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Artificial Carbohydrate Antigens: The Synthesis of Glycopeptidic Haptens with T_N Specificity[1]

BERNARD FERRARI AND ANDRE A. PAVIA[2]

Laboratoire de Chimie Bioorganique, Faculté des Sciences, 33, rue Louis Pasteur, 84000 Avignon, France

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The synthesis of *O*-(2-acetamido-2-deoxy-α-D-galactopyranosyl)-*N*-acetyl-L-serine 8-methoxycarbonyloctanamide and *O*-(2-acetamido-2-deoxy-α-D-galactopyranosyl)-*N*-8-methoxycarbonyloctanoyl-L-serine methylamide is reported. These compounds represent the antigenic determinant of cell-membrane glycoproteins carrying T_N specificity. Both glycopeptides are functionalized for the purpose of preparing artificial antigens by attachment to immunogenic carriers and solid supports. Different schemes are needed for the synthesis of the haptens.

INTRODUCTION

The MN blood group specificity is carried by a tetrasaccharide bound to glycophorin, the main erythrocyte membrane glycoprotein, by an *O*-glycosidic linkage involving *N*-acetyl-α-D-galactosamine and L-serine and/or L-threonine (*1–3*). This linkage is also present in numerous other glycoproteins, including cell-membrane glycoproteins (*4–7*), blood-group substances (*4, 8*), immunoglobulins (*4, 9*), and antifreeze glycoproteins (*4, 10*).

We have previously described (*11*) the synthesis of derivatives of *O*-(2-acetamido-2-deoxy-α-D-galactopyranosyl)-L-serine (α-D-GalNac-Ser) and the analogous L-threonine compound (α-D-GalNac-Thr). α-D-GalNac bound to Ser or Thr represents the antigenic determinant of membrane glycoproteins carrying the so-called T_N specificity. In addition they are supposed to be the biosynthetic precursors of the T-antigenic determinants, namely, *O*-(3-*O*-(β-D-galactopyranosyl)-2-acetamido-2-deoxy-α-D-galactopyranosyl)-L-serine (β-D-Gal-(1 → 3)-α-D-Gal Nac-Ser) and the analogous L-threonine compound (β-D-GalNac-(1 → 3)-α-D-Gal Nac-Thr).

Our interest has focused on T_N and T antigens after the claim (*12, 13*) that the latter were present at the surface of human breast cancer cells. Furthermore the level of anti-T antibodies that are present in healthy human serum is severely decreased in cancer breast patients. In addition the presence of T_N antigens at the surface of erythrocytes is correlated with hematological disorders such as leucopenia and thrombopenia (*14, 15*).

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[2] To whom inquiries should be addressed.

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T_N antigen was chosen as the first step of a project aimed at providing (i) a substrate for galactosyl transferases with the hope of gaining a better understanding of the biosynthesis of glycoproteins carrying this type of oligosaccharide moiety, and (ii) an artificial antigen in order to obtain anti-T_N (and anti-T) antibodies as well as the corresponding immunoadsorbants.

It is well known that simple haptens do not stimulate the immune system. They must be attached to a carrier in order to induce the production of antibodies by the organism. In our case the haptens were synthesized as 8-hydrazidooctanamido and 8-hydrazidooctanoyl derivatives of the corresponding glycosylamino acids. The procedure used to prepare artificial antigen follows the well-established methodology of Lemieux *et al.* (*16–23*). The carbohydrate–protein conjugates that have been most employed are galactosyl and lactosyl bovine serum albumin. The reaction sequence for the synthesis of these conjugates is described in the literature (*16–23*).

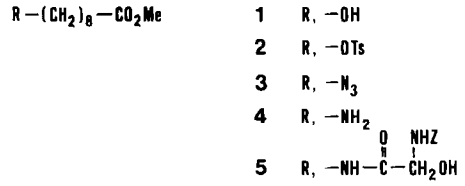
The immunogenicity of artificial antigens obtained in this way has been verified both with respect to the level of humoral response (*18*) and the carbohydrate specificity of these antibodies (*18, 24*).

RESULTS

In order to improve the specificity of the anti-glycosyl antibodies raised against such artificial antigens, the 2-acetamido-2-deoxy-α-D-galactopyranose molecule was bound to serine and the latter condensed alternatively on both sides of the amino acid moiety with the aliphatic chain. Furthermore, in the case of the attachment of the aliphatic "arm" to the carboxylic function of the hapten, the amino group of the amino acid was converted to its *N*-acetyl derivative. Conversely a methylamido derivative was used to protect the carboxylic function of the *N*-lengthened hapten. Both protecting groups were chosen because of their stability to the acidic and basic conditions required in subsequent steps. In addition they represent a close analogy to the peptide bond.

