.5 chloroform); ^{13}C-n.m.r. ($CDCl_3$): δ 174.1 and 170.3 (2 CO), 101.9, 99.9 and 97.7 (C-1, C-1′ and C-1″), 94.9 (OCH_2O), and 16.6 (C-6″).

Compound **24** was further characterized by hydrogenolysis to the H type 2 hapten (**22**) under the same conditions already described for the preparation of **22** from **18**.

*8-Methoxycarbonyloctyl 2-acetamido-3-*O*-acetyl-6-*O*-benzyloxymethyl-2-deoxy-4-*O*-[3,4,6-tri-*O*-benzyl-2-*O*-(2,3,4-tri-*O*-benzyl-α-*L*-fucopyranosyl)-β-*D*-galactopyranosyl]-β-*D*-glucopyranoside* (**25**). — Compound **24** (540 mg) was taken up in 1:3 acetic anhydride–pyridine (4 mL). The mixture was kept overnight at room temperature and then evaporated. Toluene was evaporated several times from the residue to give **25** as a colorless syrup (530 mg, 93%, $[\alpha]_D$ −44°), homogenous by t.l.c.; ^{1}H-n.m.r.: δ 5.66 (d, $J_{1,2}$ 4 Hz, H-1″) and 1.89, 1.86 (2s, $NHCOCH_3$ and $OCOCH_3$); ^{13}C-n.m.r.: δ 174.26, 171.15 and 169.92 (3 CO), 101.35 and 100.97 (C-1 and C-1′), 97.44 (C-1″), 95.01 (OCH_2O), 23.25 ($NHCOCH_3$), 20.64 ($OCOCH_3$), and 16.49 (C-6″).

*8-Methoxycarbonyloctyl 2-acetamido-3-*O*-acetyl-2-deoxy-4-*O*-[3,4,6-tri-*O*-benzyl-2-*O*-(2,3,4-tri-*O*-benzyl-α-*L*-fucopyranosyl)-β-*D*-galactopyranosyl]-β-*D*-glucopyranoside* (**26**). — A solution of compound **25** (380 mg, 0.28 mmol) in dry dichloromethane

(30 mL) was kept cooled to −45° (bath temperature) while a saturated solution of hydrogen bromide in acetic acid (~30%, 0.15 mL, ~0.5 mmol) was added. After 5 min at −45°, pyridine (0.5 mL) was added to interrupt the reaction and the mixture was allowed to reach room temperature. Washing with 2M sulfuric acid and aqueous sodium hydrogencarbonate, drying, and evaporation yielded a syrup that appeared (t.l.c.) to contain starting material plus a compound having a lower R_F value in approximately equal proportions. Chromatography on a column of silica gel (50 g), with 1:1 toluene–ethyl acetate, first eluted starting material **25** (140 mg, 35%) and then pure **26** (160 mg, 46%) as a colorless syrup; ^{13}C-n.m.r. δ: 101.77, 101.07 (C-1 and C-1′), 97.42 (C-1″), 61.22 (C-6), 53.75 (C-2), 51.42 (OCH_3), 23.27 ($NHCOCH_3$), 20.68 ($OCOCH_3$), and 16.49 (C-6″).

