2-SUBSTITUTED METHYL α-D-GALACTOPYRANOSIDES: SYNTHESIS AND BINDING AFFINITY FOR THE A AND B SUBUNITS OF THE Griffonia simplicifolia I ISOLECTINS

ROKURO KAIFU*, LISA C. PLANTEFABER, AND IRWIN J. GOLDSTEIN
Department of Biological Chemistry, The University of Michigan, Ann Arbor, Michigan 48109 (U.S.A.)
(Received September 7th, 1981; accepted for publication, December 28th, 1984)

ABSTRACT

The binding affinities of the N-acetyl, N-trifluoroacetyl, N-propionyl, N-formyl, N-benzoyl, N-p-nitrobenzoyl, N-p-aminobenzoyl, and N-methyl derivatives of methyl 2-amino-2-deoxy-α-D-galactopyranoside and the 2-O-acetyl, -benzoyl, -benzyl, and -methyl derivatives of methyl α-D-galactopyranoside for the A and B subunits of the Griffonia simplicifolia I isolectins have been determined by hapten inhibition analysis of a galactomannan-isolectin precipitation system. Models for these carbohydrate–protein interactions are presented together with an interpretation of the results on the basis of electronic and steric effects.

INTRODUCTION

A lectin which specifically agglutinates human type-B erythrocytes has been found in extracts of G. simplicifolia, and its activity was inhibited by D-galactose, D-galactose-containing oligosaccharide, and type-B substance. This blood-group B-active lectin was purified by affinity chromatography and characterized as the first α-D-galactosyl-binding lectin. Further investigation revealed that the lectin consists of five isolectins, which are tetrameric glycoproteins composed of various proportions of two unique subunits (A and B) that have been designated GS I-A, A,B, A,B,B, and B. Both the A and B subunits have identical affinities for α-D-galactopyranosyl groups, but the A subunit also exhibits a pronounced affinity for 2-acetamido-2-deoxy-α-D-galactopyranosyl units (K_{assoc} = 1.87 \times 10^5 M^{-1}), whereas the B subunit shows extremely poor binding affinity (K_{assoc} = 1.26 \times 10^2 M^{-1}) toward this amino sugar. Both the A and B subunits possess loci which interact with the hydroxyl groups at C-1(α), C-3, C-4, and C-6. In order to determine the binding specificity of the loci in both subunits for the O-2 substituent of methyl α-D-galactopyranosides, various 2-substituted methyl α-D-galacto-
RESULTS AND DISCUSSION

Methyl 2-O-acyl-α-D-galactopyranoside derivatives were prepared from methyl α-D-galactopyranoside (1) via methyl 3,4-O-isopropylidene-6-O-trityl-α-D-galactopyranoside (3). Removal of the isopropylidene and trityl groups in acidic media whilst an acyl group is present at O-2 may cause anomerisation. In order to ensure the α configuration of the products, methyl 2-O-acyl-α-D-galactopyranosides were also prepared by routes in which cleavage of protecting groups was carried out by hydrogenation.

Tritylation of methyl 3,4-O-isopropylidene-α-D-galactopyranoside7 (2), followed by treatment of the product 3 with pyridine-acetic anhydride, afforded methyl 2-O-acetyl-3,4-O-isopropylidene-6-O-trityl-α-D-galactopyranoside (4). The trityl and isopropylidene groups were removed from 4 by heating for 2 h at 95° in aqueous 80% acetic acid, to give 38% of crystalline methyl 2-O-acetyl-α-D-galactopyranoside (5). An alternative route involved benzylation of 1,2:5,6-di-O-isopropylidene-α-D-galactofuranose8 (6) and hydrolysis of the product (7) with aqueous 90% trifluoroacetic acid, to afford 3-O-benzyl-D-galactose (8). Treatment of 8 with boiling, methanolic 4% hydrogen chloride for 2 days gave methyl 3-O-benzyl-α-D-galactopyranoside (9), in admixture with other isomeric glycosides. Compounds 7-9 were amorphous and used without further purification in subsequent reactions. Benzylidenation of 9 in the usual manner gave 14% (based on 7) of crystalline methyl 3-O-benzyl-4,6-O-benzylidene-α-D-galactopyranoside (10), the α configuration of which was confirmed by the J1,2 value of 3.5 Hz. Treatment of 10 with pyridine–acetic anhydride followed by catalytic hydrogenolysis of the resulting acetate (11) gave 5. Compound 5 was prepared previously9 by partial acetylation of
methyl 4,6-\(O\)-benzylidene-\(\alpha\)-D-galactoside followed by removal of the benzylidene group, but it was not crystallized.