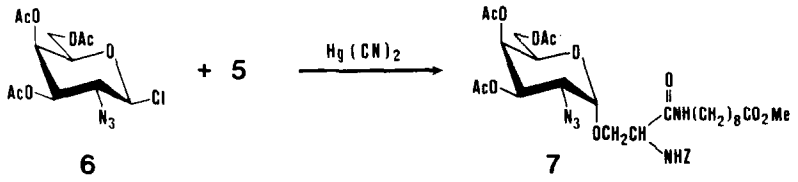
The reaction sequence for the synthesis of these carbohydrate protein conjugates involves the preparation of the glycosylamino acid unit, condensation with the appropriate aliphatic "arm" (either on the C-terminal or N-terminal side), formation of the hydrazide, conversion to the azide, and finally coupling of the azide to the bovine serum albumin. Previous attempts to apply the above scheme to the synthesis of the hapten **10** showed that the attachment of the methoxycarbonyl derivative at the carboxylic side proceeded with very poor yield. Hence we decided to begin with the condensation of methyl-9-aminononanoate with serine. Methyl-9-aminononanoate (**4**) was obtained in quantitative yield by treatment of 8-methoxycarbonyloctanol (**1**) with paratoluenesulfonyl chloride, conversion into the azido derivative **3** and hydrogenation in the presence of palladium-on-charcoal as catalyst.

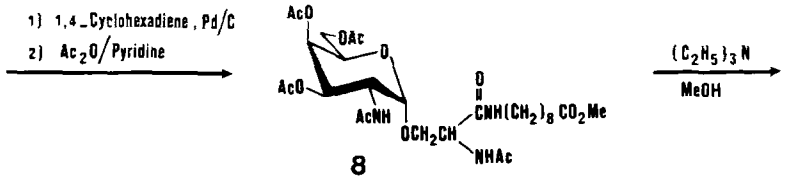
Compound **4** was treated with *N*-benzyloxycarbonyl-L-serine in the presence of isobutylchloroformate to provide **5** in 20% yield. This yield contrasts sharply with the 80% yield obtained when 8-methoxycarbonyloctanoic anhydride was con-

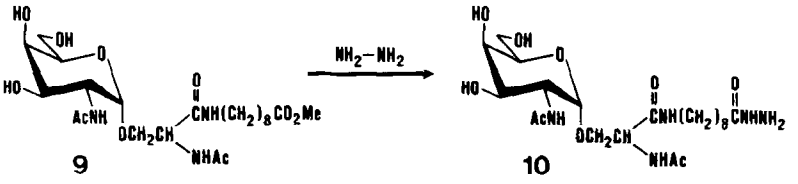


densed with the amino group of serine. Neither the condensation with dicyclohexylcarbodiimide, nor the activation of the carboxyl group with either B.O.P. reagent, 2-nitrophenol, or pentachlorophenol significantly improved the yield. This behavior must be ascribed to a lack of reactivity of compound **4**, since condensation of *N*-benzyloxycarbonyl-L-serine with glycine methyl ester proceeded easily. In view of the highly hydrophobic character of compound **4**, a possible, although highly speculative explanation might be related to the existence of micellar structures that mask the amino group.

Glycosylation of compound **5** with 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-β-D-galactopyranosyl chloride (**6**) was accomplished as previously described (*11*). *O*-(3,4,6 - Tri - *O* - acetyl - 2 - azido - 2 - deoxy - α - D - galactopyranosyl) - *N* - benzyloxycarbonyl-L-serine 8-methoxycarbonyloctanamide (**7**) was obtained in 55% yield. The α-anomeric configuration of **7** was established by proton magnetic resonance (H-1 1, 5.1 ppm, J_{12} = 3.7 Hz; H-2, 3.97 ppm, J_{23} = 10.5 Hz).







Concomitant removal of the benzyloxycarbonyl protecting group and reduction of the azido substituent was achieved by treatment of compound **7** with 1,4-cyclohexadiene in the presence of 10% palladium-on-charcoal (*25*). The diamino intermediate was acetylated without isolation with acetic anyhydride in pyridine to give the 8-methoxycarbonyloctanamide derivative of *O*-(3,4,6-tri-*O*-acetyl-2-acetamido-2-deoxy-α-D-galactopyranosyl)-*N*-acetyl-L-serine (**8**) in 87% yield. Compound **8** was deblocked by treatment with triethylamine in methanol to provide **9**, which was in turn converted to the hydrazide derivative **10** in 87% yield. When necessary, the hydrazide is converted by standard procedure (*16*) to the corresponding acyl azide which is used without isolation to acylate amino groups such as those of the L-lysine residues of bovine serum albumin.

