ARTIFICIAL ANTIGENS AND AFFINITY SORBENTS WITH GROUPS SPECIFICITIES Le^a, Le^b, and Le^d

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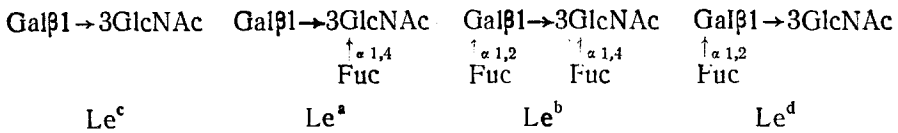
Oligosaccharides of the Lewis system have been synthesized in the form of 3-aminopropylglycosides, from which neoglycoproteins and affinity sorbents have been obtained. All three oligosaccharides were obtained from one and the same precursor.

The carbohydrate determinants, of the Lewis system, Le^a, Le^b, and Le^d (H type I) form an important system of widely distributed antigens.



The Lewis antigens Le^a and Le^b determine the corresponding blood groups, and all three antigens have been detected in the epithelial cells of the gastrointestinal tract and also in biological fluids such as saliva, gastric juice, and milk [1]. In some cases, the same antigens are tumor-associated [2, 3]. The antigens of the Le system have been found in receptors for the epidermal growth factor [4], and their role in kidney transplantation is being investigated [5].

The investigation of the function of the Le^a, Le^b, and Le^d antigens on the surface of the cell is complicated by the high structural affinity of the antigenic determinants and requires highly specific reagents for their discrimination. As the practice of recent years has shown, such reagents can only be antibodies carefully characterized with respect to specificity – monoclonal [6], or polyclonal purified with the aid of immunosorbents [7]. In a study of the specificity of antibodies, a sufficiently broad set of oligosaccharides is necessary since their absence may lead to erroneous conclusions concerning specificity [8, 9]. It is obvious that the preparation of an exhaustive series of oligosaccharides related in structure (artificial antigens) is impossible without oligosaccharide synthesis.

In the present work a convenient synthesis of the Le^a, Le^b, and Le^d oligosaccharides from a common disaccharide precursor is proposed. The oligosaccharides were synthesized in a form ready for immobilization, namely, in the form of glycosides of an aminopropyl "prespacer" [10], the amino group of which permits conjugates with proteins (antigens) and also with insoluble supports (immunosorbents) to be readily obtained. As has been shown previously [10, 11], derivatives of N-acetylglucosamine with two unsubstituted hydroxy groups at C-3 and C-4 are glycosylated by acetobromogalactose in position 3 with high yield and regioselectivity if the reaction is performed under Helferich's conditions (mercury cyanide + bromide; benzene/nitromethane; 50-60°C). In the present case, therefore, also, for glycosylation we took a diol – compound IV – obtained by opening the benzylidene ring in (II) with sodium cyanohydroborate [12] (see scheme, following page).

Since the solubility of compound (II) under the reaction condition was low and the actual opening process took place nonspecifically, compound (II) was acetylated. The benzylidene ring of the resulting acetate (III) opened with sodium cyanohydroborate exclusively in the direction of the 6-O-benzyl derivative (V), and none of the isomeric 4-O-benzyl ether was detected. Deacetylation of the acetate (V) gave the diol (IV) from 40% (plus a comparable

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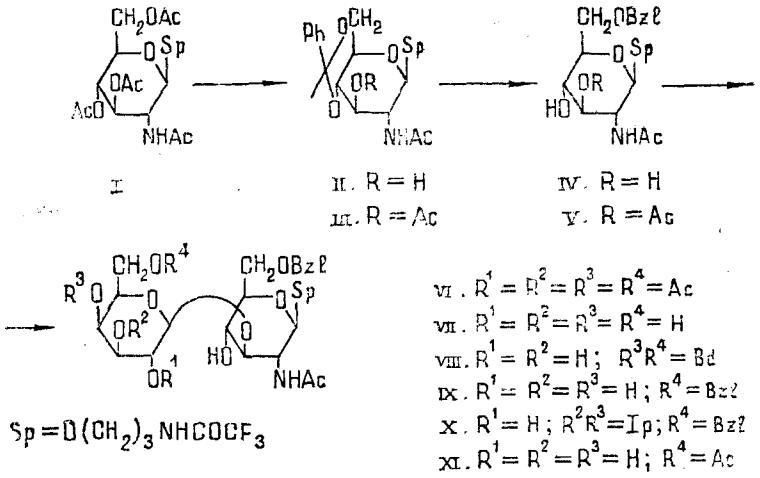


