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Note

Synthesis of the D-glucuronic acid conjugates of *N*-(4-hydroxyphenyl)- and *N*-(2-hydroxyethyl)-retinamides

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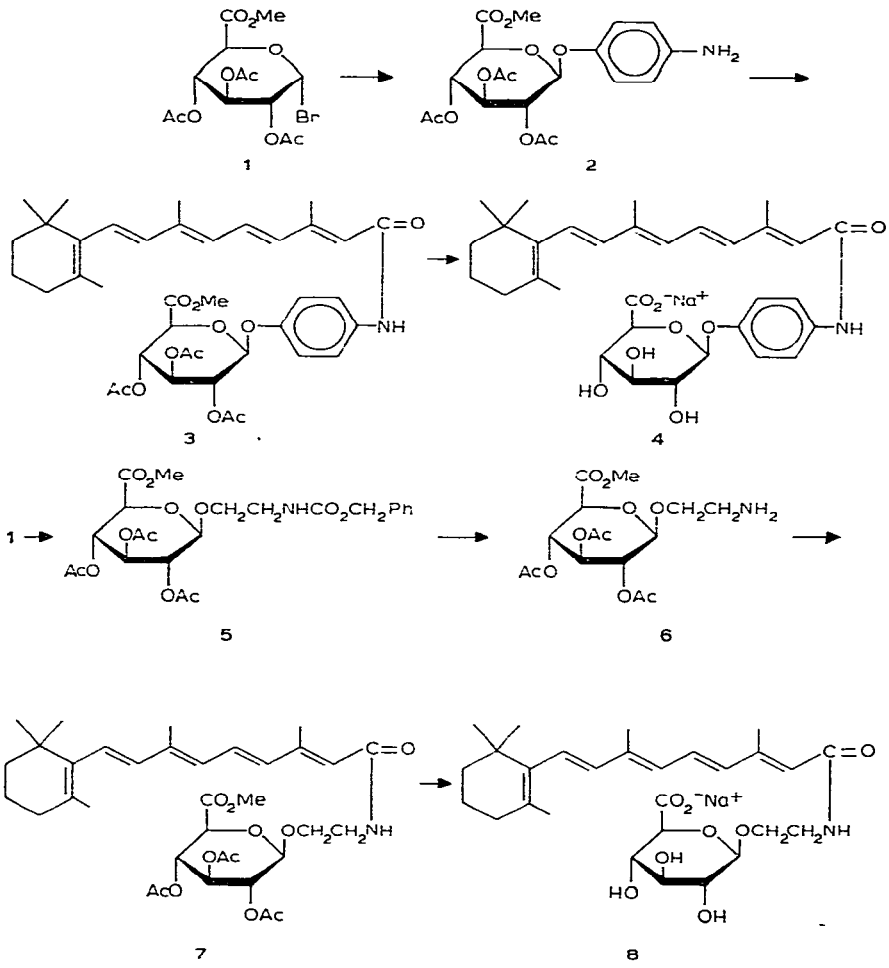
N-(4-Hydroxyphenyl)[1]- and *N*-(2-hydroxyethyl)[2]-retinamides are excellent cancer-chemopreventive agents and are less toxic than the parent acid[3,4]. Therefore, the tissue-distribution pattern and metabolism of these two potentially useful drugs is of pharmacological interest. As one of the primary metabolic conversions of retinoids, phenols, and alcohols is *O*-conjugation to D-glucuronic acid to form β-D-glucosiduronates in the microsomal fractions of liver, kidney, and intestinal mucosa[5–7], we undertook the synthesis of the β-D-glucosiduronates **4** and **8** of these retinamides. In addition to being useful as comparison compounds in metabolism studies, these compounds might be effective in the prevention of bladder cancer. The major mode of excretion of such lipophilic retinoids as these amides is in the bile[5]. However, upon conjugation to a water-solubilizing group such as a glucuronate salt, renal clearance is also possible[6,7]. Administration of the glucosiduronic conjugates may permit these retinoids to reach the bladder in sufficient concentration to prevent effectively the development of epithethial cancer there. The synthetic approach and purification methods employed in preparing these compounds are described herein.