For the synthesis of the hapten carrying the aliphatic arm on the N-terminal side of serine, we found it more advantageous to start with the glycosylation step. *N*-(Benzyloxycarbonyl)-L-serine methylamide (**11**) was obtained in quantitative yield by treatment of *N*-(benzyloxycarbonyl)-L-serine methyl ester with a commercial solution of 30% methylamine in ethanol. Glycosylation of **11** with **6** was accomplished as previously described (*11*). Pure *O*-(3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-α-D-galactopyranosyl)-*N*-benzyloxycarbonyl-L-serine methylamide (**12**) was obtained as crystalline material in 55% yield. Evidence for the α-anomeric configuration of **12** was based on proton-magnetic resonance signals at δ5.06 ppm (H-1, d, J_{12} = 3.6 Hz) and 3.68 ppm (H-2, q, J_{23} = 10.2 Hz) and optical rotation $[\alpha]_D^{22}$ +67.6°.

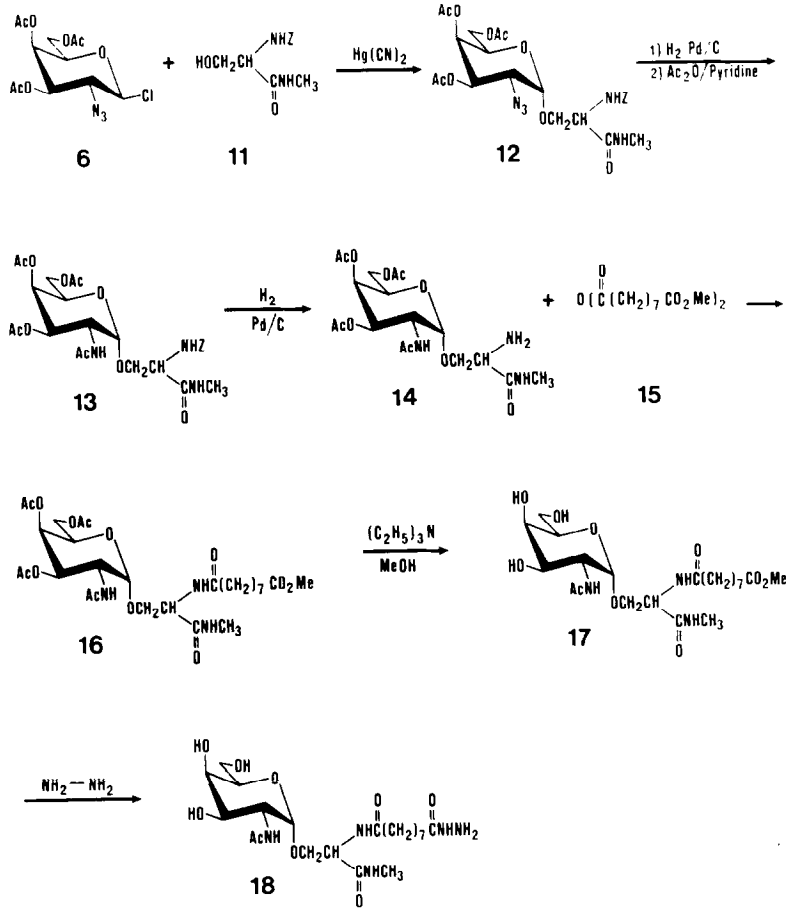
Selective hydrogenation of **12** with 5% palladium-on-charcoal in methanol gave the pure 2-amino-2-deoxy derivative in 62% yield. As previously observed, no appreciable hydrogenolysis of the benzyloxycarbonyl protecting group occurred