I

II. R = H
III. R = Ac

IV. R = H
V. R = Ac

Sp = O(CH$_2$)$_3$NHCOCF$_3$

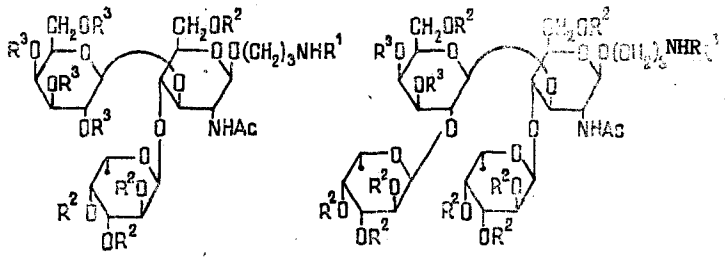
VI. R^1 = R^2 = R^3 = R^4 = Ac
VII. R^1 = R^2 = R^3 = R^4 = H
VIII. R^1 = R^2 = H; R^{3}R^4 = Bd
IX. R^1 = R^2 = R^3 = H; R^4 = Bzl
X. R^1 = H; R^{2}R^3 = Ip; R^4 = Bzl
XI. R^1 = R^2 = R^3 = H; R^4 = Ac

amount of the 4-O-Bzl isomer) to 82%. Thus, an acetyl group at O-3 directs the reductive opening of the ring to the formation of the 6-O-benzyl isomer.

The glycosylation of diol (IV) with 1.2 equivalents of acetobromogalactose at 50-60°C in a mixture of benzene and nitromethane in the presence of mercury cyanide gave, with a yield of 79%, the disaccharide (VI), having a hydroxy group at C-4 of the glucosamine residue ready for further glycosylation. The transprotection of the disaccharide (VI) necessary for the synthesis of the Le^b and Le^d derivatives was carried out in the following way. The Zemplen deacetylation of compound (VI) gave the pentaol (VII) which was converted by the action of PhCH(OMe)$_2$ in the presence of TsOH into the benzylidene derivative (VIII). The latter (in the form of the diacetate in order to increase its solubility) was cleaved with sodium cyanohydroborate and, after deacetylation, the tetraol (IX) was obtained. The action of 2,2-dimethoxypropane on the tetraol (IX) in the presence of TsOH gave the diol (X). It must be mentioned that another method of protecting the 6'-OH group, via compound (XI), proved unsuccessful, since the acetylation of the pentaol (VII) by the trimethylsilyl method [13] took place ambiguously (see also [14]).

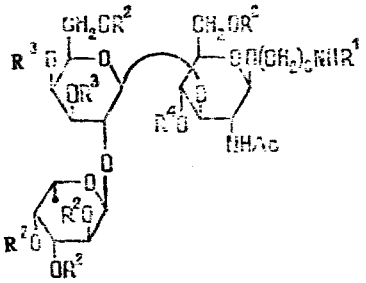
The α-fucosylation of disaccharides (VI) and (X) was carried out with 2,3,4-tri-O-benzylfucopyranosyl bromide by the method of halide ion catalysis [15]. The protected Le^a trisaccharide (XII) was obtained with a yield of 92% when a threefold excess of the fucosyl bromide was taken. The glycosylation of the diol (X) with an excess (six equivalents) of the fucosyl bromide gave the protected Le^d tetrasaccharide (XVII) with a yield of 63%. Glycosylation of the same diol (X) with two equivalents of the fucosyl bromide gave a 70% yield of the protected Le^d trisaccharide (XXI).

The elimination of the protective groups (by standard methods: see the Experimental part) led to the free oligosaccharides (XIV) and (XVIII) in the form of the (CH$_2$)$_3$NHCOCF$_3$ β-glycosides, the purity of which was checked with the aid of HPLC; the Le^d trisaccharide derivative was obtained in the form of the octaacetate (XXIV). For subsequent immobilization, the trifluoroacetyl protection was eliminated quantitatively by an anion-exchanger in the OH$^-$ form, after which the free amines (XV), (XIX), and (XXV) were either used directly or were converted into the activated OS–O(CH$_2$)$_3$NHCO(CH$_2$)$_4$CON$_3$ forms as described in [16]. Condensation of the azides (XVI), (XX), and (XXVI) with BSA and with cytochrome c was performed by the method of Inman et al. [17]



XIII. R^1 = CF$_3$CO; R^2 = Bzl; R^3 = Ac
XIV. R^1 = CF$_3$CO; R^2 = Bzl; R^3 = H
XV. R^1 = CF$_3$CO; R^2 = R^3 = H
XV. R^1 = R^2 = R^3 = H
XXII. R^1 = CO(CH$_2$)$_4$CON$_3$; R^2 = R^3 = H