Treatment of 3 with benzyol chloride–pyridine afforded 98% of the 2-benzozate (12), hydrolysis of which for 2 h at 95° in aqueous 80% acetic acid gave 54% of methyl 2-O-benzyol-\(\alpha\)-D-galactopyranoside (13). The protecting groups in 12 could also be removed by hydrogenolysis. An alternative route to 13 involved partial benzyolation of methyl 4,6-\(O\)-benzylidene-\(\alpha\)-D-galactopyranoside (14) with benzoilimidazole, following the procedure\(^{10}\) for the preparation of the corresponding benzyl \(\beta\)-D-galactoside, to give methyl 3-O-benzyol-4,6-\(O\)-benzylidene-\(\alpha\)-D-galactopyranoside (15). Treatment of 15 with dilute alkali–acetone gave 63% of the 2-benzoate 16 (cf. ref. 11). Compounds 15 and 16 have been prepared by other procedures\(^{12}\). Catalytic hydrogenolysis of 16 gave 52% of 13.

Ether derivatives of methyl \(\alpha\)-D-galactopyranoside were prepared from 3 by conventional procedures. Treatment of 3 with methyl iodide and sodium hydride in \(N,N\)-dimethylformamide gave 70% of methyl 3,4-\(O\)-isopropylidene-2-O-methyl-6-\(O\)-trityl-\(\alpha\)-D-galactopyranoside (17). Hydrolysis of 17 followed by chromatography on silica gel gave 43% of crystalline methyl 2-O-methyl-\(\alpha\)-D-galactopyranoside (18). The \(\alpha\) configuration of 18 was indicated by the J\textsubscript{1,2} value of 3 Hz. Non-crystalline 18 was previously obtained\(^{13}\) by hydrolysis of methyl 3,4-\(O\)-isopropylidene-2-O-methyl-\(\alpha\)-D-galactopyranoside.

Reaction of 3 with benzyl chloride and potassium hydroxide gave 63% of methyl 2-O-benzyol-3,4-\(O\)-isopropylidene-6-\(O\)-trityl-\(\alpha\)-D-galactopyranoside (19). Hydrolysis of 19 gave 54% of methyl 2-O-benzyol-\(\alpha\)-D-galactopyranoside (20). Overlapping peaks prevented direct determination of the \(\alpha\) configuration of 20 by n.m.r. spectroscopy, but the absence of a signal for H-1\(\beta\) in the region \(\delta\) 4.0–4.7 indirectly suggested the \(\alpha\) configuration.

\(N\)-Acyl derivatives of methyl 2-amino-2-deoxy-\(\alpha\)-D-galactopyranoside (21) were prepared conventionally. Treatment of 21 with formic acid–acetic anhydride\(^{14}\) gave the \(N\)-formyl derivative (22). Likewise, 21 with propionic anhydride and benzoic anhydride in methanol gave the \(N\)-propionyl (23, 65%) and \(N\)-benzoyl (24, 29%) derivatives; 24 was prepared previously\(^{15}\) from methyl 2-benamido-3,4,6-tri-\(O\)-benzyol-2-deoxy-\(\alpha\)-D-galactopyranoside. Treatment of 21 with \(S\)-ethyl trifluoroacetate produced the \(N\)-trifluoroacetate derivative 25. Following the method of Gorin\(^{16}\) for 2-deoxy-2-methylamino-D-galactose, methyl 3,4,6-tri-\(O\)-acetyl-2-benzyloxy carbonylamino-2-deoxy-\(\alpha\)-D-galactopyranoside (27) was treated with methyl iodide and silver oxide in \(N,N\)-dimethylformamide and the resulting \(N\)-methyl derivative 28 was subjected to Zemplén deacetylation (29). Catalytic hydrogenolysis then gave 84% of amorphous methyl 2-deoxy-2-methylamino-\(\alpha\)-D-galactopyranoside (30). Compound 30 was homogeneous by t.l.c. and the \(\alpha\) configuration was indicated by the J\textsubscript{1,2} value of 3.5 Hz. Methyl 2-deoxy-2-\(p\)-nitro- (and \(p\)-amino)benzamido-\(\alpha\)-D-galactopyranoside (33 and 34) were prepared according to published procedures\(^{17}\).

The binding affinity of the 2-substituted methyl \(\alpha\)-D-galactopyranoside
Fig. 1. Inhibition of *G. simplcifolia* I-\(A_4\) isoelectin-galactomannan interaction by methyl \(\alpha\)-D-galactopyranoside derivatives. Each tube contained \(A_4\) isoelectin (60 \(\mu\)g), guaran (8 \(\mu\)g), and inhibitor (see Table I) in a total volume of 0.5 \(\text{mL}\).