*8-Methoxycarbonyloctyl 2-acetamido-3-*O*-acetyl-2,6-dideoxy-6-iodo-4-*O*-[3,4,6-tri-*O*-benzyl-2-*O*-(2,3,4-tri-*O*-benzyl-α-*L*-fucopyranosyl)-β-*D*-galactopyranosyl]-β-*D*-glucopyranoside* (**28**). — Methanesulfonyl chloride (20 μL, 0.27 mmol) was added to a solution of the alcohol **26** (160 mg, 0.13 mmol) in pyridine (2 mL). After 6 h at room temperature, t.l.c. indicated complete conversion into a slightly faster-migrating product. A drop of water was added to the mixture to decompose the excess of methanesulfonyl chloride and the mixture was then partitioned between 1:1 diethyl ether–ethyl acetate and water. The organic layer was washed with 2M aqueous sulfuric acid and aqueous sodium hydrogencarbonate. Drying and evaporation left a chromatographically homogeneous syrup (**27**) that was taken up in dry *N*,*N*-dimethylformamide (2 mL) containing potassium iodide (106 mg, 0.64 mmol) and molecular sieve (0.5 g). After stirring for 48 h at 65°, t.l.c. indicated almost complete conversion into a faster-migrating product. The mixture was diluted with 1:1 toluene–diethyl ether and washed twice with water. Drying and evaporation gave a syrup that was chromatographed on silica gel (12 g) with 1:1 toluene–ethyl acetate. The main fraction was a syrupy iodo compound (100 mg, 58%), $[\alpha]_D$ −36° (*c* 0.5, chloroform), which appeared to be pure **28** as judged by t.l.c. and ^{13}C-n.m.r.; ^{13}C-n.m.r.: δ 174.10, 171.00 and 169.89 (3 CO), 101.27, 101.07 (C-1 and C-1′), 97.68 (C-1″), 53.55 (C-2), 51.41 (OCH_3), 23.27 ($NHCOCH_3$), 20.66 ($OCOCH_3$), and 16.56 (C-6″), 5.20 (C-6).

*8-Methoxycarbonyloctyl 2-acetamido-2,6-dideoxy-4-*O*-[2-O-(α-L-fucopyranosyl)-β-D-galactopyranosyl]-β-D-glucopyranoside* (**29**). — Compound **28** (100 mg) was taken up in 1:5 ethyl acetate–methanol (6 mL) and the solution was hydrogenated at 140 lb.in^{-2} in the presence of 5% palladium-on-carbon (100 mg). After 24 h, t.l.c. showed almost complete reaction. The filtered mixture was evaporated and chromatographed on Sephadex LH-20 (40 g) with 1:1 ethanol–water as eluent to provide, after evaporation of the appropriate fractions, a syrupy product (13 mg, 24% yield). The reasons for this low recovery were not established. The ^{1}H-n.m.r. spectrum showed the presence of 2 acetyl groups and two other *C*-methyl groups of equal intensities. The material was taken up in 0.1M methanolic sodium methoxide and kept overnight at room temperature. Neutralization with Amberlite IR-120 (H^+) and evaporation gave a residue that was purified on a column of Bio-gel P-2. A total of 7 mg (13%) of pure **29** was obtained on lyophilization of the appropriate

fractions; ^{1}H-n.m.r. (D_2O, OCH_3 peak set at δ3.984): δ 5.59 (d, $J_{1,2}$ 3.2 Hz, H-1″), 4.95 and 4.77 (2 doublets, $J_{1,2}$ 8.0 and 8.4 Hz, H-1 and H-1′), 4.57 (q, $J_{5,6}$ 7 Hz, H-5″), 2.68 (t, OCH_2), 2.33 (s, $NCOCH_3$), 1.69 (d, $J_{5,6}$ 6 Hz, 5-CH_3), and 1.52 (d, $J_{5,6}$ 7 Hz, 5″-CH_3).

*8-Methoxycarbonyloctyl 2-acetamido-6-*O*-benzyloxymethyl-2-deoxy-(3,4,6-tri-*O*-benzyl-β-*D*-galactopyranosyl)-β-*D*-glucopyranoside* (**30**). — Compound **17** (1.45 g) was treated with tris(triphenylphosphine)rhodium(I) chloride (100 mg) in 15:6:2 ethanol–toluene–water (46 mL) as described in the preparation of compound **24**. The product was applied to a column of silica gel (200 g), packed in 9:1 dichloromethane–acetone. Elution with the same solvent removed some impurities. Elution was continued with solvent mixtures containing gradually increasing proportions of acetone (up to 50%). The main band eluted was pure **30** (1.25 g, 90%). Recrystallization of the solid from ethyl acetate–diethyl ether–*n*-hexane gave the analytical sample, m.p. 101–103°, $[\alpha]_D$ −9° (chloroform).