XVII. R^1 = CF$_3$CO; R^2 = Bzl, R^{3}R^3 = Ip
XVIII. R^1 = CF$_3$CO; R^2 = R^3 = H
XIX. R^1 = R^2 = R^3 = H
XX. R^1 = CO(CH$_2$)$_4$CON$_3$; R^2 = R^3 = H



xxi. $R^1 = CF_3CO$; $R^2 = Bzl$; $R^3R^3 = Ip$; $R^4 = $ ..
xxii. $R^1 = CF_3CO$; $R^2 = R^3 = R^4 = H$
xxiii. $R^1 = CF_3CO$; $R^2 = Bzl$; $R^3R^3 = Ip$; $R^4 = H$
xxiv. $R^1 = CF_3CO$; $R^2 = R^3 = R^4 = Ac$
xxv. $R^1 = R^2 = R^3 = R^4 = H$
xxvi. $R^1 = CO(CH_2)_4CON_3$; $R^2 = R^3 = R^4 = H$

As the matrix for the affinity sorbents we used macroporous glass modified with poly-(4-nitrophenyl acrylate) [18]. Such an activated matrix permits the direct one-stage immobilization of ligands with primary amino groups, and this with high yields and simultaneously with high degrees of inclusion of the ligand (up to 50 μmole per 1 g of sorbent). Traditional approaches to the activation of the matrix (for example, the cyanogen bromide activation of Sepharose) permits high degrees of inclusion to be obtained only with a considerable excess (from a few to hundreds of equivalents) of the ligand. Approaches based on the activation of the ligand (even the best of them, such as the azide method [19]) have their own disadvantages, the main one of which consists of side reactions of the activated component. Ivanov's approach [18] is therefore the best for the immobilization of difficultly accessible ligands.

The binding of the antibodies with the support was studied for the case of the Le^a sorbent containing 7.5 μmole/g of the ligand. The nonspecific sorption of the proteins was not more than 0.5 mg/g (thanks to the polymeric coating of the inorganic substrate [18]), while the specific sorption amounted to 3-10 mg/g. The sorption of two types of anti-Le^a antibodies was tested: 1) standard anti-Le^a goat serum (obtained by immunizing goats with the saliva of nonsecreting donors of the OLe^{a+b-} group; Scientific-Research Institute of Vaccines and Sera, Leningrad); and 2) rabbit monospecific anti-Le^a antibodies against the artificial Le^a~BSA antigen.* The sorbent, taken in an amount of 100 mg per 1 ml, lowered the titer of the goat antiserum (Coombs' indirect method [22]) from 1/32 to 1/2; in the same test, the titers of control Le^b, A, B, and I antisera remained unchanged. The monospecific rabbit antibodies were bound in an amount of 3-10 mg/g, depending on the temperature and time of sorption; the activity of the antibodies before and after sorption, and also the activity of the antibodies desorbed at pH 3 (or pH 9) was checked by the immunoenzyme method in planchets sensitized with the Le^a~cytochrome conjugate or with the saliva of Le^{a+b-} donors.

The titers of the purified antibodies did not change after an adsorption–desorption–neutralization cycle, while the titer of the unpurified anti-Le^a serum rose by a factor of 2-2.5. The solvent withstood at least five sorption-desorption cycles without loss of affinity.

The Gal and GlcNAc residues were of the D-configuration, and Fuc of the L-configuration; Ac denotes acetyl, Bzl – benzyl, Bd – benzylidene, OS – oligosaccharide; Ip – isopropylidene; BSA – bovine serum albumin; and PBS – phosphate-salt buffer.

EXPERIMENTAL

PMS spectra were taken on Varian SC-300 and Bruker WM-500 instruments. HPLC was conducted on a Du Pont 8800 instrument using a 4.6 × 250 mm Zorbax 10 μm ODS column with elution by means of 3% MeCN–water at 3 ml/min with a Holochrome detector (Gilson), detection at 206 nm. TLC was performed on plates coated with silica gel 60F-254 (E. Merck), and the substances were detected with a 5% solution of sulfuric acid in methanol at 150°C. Column chromatography was carried out on silica gel 40-100 μm (Chemapol). To determine their monosaccharide compositions, the glycoconjugates were subjected to acid methanolysis and subsequent treatments as described in [20], after which the trimethylsilyl derivatives were subjected to GLC (Hewlett-Packard 5710A chromatograph, 0.25 mm × 40 m capillary column; stationary phase SE-30; carrier gas helium at 60 ml/min; flame-ionization detector); temperature conditions: 2°C/min to 100°C, 100→230°C (4°C/min), and then isothermal; mannitol was used as in-

*For the preparation and characterization of the specificity of the antibodies, see: A. Ya. Khorlin, N. V. Bovi, N. D. Gabrielyan, E. V. Targulyan, G. V. Zatevakhina, and M. L. Antimova, Immunologiya, No. 5, 65-69 (1987).

ternal standard. Melting points were determined on a Boetius instrument (GDR). Optical rotations were measured on Perkin-Elmer 141 (USA) and Polamat M (GDR) polarimeters at 20-25°C.