Because of the lability of the pentaene system of the retinoids, and the potential interaction of these double bonds with the mercury and silver salts typically used to form glucosiduronic linkages, we decided to employ a stepwise synthetic approach, first introducing the 2-hydroxyethylamino and 4-hydroxyanilino groups onto the sugar, followed by amide-bond formation with retinoic acid. The readily accessible methyl (*p*-aminophenyl 2,3,4-tri-*O*-acetyl-β-D-glucopyranosid)uronate[8] (**2**) was chosen as the starting material for the preparation of glucosiduronate **4**. Amide formation with retinoyl chloride, which had been generated *in situ* with thionyl chloride–pyridine[9], afforded the protected 4-retinamidophenyl D-glucosiduronate **3**. ^{1}H-N.m.r. spectroscopy established that the anomeric configuration[10,11] was β. Because of the decreased nucleophilicity of the aromatic amine, amide formation with retinoylimidazole[12] was not effective. Base-catalyzed removal of the methyl



ester and acetyl protecting-groups, acidification, and ion exchange afforded sodium (4-retinamidophenyl β-D-glucosid)uronate (**4**).

The synthesis of the sodium (2-retinamidoethyl β-D-glucosid)uronate (**8**) was accomplished similarly, starting with methyl (tri-*O*-acetyl-α-D-glucopyranosyl bromide)uronate[13] (**1**) and *N*-benzyloxycarbonylethanolamine[14]. While the condensation occurred in good yield (60–70%), the β: α anomer ratio did not exceed 3:2, even though a variety of solvents (benzene, acetonitrile, and nitromethane) and metal salts (freshly prepared silver carbonate, silver oxide, and mercuric cyanide)[15] were investigated[10,11] in the attempt to increase the amount of β anomer **5**. In contrast to the facile separation of the (more rigid) aryl glucosiduronic anomers in the preceding sequence, purification of the (more flexible) ethano anomers was very difficult. A combination of column chromatography, crystallization, and preparative, high-performance liquid chromatography (l.c.) finally afforded the more-polar β anomer in low yield.

Removal of the benzyloxycarbonyl group from the β anomer **5** by hydrogenolysis with palladium-on-carbon in anhydrous methanol[16] gave only low yields of the ethylamino D-glucosiduronate **6**, together with many side-products. Decreasing the basicity of the primary amino group of the product by conducting the hydrogenation in the presence of one equivalent of acetic acid removed this problem. The free amine, generated from the acetate salt by addition of one equivalent of triethylamine, was allowed to react with retinoylimidazole to form* the protected D-glucosiduronate **7**. ^{1}H-N.m.r. spectroscopy again established[10,11] that the anomeric configuration was β. Alkaline hydrolysis removed the protecting groups, to give sodium (2-retinamidoethyl β-D-glucosid)uronate.

Both water-soluble glucosiduronates were purified by preparative, reverse-phase l.c. followed by lyophilization. Analytical reverse-phase l.c. (260 nm) indicated a purity of 97% for both hygroscopic, readily oxidizable, yellow powders. ^{13}C-N.m.r. spectroscopy confirmed that only one anomer was present. The negative optical rotation values[13] support the β-D-anomeric configuration.

EXPERIMENTAL

General methods. — Melting points are uncorrected. I.r. spectra were recorded with a Perkin–Elmer 710B infrared spectrophotometer. N.m.r. spectra were obtained with a Varian A-60A or XL-100-FT spectrometer, with tetramethylsilane as the internal standard (δ 0) and solvent as specified. Signals are designated by the standard retinoid (C-1 to C-20) and sugar (C-1′ to C-5′) numbering systems, and were assigned by comparison with reported spectra for retinoids[17] and α- and β-glucosiduronates[10,11,18]. High-resolution, mass-spectral analyses were conducted with a CEC-21-110B high-resolution mass spectrometer equipped with facilities for combination g.l.c.–m.s. High-performance liquid chromatographic analyses were performed with a Waters Associates ALC 210 instrument equipped with either a 30 cm × 3.9 mm μPorasil or μBondapak/C18 column. Detection was by a Schoeffel Instrument Model 770 variable-wavelength u.v. monitor. Analyses were performed at room temperature and a flow rate of 2 mL/min. Preparative work was performed on a Waters Associates Prep LC/System 500 instrument with Prep Pak-500/silica or Bondapak/C_{18} cartridges at a flow rate of 0.2 L/min. Detection was by u.v. absorption or refractive index. U.v. spectra were obtained with a Perkin–Elmer model 575 spectrometer.