Fig. 2. Inhibition of *G. simplcifolia* I-\(B_4\) isoelectin-galactomannan interaction by methyl \(\alpha\)-D-galactopyranoside derivatives. Each tube contained \(B_4\) isoelectin (60 \(\mu\)g), guaran (8 \(\mu\)g), and inhibitor (see Table I) in a total volume of 0.5 \(\text{mL}\).
TABLE I

INHIBITION OF G. simplicifolia I ISOLECTINS A<sub>4</sub> AND B<sub>4</sub>-GALACTOMANNAN PRECIPITATION BY METHYL α-D-GALACTOPYRANOSONIDE DERIVATIVES

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Amount giving 50% inhibition (µmol)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>for A&lt;sub&gt;4&lt;/sub&gt;</td>
</tr>
<tr>
<td>Methyl α-D-galactopyranoside (1)</td>
<td>0.23</td>
</tr>
<tr>
<td>Methyl 2-O-acetyl-α-D-galactopyranoside (5)</td>
<td>0.12</td>
</tr>
<tr>
<td>Methyl 2-O-benzoyl-α-D-galactopyranoside (13)</td>
<td>0.53</td>
</tr>
<tr>
<td>Methyl 2-O-methyl-α-D-galactopyranoside (18)</td>
<td>2.1</td>
</tr>
<tr>
<td>Methyl 2-O-benzyl-α-D-galactopyranoside (20)</td>
<td>1.7</td>
</tr>
<tr>
<td>Methyl 2-amino-2-deoxy-α-D-galactopyranoside (21)</td>
<td>0.87</td>
</tr>
<tr>
<td>Methyl 2-deoxy-2-formamido-α-D-galactopyranoside (22)</td>
<td>0.043</td>
</tr>
<tr>
<td>Methyl 2-deoxy-2-propionamido-α-D-galactopyranoside (23)</td>
<td>0.023</td>
</tr>
<tr>
<td>Methyl 2-benzamido-2-deoxy-α-D-galactopyranoside (24)</td>
<td>0.072</td>
</tr>
<tr>
<td>Methyl 2-deoxy-2-trifluoroacetamido-α-D-galactopyranoside (25)</td>
<td>0.018</td>
</tr>
<tr>
<td>Methyl 2-deoxy-2-methylamino-α-D-galactopyranoside (30)</td>
<td>1.7</td>
</tr>
<tr>
<td>Methyl 2-acetamido-2-deoxy-α-D-galactopyranoside (31)</td>
<td>0.019</td>
</tr>
<tr>
<td>Methyl 2-deoxy-2-β-D-lyxo-hexopyranoside (32)</td>
<td>1.6</td>
</tr>
<tr>
<td>Methyl 2-deoxy-2-p-nitrobenzamido-α-D-galactopyranoside (33)</td>
<td>0.014</td>
</tr>
<tr>
<td>Methyl 2-deoxy-2-p-aminobenzamido-α-D-galactopyranoside (34)</td>
<td>0.084</td>
</tr>
</tbody>
</table>

derivative for the G. simplicifolia A<sub>4</sub> and B<sub>4</sub> isolecitins was examined by hapten inhibition analysis of the isolecitin–galactomannan precipitation system. The complete inhibition curves are presented in Figs. 1 and 2, and the amounts of the galactoside derivatives required for 50% inhibition, determined from the inhibition curves, are shown in Table I.

Although the 2-acylamino-2-deoxy and the 2-O-acyl derivatives of methyl α-D-galactopyranoside are better inhibitors of the A subunit than the parent galactoside, N-acyl derivatives (NHCOCF<sub>3</sub>, 25; NHAc, 31; NHCOEt, 23; NHCHO, 22; NHBz, 24; NHCH<sub>2</sub>HNO<sub>2</sub>, 33; and NH<sub>2</sub>H<sub>2</sub>NH<sub>2</sub>, 34) of methyl 2-amino-2-deoxy-α-D-galactopyranoside (21) showed greater affinity for the A<sub>4</sub> isolecitin than the corresponding 2-O-acyl derivatives (OAc 5 and OBz 13). The N-p-nitrobenzamido derivative 33, the most potent inhibitor of the A<sub>4</sub> isolecitin, was also one of the most potent inhibitors of the lima-bean lectin<sup>18,19</sup>. It is possible that the partial electronegative charge (δ<sup>-</sup>) on the carbonyl oxygen atom and the partial electropositive charge (δ<sup>+</sup>) on the carbonyl carbon atom of these derivatives interact with oppositely charged loci in the A-subunit receptor-site. This is shown in the model (Fig. 3) proposed previously<sup>6</sup>. A more refined interpretation is not possible at this time.

The N-trifluoroacetyl derivative 25 was equivalent in inhibitory potency to the N-acetyl derivative 31. Thus, the strong electronegative effect exerted by the CF<sub>3</sub> group does not diminish the binding affinity of 25 for the A subunit.

Methyl 2-deoxy-α-D-lyxo-hexopyranoside (32) and methyl 2-O-benzyl-α-D-galactopyranoside (20) are 7-times less reactive, and methyl 2-O-methyl-α-D-galac-
Fig. 3. Diagrammatic model of the carbohydrate binding-site of the GS I-A subunit, showing the δ⁺ and δ⁻ loci. A 2-acetamido-2-deoxy-α-D-galactopyranosyl group is shown occupying the carbohydrate binding-site.