Anal. Calc. for $C_{53}H_{69}NO_{14}$: C, 67.42; H, 7.32; N, 1.48. Found: C, 67.4; H, 7.36; N, 1.48.

The ^{13}C-n.m.r. spectrum of **30** in $CDCl_3$ was similar to that of the starting material **17**, except for the absence of signals expected for an allyl group.

*8-Methoxycarbonyloctyl 2-acetamido-3-*O*-allyl-6-*O*-benzyloxymethyl-2-deoxy-4-*O*-[3,4,6-tri-*O*-benzyl-2-*O*-(2,3,4-tri-*O*-benzyl-β-*D*-arabinopyranosyl)-β-*D*-galactopyranosyl]-β-*D*-glucopyranoside* (**31**). — Compound **17** (390 mg, 0.40 mmol) in dry 1,2-dichloroethane (10 mL) containing tetraethylammonium chloride (200 mg, 1.20 mmol) and 4A molecular sieve (2 g) was treated at 50° with 2,3,4-tri-*O*-benzyl-β-*D*-arabinopyranosyl chloride (1.2 mmol, prepared from 500 mg of 2,3,4-tri-*O*-benzyl-*D*-arabinose[37] by treatment with an excess of *N*-chloromethylene-*N*,*N*-dimethylammonium chloride in *N*,*N*-dimethylformamide for 30 min at room temperature). After 2 days, more of the glycosyl chloride (1.2 mmol) was added and the temperature raised to 65°. After 2 more days, the mixture was cooled to room temperature, diluted with dichloromethane, filtered, and washed with aqueous sodium hydrogencarbonate and water. Drying and evaporation left a syrup that was chromatographed on silica gel (40 g). Toluene–ethyl acetate (13:9) first eluted a main fraction (450 mg) and then a minor one (50 mg) that proved to be pure starting material (**17**).

^{13}C- And ^{1}H-n.m.r. spectroscopy, together with t.l.c. of the main fraction, showed it to be a mixture of the desired compound **31** and an unidentified product (most probably 2,3,4-tri-*O*-benzyl-*D*-arabinose) present in ~1:2 molar ratio. The yield of compound **31** was ~280 mg (52%). No further attempt at purification was made, and the mixture was used directly to prepare **32**.

*8-Methoxycarbonyloctyl 2-acetamido-6-*O*-benzyloxymethyl-2-deoxy-4-*O*-[3,4,6-tri-*O*-benzyl-2-*O*-(2,3,4-tri-*O*-benzyl-β-*D*-arabinopyranosyl-β-*D*-galactopyranosyl]-β-*D*-glucopyranoside* (**32**). — The aforementioned, highly impure preparation of compound **31** (430 mg), was taken up in 7.5:3:1 ethanol–toluene–water (11.5 mL) containing tris(triphenylphosphine)rhodium(I) chloride (30 mg) and the mixture was boiled under reflux for 2.5 h. The product was further processed as described for the prepara-

tion of **24**, and the product was applied to a column of silica gel (40 g) that was eluted with 3:2 toluene–ethyl acetate until no more impurities could be detected in the eluate. Further elution with 3:7 toluene–ethyl acetate yielded pure **32** (200 mg), as a colorless syrup, $[\alpha]_D$ −41° (chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 5.20 (d, $J_{1',2'}$ 3 Hz, H-1″); ^{13}C-n.m.r. ($CDCl_3$): δ 101.97, 99.64 (C-1 and C-1′), 98.33 (C-1″), 94.82 (OCH_2O), and 57.77 (C-5″).