Reactions and purifications of the retinoids were conducted with deoxygenated solvents and under inert gas (argon) and either subdued light or photographic red light. Retinoid intermediates were stored at −40°. Solvents were dried or distilled

*Alternative syntheses were unsuccessful. When *N*-(2-hydroxyethyl)retinamide was allowed to react with the bromo sugar **1** in the presence of silver carbonate, no glycoside was detected in the mixture. Attempted *O*-glycosidation of *N*-benzyloxycarbonylethanolamine with methyl 1,2,3,4-tetra-*O*-acetyl-β-D-glucopyranuronate in the presence of stannic chloride[10] resulted in attack on the carbamate group, and other side-reactions.

before use. T.l.c. analyses were made with analytical silica-gel plates (Analtech). Solutions were dried with sodium sulfate.

Methyl (tri-*O*-acetyl-α-D-glucopyranosyl bromide)uronate (**1**) was prepared in two steps from D-glucuronolactone by the procedure of Bollenback and co-workers[13]. Methyl (4-aminophenyl 2,3,4-tri-*O*-acetyl-α-D-glucopyranosid)uronate (**2**) was prepared by *O*-glucosiduronation of methyl (tri-*O*-acetyl-α-D-glucopyranosyl bromide)uronate with *p*-nitrophenol in the presence of silver oxide followed by reduction of the nitro group, using procedures reported by Kato and co-workers[19] and Furukawa and co-workers[8]. Recrystallized (ethanol) material, m.p. 153–154° (lit.[8] 154–156°), contained about 10% of the α anomer by l.c. *N*-Benzyloxycarbonylethanolamine was prepared from ethanolamine and benzylchloroformate by the method reported by Rose[14].

Methyl (4-retinamidophenyl tri-O-acetyl-β-D-glucopyranosid)uronate (**3**). — A solution of all-*trans*-retinoic acid (0.61 g, 2.0 mmol) in ether (10 mL) containing pyridine (0.2 g, 2.5 mmol) was cooled to −10° and treated with thionyl chloride (0.25 g, 2.0 mmol) in ether (1 mL)[9]. The mixture was allowed to warm to room temperature during 1.5 h with stirring. An orange-red solution of the (unstable) acid chloride was obtained together with a precipitate of pyridine hydrochloride. The solution was transferred all at once by syringe into a stirred suspension of **2** (0.868 g, 2.0 mmol) in benzene (7 mL) containing 0.3 g (3.8 mmol) of pyridine at room temperature, and the reaction vessel was rinsed with benzene (5 mL). The red faded to yellow within 2–3 min. The mixture was kept for 65 h, diluted with ethyl acetate (30 mL), and washed with water (3 × 30 mL), followed by 30-mL aliquots of 1% aqueous sulfuric acid, saturated sodium hydrogencarbonate, and brine (twice). The solution was dried and evaporated to a yellow gum (1.38 g). The crude product was combined with 0.78 g of material from a similar preparation and applied in benzene to a column (2.5 × 35 cm) of Merck Silica Gel 60. The column was gradient-eluted with 150-mL volumes of 20, 25–50, 60, and 75% ethyl acetate–hexane. Unreacted retinoic acid eluted first, followed by 1.08 g of the product ester as a solid yellow foam, and then 0.55 g of a mixture of the α and β anomers. The anomeric mixture (0.9 g) from two combined preparations was subjected to l.c. (2:1 hexane–EtOAc, 0.2 L/min) to yield 0.40 g more of the β anomer (total yield, 1.48 g, 59%), followed by 235 mg of the α anomer containing a trace of the β anomer. The major product was crystallized from 5 mL of 2:1 hexane–ethyl acetate to afford 1.183 g (47%) of the amide, m.p. 98–101° (dec.): ν_{max}^{mull} 3260 (NH), 1750 (OAc, CO_2Me), 1645 (C=C, CONH), 1610 (Ar), 1580, 1525 [sh (Ar)], 1415, 1220, 1170, 1045, 970, 910, 895, and 740 cm^{-1}; ^{1}H-n.m.r. ($CDCl_3$): δ 1.07 [s, 6 H, 16,17-CMe_2], 1.74 (s, 3 H, 18-CH_3), 1.4–1.7 [m, 4 H, 2,3-$(CH_2)_2$], 1.9–2.15 (4 s and m, 14 H, 3 OAc, 19-CH_3, 4-CH_2), 2.24 (s, 3 H, 20-CH_3), 3.77 (s, 3 H, CO_2Me), 4.20 (dd, 1 H, *J* 3, 1.5 Hz, H-5′), 5.05–5.40 (m, 4 H, H-1′,2′,3′,4′), 5.82 (s, 1 H, 14-HC=C), 6.05–6.4 (m, 4 H, 7,8-HC=CH, 10,12-C=CH), and 6.85–7.55 (m, 2 H, 11-C=CH, NHCO, and dd, 4 H, ArH); ^{13}C-n.m.r. ($CDCl_3$): δ 170.023, 169.381, 169.289, and 167.066 (C-6′ and 3 OCOR), 165.355 (C-15), 153.175 (Ar–O), 150.382 (C-13), 139.197 (C-9), 137.802

