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# GLYCOSIDIC DERIVATIVES OF 2-ACETAMIDO-2-DEOXY-D-GALACTOPYRANOSE SUITABLE FOR USE AS LIGANDS IN AFFINITY CHROMATOGRAPHY

MINA A. NASHED
*Department of Chemistry, Faculty of Science, Alexandria University, Alexandria (Egypt)*
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## ABSTRACT

Allyl 2-acetamido-3,4,6-tri-*O*-benzoyl-2-deoxy-α-D-galactopyranoside (**1**) was used to prepare 3-amino-2-hydroxypropyl 2-acetamido-2-deoxy-α-D-galactopyranoside (**5**) and 2-hydroxy-3-mercaptopropyl 2-acetamido-2-deoxy-α-D-galactopyranoside (**9**). Epoxidation of **1** with *m*-chloroperoxybenzoic acid gave an excellent yield (98%) of the epoxide derivative **2**, which was the key intermediate. Azide ion was used to open the epoxide ring. Zemplén *O*-debenzoylation, followed by palladium-assisted reduction of the azide group afforded the amine **5**. Similarly the xanthate ion was used to open the epoxide ring; tetrahydropyranylation, and then ester exchange, followed by mild acid hydrolysis yielded the thiol **9**.

## INTRODUCTION

Affinity-chromatography media for the isolation of soybean lectin and other *N*-acetylgalactosamine-specific proteins have been made by treating epoxy-activated Sepharose with D-galactosamine[1] or *N*-acetylgalactosamine[2] under alkaline conditions. The resulting supports are undoubtedly heterogeneous and have a low degree of derivatization.

An improved support could be made by functionalizing the allyl group of allyl 2-acetamido-3,4,6-tri-*O*-benzoyl-2-deoxy-α-D-galactopyranoside[3] (**1**) and then attaching it to the appropriately activated Sepharose. Various ways of modifying allyl groups are known to afford useful ligands[4–6].

The present work reports the preparation of two affinity ligands from allyl 2-acetamido-2-deoxy-α-D-glucopyranoside[4] by isomerization to the corresponding *N*-acetylgalactopyranoside, and then the introduction of a thiol or an amino functional group into the allyl group *via* ring opening of the derived epoxide.

## RESULTS AND DISCUSSION

Allyl 2-acetamido-3,4,6-tri-*O*-benzoyl-α-D-galactopyranoside[3] (**1**) was prepared by epimerization[3] of the glucose analogue at C-4. Treatment of **1** with *m*-

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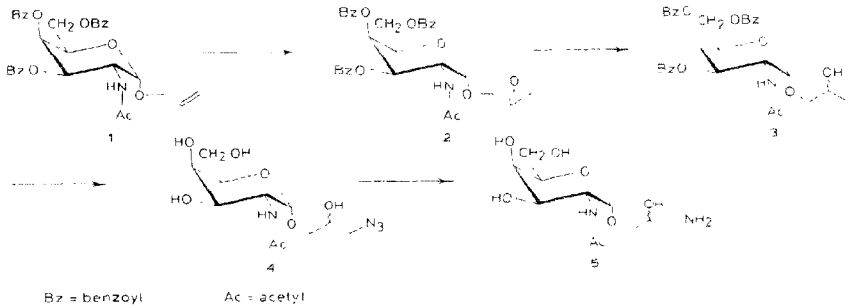
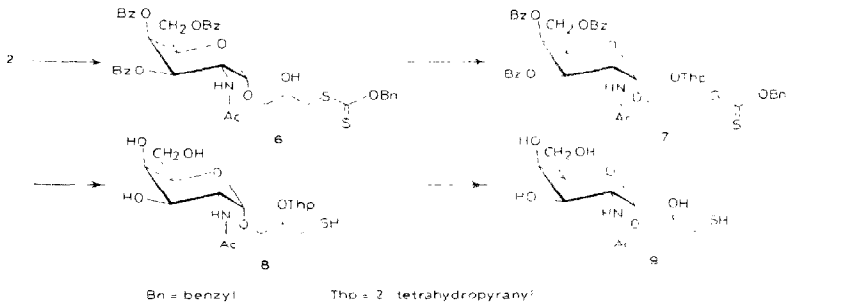
chloroperoxybenzoic acid[6,7] in benzene gave the epoxide derivative **2** in quantitative yield; $^1$H-n.m.r. showed for H-1 two doublets, at $\delta$ 5.14 and 5.12, having small spacings, indicating a diastereomeric mixture.