TABLE 1

^{1}H AND ^{13}C-NMR DATA FOR COMPOUNDS **10** AND **18**

Compound	^{1}H-NMR (D_2O), δ ppm, *J* Hz	^{13}C-NMR (D_2O), δ ppm
10	4.88, d, J_{12} = 3.5, H-1; 4.18, q, J_{23} = 10.8, H-2; 3.89, q, J_{34} = 3.0, H-3; 4.0, d, J_{45} < 0.5, H-4; 4.53, t, *J* = 5.0 CHα (Ser); 2.04, 2.06, 2s, 6H, CH_3(Nac); 2.2, t, *J* = 7.0, $-CH_2CO-$.	98.9, C-1; 50.8, C-2; 68.8, C-3; 69.5, C-4; 72.3, C-5; 62.1, C-6; 54.8, Cα(Ser); 68.4, Cβ(Ser); 22.8, 33.1 CH_3(Nac); 40.4, CH_2CO; 172.1, 175, 175.3, 194, C=O.
18	4.92, d, J_{12} = 3.5, H-1; 4.18, q, J_{23} = 10.8, H-2; 3.75, q, J_{34} = 3.0, H-3; 4.0, d, J_{45} < 0.5, H-4; 4.56, t, *J* = 5.0, CHα(Ser); 2.08, s, CH_3(Nac); 2.2, 2.34, 2t, *J* = 7.0, $-CH_2CO-$; 2.77, s, N-CH_3.	98.8, C-1; 50.8, C-2; 68.7, C-3; 69.4, C-4; 72.3, C-5; 62.1, C-6; 54.7, Cα(Ser); 68.1, Cβ(Ser); 23.0, CH_3(Nac); 36.4, N-CH_3; 172.9, 175.4, 178.4, 194, C=O.

(*11*). Conversion of the amino sugar intermediate into the acetamido derivative, *O*-(3,4,6-tri-*O*-acetyl-2-acetamido-2-deoxy-α-D-galactopyranosyl)-*N*-benzyloxycarbonyl-L-serine methylamide (**13**) was achieved in essentially quantitative yield by treatment with acetic anhydride–pyridine.



After hydrogenolysis of **13**, the free-amino glycosylamino acid derivative **14** was condensed with 8-methoxycarbonyloctanoid anhydride **15** to afford pure **16** in 80% yield. Removal of the blocking groups and treatment with hydrazine as described above, provided *O*-(2-acetamido-2-deoxy-α-D-galactopyranosyl)-*N*-8-hydrazidooctanoyl-L-serine methylamide (**18**) in 91% yield. Structure and configuration of compounds **10** and **18** were confirmed by ^1H and ^{13}C-NMR spectroscopy (see Table 1). The synthetic glycopeptidic antigens are being tested with the hope of improving the specificity of the antiglycosyl antibodies as compared with those obtained when 2-acetamido-2-deoxy-α-D-galactopyranose is not bound to serine.

EXPERIMENTAL

General Methods

Melting points were determined with a Büchi instrument and are reported uncorrected. Optical rotations were determined at room temperature with a Roussel–Jouan polarimeter. Elemental analyses were made by the Service Central de Microanalyse du CNRS division of Montpellier. TLC was performed on silica-gel plates, sprayed with 10% sulfuric acid in ethanol followed by heating at 100°C. ^{1}H-NMR spectra were recorded with either a Varian EM-390 (90 MHz) or a Bruker HFX-10 (400 MHz); ^{13}C-NMR spectra were recorded with the Bruker HFX-10 (100.575 MHz); chemical shifts are given in δ ppm for solutions in $CDCl_3$, D_2O, or CD_3OD with tetramethylsilane (0 ppm) or pyrazine (145.8 ppm) as internal standard. Infrared spectra were recorded with a Beckman-4 spectrophotometer. 8-Methoxycarbonyloctanol and 8-methoxycarbonyloctanoic acid were kindly provided by Chembiomed Ltd., Edmonton, Alberta, Canada.

Methyl-8-tosyloxynonanoate (2)

To a cold solution of 8-methoxycarbonyloctanol (1) (13.74 g, 72 mmol) in pyridine (120 ml) was added tosyl chloride (14.4 g, 75 mmol). The solution was cooled at 0°C, stirred for 12 hr then poored into ice-cold water (1 liter) and extracted with ether (1 liter). The organic layer was washed with 1 *N* hydrochloric acid (500 ml), dried (sodium sulfate), and evaporated to a clear syrup that was washed with petroleum ether to yield the pure product (2): 18.6 g (75%); ν_{max}^{KBr}, 1180 cm^{-1} (SO_2).

Anal. Calcd for $C_{17}H_{26}O_5S_1$ (342.4): C, 59.62; H, 7.65. Found: C, 59.71; H, 7.59.

^{1}H-NMR ($CDCl_3$) δ3.68 (s, 3H, OMe); 2.31 (t, J = 6 Hz, 2H, $-CH_2CO$).