and 137.328 (C-6,8), 135.491 and 134.294 (C-12, *p*-Ar), 130.354 (C-11), 129.911 and 129.624 (C-5,10), 128.539 (C-7), 121.858 and 121.452 (*m*-Ar and C-14), 117.801 (*o*-Ar), 99.733 (C-1'), 72.533, 72.036 and 71.191 (C-2',3',4'), 69.261 (C-5'), 52.915 (MeO), 39.696 (C-2), 34.315 (C-1), 33.152 (C-4), 28.985 (C-16,17), 21.742 (C-18), 20.576 and 20.489 (3 OAc), 19.289 (C-3), 13.716 (C-20), and 12.898 (C-19); λ_{max}^{EtOH} 362 nm (ε 5.24 × 10^4): m.s. calc. for $C_{39}H_{49}NO_{11}$ 707.3305, found 707.3312. The mass spectrum indicated complete absence of retinoic acid.

*Sodium (4-retinamidophenyl β-*D*-glucopyranosid)uronate* (**4**). — A solution of recrystallized **3** (1.15 g, 1.6 mmol) in 10 mL of 0.1M sodium methoxide in methanol was stirred for 18 h at room temperature. Solvent was evaporated in a stream of argon. The yellow solid was treated with 15 mL of 0.2M sodium hydroxide (3 mmol) in 1:1 methanol–water and stirred for 4.5 h at room temperature. A yellow gum separated. Addition of 0.05M sulfuric acid (20 mL, 1 mmol) converted the gum into a solid, which was dissolved in methanol (40 mL). The solution was applied to a column (4 × 40 cm) of sodium sulfonate ion-exchange resin (Amberlite IR-120) that had been washed with 1 L of 0.3M aqueous sodium hydroxide and then with 2 L of water. The salt was eluted under nitrogen during a 2-h period with 800 mL of 2:1 methanol–water. The eluate was concentrated to 300 mL by careful evaporation at 0.2 mmHg and below room temperature to minimize foaming. The residual aqueous solution was shell-frozen at −78° and lyophilized under argon at 30–60 μmHg over a 3-day period. The yellow residue was extracted with methanol (100 mL), the mixture filtered to remove sodium sulfate, and the filtrate evaporated; the volume was brought to 40 mL with methanol. The product had begun to separate at a volume of about 30 mL. The solution was applied to a reverse-phase l.c. column in three aliquots: (*1*) 8 mL of solution, which was eluted with 80% methanol–water; (*2*) 20 mL of solution, eluted with 70% methanol–water; and (*3*) 12 mL of solution, eluted with 70% methanol–water. A single, major peak was observed by refractive-index detection. The combined product-fractions (2.5 L) were concentrated to 550 mL. Much less foaming was encountered than with the crude material. Any silanol present, which could have resulted from hydrolytic cleavage of the C_{18} adsorbent during chromatography, could be removed readily by extraction with hexane. Lyophilization of the frozen residue for 4 days yielded a yellow powder (550 mg, 55% yield assuming hydration by 2 H_2O). The product was transferred in an argon-filled glove bag into vials. The product had m.p. 255° (dec., darkened >150°), $[\alpha]_D^{20}$ −56.7° (*c* 0.9, methanol); l.c. (reverse-phase, 70% methanol–water, 280 nm) t_R 1.8 (97%) and 0.8 min (3%); ν_{max}^{mull} 3350–2500 (OH), 1620 (NH, C=C), 1515 (NH, C=O), 1420, 1310, 1240, 1230, 1170, 1065, 1030, 975, 840, and 730 cm^{-1}; ^{1}H-n.m.r. (Me_2SO-d_6)*: δ 1.06 [s, 6 H, 16,17-CMe_2], 1.3–1.7 [m, 4 H, 2,3-$(CH_2)_2$], 1.73 (s, 3 H, 18-CH_3),