8-Methoxycarbonyloctyl 2-acetamido-[*2-O-(β-*D*-arabinopyranosyl)-β-*D*-galacto-pyranosyl*]*-2-deoxy-β-*D*-glucopyranoside* (**33**). — Compound **33** (190 mg) was hydrogenated overnight at 140 lb.in^{-2} in 95% ethanol (10 mL) with 5% palladium-on-charcoal (100 mg) as catalyst. Filtration and evaporation left a glass that was purified by filtration of a solution of the glass in 10% aqueous ethanol through a column of Bio-gel P-2. The yield of chromatographically homogeneous **33** was 80 mg (80%), $[\alpha]_D$ −82°, (*c* 0.5, water); ^{1}H-n.m.r. (D_2O, OCH_3 peak set at δ3.984): δ 5.70 (d, $J_{1',2'}$ 4 Hz, H-1″), 4.83, 4.79 (2 doublets, $J_{1,2}$ 8 Hz, H-1 and H-1′), 2.68 (t, $COCH_2$), and 2.32 (s, $NHCOCH_3$); ^{13}C-n.m.r. (D_2O, external Me_4Si): δ 177.71 and 174.33 (2 C=O), 101.16 (C-1), 100.68 (C-1′), 99.83 (C-1″), 63.52 (C-5″), 61.23 (C-6′), 60.33 (C-6), 55.46 (C-2), 52.21 (OCH_3), and 22.43 ($NHCOCH_3$).

8-Methoxycarbonyloctyl 2-acetamido-6-O-benzyloxymethyl-2-deoxy-4-O-[*3,4,6-tri-O-benzyl-2-O-(2,3,4-tri-O-benzyl-α-*L*-fucopyranosyl)-β-*D*-galactopyranosyl*]*-3-O-(2,3,4-tri-O-benzyl-α-*L*-fucopyranosyl)-β-*D*-glucopyranoside* (**34**). — A solution of 2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl bromide (4.7 mmol) in dry dichloromethane (15 mL) was added to a solution of the diol **30** (0.90 g, 0.95 mmol) in dry dichloromethane (25 mL) containing tetraethylammonium bromide (0.50 g, 2.38 mmol), *N*,*N*-dimethylformamide (1.0 mL) and 4A molecular sieve (10 g). The mixture was stirred for 3 days at room temperature and then processed as described in the preparation of compound **21**. The crude syrup was chromatographed on a column of silica gel (250 g) with 4:1 toluene–acetone as eluent. The appropriate fractions were collected and evaporated, and the residue was re-chromatographed on a column of silica gel (150 g) with 7:3 toluene–ethyl acetate. Pure **34** (1.27 g, 75%) was obtained as a colorless syrup, $[\alpha]_D$ −66° (*c* 0.5, chloroform).

*8-Methoxycarbonyloctyl 2-acetamido-2-deoxy-3-*O*-(α-*L*-fucopyranosyl)-4-*O*-*[*2-O-(α-*L*-fucopyranosyl)-β-*D*-galactopyranosyl*]*-β-*D*-glucopyranoside* (**35**). — A solution of compound **34** (0.29 g) in 1:9 ethyl acetate–ethanol (10 mL) was hydrogenated at 140 lb.in^{-2} overnight, with palladium-on-charcoal (5%, 0.2 g) as catalyst. A total of 0.12 g (87%) of chromatographically homogeneous product was obtained, $[\alpha]_D$ −104° (*c* 0.5, water). The compound was best characterized by ^{1}H- and ^{13}C-n.m.r. spectroscopy (see Table I and Fig. 2).