Fig. 4. Diagrammatic model of the carbohydrate binding-site of the GS I-B subunit, showing its interaction with (a) an α-D-galactopyranosyl group, (b) a 2-amino-2-deoxy-α-D-galactopyranosyl group, (c) a 2-O-acetyl-α-D-galactopyranosyl group, and (d) a 2-acetamido-2-deoxy-α-D-galactopyranosyl group.

topyranoside (18) is 10-times less potent an inhibitor of the A₄-guara precipitation system than methyl α-D-galactopyranoside. These results may be explained by postulating an interaction (probably a hydrogen bond) between HO-2 and a locus in the combining site of the A subunit; the 2-deoxy and the 2-ether derivatives cannot donate hydrogen bonds.

Interaction of the series of 2-substituted methyl α-D-galactosides with the B₄ isolectin may also be interpreted in terms of our previous model with the added feature of a hydrogen bond-forming locus (electronegative charge). Indeed, the potent inhibition by methyl α-D-galactopyranoside (1) and methyl 2-amino-2-deoxy-α-D-galactopyranoside (21) may be explained by the formation of hydrogen bonds between the C-2 OH and NH groups, respectively, and the electronegative charge [partial (δ⁻) or formal] in the B-subunit receptor-site (Fig. 4a,b). Conversion of the C-2 amino group in 21 to MeNH in 30 decreased the inhibitory potency 19-fold, by a reduction of the partial electropositive charge (δ⁺) of the N-bound H atom and/or steric hindrance due to the methyl group.

The 50-fold decrease in binding affinity of methyl 2-deoxy-α-D-lyxo-hexopyranoside (32) may be ascribed to the fact that the C-2 deoxy group cannot
be involved in hydrogen bonding at the receptor site of the B subunit.

Similarly, the inability to donate hydrogen bonds and the steric hindrance caused by the alkyl and aralkyl groups account for the exceptionally low affinity of the 2-O-methyl (18) and 2-O-benzyl (20) derivatives of methyl α-D-galactopyranoside for the B₄ isolecitin. It required concentrations of 80 μM 20 and 92 μM 18, compared to only 0.12 μM methyl α-D-galactopyranoside, to inhibit B₄-galactomannan precipitation by 50%.

Although the 2-acetate (5) and the 2-benzoate (13) of methyl α-D-galactopyranoside are ~20-times less potent inhibitors than the parent galactoside, they are 40-times more potent than the 2-O-methyl (18) and 2-O-benzyl (19) and (20) derivatives. This finding suggests a mode of binding in which the partial electro-positive charge (δ⁺) at the carbonyl carbon atom interacts with a corresponding negative charge in the B-subunit receptor-site as shown in Fig. 4c. This site may be the same site that is involved in hydrogen-bond formation with HO-2 of methyl α-D-galactopyranoside.

The 2-acylamino-2-deoxy derivatives, on the other hand, may bind to the B-subsite receptor via a different mode (Fig. 4d) in which the N-H group interacts with the electronegative locus in the B-subunit binding-site. Steric effects also probably play a role in this binding process, inasmuch as the N-formyl derivative 22 is 5-times more potent an inhibitor than the N-acetyl (31) and N-propionyl (23) derivatives.

The strong binding affinity of the N-trifluoroacetyl derivative 25 may be due to enhancement of the δ⁺ charge on the carbonyl carbon by the CF₃ group promoting interaction with the electronegative locus in the B binding-site in a manner analogous to that of the N-acetyl derivative (Fig. 4d).

EXPERIMENTAL

General. — Melting points were determined with a Fisher–Johns apparatus and are uncorrected. I.r. spectra (KBr discs) were recorded on a Perkin–Elmer 283 spectrophotometer. N.m.r. spectra (internal Me₄Si, unless stated otherwise) were recorded with a Varian T-60 n.m.r. spectrometer. Silica gel G (Merck) was used for column chromatography and t.l.c. (detection by charring with sulfuric acid). Microanalyses were performed by Spang Microanalytical Laboratory (Eagle Harbor, Michigan).

Griffonia simplicifolia I-A, isolecitin was purified according to the procedure of Murphy and Goldstein. Griffonia simplicifolia I-B₄ isolecitin was isolated by the method of Delmotte and Goldstein. Cassia alata galactomannan was available in this laboratory.