The mercury cyanide, tetraethylammonium bromide and toluene-4-sulfonic acid were dried in vacuum at 0.5 mm Hg/25°C for 0.5-2 h immediately before being used in the reaction. The sodium cyanohydroborate was a product of E. Merck, the Amberlyst A-26 from Fluka, and the BSA and cytochrome c from Sigma. The optical densities of the protein solutions were measured on a Pye Unicam PU 8610 instrument at 280°C. The IEA results were recorded on a Titertec Multiskan MCC photometer at 492 nm. The IEA was performed on Nunc polystyrene planchets, and the antiimmunoglobulin conjugates were obtained in the N. F. Gamalei Scientific-Research Institute of Epidemiology and Microbiology of the USSR Academy of Medical Sciences. The antisera and erythrocytes were obtained in the Central Scientific-Research Institute of Hematology and Blood Transfusion (Moscow). The activated macroporous glass was kindly supplied by V. P. Zubov and A. E. Ivanov (Institute of Bioorganic Chemistry of the USSR Academy of Sciences).

3-(Trifluoroacetamido)propyl 2-Acetamido-4,6-O-benzylidene-2-deoxy-β'-D-glucopyranoside (II). A solution of 11.5 g (23 mmole) of glycoside (I) [21] in 250 ml of absolute methanol was treated with 0.2 ml of 1 M sodium methanolate in methanol. After 2 h, the solution was evaporated to dryness; the residue was suspended in 150 ml of acetonitrile, 7 g (46 mmole) of benzaldehyde dimethylacetal was added, the mixture was heated to 50°C, and then 40 mg of toluene-4-sulfonic acid was added. The crystalline precipitate that deposited after cooling was separated off, giving 10 g (94%) of derivative (II), mp 271-272°C (ethanol), $[\alpha]_D$ −72° (c 0.7; DMSO). PMR (DMSO-d_6): 1.70 m (2H, CCH_2C), 1.83 s (3H, Ac), 4.48 d (1H, $J_{1,2}$ = 8.0 Hz, H-1), 5.29 d (1H, J = 5 Hz, OH), 5.61 s (1H, PhCH), 7.4 m (5H, Ph), 7.84 d (1H, J = 8.5 Hz, NH), 9.40 m (1H, $NHCOCF_3$). Found, %: C 51.94, H 5.43, N 6.00. $C_{20}H_{25}F_3N_2O_7$. Calculated, %: C 51.95, H 5.41, N 6.06.

3-(Trifluoroacetamido)propyl 2-Acetamido-3-O-acetyl-6-O-benzyl-2-deoxy-β-D-glucopyranoside (V). A solution of 3.9 g (8.4 mmole) of compound (II) in 100 ml of pyridine was treated with 20 ml of acetic anhydride, and the mixture was stirred for 20 h. The resulting suspension of the acetate (III) was evaporated to dryness and the residue was suspended in 350 ml of oxolane, to which 17 g of 3 Å sieve and then 4.8 g (76 mmole) of sodium cyanohydroborate were added, whereupon the acetate (III) dissolved completely. Then, at 20°C, an ethereal solution of hydrogen chloride was added until a vigorous reaction with the evolution of gas began. After 8 h, the solution was filtered and was evaporated to 1/5 of its initial volume, 500 ml of chloroform and 200 ml of water was added, the aqueous layer was extracted with ml of chloroform, the combined organic phase was washed with water and evaporated to dryness, and the residue was deposited on a column of silica gel (3 × 30 cm) from which 5% of methanol in chloroform eluted 3.5 g (82%) of compound (V), mp 107-108°C (chloroform–ether), $[\alpha]_{546}$ −93° (c 0.6, $CHCl_3$). PMR ($CDCl_3$): 1.74 m (2H, CCH_2C), 1.87 s (3H, NAc), 2.02 s (3H, OAc), 3.20 m (2H, NCH_2), 3.25 d (1H, J = 3.2 Hz, OH), 3.50 m (1H, H-5), 3.62 m (1H, H-4), 3.83 m (1H, H-2), 4.42 d (1H, J = 9.0 Hz, H-1). 4.96 dd (1H, $J_{2,3}$ = 8.0 Hz, $J_{3,4}$ = 8.0 Hz, H-3), 6.12 d (1H, J = 8.0 Hz, NH). 7.25 m (2H, Ph), 7.53 m (1H, $NHCOCF_3$). Found, %: C 52.21, H 5.72, N 5.45. $C_{22}H_{29}F_3N_2O_8$. Calculated, %: C 52.17, H 5.73, N. 5.53.