*8-Methoxycarbonyloctyl 2-acetamido-3-O-allyl-4-O-(2-O-benzoyl-3,4,6-tri-O-benzyl-β-*D*-galactopyranosyl)-2-deoxy-6-O-(2,2,2-trichloroethoxy)methyl-β-*D*-gluco-pyranoside* (**36**). — A solution of the alcohol **11** (4.37, 7.38 mmol) and the bromide **2** in 1:1 nitromethane–toluene (30 mL) was stirred and cooled to −20° in the presence of 4A molecular sieve (5 g). A solution of silver trifluoromethanesulfonate (3.08 g, 12 mmol) and *sym*-collidine (0.90 mL, 6.8 mmol) in 1:1 nitromethane–toluene

(16 mL) was then added, and stirring was continued for 5 min at −20° and for a further 30 min without cooling. More *sym*-collidine (1.0 mL) was then added and the mixture was diluted with diethyl ether (200 mL) and filtered. Washing the filtrate with aqueous sodium thiosulfate, water, 2M sulfuric acid, and aqueous sodium hydrogencarbonate, followed by evaporation gave a colorless syrup (12 g) that was chromatographed on a column of silica gel (500 g) with 1:1 toluene–ethyl acetate as eluent. The syrupy material crystallized on storage; 2.4 g (29%). Recrystallization from diethyl ether–*n*-hexane gave colorless needles, m.p. 87–89°, $[\alpha]_D$ −6° (*c* 0.5, chloroform). ^{13}C-n.m.r. ($CDCl_3$): δ 174.2, 170.0 and 165.8 (3 C=O), 134.9 and 116.5 (CH_2=CH), 100.2 and 100.0 (C-1 and C-1′), 96.8 (CCl_3), and 96.0 (OCH_2O).

Anal. Calc. for $C_{58}H_{72}Cl_3NO_{15}$: C, 61.67; H, 6.38; N, 1.24. Found: C, 61.67; H, 6.36; N, 1.10.

*8-Methoxycarbonyloctyl 2-acetamido-4-*O*-(2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-β-*D*-galactopyranosyl)-2-deoxy-6-*O*-(2,2,2-trichloroethoxy)methyl-β-*D*-glucopyranoside* (**37**). — Tris(triphenylphosphine)rhodium(I) chloride (250 mg) was added to a solution of compound **36** (2.2 g) in 40:16:15 ethanol–toluene–water (71 mL) and the mixture was boiled under reflux until t.l.c. (ethyl acetate) showed complete disappearance of the starting material (3.5 h). The mixture was then processed as already described for the preparation of **24** to provide a brown syrup that was chromatographed on a column of silica gel (200 g). Elution with ethyl acetate provided **37** as a chromatographically homogeneous syrup (1.67 g, 78%). Its ^{13}C- and ^{1}H-n.m.r. spectra (in $CDCl_3$) resembled those of compound **36** except for the absence of signals for an allyl group.

*8-Methoxycarbonyloctyl 2-acetamido-4-*O*-(2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-β-*D*-galactopyranosyl)-2-deoxy-3-*O*-(2,3,4-tri-*O*-benzyl-α-*L*-fucopyranosyl)-6-*O*-(2,2,2-trichloroethoxy)methyl-β-*D*-glucopyranoside* (**38**). — A solution of 2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl bromide (3.8 mmol) in dry dichloromethane (10 mL) was added to a stirred solution of the alcohol **37** (1.67 g, 1.53 mmol) and tetraethyl ammonium bromide (0.80 g, 3.83 mmol) in dry dichloromethane (40 mL) containing 4A molecular sieve (10 g) and *N*,*N*-dimethylformamide (2 mL). After 3 days at room temperature, methanol (2 mL) was added (to decompose the excess of bromide) and the mixture was stirred for another 6 h. The mixture was filtered, and the filtrate washed with aqueous sodium hydrogencarbonate and evaporated to a syrup that was applied to a column of silica gel (200 g). Elution with 9:1 dichloromethane–diethyl ether removed fast-moving impurities and subsequent elution with 7:3 dichloromethane–diethyl ether provided a colorless foam (1.85 g, 80%), $[\alpha]_D$ −33° (*c* 0.5, chloroform). Further development with 7:3:3 dichloromethane–diethyl ether–ethyl acetate eluted the starting material (**37**, 0.22 g, 13%); ^{13}C-n.m.r. ($CDCl_3$): δ 174.18, 169.99 and 165.06 (3 C=O), 100.17, 100.00, 97.05 (double intensity) and 96.45 (C-1, C-1′, C-1″, CCl_3, OCH_2O), and 16.43 (C-6″).