*^{1}H-N.m.r. spectra for compounds **4**, **6**, and **8** were taken in Me_2SO-d_6 to enhance solubility and sharpen signals. Perhaps because of differences in solvation, the positions of signals for H-1' and H-5' did not agree with those reported for β-glucosiduronates in $CDCl_3$ (refs. 10 and 11). Similar shift-differences were found in the spectra of compound **5** in these solvents.

2.01 (s, 3 H, 19-CH_3), 2.37 (s, 3 H, 20-CH_3), 2.53 (m, 2 H, 4-CH_2), 3.2–3.6 (broad s and m, 4 H), 3.0–4.0 and 5.0–5.7 (broad ms, 2 H and 4 H, OH and H_2O), 4.8 (broad d, 1 H, *J* 4 Hz), 6.07 (broad s, 1 H, 14-HC=C), 6.2–6.5 (m, 5 H, 7,8-HC=CH, 10,12-C=CH, OH-exchanged D_2O), 6.85–7.15 (m, 3 H, 11-C=CH, and 2 H, ArH), 7.57 (d, 2 H, *J* 9 Hz, ArH), and 10.02 (broad s, 1 H, NHCO, exchanged D_2O on heating); ^{13}C-n.m.r. (Me_2SO-d_6): δ 173.316 (C-6′), 164.534 (C-15), 153.379 (Ar–O), 147.630 (C-13), 137.792, 137.274, 137.123 and 136.247 (C-9,6,8,12), 136.109 (*p*-Ar), 134.032 (C-11), 130.117 and 129.213 (C-5,10), 127.345 (C-7), 123.228 (C-14), 120.369 (*m*-Ar), 116.778 (*o*-Ar), 101.104 (C-1′), 76.644 (C-3′), 74.369 and 73.142 (C-2′,4′), 71.975 (C-5′), 39.285 (C-2), 33.832 (C-1), 32.657 (C-4), 28.775 (C-16,17), 21.473 (C-18), 18.823 (C-3), 13.249 (C-20), and 12.522 (C-19); λ_{max}^{MeOH} 360 (ε 4.76 × 10^4 if 2 H_2O), 233 nm (1.46 × 10^4 if 2 H_2O); m.s. [tetrakis(trimethylsilyl) derivative], calc. for $C_{44}H_{73}NO_8Si_4$ 855.4413, found 855.4362.