3-(Trifluoroacetamido)propyl 2-Acetamido-6-O-benzyl-3-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-2-deoxy-β-D-glucopyranoside (VI). A solution of 1.32 g (2.6 mmole) of the acetate (V) in 50 ml of absolute methanol was treated with one drop of 0.5 M sodium methanolate in methanol, and after a day it was evaporated to dryness. The residue (the diol (IV)) was dissolved in a mixture of 15 ml of nitromethane and 15 ml of benzene, and this solution was treated with 3 g of 4 Å sieve and 760 mg (3 mmole) of mercury cyanide. The mixture was kept at 20°C for 3 h and was then heated to 50°C and, in the course of 1 h, 1.2 g (3 mmole) of acetobromogalactose in 20 ml of benene was added at 50-60°C and this temperature was maintained for another 2 h, after which the mixture was cooled, diluted twofold with chloroform, and filtered, and the filtrate was washed with saturated aqueous sodium bicarbonate and was evaporated. The residue was deposited on a column of silica gel (2 × 15 cm), and a 2 → 5% gradient of methanol in chloroform eluted 1.62 g (79%) of the disaccharide (VI) with mp 90-91°C (chloroform–ether), $[\alpha]_{546}$ +11° (c 1; $CHCl_3$). PMR ($CDCl_3$): 1.83 m (2H, CCH_2C), 1.99 s, 2.01 s, 2.02 s, 2.09 s, 2.16 s (15H, 5Ac), 4.79 d (1H, $J_{1',2'}$ = 7.5 Hz, H-1'), 6.26 d (1H, J = 8.5 Hz, NH), 7.34 m (5H, Ph), 7.63 m (1H, $NHCOCF_3$). Found, %: C 51.45, H 5.68, N 3.49. $C_{34}H_{45}F_3N_2O_{16}$. Calculated, %: C 51.39, H 5.67, N 3.53.

3-(Trifluoroacetamido)propyl 2-Acetamido-6-O-benzyl-3-O-(4,6-O-benzylidene-β-D-galactopyranosyl)-2-deoxy-β-D-glucopyranoside (VIII). A solution of 3.97 g (5 mmole) of the acetate

(VI) in 200 ml of absolute methanol was treated with 0.1 ml of 0.5 M sodium methanolate in methanol, and after 15 h it was evaporated to dryness. The residue was dissolved in 300 ml of acetonitrile at 50°C, and to this solution were added 3 g (20 mmole) of benzaldehyde dimethylacetal, and then 100 mg of toluene-4-sulfonic acid and the mixture was kept at 50°C for 30 min; after this, it was cooled, neutralized with 0.5 ml of triethylamine, and evaporated. The residue was recrystallized from ethanol, giving 2.7 g (76%) of compound (VIII), mp 250-252°C, $[\alpha]_D$ −27° (c 0.15; DMSO). PMR (DMSO-d_6): 1.66 m (2H, CCH_2C), 1.75 s (3H, Ac), 4.44 d (1H, J = 8.0 Hz, H-1), 5.50 s (1H, PhCH), 7.2-7.4 m (10H, 2Ph), 7.72 d (1H, J = 7 Hz, NH), 9.19 m (1H, CF_3CONH). Found, %: C 55.50, H 5.71, N 3.91. $C_{33}H_{41}F_3N_2O_{12}$. Calculated, %: C 55.46, H 5.74, N 3.92.