Hapten inhibition analysis. — A modification of the precipitin reaction described by So and Goldstein was employed. To 3-mL centrifuge tubes containing increasing quantities of methyl α-D-galactopyranoside derivative in salt-Pi buffer [0.1 M phosphate (pH 7.2), 0.1 M NaCl, 300 μL] and galactomannan (8 μg) in
44 R. KAIFU, L. C. PLANTEFAKER, I. J. GOLDSTEIN

salt-P, (100 μL) was added lectin (60 μg) in salt-P, buffer (100 μL). Tubes were
incubated for 24 h at 25° and centrifuged, and the pellets were washed with salt-P,
buffer (300 μL). The precipitates were dissolved in 0.05M sodium hydroxide and
protein was determined by a semimicro Lowry procedure. If the precipitates were
not completely dissolved, the alkaline solutions were shaken for 30 s at 50°.

*Methyl 3,4-O-isopropylidene-6-O-trityl-α-d-galactopyranoside (3).* — Trityl
chloride (3.06 g) was stirred with a solution of methyl 3,4-O-isopropylidene-α-d-
galactopyranoside7 (2, 1.34 g) in pyridine (5 mL) for 40 h at room temperature.
The mixture was then filtered and poured into iced-water (120 mL), the precipitate
was washed with water, and a solution in ether was dried (Na2SO4) and concen-
trated. The syrupy residue was eluted from a column of silica gel with chloroform-
methanol (99:3:0.7). Fractions containing 3 were collected and concentrated. Addi-
tion of water to the residue gave an amorphous solid (2 g, 72%) which crystallized
from hexane to give 3, m.p. 111–113°, [α]D3 +52° (c 1, chloroform); Rf 0.55 (1:1
benzene–ethyl acetate). N.m.r. data (CDCl3); δ 1.30, 1.42 (2 s, 6 H, CMe), 3.44
(s, 3 H, OMe), 4.71 (d, 1 H, J1,2 4 Hz, H-1), and ~7.3 (15 H, 3 Ph).

*Anal. Calc.* for C32H35O7: C, 73.09; H, 6.77. Found: C, 73.19; H, 6.79.
The acetate (4) of 3 had m.p. 87–90° (from ethanol), [α]D3 +78° (c 1,
chloroform); Rf 0.29 (4:1 hexane–ethyl acetate); νmax 1750 cm⁻¹ (OAc).

The benzoate (12) of 3 was amorphous and had [α]D3 +73° (c 1, chloroform);
Rf 0.59 (4:1 hexane–ethanol acetate); νmax 1731 cm⁻¹ (ester).

*Anal. Calc.* for C34H35O8: C, 74.46; H, 6.25. Found: C, 74.50; H, 6.16.

*Methyl 3-O-benzyl-4,6-O-benzylidene-α-D-galactopyranoside (10).* — A mix-
ture of 1,2:5,6-di-O-isopropylidene-α-D-galactofuranose8 (6.2 g), benzyl chloride
(7.6 g), and potassium hydroxide powder (4.03 g) was stirred for 3 h at 120°, then
cooled, diluted with water (50 mL), and extracted with chloroform (4 × 20 mL).
The combined extracts were washed successively with aqueous pyridine, 2% hydro-
chloric acid, saturated aqueous sodium hydrogen carbonate, and water, dried
(MgSO4), and concentrated. The residue was eluted from a column (1 × 12 cm) of
silica gel with chloroform. Fractions having Rf 0.1 (t.l.c., chloroform) were com-
bined and concentrated to give the 3-O-benzyl derivative 7 (2.2 g). N.m.r. data
(CDCl3); δ 1.35, 1.40, 1.52 (3 s, 12 H, 2 Me2C), 5.85 (d, 1 H, J1,2 4 Hz, H-1), and
7.35 (5 H, Ph).

A solution of 7 (2.2 g) in aqueous 90% trifluoroacetic acid (20 mL) was stored
for 15 min at room temperature, and then concentrated to give amorphous 3-O-
benzyl-D-galactose (8, 1.5 g), Rf 0.18 (chloroform–methanol, 1:1).

A solution of 8 (1.5 g) in methanolic 4% hydrogen chloride (50 mL) was
boiled under reflux for 2 days, then neutralized with silver carbonate, filtered, and
concentrated. The residue, which contained three components (t.l.c.; chloroform–
methanol, 9:1), including methyl 3-O-benzyl-α-D-galactopyranoside (9), was
stirred with benzaldehyde (5 mL) and zinc chloride (1.4 g) overnight at room tem-
perature. The mixture was poured into ice-water (30 mL) and hexane (20 mL), and
the precipitate was collected, washed with hexane and ice-water, and eluted from a column of silica gel with chloroform–methanol (99.3:0.7). Fractions having \( R_F \) 0.57 (chloroform–methanol, 40:1) were combined and concentrated, and the residue was crystallized from ethanol to give 10 (430 mg, 14% based on 7), m.p. 194°, \([\alpha]_{D}^{23} +186^\circ \) (c 1, chloroform). N.m.r. data (CDCl\(_3\)): \( \delta \) 3.42 (s, 3 H, OMe), 4.91 (d, 1 H, \( J_{\alpha,2} \) 3.5 Hz, H-1), and 7.34 (10 H, 2 Ph).