*Methyl (2-aminoethyl tri-*O*-acetyl-β-*D*-glucopyranosid)uronate* (**6**). — A representative experiment is reported. To a mechanically stirred solution of 4.0 g (10.1 mmol) of methyl (tri-*O*-acetyl-α-D-glucopyranosyl bromide)uronate (**1**) and 1.99 g (10.2 mmol) of *N*-benzyloxycarbonylethanolamine in dry benzene (40 mL) were added 1.93 g (7.0 mmol) of silver carbonate and 2.0-g portions of molecular sieves 4-A and Drierite. This mixture was stirred for 20 h, filtered (filter-rinsed with benzene), and the filtrate diluted with benzene to 250 mL, washed with M sodium hydroxide (2 × 40 mL) and brine (40 mL), and dried. Evaporation afforded an almost colorless oil, the n.m.r. spectrum of which indicated a 1 : 1 mixture of α and β anomers. The oil was chromatographed on silica gel (300 g) with 70% ethyl acetate–pentane (40-mL fractions) to afford 3.2 g of product in fractions 24–27. Fractions 24 and 25 contained 2.04 g of an oil that, after l.c. with 50% ethyl acetate–hexane and crystallization from 40% ethyl acetate–hexane, gave 0.96 g (19% yield) of methyl (*N*-benzyloxycarbonylaminoethyl tri-*O*-acetyl-β-D-glucopyranosid)uronate (**5**), m.p. 84–86.5°, $[\alpha]_D^{21}$ −18.9° (*c* 1, methanol); $\nu_{max}^{CHCl_3}$ 1760 (CO_2R), 1720, 1370, and 1030 cm^{-1}; ^{1}H-n.m.r. ($CDCl_3$): δ 2.02 (s, 9 H, OAc), 3.42 (t, 2 H, *J* 5 Hz, OCH_2), 3.55–4.15 (ms, 3 H, H-5′, CH_2N), 3.80 (s, 3 H, CO_2Me), 4.55 (d, 1 H, *J* 6.5 Hz, H-1′), 4.85–5.3 (m, 4 H, 3 HCO, NH), 5.92 (s, 2 H, CH_2Ar), and 7.33 (s, 5 H, ArH).

Anal. Calc. for $C_{23}H_{29}NO_{12}$: C, 54.01; H, 5.72; N, 2.74. Found: C, 53.95; H, 5.71; N, 2.72.

Fractions 26 and 27 weighed 1.2 g and on crystallization afforded 0.98 g of β anomer 90% pure by analytical l.c. and ^{1}H-n.m.r. The anomeric purity did not increase on seeding and recrystallization.

To a solution of pure **5** (0.511 g, 1 mmol) and acetic acid (57 μL 1 mmol) in 100 mL of anhydrous methanol was added 125 mg of 5% palladium-on-carbon (M.C.B.). This mixture was stirred under hydrogen for 2 h, filtered through Celite, and evaporated under diminished pressure overnight to afford 378 mg (98% crude yield) of a white solid; $[\alpha]_D^{21}$ −9.7° (*c* 10, methanol); ^{1}H-n.m.r. (Me_2SO-d_6): δ 1.92 (s, 3 H, OAc), 2.07, 2.08, and 2.10 (3 s, 9 H, OAc), 2.8–3.0 (m, 2 H, CH_2N), 3.6–3.7 (m, 2 H,

CH_2O), 3.75 (s, 3 H, CO_2Me), 4.53 (d, 1 H, *J* 10 Hz, H-1'), 4.1–5.5 (ms, 4 H, HCO), and 5.6 (broad m, 3 H, NH_3^+). The acetate salt of methyl (2-aminoethyl tri-*O*-acetyl-β-D-glucopyranosid)uronate was used without further purification in the next reaction.

Anal. Calc. for $C_{17}H_{27}NO_2 \cdot 1.5\ H_2O$: C, 44.02; H, 6.51; N, 3.02. Found: C, 43.98; H, 6.20; N, 3.03.

The free amine **6** was isolated and characterized from one hydrogenation performed in anhydrous methanol[14], followed by evaporation, trituration of the residue, and crystallization from chloroform (33% yield); fine white crystals, m.p. 152° (dec.); $\nu_{max}^{CHCl_3}$ 1765 (C=O) and 1020 cm^{-1}; ^{1}H-n.m.r. (Me_2SO-d_6)*: δ 2.03, 2.07, and 2.10 (3 s, 9 H, OAc), 3.02 (t, 2 H, *J* 5 Hz, CH_2N), 3.37 (s, 3 H, CO_2Me), 3.73 (m, 2 H, CH_2N), 3.92 (t, 2 H, *J* 5 Hz, CH_2O), 4.12–5.1 (ms, 5 H); m.s. calc. for $C_{15}H_{24}NO_{10}$ (M + H) 378.1400, found 378.1354; calc. for $C_{13}H_{20}NO_8$ (M − CO_2-CH_3) 318.1189, found 318.1180.