Methyl-9-azidononanoate (3)

Compound 2 (4.90 g, 14.35 mmol) was dissolved in anhydrous DMF (40 ml) and NaN_3 (1.05 g, 16 mmol) was added. The solution was stirred at 60°C for 30 min and concentrated to a residue that was dissolved in ether (400 ml), extracted with ice-cold water (350 ml), and dried over sodium sulfate. Concentration gave a syrup that was filtered on silica gel with CH_2Cl_2 as eluant. Compound 3 was obtained chromatographically pure, 3.04 g (99%): ν_{max}^{KBr} 2150 cm^{-1} (N_3); ^{1}H-NMR ($CDCl_3$) δ3.68 (s, 3H, OMe), 2.31 (t, J = 6 Hz, 2H, $-CH_2CO-$).

Methyl-9-aminononanoate (4)

Compound 3 (710 mg, 3.32 mmol) in anhydrous CH_3OH (20 ml) was hydrogenated at atmospheric pressure in the presence of 5% palladium-on-charcoal catalyst (150 mg) until TLC showed no starting material (2 hr). The suspension was filtered through a bed of celite and the filtrate evaporated to a clear syrup, 620 mg (98%). This was treated immediately in the next step.

N-Benzyloxycarbonyl-L-serine 8-Methoxycarbonyloctanamide (**5**)

A solution of Z-L-Ser (11.96 g, 50 mmol) in THF (100 ml) was cooled to −10°C, neutralized with *N*-methylmorpholine (5 g, 50 mmol) and treated with isobutylchloroformate (7.16 g, 52 mmol). After 15 min, a solution of compound **4** (8 g, 42 mmol) in CH_2Cl_2 (50 ml) was added. The mixture was stirred overnight at room temperature then concentrated to a syrup that was dissolved in EtOAc (800 ml), washed with 1 *N* hydrochloric acid (400 ml) and water (400 ml). The organic layer was dried (sodium sulfate), filtered, and evaporated to give a white foam. Pure product (2.11 g, 11%) was obtained by passing this mixture through a silica-gel column, using EtOAc as eluant. $[\alpha]_D^{22}$ −13° (*c* 1.0, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ7.33 (s, Ph), 5.11 (s, $OCOCH_2$), 3.66 (s, 3H, OMe), 3.21 (m, 2H, $-CH_2N-$), 2.3 (t, J = 6.5 Hz, 2H, $-CH_2CO-$).

O-(3,4,6-Tri-O-acetyl-2-azido-2-deoxy-α-D-galactopyranosyl)-N-benzyloxycarbonyl-L-serine 8-Methoxycarbonyloctanamide (**7**)

A solution of compound **5** (2.01 g, 4.92 mmol) in a mixture of nitromethane and benzene (2 : 1, v/v, 20 ml) was shaken for 30 min in the presence of crushed 4-Å molecular sieves (4 g) and mercuric cyanide (2.1 g, 8.3 mmol). The suspension was stirred under a nitrogen atmosphere at 55°C and 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-β-D-galactopyranosyl chloride (**6**) (*11, 26*) (3 g, 8.6 mmol) was added over 8 hr. After 24 hr, TLC revealed no starting material, the solution was evaporated to a syrup that was taken up in $CHCl_3$ (30 ml), the suspension kept for 2 hr at 0°C, and the crystalline mercuric salt filtered off. The filtrate was evaporated and the resulting syrup chromatographed on a column of silica gel (150 mg) with 7 : 3 (v/v) CH_2Cl_2/ether to give the pure product (1.95 g, 55%): ν_{max}^{KBr} 2120 cm^{-1} (N_3).

Anal. Calcd for $C_{33}H_{47}N_5O_{13}$ (721.7): C, 54.91; H, 6.56; N, 9.70. Found: C, 54.85; H, 6.70; N, 9.76.

^{1}H-NMR ($CDCl_3$) δ7.34 (s, Ph), 6.54 (t, J = 6 Hz, NH), 5.79 (d, J = 7.5 Hz, NH Ser), 5.4 (d, $J_{45} < 0.5$ Hz, H-4), 5.28 (q, J_{34} = 3 Hz, H-3), 5.1 (d, J_{12} = 3.7 Hz, H-1), 3.97 (q, J_{23} = 10.5 Hz, H-2), 3.63 (s, 3H, OMe), 3.28 (m, 2H, $-CH_2N-$), 2.18 (t, J = 3.7 Hz, $-CH_2CO-$).