* Anal. Calc. for C\(_{37}\)H\(_{26}\)O\(_5\): C, 67.73; H, 6.50. Found: C, 67.84; H, 6.46.

The acetate (11) of 10 had m.p. 104°, \([\alpha]_{D}^{23} +154^\circ \) (c 1, chloroform); \( R_F \) 0.73 (40:1 chloroform–methanol); \( \nu_{\text{Br}} \max \) 1750 cm\(^{-1}\) (ester).


**Methyl 2-O-acetyl-\( \alpha \)-D-galactopyranoside (5).** — (a) *From 4.* A mixture of 4 (850 mg) and 80% acetic acid (80 mL) was heated at \(-100^\circ\) for 2 h and concentrated, and the residue was washed with hexane and then dissolved in a small amount of chloroform. Addition of hexane gave a precipitate which was eluted from a column of silica gel with chloroform–methanol (94:6). The fractions containing 5 (\( R_F \) 0.27; chloroform–methanol, 9:1) were combined and concentrated. Crystallization of the residue from ethyl acetate gave 5 (150 mg, 39%), m.p. 82-82.5°; \( \nu_{\text{Br}} \max \) 1730 cm\(^{-1}\) (ester). N.m.r. data (CD\(_3\)OD): \( \delta \) 2.07 (s, 3 H, OAc), 3.37 (s, 3 H, OMe), and 5.07 (d, 1 H, H-2).

* Anal. Calc. for C\(_9\)H\(_{18}\)O\(_7\): C, 45.76; H, 6.83. Found: C, 45.64; H, 6.76.

(b) *From 11.* A solution of 11 in methanol (10 mL) was shaken with hydrogen at atmospheric pressure in the presence of 20% Pd/C for 20 h at room temperature, filtered, and concentrated. Crystallization of the residue from ethyl acetate gave 5 (40 mg, 68%), m.p. 81–82.5°.

**Methyl 3-O-benzoyl-4,6-O-benzylidene-\( \alpha \)-D-galactopyranoside (15).** A solution of benzoyl chloride (7.03 g, 50 mmol) in freshly purified chloroform (25 mL) was added dropwise to a stirred solution of imidazole (6.81 g, 100 mmol) in chloroform (75 mL) during 5 min at 5°. The precipitated imidazole hydrochloride was collected and washed with chloroform (25 mL). The combined filtrate and washings were added to a solution of methyl 4,6-O-benzylidene-\( \alpha \)-D-galactopyranoside\(^23\) (14; 14.11 g, 50 mmol) in chloroform (220 mL) and the mixture was heated under reflux for 18 h. The mixture was washed successively with saturated aqueous sodium hydrogencarbonate and water, dried (Na\(_2\)SO\(_4\)), and concentrated. The residue was eluted from a column of silica gel with chloroform. Fractions containing 15 (\( R_F \) 0.58; benzene–ethyl acetate, 1:1) were combined and concentrated. Recrystallization of the residue (9 g, 47%) from ether–light petroleum gave 15, m.p. 137–139°; lit.\(^12\) m.p. 137–139°.

**Methyl 2-O-benzoyl-4,6-O-benzylidene-\( \alpha \)-D-galactopyranoside (16).** — A solution of 15 (3 g) in acetone (50 mL) was stirred with 0.05M sodium hydroxide (50 mL) for 2 min at room temperature, diluted with ice–water (100 mL), and cooled in an ice-bath for 5 min. The precipitate was collected and washed with water to yield 16 (1.9 g, 63%). Recrystallization from chloroform–ether gave crystals, m.p. 201–203°; lit.\(^12\) m.p. 202–204°.
Methyl 2-O-benzoyl-α-D-galactopyranoside (13). — (a) From 12. A solution of 12 in 80% acetic acid (80 mL) was heated for 2 h at 95° and then concentrated to a syrup which was crystallized from ethanol to give 13 (207 mg, 54%), m.p. 171-173°, [α]D +170° (c 1, methanol); Rf 0.80 (2:1 chloroform–methanol); νmaxKBr 1720 cm⁻¹ (ester). N.m.r. data (CD3OD): δ 3.39 (s, 3 H, OMe) and 8.1 (5 H, Ph).

Anal. Calc. for C14H18O7: C, 56.37; H, 6.08. Found: C, 56.27; H, 6.11.

(b) From 16. A solution of 16 (400 mg) in methanol (30 mL) was stirred with 10% Pd/C (60 mg) in an atmosphere of hydrogen for 3 h at room temperature, filtered, and concentrated. Addition of ethanol to the residue gave 13 (200 mg, 52%), m.p. 171-173°.