*8-Methoxycarbonyloctyl 2-acetamido-4-*O*-(2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-β-*D*-galactopyranosyl)-2-deoxy-3-*O*-(2,3,4-tri-*O*-benzyl-α-*L*-fucopyranosyl)-β-*D*-glucopyranoside* (**39**). — Zinc dust (1.50 g) and silver carbonate (0.20 g) were added, at room

temperature, to a stirred solution of compound **38** (1.50 g) in acetic acid (25 mL). After 15 min, the mixture was diluted with dichloromethane and filtered. The filtrate was washed with water and aqueous sodium hydrogencarbonate and then evaporated to a syrup that was chromatographed on silica gel (120 g) with 7:3 dichloromethane–ethyl acetate as eluent. Starting compound (0.16 g, 11%) was first eluted. Further development with 7:3:1 dichloromethane–ethyl acetate–ethanol gave a chromatographically homogeneous glass (0.88 g, 66%); ^{13}C-n.m.r. ($CDCl_3$): δ 174.19, 169.97 and 164.89 (3 C=O), 100.18, 99.78 and 97.18 (C-1, C-1′, C-1″), and 16.37 (C-6″). As the spectrum indicated high purity, this intermediate was not further characterized.

*8-Methoxycarbonyloctyl 2-acetamido-2-deoxy-3-*O*-(α-*L*-fucopyranosyl)-4-*O*-(β-*D*-galactopyranosyl)-β-*D*-glucopyranoside* (**41**). — Compound **39** (0.83 g) in 95% ethanol (25 mL) was hydrogenated at 140 lb.in^{-2} and room temperature for 24 h with palladium-on-charcoal (5%, 0.4 g) as catalyst. Filtration and evaporation of the filtrate gave **40** as a glass (0.44 g), homogeneous on t.l.c. (12:3:3:2 ethyl acetate–acetic acid–methanol–water), which was taken up in dry methanol containing sodium methoxide. After 10 min at room temperature, t.l.c. indicated complete conversion into a single compound having a lower R_F value. Neutralization with methanol-washed Amberlite IR-120 (H^+), filtration, and evaporation gave a residue that was partitioned between water and diethyl ether. The aqueous layer was washed with diethyl ether and lyophilized to give a white powder (0.34 g, 83%), $[\alpha]_D$ −64° (*c* 0.5, water). The ^{1}H- and ^{13}C-n.m.r. parameters are presented in Table I. The ^{1}H-n.m.r. spectrum is presented in Fig. 2.

*8-Methoxycarbonyloctyl 2-acetamido-3,6-di-*O*-benzyloxymethyl-2-deoxy-4-*O*-(3,4,6-tri-*O*-benzyl-β-*D*-glucopyranosyl)-β-*D*-glucopyranoside* (**44**). — Compound **8** (633 mg, 1.00 mmol) was dissolved in 1:1 benzene–nitromethane (15 mL) containing mercuric cyanide (0.52 g, 2.06 mmol) and powdered calcium sulfate (Drierite, 0.6 g). The *gluco* bromide (**3**, 0.81 g, 1.56 mmol) was added to the mixture kept at 55° and stirring was continued for 44 h. Conventional processing of the mixture, followed by chromatography, with 2:2:1 ethyl acetate–diethyl ether–*n*-hexane as eluent, provided a fraction (R_F 0.54, 514 mg). The ^{1}H-n.m.r. spectrum was consistent with that of a mixture of the disaccharide derivatives **42** and **43**. *O*-Deacetylation, with sodium methoxide in methanol, followed by chromatography (1:5 ethyl acetate–*n*-hexane), provided in the first fraction the title compound (**44**, R_F 0.50) as a clear syrup (331 mg, 31%); ^{1}H-n.m.r. ($CDCl_3$): δ 7.44–7.14 (m, 25 H), 5.69 (d, 1H, NH), 4.99–4.34 (m, 16H, with H-1′ at 4.72, $J_{1',2'}$ 8.5 Hz and H-1 at δ 4.51, $J_{1,2}$ 7.5 Hz), 4.08–3.36 (m, 17H, with OCH_3 at δ 3.68), 3.10 (1H, OH), 2.28 (t, 2H, CH_2CO), 1.75 (s, 3H, $NHCOCH_3$), and 1.70–1.20 (m, 12H).