O-(3,4,6-Tri-O-acetyl-2-acetamido-2-deoxy-α-D-galactopyranosyl)-N-acetyl-L-serine 8-Methoxycarbonyloctanamide (**8**)

Compound **7** (360 mg, 0.5 mmol) was dissolved in ethanol (7 ml). A gentle stream of nitrogen was passed through the reaction mixture, 10% palladium-on-charcoal catalyst (500 mg) was added, followed by the addition of 1,4-cyclohexadiene (1.4 ml). After 2 hr at 25°C the suspension was filtered through a bed of celite and the filtrate evaporated to give a clear syrup. This was taken immediately with a mixture of pyridine (10 ml) and Ac_2O (2.5 ml) and let stand overnight at 5°C. The solvent was removed *in vacuo*, the residue dissolved in CH_2Cl_2 (250 ml), and the solution washed with cold 1 *N* hydrochloric acid (100 ml), water (100 ml), saturated aqueous $NaHCO_3$ (100 ml), then water (100 ml). The organic layer was dried and evaporated, the resulting syrup being passed through a column of silica

gel. Elution with 95:5 (v/v), EtOAc/CH_3OH gave the pure product, 230 mg (72%).

Anal. Calcd for $C_{29}H_{47}N_3O_{13}$ (645.7): C, 53.94; H, 7.33; N, 6.50. Found: C, 54.01; H, 7.35; N, 6.62.

^{1}H-NMR ($CDCl_3$) δ6.9 (t, J = 6 Hz, NH), 6.4 (d, J = 7.5 Hz, NH Ser), 5.33 (d, $J_{45} \simeq$ 0.5 Hz, H-4), 5.1 (q, J_{34} = 3 Hz, H-3), 4.88 (d, J_{12} = 3.4 Hz, H-1), 4.5 (m, J_{23} = 10.8 Hz, H-2), 3.66 (s, 3H, OMe), 3.23 (m, 2H, $-CH_2-N$), 2.3 (t, J = 7 Hz, 2H, $-CH_2CO-$).

O-(2-Acetamido-2-deoxy-α-D-galactopyranosyl)-N-acetyl-L-serine 8-Methoxycarbonyloctanamide (**9**)

Compound **8** (200 mg, 0.30 mmol) was dissolved in anhydrous CH_3OH (10 ml) and $(C_2H_5)_3N$ (0.8 ml) was added. After 5 hr at room temperature the reaction was complete (TLC). The solvent was removed *in vacuo*, the residue taken in CH_3OH and shaken with prewashed low acid resin (5 ml, Amberlite IRC 50). Removal of the resin and concentration of the solution gave a white solid. This was washed with ether and dried to give the analytically pure product, 140 mg (87%): mp 212–215°C; $[\alpha]_D^{22}$ +94.8° (*c* 0.5, CH_3OH).

Anal. Calcd for $C_{23}H_{41}N_3O_{10}$, H_2O (537.6): C, 51.38; H, 8.06; N, 7.81. Found: C, 51.45; H, 8.02; N, 7.73.

^{1}H-NMR (CD_3OD) δ4.76 (d, J_{12} = 3.5 Hz, H-1), 4.23 (q, J_{23} = 10.8 Hz, H-2), 3.59 (s, 3H, OMe), 3.2 (m, 2H, $-CH_2-N$), 2.22 (t, J = 7 Hz, $-CH_2CO-$), 1.95 (s, 6H, N-Ac).

O-(2-Acetamido-2-deoxy-α-D-galactopyranosyl)-N-acetyl-L-serine 8-Hydrazidooctanamide (**10**)

A solution of compound **9** (140 mg, 0.26 mmol) in a mixture of CH_3OH (6 ml) and 85% aqueous hydrazine (4 ml) was shaken for 6 hr. After TLC revealed no starting material, the solution was concentrated and the remaining traces of hydrazine were eliminated by coevaporation with toluene. The resulting yellow solid was washed several times with acetone then ether. The hapten **10** was obtained pure as a white solid, 120 mg (86%); mp 220–222°C; $[\alpha]_D^{22}$ +88.6° (*c* 1, H_2O).

Anal. Calcd for $C_{22}H_{41}N_5O_{10}$, H_2O (537.6): C, 49.15; H, 8.06; N, 13.02. Found: C, 49.06; H, 7.89; N, 12.93.

For ^{1}H-NMR and ^{13}C-NMR see Table 1.

N-Benzyloxycarbonyl-L-serine Methylamide (**11**)

Z-L-Ser-OMe (2.4 g, 9.4 mmol) was dissolved in a solution of 33% methylamine in absolute ethanol (60 ml) (Fluka 65590). The reaction was shaken for 2 hr and then concentrated to a yellow solid. Several evaporations of CH_3OH were performed to eliminate the traces of methylamine. Crystallization from EtOAc gave the pure compound as white needles, 1.9 g (80%); mp 121–123°C (lit.[27] mp 129°C).