Methyl (2-retinamidoethyl tri-O-acetyl-β-D-glucopyranosid)uronate (7). — To a degassed solution of all-*trans*-retinoic acid (300 mg, 1.0 mmol) dissolved (slight warming necessary) in *N*,*N*-dimethylformamide (2 mL, dried over molecular sieves 4-A) was added 180 mg (1.1 mmol) of *N*,*N*'-carbonyldiimidazole[12]. The solution was again degassed under argon and stirred for 30 min at room temperature. Next a degassed solution of 328 mg (0.75 mmol) of the crude acetate of amine **6** and 0.139 mL (1.0 mmol) of triethylamine (dried over potassium hydroxide pellets) in 1 mL of *N*,*N*-dimethylformamide was added, followed by *N*,*N*-dimethylformamide (1 ml) as a rinse. After degassing, the yellow-orange solution was stirred for 20 h at room temperature, diluted with ethyl acetate (75 mL), washed with 20-mL portions of water (twice), 1% sulfuric acid, water, saturated sodium hydrogencarbonate, and brine, and dried. Evaporation afforded a yellow-orange gum, R_F (25% ethyl acetate–chloroform) 0.1, 0.38 (amide), 0.48 (retinoic acid), 0.60. The gum was chromatographed (30 g of Merck Silica Gel 60, 25% ethyl acetate–chloroform, 10-mL fractions) to afford 0.38 g of amide as a yellow solid; l.c. (50% ethyl acetate–hexane, 280 nm) t_R 7–8 (8%), 13 (84%), and 15 min (8%). As crystallization from 50% ethyl acetate–hexane afforded material enriched in the impurities of lower l.c. retention-time, the amide was purified by preparative l.c. (40% ethyl acetate–hexane, 500-mL fractions, 260 nm) and crystallization from ethyl acetate–hexane to afford 166 mg (29% yield) of yellow powder, m.p. 103–105°; l.c. t_R 7–8 (1%) and 13 min (99%); $\nu_{max}^{CHCl_3}$ 1750 (CO_2R), 1660 (CONR), 1370, and 1030 cm^{-1}; ^{1}H-n.m.r. ($CDCl_3$): δ 1.06 [s, 6 H, 16,17-CMe_2], 2.01 (s, 3 H, 20-CH_3), 2.05 (s, 9 H, OAc), 2.36 (s, 3 H, 19-CH_3), 3.4–3.65 (m, 2 H, CH_2N), 3.78 (s, 3 H, CO_2Me), 3.66–3.9 (m, 2 H, CH_2O), 4.08 (m, 1 H, H-5'), 4.60 (d, 1 H, *J* 7.0 Hz, H-1'), 4.9–5.35 (m, 3 H, H-2',3',4'), 5.72 (s, 1 H, 14-C=CH), 6.0–6.4 (m, 5 H, 7,8-HC=CH, 10,12-C=CH, and NH), and 6.92 (dd, 1 H, *J* 11, 12 Hz, 11-C=CH); ^{13}C-n.m.r. ($CDCl_3$): δ 169.995, 169.408, 169.408 (*O*C*O*Me), 167.244 and 167.108 (*C*O_2Me and CON), 148.784 (C-13), 138.682 (C-9), 137.778 (C-8), 137.387 (C-6), 135.664 (C-12), 129.768 (C-11), 129.632 (C-5,10), 128.201 (C-7), 121.345 (C-14), 101.025 (C-1'), 72.392, 71.922, 71.423 (C-2',3',4'), 69.942 (OCH_2), 62.298 (C-5'), 52.953 (CO_2CH_3), 39.665 (C-2), 39.089 (NCH_2), 34.290 (C-1), 33.108

(C-4), 28.965 (C-16,17), 21.726 (C-18), 20.664, 20.581 and 20.471 ($OCOCH_3$), 19.268 (C-3), 13.571 (C-20), and 12.853 (C-19); m.s. calc. for $C_{35}H_{49}NO_{11}$ 659.3305, found 659.3352.

