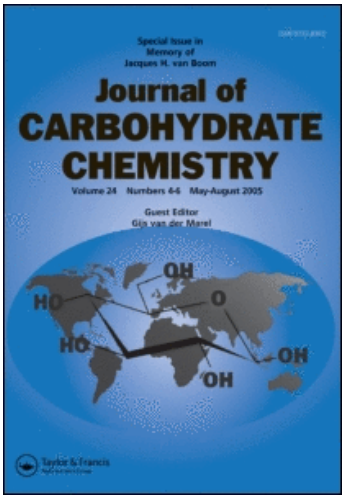
This article was downloaded by:
On: *23 January 2011*
Access details: *Access Details: Free Access*
Publisher *Taylor & Francis*
Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



Journal of Carbohydrate Chemistry

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713617200

Preparation of 4-Retinamidophenyl- and 4-Retinamidobenzyl-*C*-glycosyl and *C*-Glucuronosyl Analogues of the Glucuronide of 4-Hydroxyphenyl-Retinamide as Potential Stable Cancer Chemopreventive Agents

M. J. Panigot[a]; K. A. Humphries[a]; R. W. Curley Jr.[a]

[a] Division of Medicinal Chemistry and Pharmacognosy College of Pharmacy, The Ohio State University, Columbus, OH

To cite this Article Panigot, M. J. , Humphries, K. A. and Curley Jr., R. W.(1994) 'Preparation of 4-Retinamidophenyl- and 4-Retinamidobenzyl-*C*-glycosyl and *C*-Glucuronosyl Analogues of the Glucuronide of 4-Hydroxyphenyl-Retinamide as Potential Stable Cancer Chemopreventive Agents', Journal of Carbohydrate Chemistry, 13: 2, 303 — 321

To link to this Article: DOI: 10.1080/07328309408009195

URL: http://dx.doi.org/10.1080/07328309408009195

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: http://www.informaworld.com/terms-and-conditions-of-access.pdf

This article may be used for research, teaching and private study purposes. Any substantial or systematic reproduction, re-distribution, re-selling, loan or sub-licensing, systematic supply or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

J. CARBOHYDRATE CHEMISTRY, 13(2), 303-321 (1994)

PREPARATION OF 4-RETINAMIDOPHENYL- AND 4-RETINAMIDOBENZYL-*C*-GLYCOSYL AND *C*-GLUCURONOSYL ANALOGUES OF THE GLUCURONIDE OF 4-HYDROXYPHENYL-RETINAMIDE AS POTENTIAL STABLE CANCER CHEMOPREVENTIVE AGENTS

M.J. Panigot, K.A. Humphries and R.W. Curley, Jr.*

Division of Medicinal Chemistry and Pharmacognosy
College of Pharmacy, The Ohio State University, Columbus, OH 43210

Received March 5, 1993 - Final Form October 21, 1993

Downloaded At: 09:43 23 January 2011

ABSTRACT

Glucuronide metabolites of retinoic acid and its analogues have been suggested to be active cancer chemopreventive analogues of the parent molecules. However, these metabolites are susceptible to ß-glucuronidase and acid-catalyzed cleavage and it is not clear whether these carbohydrate conjugates must be hydrolyzed back to the parent molecule to show activity. Thus, the multistep syntheses of stable *C*-glycosyl and *C*-glucuronosyl analogues of the known glucuronide metabolite of the breast cancer chemopreventive agent 4-hydroxyphenylretinamide (4-HPR) are outlined. The chemical and enzymatic stability of these compounds has been evaluated relative to the glucuronide of 4-HPR.

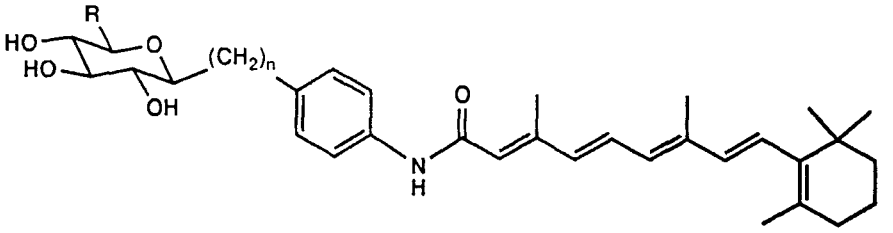
INTRODUCTION

Recent evidence suggests that the glucuronide conjugates of retinoids may be active metabolites of the parent compounds.[1] The retinoids as a class are derived from retinoic acid (1) which is itself a metabolite of vitamin A (retinol). The retinoids have generated much interest as dermatologic agents and as cancer chemopreventive or chemotherapeutic compounds.[2] The retinamide *N*-4-hydroxyphenylretinamide (2) is a synthetic analogue of 1 which has shown promise as a less toxic

Copyright © 1994 by Marcel Dekker, Inc.

analogue of **1** with particular utility as a breast cancer chemopreventive.[3] It has been shown that **2** concentrates in the mammary epithelium of female rats along with certain metabolites of **2**.[4] Among the metabolites of **2** formed *in vivo* is its glucuronide **3**.[5] We have recently shown that **3** has reduced toxicity and greater activity than **2** as an antiproliferative in MCF-7 human mammary tumor cells in culture.[6] However, it remains unclear whether these conjugates function as is, whether they must be hydrolyzed back to the parent molecule to exert their effects, or whether the glucuronic acid moiety is required as the conjugating carbohydrate. For example, it has recently been found that the glucoside of **1** shows similar biological activity to the glucuronide of **1**.[7] Based on our experience with Grignard additions to glycopyranosyl halides,[8] we have prepared some stable glycopyranosyl and glycopyranosiduronosyl benzene and phenylmethane analogues of **3** to evaluate the above questions. The preparation of these analogues (**4-9**) as well as their properties are described herein.