Evaporation of the next fraction (R_F 0.38) provided 8-methoxycarbonyloctyl 2-acetamido-3,6-di-*O*-benzyloxymethyl-2-deoxy-4-*O*-(3,4,6-tri-*O*-benzyl-α-D-glucopyranosyl)-β-D-glucopyranoside (**45**, 119 mg, 11%); ^{1}H-n.m.r. ($CDCl_3$): δ 5.12 (d, $J_{1',2'}$ 3 Hz, 1H, H-1′). This compound was not investigated further.

*8-Methoxycarbonyloctyl 2-acetamido-3,6-di-*O*-benzyloxymethyl-2-deoxy-4-*O*-[3,4,6-tri-*O*-benzyl-2-*O*-(2,3,4-tri-*O*-benzyl-α-*L*-fucopyranosyl)-β-*D*-glucopyranosyl]-

β-D-*glucopyranoside* (**46**). — The alcohol **44** (245 mg, 0.23 mmol) was engaged in a bromide-ion-catalyzed reaction under the conditions described for the preparation of the *α-fuco* compound (**18**), except that a further addition of tri-*O*-benzyl-α-L-fucopyranosyl bromide (0.5 mmol) was made after 15 h. After an additional 24 h, the mixture was filtered, washed, and evaporated to leave a dark syrup. Decolorization over a bed of alumina, with 1:1 dichloromethane–ethyl acetate, followed by column chromatography on silica gel eluting with 4:1 dichloromethane–ethyl acetate, provided a clear syrup (R_F 0.38, 193 mg, 57%), $[\alpha]_D$ −13.0° (*c* 0.2, chloroform); ^{1}H-n.m.r. ($CDCl_3$): *δ* 7.45–7.04 (m, 40 H), 5.74 (d, 1H, NH), 5.57 (d, $J_{1'',2''}$ 3.5 Hz, 1H, H-1″), 4.98–4.42 (m, 22 H), 4.30 (q, $J_{5'',6''}$ 6.5 Hz, 1 H, H-5″), 4.08–3.34 (m, 20 H, with OCH_3 at *δ* 3.67), 2.28 (t, 2 H, CH_2CO), 1.79 (s, 3 H, $NHCOCH_3$), and 1.70–1.19 (m, 15 H, with 3 × H-6″ at *δ* 1.20); ^{13}C-n.m.r. ($CDCl_3$): *δ* 174.16 ($COOCH_3$), 170.10 ($NHCOCH_3$), 101.30 (C-1), 100.50 (C-1′), 97.99, 96.23 and 95.24 (C-1″ and 2 OCH_2O), 55.73 (C-2), 51.32 (OCH_3), 34.13 (CH_2CO), 23.44 ($NHCOCH_3$), and 16.80 (C-6″).

Anal. Calc. for $C_{88}H_{105}NO_{19}$: C, 71.38; H, 7.15; N, 0.95. Found: C, 71.22; H, 7.27; N, 1.04.