The epoxide ring in compound **2** was first opened by azide ion[8]. Treatment of compound **2** with 2 molar proportions of sodium azide in 2-methoxyethanol in the presence of ~2 molar proportions of ammonium chloride gave compound **3** in excellent yield. $^1$H-N.m.r. spectroscopy showed a new, $D_2O$-exchangeable, signal at $\delta$ 2.13, which was assigned to the *OH* group at C-2. Zemplen *O*-debenzoylation of compound **3** followed by reduction with palladium–charcoal under hydrogen at atmospheric pressure and at room temperature afforded compound **5** in 94% yield. Its structure was confirmed by $^1$H-n.m.r. spectroscopy, which showed a broad signal at $\delta$ 4.06 ($D_2O$-exchangeable, $NH_2$) and an upfield shift of aglycon H-3 resonance to $\delta$ 0.96, which appeared as a doublet (spacing 6.6 Hz).

Benzylxanthate ion was used for introduction of the thiol group. Treatment of compound **2** with potassium benzylxanthate[9] in the presence of ammonium



chloride in ethanol–water (10:0.3, v/v) for 30 min at room temperature gave compound **6**. Its $^1$H-n.m.r. spectrum showed additional signals in the aromatic region and a broad, $D_2O$-exchangeable signal at $\delta$ 1.41 (O*H* at C-2). Efforts to deprotect compound **6** with sodium methoxide in methanol failed to give the thiol, presumably because of interaction of the neighboring hydroxyl group with the xanthate function. Accordingly, the hydroxyl group was protected by the tetrahydropyran-2-yl group. Treatment of compound **6** with 2,3-dihydro-4-*H*-pyran and a catalytic amount of *p*-toluenesulfonic acid in chloroform for ~15 min at room temperature yielded compound **7**. The structure of **7** was established by $^1$H-n.m.r. spectroscopy, which showed a multiplet at $\delta$ 1.91–1.39 for the tetrahydropyranyl group with disappearance of the O*H* signal. Saponification of compound **7** with 0.5M sodium methoxide at room temperature under nitrogen proceeded readily, and then hydrolysis with acetic acid furnished compound **9** in 85% yield. The structure of **9** was confirmed by $^1$H-n.m.r. spectroscopy, which showed two triplets, at $\delta$ 1.33 and 1.19, with spacing 7.5 Hz, characteristic[10] of an S*H* signal.

Preliminary experiments showed that thiol derivative **9** may be readily coupled with epoxy-activated Sepharose 6B to give an affinity matrix having high capacity for specific adsorption of soybean lectin.

## EXPERIMENTAL

*Instrumental and chromatographic procedures.* — Optical rotations were measured with a Perkin–Elmer Model 141 polarimeter. Proton magnetic resonance spectra were recorded with a Bruker WH-270 spectrometer. Chemical shifts were referenced to tetramethylsilane as the internal standard. Elemental analyses were performed by Galbraith Laboratories, Inc., Knoxville, TN, U.S.A.

Thin-layer chromatography (t.l.c.) was performed on glass plates coated with Silica Gel G (Merck). Spots were detected by charring with sulfuric acid. For column chromatography, Silica Gel 60, particle size 63–200 $\mu$m (Merck) was used. In both procedures, the solvents employed were mixtures of acetone and chloroform for compounds of low polarity, or methanol and chloroform for more-polar compounds.

*2,3-Epoxypropyl 2-acetamido-3,4,6-tri-*O*-benzoyl-2-deoxy-α-*D*-galactopyranoside* (**2**). — Allyl 2-acetamido-3,4,6-tri-*O*-benzoyl-2-deoxy-α-D-galactopyranoside[3] (**1**) showed $^1$H-n.m.r. ($CDCl_3$): $\delta$ 8.10–7.32 (m, 15 H, Ph-H), 5.90 (d, 1 H, $J_{NH,2}$ 9.0 Hz, $D_2O$-exchangeable, N*H*), 5.88 (doublet of broad lines, 1 H, $J_{3,4}$ 3.3 Hz, $J_{4,5}$ <1 Hz, H-4), 5.85–5.70 (m, 1 H, -C*H*=), 5.40 (dd, 1 H, $J_{2,3}$ 11.6 Hz, $J_{3,4}$ 3.3 Hz, H-3), 5.25–5.15 (m, 2 H, -CH=C$H_2$), 4.70 (d, 1 H, $J_{1,2}$ 3.0 Hz, H-1), 4.60–3.50 (m, 6 H, OC$H_2$CH=, and sugar C*H*), and 1.80 (s, 3 H, C$H_3$CO). Compound **1** (ref. 3) (4 g, 7 mmol) was dissolved in benzene (50 mL) containing 3-chloroperoxybenzoic acid (4 g, 23 mmol) and the solution was boiled under reflux for 1.5 h, at which time t.l.c. of the mixture showed complete conversion of the starting material. The mixture was cooled and successively washed with saturated