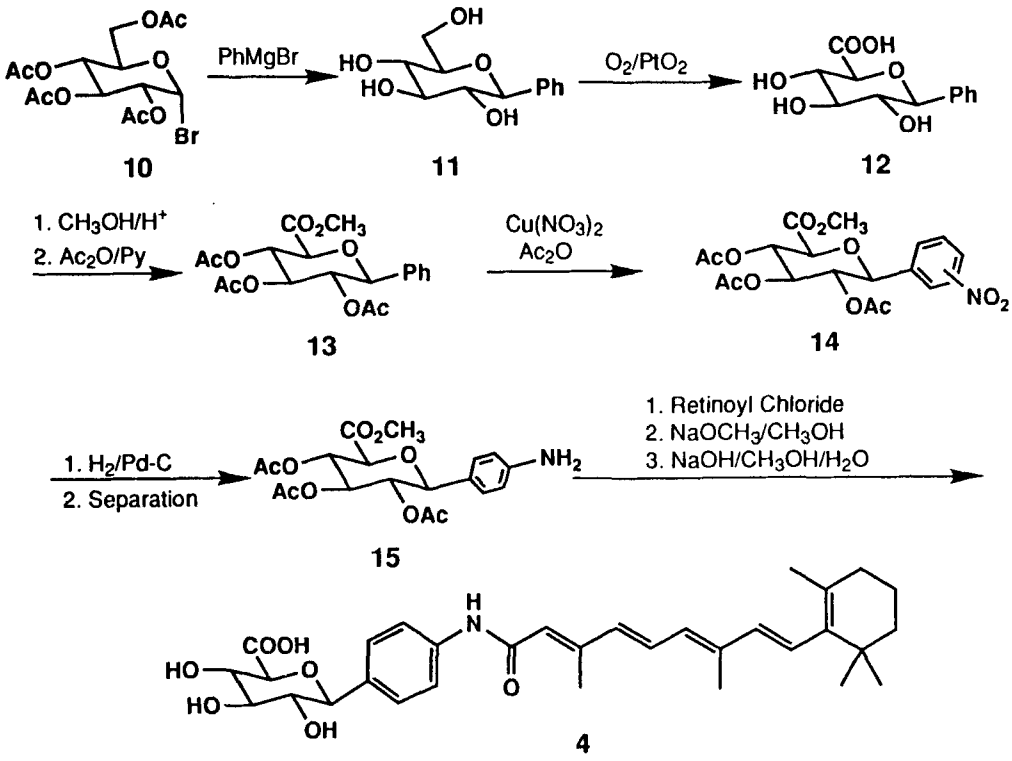
Downloaded At: 09:43 23 January 2011



4 n = 0, R = CO_2H.
5 n = 0, R = CH_2OH
6 n = 0, R = H
7 n = 1, R = CO_2H
8 n = 1, R = CH_2OH
9 n = 1, R = H

RESULTS AND DISCUSSION

The strategy for the synthesis of **4** is shown in Scheme 1. Alkylation of (2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl) bromide (**10**) with phenylmagnesium bromide by the procedure of Bonner and Hurd[9] produced (ß-D-glucopyranosyl)benzene (**11**) which was isolated as its tetraacetate. After deacetylation with methanolic potassium carbonate, the *C*-glucosyl compound



Scheme 1. Synthesis of **4**.

Downloaded At: 09:43 23 January 2011

was oxidized to the *C*-glucuronosyl analogue (**12**) with oxygen and platinum(IV) oxide (Adams' catalyst).[10] The *C*-glucuronosyl compound was reprotected as its methyl ester and acetylated by standard methodology to give **13**. Treatment of the *C*-glucuronosyl compound with copper(II) nitrate and acetic anhydride by a variation of the method of Craig and Bonner[11] gave rise to a 1:1 mixture of methyl *C*-glucuronosyl-2- and -4-nitrobenzene triacetates (**14**) which were not separated. These crude protected nitrophenyl compounds were reduced to the corresponding anilines by catalytic hydrogenation and then separated chromatographically to give *p*-isomer **15**. The protected **15** was retinoylated and deprotected by a procedure analogous to that used by Dawson and Hobbs[12] in their synthesis of **3**. This synthesis of **4**, starting from **10**, proceeded in 1% overall yield over ten steps. The primary difficulties encountered in this

synthesis stemmed from the selective oxidation of the hydroxymethyl group to the uronic acid functionality and isolation of the amphiphilic **4**. For these reasons and to explore the importance of the carbohydrate moiety on biological properties, *C*-glycosyl compounds not containing the uronic acid function were prepared as well.

Preparation of **5** followed an analogous scheme after omission of the laborious oxidation of the 6-hydroxymethyl group to the carboxylic acid. Thus, the tetraacetate of **11** was nitrated, reduced, retinoylated, and deprotected giving the desired **5** in overall yields somewhat better than **4** (2.2%).

Arylation of (2,3,4-tri-*O*-acetyl-ß-D-xylopyranosyl) chloride (**16**)[13] with phenylmagnesium bromide followed by reacetylation gave (2,3,4-tri-*O*-acetyl-ß-D-xylopyranosyl)benzene. Again, nitration, reduction, retinoylation, and deacetylation analogous to the steps used to prepare **5** provided **6** smoothly in 4% overall yield.

Downloaded At: 09:43 23 January 2011

Due to the difficulties we encountered in achieving the desired reaction of benzylmagnesium chloride with **10**,[8] an alternative substrate was used for the synthesis of **7** and **8**. Reaction of (2,3,4,6-tetra-*O*-benzyl glucopyranosyl) bromide[14] with benzyl Grignard reagent gave the desired *C*-glucosyl phenylmethane.[8] The perbenzylated *C*-glucosyl product was deprotected by catalytic hydrogenation and the crude tetrol was peracetylated to give the tetraacetate of (ß-D-glucopyranosyl)phenylmethane. From this stage the chemistry employed paralleled the appropriate paths used to produce **4** and **5** and yielded **7** and **8** smoothly in yields comparable to those for **4** and **5**, respectively.

Benzylmagnesium chloride, however, reacted in the more usual manner with **16** using the procedure of Noyori *et al.*[15] The resulting crude triol was acetylated directly to give (ß-D-xylopyranosyl)phenylmethane as its triacetate. Nitration, reduction, retinoylation, and deacetylation were done analogously to the preparation of **6** to yield **9** in 2.6% overall yield.

One of the potential advantages of *C*-glycosyl compounds in biological systems is their greater hydrolytic stability and their resistance to enzymatic cleavage to the free sugar and the aglycone. Both of these hydrolytic pathways are available to glucuronides. To test the relative stability of the *C*-glucopyranosiduronosyl linkage toward acidic conditions, samples of **3** and **4** were treated with 0.1N methanolic HCl at 37 °C for 2 h. After this time the glucuronide had undergone *ca.* 20%

Table 1. Activity of Assayed Compounds with Respect to ß-Glucuronidase[a]

Compound	$IC_{50}(\mu M)$[b]
3	184.5[c]
4	267
7	236
D-glucaro[1,4]lactone	8.4

a. Conditions used for the assay: 400 µg of the glucuronide of 2(3), 1.4 mL MeOH, 2.0 mL of distilled water, 0.6 mL of an aqueous solution of bovine serum albumin, 0.2 mL of an aqueous solution of bovine liver ß-glucuronidase (1 mg/mL corresponding to 624 Fishman units/mL), 0.6 mL of 1 M acetate buffer, pH = 4.5, incubation in polypropylene bottles at 37 °C for at least 2 h.

b. Determined by the amount of 2 liberated as measured by HPLC analysis.

c. Km of enzyme for 3 as substrate

Downloaded At: 09:43 23 January 2011

solvolysis to liberate 2 as determined by HPLC while 4 remained intact. Thus, it would appear that as expected these analogues show the desired stability toward acid-catalyzed hydrolysis.

Cleavage of glucuronides to glucuronic acid and the aglycone by ß-glucuronidase appears to be a general reaction for glucuronides, regardless of the structure of the aglycone. In similar ß-glucosidase-mediated hydrolyses of glucosides to glucose and the aglycone, *C*-glucosyl phenylmethane and *C*-glucosyl benzene have found use as enzyme inhibitors. Here, the C-glucosyl phenylmethane[16] acts as a slightly more potent inhibitor than the C-glucosyl benzene.[17] We have found analogously that compounds 4 and 7 appear to act as competitive inhibitors of ß-glucuronidase-mediated hydrolysis of 3 to 2 and glucuronic acid with 7 being slightly more effective than 4. Table 1 presents the measured IC_{50} values for 4 and 7 as well as the Km value of the enzyme for 3 under the experimental conditions. While the *C*-glucuronosyl compounds were less potent inhibitors than the best known inhibitor, D-glucaro[1,4]lactone, few synthetic inhibitors of this enzyme are known and most other natural inhibitors are far weaker.[18,19] Thus, these compounds may be useful as ß-glucuronidase inhibitors.