Methyl 3,4-O-isopropylidene-2-O-methyl-6-O-trityl-α-D-galactopyranoside (17). — To a stirred solution of 3 (928 mg) in N,N-dimethylformamide (30 mL) was added sodium hydride (360 mg). After stirring for 1 h, methyl iodide (1.7 mL) was gradually added and stirring was continued overnight at room temperature. Methanol (8 mL) was added and, after 10 min, the mixture was filtered through Celite and concentrated. A solution of the residue in chloroform (80 mL) was washed with water (4 × 60 mL), dried (Na2SO₄), and concentrated. The residue was crystallized from ethanol to give 17 (670 mg, 70%), m.p. 125-126°, [α]D +63° (c 1, chloroform); Rf 0.34 (3:1 hexane–ethyl acetate). N.m.r. data (CDCl3): δ 1.33, 1.47 (2 s, 6 H, Me₂), 3.43 (s, 3 H, MeO-1), 3.53 (s, 3 H, MeO-2), 4.82 (d, 1 H, J1,2 3.5 Hz, H-1), and ~7.33 (15 H, 3 Ph).


Methyl 2-O-methyl-α-D-galactopyranoside (18). — A solution of 17 (600 mg) in aqueous 80% acetic acid (40 mL) was heated for 2 h at 95° and then concentrated, and the residue was dissolved in a small amount of chloroform and precipitated with hexane. After two further similar treatments, a solution of the precipitate in chloroform was applied to a column of silica gel (100 mL) and eluted with dichloromethane–methanol (94:6). The fractions containing 18 (Rf 0.63; chloroform–methanol, 2:1) were combined and concentrated. Crystallization of the residue (110 mg, 43%) from ethyl acetate afforded 18, m.p. 81-83°, [α]D +170° (c 0.8, methanol). N.m.r. data (CDCl3): δ 3.42 (s, 3 H, MeO-1), 3.50 (s, 3 H, MeO-2), and 4.95 (d, 1 H, J1,2 3 Hz, H-1).


Methyl 2-O-benzyl-3,4-O-isopropylidene-6-O-trityl-α-D-galactopyranoside (19). — Potassium hydroxide powder (706 mg) was added to a solution of 3 (696 mg) in benzyl chloride (1.33 g), and the mixture was stirred for 2 h at 120°, cooled, diluted with ice–water (50 mL), and extracted with chloroform (4 × 10 mL). The combined extracts were washed with water (4 × 15 mL), dried (Na₂SO₄), and concentrated. Recrystallization of the residue from methanol gave 19 (520 mg, 63%), [α]D +41° (c 1. chloroform); Rf 0.63 (3:1 hexane–ethyl acetate). N.m.r. data (CDCl3): δ 1.33 (6 H, CMe₂), 3.37 (s, 3 H, OMe), and ~7.33 (20 H, 4 Ph).

Methyl 2-O-benzyl-α-D-galactopyranoside (20). — A solution of 19 (440 mg) in aqueous 80% acetic acid (20 mL) was heated for 2 h at 95° and then concentrated. The residue was dissolved in a small amount of chloroform (~0.3 mL), hexane was added, and the precipitate was washed with hexane, dissolved in chloroform, and applied to a column of silica gel (150 mL). The column was eluted with chloroform–methanol (97:3) and the fractions containing 20 ($R_f$ 0.33; 9:1 chloroform–methanol) were combined and concentrated to give 20 (120 mg, 54%). Recrystallization from methanol–ether–hexane gave plates, m.p. 122–123°, $[\alpha]_{D}^{23} +96^\circ$ (c 0.56, methanol). N.m.r. data (CDCl$_3$): δ 3.34 (s, 3 H, OMe) and 7.37 (5 H, Ph).


Methyl 2-deoxy-2-formamido-α-D-galactopyranoside (22). — To a stirred solution of methyl 2-amino-2-deoxy-α-D-galactopyranoside (21) in formic acid (2.5 mL) was slowly added acetic anhydride (0.93 mL) at 0°. After storage for 3 h at room temperature, the mixture was concentrated and a solution of the residue in water (3 mL) was adjusted to pH 11 with 2M sodium hydroxide and maintained there for 40 min. The solution was treated with Amberlite IR-120 (H$^+$) resin and concentrated, and the residue was crystallized from ethanol to give 22 (40 mg, 14%), m.p. 163–165°, $[\alpha]_{D}^{23} +179^\circ$ (c 0.7, methanol); $R_f$ 0.66 (60:35:6 chloroform–methanol–water); $\nu_{\text{max}}^{KBr}$ 1637 and 1549 cm$^{-1}$ (amide).

Anal. Calc. for C$_{16}$H$_{19}$NO$_5$: C, 43.44; H, 6.84; N, 6.33. Found: C, 43.58; H, 6.80; N, 6.24.