sodium sulfite, 5% sodium hydrogencarbonate, and water. Evaporation of the solvent gave **2** as an amorphous solid; 5.0 g (98%). For identification, a sample was purified on a column of silica gel to yield the title compound as a glassy foam, $[\alpha]_D^{25}$ +104°, $[\alpha]_{436}^{25}$ +211° (*c* 0.97, chloroform); $^1$H-n.m.r. ($CDCl_3$) similar to that of **1**, except for loss of the vinyl proton (m, 1 H, -C*H*=), and changes in the signals for H-1 because of formation of diastereoisomers: δ 5.14 and 5.42 (total 1 H, $J_{1,2}$ 3.7 Hz, H-1), and upfield shift of -CH=C*H*$_2$ to δ 2.85 and 2.57 (2 m, -CH-C*H*$_2$).

*Anal.* Calc. for $C_{35}H_{31}NO_{10}$ (589.60): C, 65.19; H, 5.30; N, 2.38. Found: C, 64.70; H, 5.50; N, 2.25.

*3-Azido-2-hydroxypropyl 2-acetamido-3,4,6-tri-O-benzoyl-2-deoxy-α-D-galactopyranoside* (**3**). — To a solution of compound **2** (2.3 g, 3.9 mmol) in 2-methoxyethanol (35 mL) and water (10 mL), ammonium chloride (0.46 g, 8.6 mmol) and sodium azide (0.51 g, 7.9 mmol) were added. The mixture was boiled under reflux for 0.5 h, whereupon t.l.c. showed the reaction to be complete. Water was added to the cooled solution and the product was obtained by conventional chloroform extraction to give a quantitative yield of the title compound. Analytically pure **3** was obtained by chromatography on a column of silica gel; $[\alpha]_D^{25}$ +94.6°, $[\alpha]_{436}^{25}$ +193° (*c* 1.78, chloroform), $^1$H-n.m.r. ($CDCl_3$) similar to that of **2**, except for new signals at δ 2.13 (t, two unresolved doublets, total 1 H, $D_2O$-exchangeable, OH), 1.94 and 1.83 (2 singlets, total 3 H, C*H*$_3$CO) (epimeric mixture at C-2 of the propyl group).

*Anal.* Calc. for $C_{32}H_{32}N_4O_{10}$ (632.63): C, 60.75; H, 5.10. Found: C, 60.47; H, 5.25.

*3-Azido-2-hydroxypropyl 2-acetamido-2-deoxy-α-D-galactopyranoside* (**4**). — Compound **3** (2.0 g) in anhydrous methanol (30 mL) was treated with 0.5M sodium methoxide (1 mL), and the mixture was boiled for 30 min under reflux. The cooled solution was deionized with Rexyn 101 ($H^+$) resin, the suspension was filtered, and the filtrate and washings were evaporated to dryness to furnish 0.90 g (91%) of **4**. Analytically pure sample was obtained by chromatography on a column of silica gel; $[\alpha]_D^{25}$ +166°, $[\alpha]_{436}^{25}$ +332° (*c* 0.82, methanol); $^1$H-n.m.r. ($Me_2SO$-$d_6$) similar to that of **3**, except for loss of aromatic protons, and the upfield shift of the sugar-ring protons.

*Anal.* Calc. for $C_{11}H_{20}N_4O_7$ (320.30): C, 41.25; H, 6.29. Found: C, 41.66; H, 6.57.

*3-Amino-2-hydroxypropyl 2-acetamido-2-deoxy-α-D-galactopyranoside* (**5**). — To a solution of compound **4** (0.5 g) in methanol (40 mL), palladium-on-charcoal catalyst (10% Pd, 30 mg) was added. The solution was stirred overnight under hydrogen at room temperature and atmospheric pressure. The catalyst was removed by filtration through Celite, and the filtrate and washings were evaporated to dryness to yield 0.43 g (94%) of **5**; $^1$H-n.m.r. ($Me_2SO$-$d_6$) similar to that of **4**, except for a new signal at δ 4.06 (bs, 2 H, $D_2O$-exchangeable, N*H*$_2$) and an upfield shift of the methylene group to δ 0.96 (d, 2 H, *J* 6.6 Hz, -C*H*$_2$NH$_2$).