Preliminary results of the activity of **4–9** as antiproliferative agents in the MCF-7 human mammary tumor cell culture model show that the analogues, particularly **4** and **7**, are promising. To further assess the properties of these analogues, competitive binding to the nuclear retinoic acid receptors is being evaluated. Preliminary results show that the *C*-glycosyl and *C*-glycosyluronates show binding ability which is superior to that of the phenol **2** or its glucuronide **3** and parallels their antiproliferative effects above. Full details of the biological activity of these compounds will be presented at a later time.

Thus, the synthesis of *C*-glucuronosyl and *C*-glycosyl analogues of the known glucuronide metabolite of **2** have been demonstrated in modest yields. These compounds are active as inhibitors of ß-glucuronidase and are hydrolytically stable. Additionally, these compounds have biological activity as antiproliferative agents in the human mammary tumor cell culture model which may result from receptor regulation. Further investigation of the properties of these compounds will be forthcoming and should allow us to explore whether these carbohydrate conjugate metabolites are merely "pro-drugs" or whether they may function as intact molecules.

Downloaded At: 09:43 23 January 2011

EXPERIMENTAL

General Melting points were determined on a Thomas-Hoover apparatus and are uncorrected. NMR spectra were recorded on an IBM AC250 spectrometer operating at 250 MHz for ^{1}H in $CDCl_3$ solutions unless otherwise noted, and referenced to residual $CHCl_3$ at δ 7.24 ppm for ^{1}H or δ 77.0 ppm for ^{13}C. Representative ^{1}H and ^{13}C NMR assignments are provided for compounds **4** and **8**. UV spectra were recorded with a Beckman DU-40 spectrophotometer. Infrared spectra were recorded using an Analect RFX-40 FTIR spectrophotometer. Electron impact high resolution mass spectra were obtained using a Kratos MS-30 spectrometer. TLC was performed on 0.25 mm silica gel 60 F_{254} precoated aluminum plates from EM Science and visualized with fluorescence quenching of 254 nm light. Normal phase column chromatography was performed on silica gel (70-230 mesh, EM Science). Reversed-phase column chromatography was performed on octadecylsilyl (RP-18) coated 40 μm silica gel particles (J.T. Baker). HPLC analysis of the stability of glucuronosyl compounds was performed on a Beckman Model 332

gradient liquid chromatograph system equipped with a Beckman Model 164 variable wavelength ultraviolet detector and fitted with a 5 μm, spherical particle RP-18 column (Zorbax-ODS, 250 x 4.6 mm) using a solvent mixture of 9:1 MeOH/H_2O, both containing 10 mM NH_4OAc at a flow rate of 1 mL/min. The presence of free **2** was determined by comparison of its retention time with an authentic sample. Glycosyl halides were prepared according to published procedures.[9,13,14b] All other reagents were used as obtained.

(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)benzene (11-tetraacetate). Compound **10** (5 g, 12.1 mmol) was dissolved in 125 mL of ether and added over 1 h to phenylmagnesium bromide prepared from 5.6 g (0.23 g-atom) of magnesium turnings and 25 mL (237 mmol) of bromobenzene in 250 mL of ether. The mixture was heated at reflux for 6 h then poured into 400 mL of H_2O. Glacial acetic acid (20 mL) was added to dissolve the magnesium salts. The mixture was shaken and the layers were separated. The aqueous layer was concentrated to dryness and crude **11** was treated with 70 mL of acetic anhydride and 100 mL of pyridine overnight. The mixture was poured into 750 mL of H_2O and the resulting precipitate was isolated by filtration and recrystallized from 2-propanol to yield 2.236 g (45%) of the tetraacetate of **11**: mp 149-150 °C; IR (KBr) 3043, 2996, 2938, 2879, 1745, 1461, 1428, 1411, 1382, 1369, 1313, 1253, 1238, 1222, 1162, 1141, 1114, 1087, 1076, 1043, 1035, 981, 921, 904, 771, 705, 617; UV (MeOH) λ 256 nm (log ε 2.44), λ 217 nm (log ε 2.87); ^1H NMR: δ 1.77 (s, 3H), 1.98 (s, 3H), 2.04 (s, 3H), 2.06 (s, 3H), 3.8-3.9 (m, 1H), 4.15 (dd, 1H, J=2.1 Hz, 13 Hz), 4.28 (dd, 1H, J=5 Hz, 13 Hz), 4.38 (d, 1H, J=9.7 Hz), 5.13 (t, 1H, J=9.7 Hz), 5.23 (t, 1H, J=9.7 Hz), 5.34 (t, 1H, J=9.7 Hz), 7.32 (m, 5H); ^{13}C NMR: δ 20.24, 20.52, 20.62, 62.40, 68.77, 72.71, 74.32, 76.19, 80.26, 127.12, 128.36, 128.83, 130.31, 166.70, 169.38, 170.22, 170.54; HRMS calcd for $C_{20}H_{24}O_9$ 408.1420, found 408.1427.

1-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)-2- and -4-nitrobenzenes. The tetraacetate of **11** (5.63 g, 13.8 mmol) was dissolved in 150 mL of acetic anhydride and copper(II) nitrate (25.58 g, 110.4 mmol) was added. The mixture was heated to 100 °C for 1 h then poured into 400 mL of ice water. The organic soluble material was extracted with ether (2 x 200 mL). The ether extracts were washed with water (2 x 100 mL) and saturated aqueous $NaHCO_3$, dried ($MgSO_4$), and concentrated to dryness to yield 6.11 g (97%) of a 1:1 mixture of *o*- and *p*-nitration products. This

Downloaded At: 09:43 23 January 2011

mixture of compounds was immediately taken to the next step in the reaction sequence.

4-(2,3,4,6-Tetra-*O*-acetyl-ß-D-glucopyranosyl)aniline. The isomeric nitroaromatics from above (1.046 g) were dissolved in 150 mL of methanol and *ca.* 100 mg of 10% palladium on carbon was added. The mixture was shaken under 40 psi of hydrogen for 1.5 h. The catalyst was removed by filtration, the filtrate was concentrated to dryness, and the residue was chromatographed on silica gel using 1:1 ethyl acetate/hexanes as elutant to give 372.2 mg (38%). A sample of this material was recrystallized from 2-propanol: mp 110-111°C; IR (KBr) 3477, 3388, 2967, 2938, 2871, 1741, 1623, 1523, 1442, 1430, 1409, 1380, 1216, 1143, 1118, 1108, 1089, 1039, 977, 921, 833, 688, 638, 620, 609; UV (MeOH) λ 286 nm (log ε 3.29), λ 242 nm (log ε 4.11); ^{1}H NMR: δ 1.78 (s, 3H), 1.98 (s, 3H), 2.03 (s, 3H), 2.05 (s, 3H), 3.68 (br s, 2H), 3.75-3.85 (m, 1H), 4.11 (dd, 1H, J=12.3 Hz, 2.3 Hz), 4.26 (d, 1H, J=10 Hz), 4.2-4.3 (m, 1H), 5.12 (t, 1H, J=9.7 Hz), 5.19 (t, 1H, J=9.7 Hz), 5.29 (t, 1H, J=9.7 Hz), 6.6 (m, 2H), 7.1 (m, 2H); HRMS calcd for $C_{20}H_{25}NO_9$ 423.1529, found 423.1538.