3-(Trifluoroacetamido)propyl 2-Acetamido-6-O-benzyl-3-O-(6-O-benzyl-3,4,-O-isopropylidene-β-D-galactopyranosyl)-2-deoxy-β-D-glucopyranoside (X). A solution of 720 mg (1 mmole) of compound (VIII) in 40 ml of pyridine was treated with 10 ml of acetic anhydride, and after 15 h the mixture was evaporated to dryness. The residue was dissolved in 70 ml oxolane, and the solution was treated with 5 g of 3 Å sieve and 1.26 g (20 mmole) of sodium cyanohydroborate. After 2 h, an ethereal solution of hydrogen chloride was added to this mixture at 20°C until a vigorous reaction with the evolution of gas began. After 8 h, 3 ml of acetic acid and 200 ml of chloroform were added and the mixture was filtered, washed twice with water, and evaporated. The residue was deposited on a column of silica gel (2 × 15 cm), and elution with a 1 → 5% gradient of methanol in chloroform yielded 620 mg of 2',3',4-tri-O-acetyl-(IX) with R_f 0.4 ($CHCl_3$–MeOH) (9:1)); an amorphous substance. It was dissolved in 70 ml of absolute methanol, the solution was treated with 1 drop of 0.5 M sodium methanolate in methanol, and, after being left for 15 h, it was evaporated to dryness. The residue (substance (IX)) was dissolved in a mixture of 20 ml of dichloromethane and 2 ml of 2,2-dimethoxypropane, and 10 mg of toluene-4-sulfonic acid was added at 20°C; after 10 min, the solution was neutralized with 0.2 ml of triethylamine and was evaporated to dryness at 20°C. The residue was deposited on a column of silica gel (2 × 15 cm), and elution with a 2 → 5% gradient of methanol in chloroform yielded 480 mg (63%, calculated on the (VIII)) of amorphous (X), $[\alpha]_{546}$ −45° (c 0.45; $CHCl_3$). PMR ($CDCl_3$): 1.23 s (3H, CH_3), 1.43 s (3H, CH_3), 1.84 m (2H, CCH_2C), 1.92 s (3H, Ac), 6.53 d (1H, J = 8 Hz, NH), 7.3 m (10H, 2Ph). Found, %: C 57.02, H 6.13. $C_{36}H_{47}F_3N_2O_{12}$. Calculated, %: C 57.14, H 6.22.

3-(Trifluoroacetamido)propyl 2-Acetamido-6-O-benzyl-3-O-(β-D-galactopyranosyl)-4-O-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-2-deoxy-β-D-glucopyranoside (XIII). A mixture of 640 mg (0.8 mmole) of the disaccharide (VI), 0.48 ml (3 mmole) of ethyldiisopropylamine, 5 g of 4 Å sieve, and 840 mg (4 mmole) of tetraethylammonium bromide in 10 ml of dichloromethane and 5 ml of DMFA was kept in the dark at 20°C for 15 h, and then a solution of 2.4 mmole of 2,3,4-tri-O-benzyylfucopyranosyl bromide [10] in 10 ml of dichloromethane was added in one portion. The mixture was kept in the dark for 96 h, and then 3 ml of methanol was added and, after 2 h, it was diluted with 200 ml of chloroform and filtered, and the filtrate was washed with water, with saturated sodium bicarbonate solution, and with water again and was dried with calcium chloride and evaporated to dryness. The residue was deposited on a column of silica gel (4 × 40 cm), and elution with a 0 → 30% gradient of methanol in chloroform yielded 890 mg (92%) of the trisaccharide (XII); amorphous. Compound (XII) was dissolved in 70 ml of absolute methanol, 1 drop of a 1 M solution of sodium methanolate in methanol was added and, after 24 h, the mixture was treated with cation-exchange resin (IR-120, H^+) and was filtered and evaporated. This gave 810 mg (97%) of the trisaccharide (XIII), with mp 97-98°C (ether), $[\alpha]_{546}$ −63° (c 1; $CHCl_3$). PMR ($CDCl_3$): 1.08 d (3H, J = 6.6 Hz, CH_3 of fucose), 1.72 m (2H, CCH_2C), 1.75 s (3H, Ac), 5.02 d (1H, $J_{1'',2''}$ = 3.5 Hz, H-1''), 6.96 d (1H, J = 7 Hz, NH), 7.3 m (20H, 4Ph), 7.48 m (1H, $NHCOCF_3$). Found, %: C 60.93, H 6.24, N 2.55. $C_{53}H_{65}F_3N_2O_{16}$. Calculated, %: C 61.04, H 6.24, N 2.69.

3-(Trifluoroacetamido)propyl 2-Acetamido-4-(α-L-fucopyranosyl)-3-O-(β-D-galactopyranosyl)-2-deoxy-β-D-glucopyranoside (XIV). The trisaccharide (XIII) (52 mg, 0.05 mmole) was subjected to hydrogenolysis in 50 ml of ethanol in the presence of 50 mg of 10% Pd-C at 25°C for 72 h. The solution was filtered and evaporated and the residue was deposited on a column of Sephadex G-25 (2 × 50 cm), after which elution with water gave 30 mg (88%) of the trisaccharide (XIV), identical with that obtained previously [10].