8-Methoxycarbonyloctyl 2-acetamido-2-deoxy-4-O-[*2*-O-(α-L-*fucopyranosyl*)-*β*-D-*glucopyranosyl*]-*β*-D-*glucopyranoside* (**47**). — Compound **46** (162 mg, 0.109 mmol) was hydrogenated for 48 h under the conditions described for the preparation of **22**. The yield of white, freeze-dried powder (R_F 0.60 in 4:1 2-propanol–water) was 65 mg (85%); $[\alpha]_D$ −78.7° (*c* 0.5, water); ^{1}H-n.m.r. (D_2O, internal acetone set at *δ* 2.48): *δ* 5.59 (d, $J_{1'',2''}$ 3.0 Hz, H-1″), 4.83 (d, $J_{1',2'}$ 8.0 Hz, H-1′), and 4.75 (d, *J* 8.0 Hz, H-1); ^{13}C-n.m.r. (D_2O, internal 1,4-dioxane set at *δ* 67.40): *δ* 101.88 (C-1), 101.04 (C-1′), 100.19 (C-1″), and 16.12 (C-6″).

N-*Deacetylation.* — The hapten (5 mg) was dissolved in dimethyl sulfoxide (2.0 mL) and 10M sodium hydroxide (0.4 mL). Thiophenol (50 μL) was added and the resulting mixture was heated for 65 h at 120° in a stainless-steel autoclave. After cooling, the mixture was diluted with water (10 mL) and made neutral with acetic acid prior to evaporation to dryness. In each experiment, the residue was triturated 4 times with diethyl ether. The white, solid residue was dissolved in 10% aqueous ethanol (0.5 mL) and the solution subjected to gel filtration by passage through a column (1.5 × 30 cm) of Bio-gel P-2 (Bio-Rad Laboratories, Richmond, CA), using the same solvent as eluent. In all instances, a second filtration was sufficient to remove all but traces of sodium acetate. In all cases, the ^{1}H-n.m.r. spectra (400 MHz, D_2O) of the amino acid was virtually the same as that of the starting material, except for the absence of signals for methoxyl and acetamido groups. The H-2 (*β*DGlcNH_2 residue) signal appeared as a doublet of doublets, with spacings near 8.5 and 9.5 Hz, in the region 3.1–3.3 p.p.m. The products, **23**, **48**, **49**, and **50**, were used in the inhibition tests (Table II) without further purification.

Hemagglutinations. — The lectin 1 of *Ulex europaeus* (DADE Division, Miami, FL), which is a specific agglutinin of O human red cells[54], was used as the agglutinin. The cells used were freshly prepared from an OLe^{a+b-} donor by collecting the whole

blood in a tube that contained EDTA to prevent clotting. The red cells were collected by centrifugation and washed 4 times with phosphate buffer (PBS, pH 7.2). A 2% suspension of the cells in the buffer solution was then prepared. The reference agglutination was established by mixing 50 μL of the cell suspension with a portion of the solution of the lectin in PBS made up to a total volume of 50 μL and to which 50 μL of PBS was added. The amount of lectin solution found to cause a ++ level of agglutination[55] on being kept at room temperature for 1 h was selected as the reference agglutination. In order to observe the agglutination, the mixture was centrifuged at 500*g* for 30 sec and agglutination estimated by observing how the packed red cells separated on gentle agitation.

The same general procedure was used to measure the inhibition of the agglutination by the various haptens listed in Table II, except that the inhibitor was present in various amounts. In practice, two-fold serial dilutions of the solution of the inhibitor were prepared by starting with a solution of the inhibitor containing 640 μg of it per 500 μL of PBS. To measure the inhibition, 50 μL of each of these solutions was added to the 50 μL of lectin solution and the resulting solution was kept at room temperature for 1 h. The 50 μL of 2% red-cell suspension was then added and the mixture kept for 1 h at room temperature and then examined, as already described, for evidence of agglutination. The inhibitory potency of haptens was expressed as the minimum amount giving complete inhibition of the agglutination. These amounts are reported in Table II.

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