*Sodium (2-retinamidoethyl β-*D*-glucopyranosid)uronate* (**8**). — To a degassed solution of **7** (351 mg, 0.53 mmol) in anhydrous methanol (12 mL) was added degassed 5M sodium hydroxide (0.532 mL, 2.66 mmol). After degassing, the orange solution was stirred for 1.5 h at room temperature, acetic acid (0.305 mL, 0.53 mmol) was added, and stirring was continued for 0.5 h. The mixture was evaporated at low temperature and combined with material obtained from hydrolysis of 137 mg (0.21 mmol) of the protected β-D-glucosiduronate. This yellow solid was dissolved in 25 mL of 60% methanol–water and purified by preparative, reverse-phase l.c. (60% methanol–water, 450-mL fractions, refractive-index detection). Fractions 7–10 contained product. Fraction 7, after concentration and lyophilization, afforded 14 mg of yellow powder; l.c. (reverse-phase, 60% methanol–water, 260 nm) t_R 0.5 (2.1%) and 4.5 min (97.9%). Fractions 8–10 were combined, concentrated, and lyophilized under argon to give 115 mg of yellow powder; l.c. t_R 0.50 (2.4%) and 4.5 min (97.6%). The ^{1}H-n.m.r. spectrum indicated that this powder contained 3 molecules of water/molecule of glucosiduronate; therefore, the hydrolysis yield was 27%. As recovery from the preparative column is poor on small-scale separations, the yield should be improved if this procedure were repeated on a larger scale. To prevent oxidation, it was necessary to conduct manipulations in an argon-filled glove bag. The sodium glucosiduronate was a highly hygroscopic, readily oxidized, pale-yellow powder; $[\alpha]_D^{20}$ +16.0° (*c* 0.5, methanol); ν_{max}^{KBr} 3350, 1680–1510, 1430, 1370, and 1060 cm^{-1}; ^{1}H-n.m.r. (Me_2SO-d_6)*: δ 1.04 [s, 6 H, 16,17-CMe_2], 1.4–1.8 [m, 4 H, 2,3-$(CH_2)_2$], 1.70 (s, 3 H, 18-C=CCH_3), 1.98 (s, 3 H, 20-C=CCH_3), 2.02 (m, 2 H, 4-CH_2), 2.30 (s, 3 H, 19-C=CCH_3), 2.85–3.9 (broad m, 8 H, NCH_2CH_2O, and 4 H), 3.34 (s, 6 H, H_2O), 4.12 (d, 1 H, *J* 7 Hz), 4.7–5.2 (broad m, 2 H, OH), 6.05–6.4 (m, 5 H, 7,8-HC=CH, 10,12,14-C=CH), 6.75–7.0 (m, 1 H, 11-HC=C), and 6.98 (s, 1 H, OH); ^{1}H-n.m.r. (Me_2SO-d_6–D_2O) δ 1.02 [s, 6 H, 16,17-CMe_2], 1.35–1.65 [m, 4 H, 2,3-$(CH_2)_2$], 1.68 (s, 3 H, 20-CH_3), 2.0 (m, 2 H, 4-CH_2), 2.26 (s, 3 H, 19-CH_3), 2.85–3.6 (m, 7 H), 4.16 (d, 1 H, *J* 6 Hz, H-5′), 6.0 (s, 1 H, 14-C=CH), 6.15–6.45 (m, 4 H, 7,8-HC=CH, 10,12-C=CH), and 6.9 (m, 1 H, 11-C=CH); ^{13}C-n.m.r. (Me_2SO-d_6): δ 173.025 (6′-CO_2Na), 166.335 (C-15 CON), 145.790 (C-13), 137.240, 137.240, and 137.092 (C-6,8,9), 136.626 (C-12), 130.114 (C-11), 129.061 and 128.481 (C-5,10), 126.961 (C-7), 123.457 (C-14), 103.379 (C-1′), 76.583 (C-3′), 73.460 and 73.257 (C-2′,4′), 72.117 (C-5′), 69.606 (OCH_2), 39.191 and 38.959 (C-2 and NCH_2), 33.798 (C-1), 32.538 (C-4), 28.746 (C-16,17), 21.422 (C-18), 18.703 (C-3), 12.985 (C-20), and 12.484 (C-19); λ_{max}^{EtOH} 348 nm (ε 3.5 × 10^4); m.s. [tetrakis(trimethylsilyl) derivative] calc. for $C_{40}H_{73}NO_8Si_4$ 807.4413, found 807.4377. The ^{1}H-n.m.r. spectrum of the salt has the H-14 signal shifted downfield. Hydrogen-bonding interaction between the amide and the sugar hydroxyl protons may have caused this shift. After exchange with D_2O, the H-14 signal resumed its typical position at δ 6.0.

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