3-(Trifluoroacetamido)propyl 2-Acetamido-6-O-benzyl-3-O-[6-O-benzyl-2-O-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl-3,4-O-isopropylidene-β-D-galactopyranosyl-4-O-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-2-deoxy-β-D-glucopyranoside (XVII). A mixture of 350 mg (0.46 mmole) of the diol (X), 0.48 (3 mmole) of diisopropylethylamine, 8 g of 4 Å sieve, and 1.05 g (5 mmole)

of tetraethylammonium bromide in 30 ml of dichloromethane and 30 ml of DMFA was kept in the dark at 20°C for 2 h, and then a solution of 3 mmole of 2,3,4-tri-O-benzylfucopyranosyl bromide in 10 ml of dichloromethane was added in one portion. After 120 h, 3 ml of methanol was added and the mixture was kept for 2 h and was then diluted with 300 ml of chloroform and was filtered; the filtrate was washed with water, with saturated sodium bicarbonate solution, with water again, and was dried and evaporated. The residue was deposited on a column (3 × 40 cm) and elution with a 5 → 40% gradient of ethyl acetate in benzene gave 460 mg (63%) of the tetrasaccharide (XVII), mp 71°C (ether–hexane), $[\alpha]_{546}$ −49° (c 0.92; $CHCl_3$). PMR ($CDCl_3$): 1.03 d (3H, J = 6 Hz, CH_3 of fucose), 1.04 d (3H, J = 7 Hz, CH_3 of fucose), 1.27 s (3H, CH_3CCH_3), 1.38 s (3H, CH_3CCH_3), 1.54 s (3H, Ac), 1.59 m (2H, CCH_2C), 3.03 m (1H, H-2), 3.26 m (2H, NCH_2), 3.46 m (2H, OCH_2), 4.94 d (1H, J = 4 Hz, H-1 of fucose), 5.03 d (1H, J = 8.0 Hz, H-1), 5.49 d (1H, J = 4 Hz, H-1 of fucose), 6.58 d (1H, J = 6 Hz, NH), 7.2 m (40H, 8Ph). Found, %: C 68.20, H 6.54. $C_{90}H_{103}F_3N_2O_{20}$. Calculated, %: C 68.01, H 6.49.

3-(Trifluoroacetamido)propyl 2-Acetamido-4-O-(α-L-fucopyranosyl)-3-O-[2-O-(α-L-fucopyranosyl)-β-D-galactopyranosyl]-2-deoxy-β-D-glucopyranoside (XVIII). A solution of 80 mg (0.05 mmole) of the tetrasaccharide (XVII) in 20 ml of 80% acetic acid was kept at 60°C for 2 h and was then evaporated to dryness. The residue was dissolved in a mixture of 50 ml of ethanol and 10 ml of acetic acid and was subjected to hydrogenolysis at 20°C in the presence of 50 mg of 10% Pd-C for 96 h. The solution was filtered and evaporated, the residue was deposited on a column of Sephadex G-25 (2 × 50 cm), and elution with water gave 34 mg (83%) of the tetrasaccharide (XVIII), homogeneous according to HPLC, $[\alpha]_D$ −120° (c 0.23; MeOH). PMR (D_2O): 1.28 m (6H, $2CH_3$ of fucose), 1.83 m (2H, CCH_2C), 2.07 s (3H, Ac), 4.32 m (1H, H-5 of fucose), 4.41 d (1H, $J_{1',2'}$ = 9 Hz, H-1'), 5.02 d (1H, J = 3.5 Hz, H-1 of fucose), 5.15 d (1H, J = 3.5 Hz, H-1 of fucose). Found, %: C 44.80, H 6.15. $C_{31}H_{51}F_3N_2O_{20}$. Calculated, %: C 44.93, H 6.16.