*Anal.* Calc. for $C_{11}H_{22}N_2O_7$ (294.30): N, 9.52. Found: N, 9.28.

*3-(Benzyloxythiocarbonyl)thio-2-hydroxypropyl 2-acetamido-3,4,6-tri-O-benzoyl-2-deoxy-α-D-galactopyranoside* (**6**). — To compound **2** (2.8 g, 4.8 mmol) in ethanol–water (10:0.3, v/v, 100 mL), ammonium chloride (0.7 g, 13 mmol) and potassium benzylxanthate[9] (1.6 g, 7.2 mmol) were added. After being stirred for 30 min at room temperature, the mixture was poured into water, and the product was recovered by conventional extraction with chloroform. The syrup obtained was purified by chromatography on a column of silica gel to give 3.2 g (86%) of pure **6** as a glassy foam, $[\alpha]_D^{25}$ +81.8°, $[\alpha]_{436}^{25}$ +171° (*c* 0.4, chloroform); $^1$H-n.m.r. ($CDCl_3$) similar to that of **2**, except for additional signals at δ 8.17–7.21 (now 20 H, $C_6H_5$), 4.56 [m, 2 H, $PhCH_2OC(S)$], and appearance of a $D_2O$-exchangeable signal at 1.41 (bs, 1 H, OH).

*Anal.* Calc. for $C_{40}H_{39}NO_{11}S_2 \cdot 0.5\ H_2O$ (782.88): C, 61.37; H, 5.15; N, 1.79; S, 8.19. Found: C, 61.16; H, 5.27; N, 2.00; S, 8.32.

*3-(Benzyloxythiocarbonyl)thio-2-O-(tetrahydropyran-2-yl)propyl 2-acetamido-3,4,6-tri-O-benzoyl-2-deoxy-α-D-galactopyranoside* (**7**). — Compound **6** (0.5 g, 0.65 mmol) in chloroform (7 mL) was treated with 2,3-dihydro-4*H*-pyran (0.3 mL) and *p*-toluenesulfonic acid (6 mg), and kept at room temperature. When the reaction was complete (15 min), the solution was washed with aqueous sodium hydrogencarbonate and water. Chromatography of the syrup on a column of silica gel furnished 0.45 g (81%) of the pure title compound as a glass, $[\alpha]_D^{25}$ +91.0°, $[\alpha]_{436}^{25}$ +188° (*c* 0.88, chloroform); $^1$H-n.m.r. ($CDCl_3$) similar to that of **6**, except for loss of the O*H* signal and the appearance of signals at δ 1.91–1.39 (m, tetrahydropyranyl-*H*).

*Anal.* Calc. for $C_{45}H_{47}NO_{12}S_2 \cdot 0.5\ H_2O$ (867.01): C, 62.34; H, 5.58; N, 1.62; S, 7.40. Found: C, 62.11; H, 5.70; N, 1.57; S, 7.19.

*2-Hydroxy-3-mercaptopropyl 2-acetamido-2-deoxy-α-D-galactopyranoside* (**9**). — To a solution of compound **7** (0.5 g, 0.58 mmol) in methanol (5 mL), 0.05M sodium methoxide (3 mL) was added and the mixture was stirred at room temperature under nitrogen for 1 h. The solution was deionized with Rexyn 101 ($H^+$) ion-exchange resin, the suspension was filtered, and the filtrate was treated with acetic acid–water (3 mL; 3:2, v/v). The solution was boiled under reflux for 1 h, and then the solvents were evaporated off and the crude syrup was chromatographed on a column of silica gel to yield 170 mg (85%) of the title compound, $[\alpha]_D^{25}$ +141° (*c* 2.22, methanol); $^1$H-n.m.r. ($D_2O$, TSP as internal reference) similar to that of **7**, except for loss of the aromatic protons, Ph-C$H_2$, tetrahydropyranyl-*H*, and upfield shift of sugar-C*H*, and the appearance of signals at δ 2.83–2.65 (m, 2 H, $CH_2SH$), and δ 1.33 and 1.19 (2t, total 1 H, *J* 7.5 Hz, $D_2O$-exchangeable, S*H*).

*Anal.* Calc. for $C_{11}H_{21}NO_7S \cdot 2\ H_2O$ (347.38): C, 38.03; H, 7.25; S, 9.23. Found: C, 38.33; H, 7.48; S, 9.31.

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