1-(2,3,4,6-Tetra-*O*-acetyl-ß-D-glucopyranosyl)-4-retinamidobenzene. Retinoyl chloride was prepared from 192 mg (0.64 mmol) of retinoic acid dissolved in 10 mL of ether containing 100 μL of pyridine and cooled to 0 °C. Thionyl chloride (47 μL, 0.64 mmol) was added. The mixture was allowed to come to room temperature over 90 min and the acetylated *C*-glycosyl aniline (270 mg, 0.64 mmol) was added in 10 mL of a 9:1 mixture of benzene and pyridine. The mixture was stirred at room temperature for 40 h, diluted with 100 mL of ethyl acetate, and extracted with 100 mL of H_2O, 2 x 100 mL of 1% H_2SO_4, and 2 x 100 mL of saturated aqueous $NaHCO_3$ solution. The organic product was dried ($MgSO_4$), concentrated, and chromatographed with 1:1 ethyl acetate/hexanes as elutant to yield 247 mg (54%) of the retinamide: ^{1}H NMR: δ 1.01 (s, 6H), 1.4-1.7 (m, 4H), 1.73 (s, 3H), 1.98 (s, 3H), 1.99 (s, 3H), 2.02 (s, 3H), 2.03 (s, 3H), 2.40 (s, 3H), 3.8-3.9 (m, 1H), 4.35 (d, 1H, J=9.3 Hz), 5.08 (t, 1H, J=9.3 Hz), 5.20 (t, 1H, J=9.3 Hz), 5.32 (t, 1H, J=9.3 Hz), 5.76 (s, 1H), 6.0-6.3 (m, 4H), 6.9-7.1 (m, 3H), 7.4 (br s, 1H), 7.5-7.6 (m, 2H).

1-(ß-D-glucopyranosyl)-4-retinamidobenzene (5). The acetylated C-glucosyl retinamide (247 mg, 0.35 mmol) was dissolved in 20 mL of methanol and 2 g of K_2CO_3 added. The mixture was stirred at room temperature for 18 h, concentrated, and chromatographed successively on

Downloaded At: 09:43 23 January 2011

RP-18 with 1:1 methanol/water, 3:1 methanol/water, and 100% methanol to yield 47.7 mg of **5** (25%): IR (KBr) 3407, 2954, 2923, 2861, 1650, 1604, 1577, 1517, 1488, 1442, 1411, 1359, 1311, 1257, 1159, 1085, 1045, 966, 835, 700, 644, 620; UV (MeOH) λ_{max} 351 nm (log ε 4.13); ^{1}H NMR: δ 1.01 (s, 6H), 1.4-1.7 (m, 4H), 1.72 (s, 3H), 2.02 (br s, 5H), 2.40 (s, 3H), 3.3-3.9 (m, 6H), 4.13 (d, 1H, J=9.3 Hz), 6.0-6.4 (m, 5H), 7.02 (dd, 1H, J=11.4, 15.0 Hz), 7.2-7.3 (m, 2H), 7.5-7.6 (m, 2H), 9.3 (br s, 1H); FAB MS calcd from $C_{32}H_{43}NO_6$ 537.31, found 537.47.

(Methyl 2,3,4-Tri-*O*-acetyl-ß-D-glucopyranosyluronate)benzene (13). The peracetylated **11** (1.45 g, 3.55 mmol) was stirred with 500 mg of K_2CO_3 in 50 mL of methanol overnight. Methanol was removed under reduced pressure and the residue was dissolved in 250 mL of H_2O containing 1 g of freshly reduced platinum(IV) oxide. Oxygen was bubbled in with heating to 80 °C for 24 h. The catalyst was removed by filtration and the filtrate concentrated to dryness. Crude **12** was heated to reflux in 200 mL of 1% H_2SO_4 in methanol for 6 h. Excess H_2SO_4 was neutralized with $NaHCO_3$ and methanol was removed. The residue was reacted with 70 mL each of acetic anhydride and pyridine for 16 h then poured into 1 L of ice water and the product was isolated by filtration and recrystallized from 2-propanol to yield 724 mg (52%) of **13**: mp 178-180 °C; IR (KBr) 3045, 3004, 2958, 1758, 1498, 1459, 1444, 1378, 1369, 1330, 1290, 1280, 1241, 1218, 1162, 1145, 1089, 1052, 1029, 979, 919, 892, 867, 769, 701, 607; UV (MeOH) λ 256 nm (log ε 2.31); λ 218 nm (log ε 2.85); ^{1}H NMR: δ 1.79 (s, 3H), 2.02 (s, 3H), 2.05 (s, 3H), 3.75 (s, 3H), 4.15 (m, 1H), 4.4 (d, 1H, J=9.3 Hz), 5.15 (m, 1H), 5.35 (m, 2H), 7.3-7.45 (m, 5H); ^{13}C NMR: δ 20.24, 20.44, 20.55, 52.68, 69.85, 72.42, 73.63, 76.86, 80.39, 127.27, 128.45, 129.05, 135.71, 167.38, 168.64, 170.14; HRMS calcd for $C_{19}H_{22}O_9$ 394.1264, found (M + 1) 395.1317.

(Methyl 2,3,4-tri-*O*-acetyl-ß-D-glucopyranosyluronate)-2- and -4-nitrobenzenes (14). Compound **13** (900 mg, 2.28 mmol) was suspended in 20 mL of acetic anhydride and 4.6 g (19.8 mmol) of copper(II) nitrate was added. The mixture was heated to 100 °C for 2 h then poured into 150 mL of ice water. The aqueous phase was extracted with 2 x 75 mL of ether. The combined ether extracts were washed with 4 x 50 mL of saturated $NaHCO_3$, dried ($MgSO_4$) and solvent removed. The product was purified by flash chromatography using 1:1 ethyl acetate/hexanes as elutant to yield 871 mg (87%) of a 1:1 mixture of *o*- and *p*-nitration products **14**. This mixture was reduced directly without further characterization.

Downloaded At: 09:43 23 January 2011

4-(Methyl 2,3,4-tri-*O*-acetyl-β-D-glucopyranosyluronate)aniline (15). Compound **14** (1.03 g, 2.35 mmol) was added to 100 mL of methanol containing 60 mg of 10 % palladium on carbon. The mixture was shaken under 40 psi of hydrogen for 2 h, filtered, concentrated, and chromatographed with 1:1 ethyl acetate/hexane as elutant to yield 269 mg (28%) of **15**: mp 200-202 °C; IR (KBr) 3450, 3361, 3232, 3023, 2996, 2654, 2925, 2856, 1727, 1631, 1614, 1590, 1519, 1496, 1442, 1367, 1228, 1180, 1132, 1108, 1054, 1033, 981, 966, 906, 889, 836, 771, 686, 649; UV (MeOH) λ 286 nm (log ε 3.19), λ 244 nm (log ε 3.99), λ 207 nm (log ε 4.00); ^{1}H NMR: δ 1.78 (s, 3H), 2.00 (s, 3H), 2.04 (s, 3H), 3.72 (s, 3H), 4.1-4.2 (m, 1H), 4.30 (m, 1H, J=9.7 Hz), 5.1-5.2 (m, 1H), 5.25-5.4 (m, 2H), 6.6-6.7 (m, 2H), 7.1-7.2 (m, 2H); ^{13}C NMR: δ 20.25, 20.36, 20.49, 52.52, 69.95, 72.20, 73.79, 76.79, 80.41, 114.81, 125.15, 128.58, 147.23, 167.70, 168.60, 169.15, 170.05; HRMS calcd for $C_{19}H_{23}NO_9$ 409.1383, found 409.1384.