3-(Trifluoroacetamido)propyl 2-Acetamido-4,6-di-O-acetyl-3-O-[3,4,6-tri-O-acetyl-2-O-(2,3,4-tri-O-acetyl-α-L-fucopyranosyl)-β-D-galactopyranosyl]-2-deoxy-β-D-glucopyranoside (XXIV). A mixture of 227 mg (0.3 mmole) of the diol (X), 0.1 ml (0.6 mmole) of diisopropylethylamine, 3 g of 4 Å sieve, and 210 mg (1 mmole) of tetraethylammonium bromide in 20 ml of dichloromethane and 20 ml of DMFA was kept in the dark for 2 h, and then 0.6 mmole of 2,3,4-tri-O-benzylfucopyranosyl bromide in 20 ml of dichloromethane was then added in one portion. After 72 h, 0.5 ml of methanol was added and the mixture was kept for 2 h, and it was then diluted with 150 ml of chloroform and filtered; the filtrate was washed with water, with saturated sodium bicarbonate solution, and with water again, and was dried and evaporated to dryness. The residue was deposited on a column of silica gel, and elution with a 10 → 60% gradient of ethyl acetate in benzene yielded 246 mg (70%) of the trisaccharide (XXIII). PMR ($CDCl_3$): 1.07 d (3H, J = 6.6 Hz, CH_3 of fucose), 1.38 s (3H, CH_3CCH_3), 1.48 s (3H, CH_3CCH_3), 1.68 m (2H, CCH_2C), 1.75 s (3H, Ac), 5.28 d (1H, J = 4 Hz, H-1"), 5.88 d (1H, J = 9 Hz, NH), 7.3 m (25H, 5Ph). The trisaccharide obtained was dissolved in 60 ml of 80% acetic acid and the solution was kept at 60°C for 2 h and was then evaporated to dryness. The residue was subjected to hydrogenolysis in a mixture of 70 ml of ethanol and 7 ml of acetic acid in the presence of 100 mg of 10% Pd-C at 25°C for 72 h. The solution was filtered and evaporated, the residue was dissolved in 20 ml of pyridine, and this solution was treated with 5 ml of acetic anhydride. After 15 h, it was evaporated to dryness and the residue was deposited on a column of silica gel (2.5 × 40 cm) from which a 1 → 6% gradient of methanol is chloroform eluted 173 mg (81%) of the octaacetate (XXIV); amorphous, $[\alpha]_{546}$ −50° (c 1; $CHCl_3$). PMR ($CDCl_3$): 1.18 d (3H, J = 7 Hz, CH_3 of fucose), 1.87 m (2H, CCH_2C), 1.96-2.16 9s (27H, 9Ac), 5.47 d (1H, $J_{1'',2''}$ = 3.5 Hz, H-1"), 6.83 d (1H, J = 6.5 Hz, NH), 7.25 m (1H, $NHCOCF_3$). Found, %: C 48.20, H 5.62, N 2.70. $C_{41}H_{57}F_3N_2O_{24}$. Calculated, %: C 48.33, H 5.64, N 2.75. The octaacetate of the Le^a glycoside [10], differing considerably in its R_f value, was isolated in trace amounts.

Neoglycoproteins. The activated derivatives (acyl azides) (XVI), (XX), and (XXVI) were obtained by the following successive treatments: a) acylation of the amines (XV), (XIX), and (XXV) with the aid of $F_5C_6OOC(CH_2)_4COOEt$; b) treatment with hydrazine hydrate in ethanol; and c) treatment of the hydrazides with N_2O_4 as described in detail by Lemieux et al. [16]. The azides were condensed with BSA and cytochrome c by the method of Inman et al. [17], giving neoglycoproteins with a carbohydrate content of 4-14 wt.%.

Affinity Sorbents. Activated macroporous glass [18] (500 mg) was washed several times with DMFA (redistilled over calcium hydride and then over ninhydrin) and was suspended in 2 ml of DMFA, after which an aminoligand (XV), (XIX), or (XXV) was added in an amount of

2.5-10 μmole. The mixtures were kept in the dark for 24 h with periodic shaking and the completeness of the reaction was checked spectrophotometrically (from the 4-nitrophenol liberated); then 200 μl of ethanolamine was added and the mixture was kept for another 15 h, after which the sorbent was washed with DMFA, with ethanol, and with water. Before the sorption of the antibodies, the sorbent was washed with PBS.

Sorption–Desorption. A 0.8 × 7 cm column was filled with 100 mg of sorbent, which was washed with 3-4 volumes of PBS and then with 1% NH_4OH, and again with PBS. Then, at 4°C, 1 ml of serum was passed into the closed system with the aid of a peristaltic pump for 1 h, the sorbent was washed with PBS until protein had disappeared from the eluate, and the antibodies were eluted with a 1% solution of NH_4OH (pH 9) or a glycine–HCl buffer solution (pH 3), and the eluate was neutralized to pH 7. Under these conditions, complete sorption of rabbit antiserum (previously subjected to affinity purification) took place; standard goat anti-Le^a serum was enriched 2- to 2.5-fold; and the amount of specific antibodies determined by the immunoenzyme method scarcely decreased, while the amount of total protein (absorption at 280 nm) fell by a factor of 2-2.5.

CONCLUSIONS

1. The Le^a, Le^b, and Le^d oligosaccharides have been synthesized in the form of their 3-trifluoroacetamidopropyl glycosides from a common precursor.

2. The immobilization of the 3-aminopropyl glycosides on proteins has given artificial antigens, and their immobilization on activated macroporous glass has given affinity sorbents with Le^a, Le^b, and Le^d affinities.

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