O-(3,4,6-Tri-O-acetyl-2-azido-2-deoxy-α-D-galactopyranosyl)-N-benzyloxycarbonyl-L-serine Methylamide (**12**)

A solution of methylamide **11** (5 g, 19.82 mmol) in a mixture of nitromethane (15 ml), benzene (5 ml), and acetonitrile (5 ml) was stirred at 60°C under a nitrogen atmosphere in the presence of dry mercury cyanide (1.4 g, 5.5 mmol). Compound **6** (*11, 26*) (3 g, 8.6 mmol) was added by fractions over 10 hr and the reaction continued for 15 hr. After CH_2Cl_2 (100 ml) was added, the mixture was filtered on a bed of celite and the filtrate evaporated. The residue was taken up with $CHCl_3$ (50 ml), the suspension kept for 3 hr at 0°C and the crystalline mercuric salts filtered off. Solvent removal left a brown syrup (12 g) that was passed through a column of silica gel and eluted with 7 : 3 : 1 (v/v) CH_2Cl_2/ether/acetone. The pure compound **12** was obtained as a white solid, 5.85 g (52%); mp 55–60°C; $[\alpha]_D^{22}$ +67.6° (*c* 1, $CHCl_3$).

Anal. Calcd for $C_{24}H_{31}N_5O_{11}$ (565.5): C, 50.97; H, 5.52; N, 12.38. Found: C, 50.64; H, 5.52; N, 12.09.

^{1}H-NMR ($CDCl_3$) δ7.33 (s, Ph), 6 (d, J = 7.5 Hz, NH Ser), 5.36 (d, $J_{45} < 0.5$ Hz, H-4), 5.26 (q, J_{34} = 3 Hz, H-3), 5.06 (d, J_{12} = 3.6 Hz, H-1), 3.68 (q, J_{23} = 10.2 Hz, H-2), 2.81 (d, J = 5 Hz 3H, N-CH_3).

O-(3,4,6-Tri-O-acetyl-2-acetamido-2-deoxy-α-D-galactopyranosyl)-N-benzyloxycarbonyl-L-serine Methylamide (**13**)

Compound **12** (880 mg, 1.55 mmol) was hydrogenated at atmospheric pressure in anhydrous CH_3OH (25 ml) in the presence of 5% palladium-on-charcoal as catalyst (700 mg). After 4 hr, TLC showed no remaining starting material, the suspension was filtered on a bed of celite and washed with CH_3OH. Evaporation of the combinated filtrates gave a white foam (700 mg) that was immediately treated with a mixture of pyridine (35 ml) and Ac_2O (2.2 ml) for one night at 0°C. The solution was concentrated *in vacuo,* the residue taken up in CH_2Cl_2 (150 ml) and washed successively with ice-cold water (100 ml), cold 1 *N* hydrochloric acid (100 ml), saturated aqueous $NaHCO_3$ (50 ml), and finally ice-cold water (50 ml). Drying of the organic layer (Na_2SO_4) and removal of the solvent left a clear syrup (700 mg). Purification was performed by passage through a column of silica gel with 6 : 4 (v/v) CH_2Cl_2/acetone as eluant. Compound **13** was obtained as a white solid, 550 mg (61%); mp 125–130°C; $[\alpha]_D^{22}$ +57.9 (*c* 1, $CHCl_3$).

Anal. Calcd for $C_{26}H_{35}N_3O_{12}$ (581.5): C, 53.69; H, 6.06; N, 7.22. Found: C, 53.48; H, 6.05; N, 7.23.

^{1}H-NMR ($CDCl_3$) δ7.33 (s, Ph), 6.5 (d, J = 9 Hz, NH-Ac), 6.3 (d, J = 7.5 Hz, NH Ser), 5.33 (d, $J_{45} < 0.5$ Hz, H-4), 5.1 (q, J_{34} = 3 Hz, H-3), 4.88 (d, J_{12} = 3.3 Hz, H-1), 2.8 (d, J = 5 Hz, 3H, N-CH_3).

O-(3,4,6-Tri-O-acetyl-2-acetamido-2-deoxy-α-D-galactopyranosyl)-L-serine Methylamide (**14**)

Compound **13** (1.1 g, 1.89 mmol) was hydrogenated in absolute ethanol at atmospheric pressure in the presence of 10% palladium-on-charcoal catalyst (1 g).

After overnight the reaction was complete (TLC). The mixture was filtered on a bed of celite and the filtrate concentrated to a clear syrup (850 mg) that was immediately used in the next step.