1-(Methyl 2,3,4-tri-*O*-acetyl-β-D-glucopyranosyluronate)-4-retinamidobenzene. Retinoyl chloride was prepared from retinoic acid (264 mg, 0.88 mmol), 73 μL of pyridine, and 65 μL (0.88 mmol) of thionyl chloride in 10 mL of ether at 0 °C. The mixture was allowed to warm to room temperature over 1 h. Compound **15** (360 mg, 0.88 mmol) was added in 15 mL of benzene containing 100 μL of pyridine. The mixture was stirred at room temperature for 72 h, diluted with 100 mL of ether, and washed with 50 mL of water, 2 x 50 mL of 0.05 M H_2SO_4, and 2 x 50 mL of saturated $NaHCO_3$. The organic layer was dried (Na_2SO_4), concentrated, and purified by flash chromatography using 1:1 ethyl acetate/hexanes as elutant to yield 379.5 mg (62%): IR (KBr) 3372, 2956, 2929, 2863, 1762, 1673, 1602, 1581, 1521, 1438, 1417, 1365, 1311, 1295, 1245, 1222, 1182, 1155, 1103, 1035, 971, 889, 840; UV (MeOH) λ_{max} 362 nm (log ε 4.57), ^{1}H NMR: δ 1.01 (s, 6H), 1.4-1.6 (m, 4H), 1.78 (s, 3H), 1.98 (s, 3H), 1.99 (br s, 5H), 2.02 (s, 3H), 2.03 (s, 3H), 2.40 (s, 3H), 3.72 (s, 3H), 4.13 (d, 1H, J=9.8 Hz), 4.38 (d, 1H, J=9.8 Hz), 5.05-5.15 (m, 1H), 5.3-5.4 (m, 2H), 5.75 (s, 1H), 6.0-6.3 (m, 4H), 6.95 (dd, 1H, J=11.4 Hz, J=15.0 Hz), 7.18 (br s, 1H), 7.25-7.3 (m, 2H), 7.5-7.55 (m, 2H); ^{13}C NMR: δ 12.66, 13.52, 13.98, 19.07, 20.12, 20.23, 20.34, 21.48, 28.77, 32.93, 34.09, 39.53, 52.49, 60.15, 69.75, 72.12, 73.51, 79.75, 119.29, 121.35, 127.73, 128.27, 129.43, 129.65, 130.19, 130.61, 135.30, 137.10, 137.60, 138.92, 139.28, 150.30, 165.22, 167.43, 168.67, 169.22, 169.95; FAB MS calcd for $C_{39}H_{49}NO_{10}$ 691.33, found (M+1) 692.38.

Downloaded At: 09:43 23 January 2011

1-(ß-D-glucopyranosyluronic acid)-4-retinamidobenzene (4). The protected *C*-glucuronosyl compound (600 mg, 0.868 mmol) was suspended in 10 mL of 0.2 M sodium methoxide in methanol and stirred at room temperature overnight. To this mixture was added 600 mg (15 mmol) of NaOH and 10 mL of H_2O and the mixture was stirred another 24 h. The mixture was acidified to pH 1 with 5% HCl and extracted with 3 x 50 mL of ethyl acetate. The ethyl acetate extracts were washed with 100 mL of H_2O, dried (Na_2SO_4), concentrated, and chromatographed on RP-18 with 85:15 methanol/H_2O to yield 148 mg (31%) of **4**: mp 130 °C (dec); IR (KBr) 3330, 3043, 2960, 2923, 2863, 1729, 1654, 1602, 1577, 1519, 1490, 1442, 1415, 1359, 1313, 1257, 1207, 1174, 1160, 1091, 1020, 966, 838; UV (MeOH) λ_{max} 362 nm (log ε 4.22); ^1H ^1H NMR (acetone-d_6): δ 1.02 (s, 6H, retinoid $C(CH_3)_2$), 1.4-1.7 (m, 4H, retinoid H-2, H-3), 1.71 (s, 3H, retinoid 5-CH_3), 2.07 (br s, 5H, retinoid 9-CH_3 and H-4), 2.42 (s, 3H, retinoid 13-CH_3), 3.0 (br s, 4H, -OH), 3.46 (t, 1H, J=9.0 Hz, pyranose H-2), 3.58 (t, 1H, J=8.7 Hz, pyranose H-3), 3.75 (t, 1H, J=8.8 Hz, pyranose H-4), 3.96 (d, 1H, J=9.6 Hz, pyranose H-5), 4.22 (d, 1H, J=9.2 Hz, pyranose H-1), 6.0-6.4 (m, 5H, retinoid vinyls), 7.05 (dd, 1H, J=11.4, 15.0 Hz, retinoid H-11), 7.3-7.4 (m, 2H, aromatic), 7.6-7.7 (m, 2H, aromatic), 9.26 (br s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ 12.48, 13.25, 18.68, 21.36, 28.71, 32.54, 33.78, 48.51(retinoid aliphatics); 71.72, 74.12, 77.85, 79.64, 81.45 (pyranose); 118.45, 122.59, 127.44, 128.01, 129.27, 129.61, 129.93, 134.11, 135.89, 136.87, 137.24, 138.11, 138.87, 148.18 (retinoid vinyl and aromatic); 164.66 (amide carbonyl); 170.55 (glucuronic acid carbonyl); FAB MS calcd for $C_{32}H_{41}NO_7$ 551.29, *found* 551.30.

(2,3,4-Tri-*O*-acetyl-ß-D-xylopyranosyl)benzene. 1,2,3,4-Tetra-*O*-acetyl-ß-D-xylopyranose (5 g, 15.7 mmol) was dissolved in 200 mL of dry chloroform and $AlCl_3$ (8.4 g, 62.8 mmol) was added. The mixture was stirred at room temperature for 2 h, diluted with 200 mL of H_2O, shaken, and separated. The chloroform layer was washed with 200 mL of H_2O, 2 x 200 mL of saturated aqueous $NaHCO_3$, dried ($MgSO_4$), and concentrated. The crude **16** was dissolved in 100 mL of ether and added to a refluxing solution of phenylmagnesium bromide prepared from 18 mL (169 mmol) of bromobenzene and 4.12 g (0.169 g-atom) of magnesium turnings in 250 mL of ether. The mixture was heated at reflux for 5 h and poured into 150 mL of H_2O containing 15 mL of glacial acetic acid. The aqueous layer was concentrated to dryness and the residue was reacted with 75 mL each of

Downloaded At: 09:43 23 January 2011

acetic anhydride and pyridine for 20 h. The mixture was poured into 300 mL of H_2O, the product was isolated by filtration and recrystallized from 2-propanol to yield 1.28 g (24%): mp 161-162 °C; IR (KBr) 3035, 3016, 2987, 2960, 2948, 2892, 1752, 1498, 1459, 1434, 1398, 1376, 1317, 1251, 1232, 1124, 1083, 1062, 1051, 1035, 1002, 977, 935, 921, 910, 896, 877, 765, 700, 651, 634; UV (MeOH) λ 256 nm (log ε 2.38), λ 216 nm (log ε 2.79); ^{1}H NMR: δ 1.78 (s, 3H), 2.00 (s, 3H), 2.04 (s, 3H), 3.43 (t, 1H, J= 11 Hz), 4.28 (d, 1H, J=9.7 Hz), 4.2-4.3 (m, 1H), 5.06 (t, 1H, J=9.5 Hz), 5.1-5.2 (m, 1H), 5.31 (t, 1H, J=9.5 Hz), 7.30 (m, 5H); ^{13}C NMR: δ 20.19, 20.52, 67.16, 69.42, 72.90, 73.90, 80.99, 127.08, 128.31, 128.73, 136.60, 168.70, 169.65, 170.14; HRMS calcd for $C_{17}H_{20}O_7$ 336.1209, found (M + 1) 337.1282.