Methyl 2-deoxy-2-propionamido-α-D-galactopyranoside (23). — Propionic anhydride (34 mg, 0.26 mmol) was added to a solution of 21 (48 mg, 0.25 mmol) in methanol (2 mL) with cooling and stirring. The mixture was stored overnight at room temperature, and the product was collected and washed with methanol–hexane to give 23 (40 mg, 65%), m.p. 230°, $[\alpha]_{D}^{23} +167^\circ$ (c 0.47, methanol); $R_f$ 0.60 (2:1 chloroform–methanol); $\nu_{\text{max}}^{KBr}$ 1644 and 1557 cm$^{-1}$ (amide).

Anal. Calc. for C$_{17}$H$_{19}$NO$_5$: C, 48.19; H, 7.68; N, 5.62. Found: C, 48.31; H, 7.56; N, 5.53.

Methyl 2-benzamido-2-deoxy-α-D-galactopyranoside (24). — Benzoic anhydride (136 mg, 0.6 mmol) was added to a solution of 21 (96 mg, 0.5 mmol) in methanol (5 mL) with stirring at 0°, stored overnight at room temperature, and then concentrated. Crystallization of the residue from ethanol gave 24 (43 mg, 29%), m.p. 213–216°; lit.$^{15}$ m.p. 213–217°.

Methyl 2-deoxy-2-trifluoroacetamido-α-D-galactopyranoside (25). — S-Ethyl trifluorothioacetate (50 mg) was added to a solution of 21 (50 mg) in methanol (3 mL), the mixture was kept overnight at room temperature and then concentrated, and the residue was crystallized from ethanol–hexane. Elution of the product from a column of silica gel with chloroform–methanol (3:1) gave 25 (12 mg, 16%), m.p. 210–212°; $R_f$ 0.67 (2:1 chloroform–methanol); $\nu_{\text{max}}^{KBr}$ 1700 and 1566 cm$^{-1}$ (amide).

Anal. Calc. for C$_{9}$H$_{14}$F$_3$NO$_6$: C, 37.38; H, 4.88; N, 4.84. Found: C, 37.45; H, 4.95; N, 4.98.
Methyl 2-benzylxycarbonylamino-2-deoxy-α-D-galactopyranoside (26). — To a stirred solution of 21 (386 mg, 2 mM) in saturated aqueous sodium hydrogen-carbonate (4 mL) was added benzylxycarbonyl chloride (578 mg, 3.4 mmol) at 0° and the mixture was stored overnight in a refrigerator. Ethyl acetate (3 mL) was then added, and the product was collected, washed with water and ethyl acetate, and recrystallized from ethanol to give 26 (180 mg, 28%), m.p. 200–201°. [α]D 23 +120° (c 1, methanol); Rf 0.80 (2:1 chloroform–methanol); νmax KBr 1687 and 1548 cm⁻¹ (amide).


Methyl 2-benzylxycarbonyl(methyl)amino-2-deoxy-α-D-galactopyranoside (29). — A solution of 26 (150 mg) in acetic anhydride (2 mL) and pyridine (2 mL) was stored for 24 h at room temperature, poured into ice-water, and extracted with chloroform. The extract was washed successively with 3M hydrochloric acid, saturated aqueous sodium hydrogen carbonate, and water, dried (Na2SO4), and concentrated to give the syrupy triacetate 27.

A solution of 27 (190 mg) in N,N-dimethylformamide (1 mL) was stirred with methyl iodide (5 mL) and silver oxide (0.3 g) overnight, diluted with chloroform, filtered, and concentrated to give the syrupy 2-benzylxycarbonyl(methyl)amino derivative 28. A solution of 28 (195 mg) in methanol was treated with a catalytic amount of sodium. After 100 min at room temperature, the mixture was rapidly deionized with Amberlite IR-120 (II⁺) resin and concentrated. Crystallization of the residue from ethanol gave 29 (80 mg, 51%), m.p. 150–155°, Rf 0.37 (9:1 chloroform–methanol). N.m.r. data (CD3OD): δ 3.00 (s, 3 H, NMe), 3.34 (s, 3 H, OMe), and 7.37 (5 H, Ph).


A solution of 29 (45 mg) in 50% methanol (4 mL) was shaken overnight under hydrogen at atmospheric pressure in the presence of 10% Pd/C (25 mg), filtered, and concentrated to give methyl 2-deoxy-2-methylamino-α-D-galactopyranoside (30: 24 mg, 84%) as an amorphous residue, Rf 0.30 (3:2:1 ethyl acetate–acetic acid–water). N.m.r. data (D2O, external Me4Si): δ 2.86 (s, 3 H, NMe), 3.86 (s, 3 H, OMe), and 5.42 (d, 1 H, J1,2 3.5 Hz, H-1).

ACKNOWLEDGMENT

This research was supported by United States Public Health Service Grant GM 29470 from the National Institutes of Health.

REFERENCES

5 L. A. Murphy and I. J. Goldstein, Biochemistry, 18 (1979) 4999–5005.
2-SUBSTITUTED METHYL \(\alpha\)-D-GALACTOPYRANOSES