8-Methoxycarbonyloctanoic Anhydride (**15**)

8-Methoxycarbonyloctanoic acid (3.03 g, 15 mmol) was dissolved in anhydrous CH_2Cl_2 (60 ml), dicyclohexylcarbodiimide (3 g) was added, and the reaction stirred 1 hr at 10°C. The dicyclohexylurea was filtered off and the cold solution of the anhydride was immediately used in the next step.

*O-(3,4,6-Tri-O-acetyl-2-acetamido-2-deoxy-α-*D*-galactopyranosyl)-N-8-methoxycarbonyloctanoyl-*L*-serine Methylamine* (**16**)

The cold solution of the anhydride **15** (7.5 mmol) in CH_2Cl_2 was added to compound **14** (850 mg). The reaction was stirred 1 hr at 0°C then let at room temperature for 0.5 hr, the pH being kept neutral by addition of TEA. The solution was concentrated *in vacuo* and the resulting syrup washed with 1 : 1 (v/v) petroleum ether/ether to eliminate the acid and the anhydride in excess. The resulting white solid was recrystallized in CH_2Cl_2 as needles. The product **16** was recovered (950 mg, 80%) as a crystalline, hygroscopic material (homogeneous by TLC) whose mp could not be determined: $[\alpha]_D^{22}$ +70.5° (*c* 1, CH_3OH).

Anal. Calcd for $C_{26}H_{45}N_3O_{13}$ (631.6): C, 53.24; H, 7.18; N, 6.65. Found: C, 53.11; H, 7.22; N, 6.78.

^{1}H-NMR ($CDCl_3$) δ6.5 (d, J = 9 Hz, -NH-Ac), 5.33 (d, $J_{45} < 0.5$ Hz, H-4), 5.1 (q, J_{34} = 3Hz, J_{23} = 10.8 Hz, H-3), 4.86 (d, J_{12} = 3.3 Hz, H-1), 3.66 (s, 3H, OMe), 2.81 (d, J = 5 Hz, 3H, N-CH_3).

*O-(2-Acetamido-2-deoxy-α-*D*-galactopyranosyl)-N-8-methoxycarbonyloctanoyl-*L*-serine Methylamide* (**17**)

Compound **16** (250 mg, 0.39 mmol) was dissolved in anhydrous CH_3OH (12 ml) and TEA (1.2 ml) was added under stirring. After 5 hr at room temperature, the resulting gel was concentrated *in vacuo* and the remaining traces of TEA were removed by coevaporation with CH_3OH. The white solid was taken up in CH_3OH and stirred with prewashed low acid resin (5 ml) (Amberlite IRC 50). Removal of the resin and concentration of the filtrate gave compound **17**, which was obtained analytically pure after recrystallization in absolute ethanol, 190 mg (96%); hygroscopic; $[\alpha]_D^{22}$ +84.7° (*c* 1, CH_3OH).

Anal. Calcd for $C_{22}H_{39}N_3O_{10}$ (505.5): C, 52,56; H, 7.77; N, 8.31. Found: C, 52.32; H, 7.89; N, 7.8.

^{1}H-NMR (CD_3OD) δ4.56 (t, J = 5 Hz, CH Ser), 4.28 (q, J_{23} = 10.8 Hz, J_{12} = 3.8 Hz, H-2), 3.65 (s, 3H, OMe), 2.76 (s, 3H, N-CH_3), 2.31 (t, J = 7 Hz, CH_2-CO-), 2.28 (t, J = 7 Hz, $-CH_2-CO$), 2.01 (s, 3H, NAc).

*O-(2-Acetamido-2-deoxy-α-*D*-galactopyranosyl)-N-8-hydrazidooctanoyl-*L*-serine Methylamide* (**18**)

A solution of compound **17** (140 mg, 0.27 mmol) in a mixture of CH_3OH (4 ml)

and 85% aqueous hydrazine (4 ml), was stirred 1 hr at 0°C, then 5 hr at room temperature. The solvent was evaporated and the remaining traces of hydrazine removed by coevaporation with toluene. The resulting white solid was washed several times with acetone, 125 mg (91%); mp 196–198°C; $[\alpha]_D^{22}$ +75.6° (*c* 1, H_2O).

Anal. Calcd for $C_{21}H_{39}N_5O_9$, H_2O (523.5): C, 48.17; H, 7.89; N, 13.37. Found: C, 48.35; H, 7.45; N, 13.02.

For ^{1}H-NMR and ^{13}C-NMR see Table 1.

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