1-(2,3,4-Tri-*O*-acetyl-β-D-xylopyranosyl)-2- and -4-nitrobenzenes. The peracetylated xylosylbenzene (1.2 g, 3.57 mmol) was nitrated analogous to the nitration of **13** to yield 1.347 g (99%) of a 1:1 mixture of *o*- and *p*- xylosyl nitrobenzenes. The crude reaction mixture was reduced without further characterization.

4-(2,3,4-Tri-*O*-acetyl-β-D-xylopyranosyl)aniline. The mixture of xylosyl nitrobenzenes (1.35g, 3.54 mmol) was reduced analogously to the reduction of **14** to yield 375 mg (30%): mp 157-159 °C; IR (KBr) 3475, 3388, 3228, 3037, 3023, 3012, 2964, 2948, 2888, 1756, 1629, 1521, 1369, 1251, 1230, 1110, 1087, 1062, 1031, 985, 939, 821, 790, 703, 684, 651, 640, 613; UV (MeOH) λ 286 nm (log ε 3.35), λ 240 nm (log ε 4.06), 210 nm (log ε 3.99); ^{1}H NMR: δ 1.77 (s, 3H), 2.00 (s, 3H), 2.03 (s, 3H), 3.40 (t, 1H, J=11 Hz), 4.16 (d, 1H, J=9.3 Hz), 4.1-4.25 (m, 1H), 5.0-5.2 (m, 2H), 5.27 (t, 1H, J=9.5 Hz), 6.6 (m, 2H), 7.08 (m, 2H); ^{13}C NMR: δ 20.13, 20.40, 66.86, 69.33, 72.63, 73.89, 80.75, 114.56, 128.17, 128.89, 146.96, 168.74, 169.56, 170.02; HRMS calcd for $C_{17}H_{21}NO_7$ 351.1318, found 351.1345.

1-(2,3,4-Tri-*O*-acetyl-β-D-xylopyranosyl)-4-retinamidobenzene. The xylopyranosyl aniline (375 mg, 1.07 mmol) was retinoylated in a manner analogous to **15** to yield 500 mg (74%): mp 96-98 °C; IR (KBr) 3367, 3330, 3037, 2956, 2927, 2863, 2827, 1758, 1673, 1600, 1579, 1519, 1490, 1440, 1415, 1267, 1311, 1245, 1224, 1153, 1091, 1033, 968, 937, 908, 877, 836, 825, 790, 723, 700, 688, 649, 638; UV (MeOH) λ_{max} 363 nm (log ε 4.89); ^{1}H NMR: δ 1.01 (s, 6H), 1.4-1.7 (m, 4H), 1.70 (s, 3H), 1.98 (br s, 5H), 2.00 (s, 6H), 2.04 (s, 3H), 2.40 (s, 3H), 3.4-3.5 (m, 1H), 4.2-4.3 (m, 1H), 4.25 (d, 1H, J=9.6 Hz), 5.04 (1H, J=9.6 Hz), 5.1-5.2 (m, 1H), 5.34 (t, 1H,

Downloaded At: 09:43 23 January 2011

J=9.3 Hz), 5.75 (s, 1H), 6.0-6.3 (m, 4H), 6.97 (dd, 1H, J=14.8 Hz, 11.4 Hz), 7.16 (br s, 1H), 7.4-7.6 (m, 4H); ^{13}C NMR: δ 12.51, 13.41, 14.89, 18.96, 20.00, 20.23, 20.26, 21.35, 28.66, 32.80, 33.96, 39.43, 65.40, 66.78, 69.22, 72.70, 73.77, 80.25, 119.20, 121.39, 127.41, 128.10, 129.47, 131.60, 135.22, 136.99, 137.47, 138.72, 149.95, 165.18, 168.67, 169.43, 169.99; FAB MS calcd for $C_{37}H_{47}NO_8$ 633.33, found (M+1) 634.24.

1-(β-D-Xylopyranosyl)-4-retinamidobenzene (6). The acetylated xylosyl retinamidobenzene (480 mg, 0.76 mmol) was deacetylated by a procedure analogous to the synthesis of **5** to yield 293.5 mg (76%) of **6**: mp 198-200 °C. IR (KBr) 3313, 3043, 2923, 2861, 1650, 1160, 1091, 1064, 998, 964, 894, 825, 790, 701, 655, 640; UV (MeOH) λ_{max} 355 nm (log ε 4.39); ^1H NMR (acetone-d_6): δ 1.02 (s, 6H), 1.4-1.7 (m, 4H), 1.70 (s, 3H), 1.95 (br s, 5H), 2.42 (s, 3H), 2.83 (s, 3H, -OH), 3.2-3.5 (m, 2H), 3.5-3.6 (m, 1H), 3.8-4.3 (m, 3H) 6.0-6.4 (m, 5H), 7.08 (dd, 1H, J=11.4 Hz, 14.9 Hz), 7.25-7.3 (m, 2H), 7.6-7.65 (m, 2H), 9.8 (br s, 1H); ^{13}C NMR (acetone-d_6): δ 12.93, 13.73, 20.01, 21.91, 33.70, 34.99, 40.55, 71.14, 71.51, 76.38, 80.29, 83.55, 83.87, 119.49, 123.51, 128.85, 130.23, 130.63, 130.96, 136.04, 137.06, 138.54, 139.33, 140.41, 141.81, 149.99, 159.21, 165.80; FAB MS calcd for $C_{31}H_{41}NO_5$ 507.30, found (M+1) 508.31.

(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)phenylmethane. 2,3,4,6-Tetra-*O*-benzyl-1-*p*-nitrobenzoyl glucose (2.00 g, 2.9 mmol) was dissolved in 20 mL of dichloromethane and hydrogen bromide was bubbled in for 10 min. The precipitated *p*-nitrobenzoic acid was removed by filtration and the filtrate was concentrated to give the crude glycosyl bromide which was dissolved in 50 mL of ether and added to benzylmagnesium chloride prepared from 600 mg (0.0247 g-atom) of magnesium and 2.8 mL (24.3 mmol) of benzyl chloride. The mixture was heated at reflux for 4 h then poured into 100 mL of water containing 5 mL of acetic acid. The mixture was shaken and separated. The ether layer was washed with 2 x 50 mL of saturated $NaHCO_3$, 1 X 50 mL of brine, dried ($MgSO_4$) and concentrated. The residue was dissolved in 80 mL of glacial acetic acid containing 0.1 g of 10% palladium on carbon and shaken under 40 psi of hydrogen for 19 h. The catalyst was removed by filtration and the filtrate was concentrated to dryness. The residue was acetylated with 30 mL each of pyridine and acetic anhydride over 16 h. The reaction was poured into 100 mL of water and extracted with 2 x 50 mL of ether. The ether extracts were washed 2 x 50 mL of water, 1 x 50 mL of 5% HCl, 1 x 50 mL of saturated $NaHCO_3$, 1 x

Downloaded At: 09:43 23 January 2011

50 mL of brine, dried ($MgSO_4$), concentrated, and crystallized from 2-propanol to yield 194 mg (16%): mp 118-119 °C; IR (KBr) 3089, 3060, 3039, 2962, 2948, 2937, 2888, 1733, 1602, 1498, 1454, 1442, 1430, 1367, 1340, 1321, 1224, 1137, 1126, 1105, 1085, 1029, 975, 937, 908, 862, 754, 698, 647, 634, 613; UV (MeOH) λ 258 nm (log ε 2.32), λ 218 nm (log ε 2.88); ^{1}H NMR (acetone-d_6): δ 1.92 (s, 3H), 1.94 (s, 3H), 1.96 (s, 3H), 1.97 (s, 3H), 2.72 (dd, 2H, J=14.5 Hz, 8.4 Hz), 2.88 (dd, 1H, J=14.5 Hz, 3.2 Hz), 3.72-3.90 (m, 2H), 4.00 (dd, 1H, J=12.1 Hz, 2.4 Hz), 4.21 (dd, 1H, J=12.1 Hz, 5.8 Hz), 4.86 (t, 1H, J=9.6Hz), 4.96 (t, 1H, J=9.8 Hz), 5.22 (t, 1H, J=9.5 Hz), 7.1-7.4 (m, 5H); ^{13}C NMR: δ 20.52, 38.01, 62.36, 68.97, 72.20, 74.54, 75.65, 78.37, 126.49, 128.20, 129.42, 137.17, 169.37, 169.55, 170.25; HRMS calcd for $C_{21}H_{26}O_9$ 422.1577, found (M+1) 423.1679.

(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)-4-aminophenylmethane. The acetylated glycosyl phenylmethane (500 mg, 1.18 mmol) was nitrated and reduced by chemistry analogous to that used in the synthesis of **15** to yield 173.2 mg (33%): mp 145-146 °C; IR (KBr) 3426, 3357, 3039, 3010, 2964, 2956, 2933, 2884, 1751, 1631, 1610, 1517, 1444, 1375, 1338, 1321, 1251, 1236, 1220, 1178, 1137, 1126, 1105, 1087, 1052, 1031, 977, 910, 865, 836, 738, 698, 688, 644, 628, 611, 601; UV(MeOH) λ 237 nm (log ε 4.03), λ 206 nm (log ε 4.18); ^{1}H NMR: δ 1.95 (s, 3H), 1.96 (s, 3H), 1.98 (s, 3H), 2.02 (s, 3H), 2.6-2.7 (m, 2H), 3.5-3.6 (m, 4H), 4.02 (dd, 1H, J=2.4 Hz, 12.1 Hz), 4.20 (dd, 1H, J=5.2 Hz, 12.1 Hz), 4.88 (t, 1H, J=9.5 Hz), 5.01 (t, 1H, J=9.6 Hz), 5.13 (t, 1H, J=9.34 Hz), 6.5-6.6 (m, 2H), 6.9-7.0 (m, 2H); ^{13}C NMR: δ 20.39, 36.96, 62.28, 66.89, 72.01, 74.51, 75.44, 78.54, 114.82, 126.77, 130.15, 144.94, 169.27, 169.43, 170.14, 170.35; HRMS calcd for $C_{21}H_{27}NO_9$ 437.1686, found 437.1687.

(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)-4-retinamidophenylmethane. Retinoylation of the glycosyl aminophenylmethane (158 mg, 0.36 mmol), was performed analogous to the retinoylation of **15** to provide 133 mg (51%) of the retinamide: IR (KBr) 2925, 1754, 1670, 1652, 1600, 1575, 1560, 1517, 1436, 1365, 1311, 1222, 1155, 1031, 968; ^{1}H NMR: δ 1.01 (s, 6H), 1.4-1.7 (m, 4H), 1.72 (s, 3H), 1.98 (s, 9H), 1.99 (s, 3H), 2.02 (br s, 5H), 2.40 (s, 3H) 2.65-2.75 (m, 2H), 3.5-3.6 (m, 2H), 4.02 (dd, 1H, J=11.9, 2.7 Hz), 4.20 (dd, 1H, J=11.9, 5.2 Hz), 4.89 (t, 2H, J=9.4 Hz), 5.01 (t, 1H, J=9.4 Hz), 5.13 (t, 1H, J=9.4 Hz) 5.77 (s, 1H), 6.0-6.3 (m, 4H), 6.95 (dd, 1H, J=11.4, 15.0 Hz), 7.1-7.15 (m, 2H), 7.4-7.5 (m, 2H); ^{13}C NMR: δ 12.83, 13.71, 19.25, 20.48, 20.51, 20.56, 20.62, 21.62, 25.33,

Downloaded At: 09:43 23 January 2011

28.94, 33.11, 34.28, 37.36, 39.71, 62.32, 68.95, 72.15, 74.55, 75.66, 78.46, 119.78, 121.22, 128.53, 129.46, 129.86, 129.91, 130.33, 132.91, 135.29, 136.93, 137.24, 137.80, 139.19, 150.38, 165.10, 169.36, 169.55, 170.23, 170.47; FAB MS calcd for $C_{41}H_{53}NO_{10}$ 719.37, found (M +1) 720.6.

(ß-D-Glucopyranosyl)-4-retinamidophenylmethane (8). The acetylated glycosyl retinamidophenylmethane (130 mg, 0.18 mmol) was deacetylated under conditions used in the preparation of **5** to yield 68 mg (68%) of **8**: mp 108-109 °C; IR (KBr) 3386, 3046, 2925, 2861, 1700, 1648, 1604, 1515, 1438, 1411, 1359, 1311, 1257, 1159, 1085, 1031, 1004, 966, 630; UV(MeOH) λ_{max} 360 nm (log ε 4.59); ^{1}H NMR (acetone-d_6): δ 1.02 (s, 6H, retinoid $C(CH_3)_2$), 1.4-1.7 (m, 4H, retinoid H-2, H-3), 1.71 (s, 3H, retinoid 5-CH_3), 1.99 (br s, 5H, retinoid 9-CH_3 and H-4), 2.42 (br s, 4H, retinoid 13-CH_3), 2.6-2.7 (m, 1H, $PhCH_aH_b$), 3.0 (br s, 4H, -OH), 3.2-3.8 (m, 5H, pyranose), 6.0-6.4 (m, 5H, retinoid vinyl H), 7.02 (dd, 1H, J=11.4, 14.9 Hz, retinoid H-11), 7.2-7.3 (m, 2H, aromatic), 7.5-7.6 (m, 2H, aromatic), 9.17 (br. s, 1H, -NH); ^{13}C NMR (acetone-d_6): δ 12.92, 13.72, 19.99, 21.90, 33.68, 34.96, 40.53 (retinoid aliphatics); 38.05 ($PhCH_2$ methylene); 63.45, 72.39, 74.53, 79.94, 80.82, 81.13 (pyranose); 119.92, 123.64, 129.26, 130.45, 130.71, 130.93, 131.46, 135.14, 136.12, 138.50, 138.67, 139.19, 149.66 (retinoid vinyl and aromatic), 165.77 (carbonyl); FAB MS calcd for $C_{33}H_{45}NO_6$ 551.32, found 550.8.

(Methyl 2,3,4-tri-*O*-acetyl-ß-D-glucopyranosyluronate)phenylmethane. The protected glycosyl phenylmethane (1.0 g, 2.37 mmol) was converted to the corresponding methyl glucopyranosiduronate by analogy to the synthesis of **13**. The residue was recrystallized from 2-propanol to yield 96.6 mg (10%): mp 116-118 °C; IR (KBr) 3087, 3066, 3031, 3002, 2954, 2940, 2919, 2857, 1737, 1496, 1454, 1432, 1381, 1365, 1294, 1226, 1145, 1112, 1081, 1060, 1035, 1008, 981, 906, 858, 750, 698, 680, 624, 601; UV (MeOH) λ 257 nm (log ε 2.37), λ 215 nm (log ε 2.94); ^{1}H NMR: δ 1.89 (s, 3H), 1.98 (s, 3H), 1.99 (s, 3H), 2.78-2.94 (m, 2H), 3.70 (s, 3H), 3.66-3.74 (m, 1H), 3.90 (d, 1H, J=9.0 Hz), 4.96 (t, 1H, J=9.2 Hz), 5.1-5.25 (m, 2H), 7.15-7.3 (m, 5H); ^{13}C NMR: δ 20.39, 20.46, 20.52, 38.13, 52.57, 69.81, 71.94, 73.78, 76.36, 78.60, 126.62, 128.33, 129.40, 136.85, 167.53, 169.29, 169.45, 170.15; HRMS calcd for $C_{20}H_{24}O_9$ 408.1420, found (M+1) 409.1500.

(Methyl 2,3,4-tri-*O*-acetyl-ß-D-glucopyranosyluronate)-2- and -4-nitrophenylmethane. The peracetylated glycosyl phenylmethane (1.56 g, 3.70 mmol) was first nitrated then oxidized by procedures analogous to the

Downloaded At: 09:43 23 January 2011

synthesis of **14** and **13** to yield 1.107 g (66%). The mixture of nitroaromatics was not further characterized but taken directly to the corresponding anilines.

(Methyl 2,3,4-tri-*O*-acetyl-β-D-glucopyranosyluronate)-4-aminophenylmethane. The mixture of glycosyl nitrophenylmethanes (1.107 g, 2.44 mmol) were reduced and purified by analogy to the reduction of **14** to yield 310 mg (30%): mp 111-113 °C; IR (KBr) 3455, 3365, 3031, 3016, 2958, 2896, 1737, 1629, 1525, 1436, 1376, 1290, 1245, 1222, 1186, 1130, 1089, 1062, 1033, 983, 919, 902, 892, 865, 836, 829, 676, 601; UV (MeOH) λ 291 nm (log ε 3.78), λ 234 nm (log ε 4.06); ^{1}H NMR: δ 1.87 (s, 3H), 1.92 (s, 3H), 1.93 (s, 3H), 2.65-2.70 (m, 2H), 3.53 (br s, 3H), 3.63 (s, 3H), 3.85 (d, 1H, J=9.4 Hz), 4.87 (t, 1H, J=9.2 Hz) 5.07 (t, 1H, J=9.5 Hz), 5.15 (t, 1H, J=9.3Hz), 6.50-6.55 (m, 2H), 6.88-6.92 (m, 2H); ^{13}C NMR: δ 20.18, 20.31, 37.00, 52.33, 69.69, 71.70, 73.69, 76.09, 98.65, 114.89, 126.31, 130.09, 145.04, 167.47, 169.13, 169.29, 169.95; HRMS calcd for $C_{20}H_{25}NO_9$ 423.1529, found 423.1527.

(Methyl 2,3,4-tri-*O*-acetyl-β-D-glucopyranosyluronate)-4-retinamidophenylmethane. From retinoyl chloride prepared as for **15** and 310 mg (0.73 mmol) of the aminophenylmethane was obtained, after chromatography using 1:1 ethyl acetate/hexanes as elutant, 303 mg (59%) of the protected *C*-glucuronosyl retinamidophenylmethane: mp 80-82 °C; IR (KBr) 3374, 2954, 2927, 2863, 1756, 1671, 1600, 1581, 1517, 1490, 1438, 1411, 1365, 1311, 1243, 1220, 1182, 1155, 1110, 1062, 1033, 970, 900, 890, 825, 755; UV (MeOH) λ_{max} 358 nm (log ε 4.64); ^{1}H NMR: δ 1.01 (s, 6H), 1.4-1.7 (m, 4H), 1.70 (s, 3H), 1.95 (s, 3H), 1.98 (s, 6H), 2.02 (br s, 5H), 2.75-2.85 (m, 2H), 3.70 (br s, 4H), 3.87 (d, 1H, J=9.3 Hz), 4.94 (t, 1H, J=9.3Hz), 5.13 (t, 1H, J=9.3 Hz), 5.21 (t, 1H, J=9.3 Hz), 5.77 (s, 1H), 6.0-6.3 (m, 4H), 6.97 (dd, 1H, J=11.4, 15.0 Hz), 7.1-7.2 (m, 3H), 7.4-7.5 (m, 2H); ^{13}C NMR: δ 12.73, 13.61, 19.16, 20.26, 20.40, 21.54, 28.86, 33.02, 34.19, 37.30, 39.65, 52.41, 69.75, 71.79, 73.68, 76.14, 78.51, 119.87, 121.37, 128.37, 129.44, 129.78, 130.15, 132.40, 135.35, 137.04, 137.17, 137.71, 138.99, 150.13, 165.19, 167.49, 169.22, 169.38, 170.02; FAB MS calcd for $C_{40}H_{51}NO_{10}$ 705.35, found (M+1) 706.5.

(β-D-Glucopyranosyluronic acid)-4-retinamidophenylmethane (7). The protected retinamidophenylmethane (200 mg, 0.28 mmol) was deprotected in a manner analogous to the synthesis of **4** to yield 74.5 mg (47%) of **7**: mp 98-100 °C; IR (KBr) 3372, 3039, 2954, 2925, 2863, 1731, 1650, 1604, 1579,

Downloaded At: 09:43 23 January 2011

1515, 1490, 1440, 1411, 1359, 1311, 1257, 1159, 1091, 1031, 1004, 966, 875, 825, 696, 634; UV (MeOH) λ_{max} 358 nm (log ε 4.50); ^{1}H NMR (acetone-d_6): δ 1.03 (s, 6H), 1.4-1.7 (m, 4H), 1.71 (s, 3H), 2.01 (br s, 5H), 2.38 (br s, 4H), 2.6-2.7 (m, 1H), 3.1-3.6 (m, 4H), 3.74 (d, 1H, J=9.3Hz), 6.0-6.4 (m, 5H), 7.10 (dd, 1H, J=11.4, 15.0 Hz), 7.2-7.38 (m, 2H), 7.6-7.65 (m, 2H), 9.22 (br s, 1H); ^{13}C NMR (acetone-d_6): δ 12.89, 13.70, 19.92, 21.88, 33.63, 34.90, 37.88, 40.44, 73.01, 74.03, 79.12, 79.51, 81.65, 119.94, 123.55, 128.66, 130.16, 130.43, 130.66, 130.89, 134.68, 137.02, 138.43, 138.63, 139.14, 149.67, 165.76, 170.94; FAB MS calcd for $C_{33}H_{43}NO_7$ 565.30, found (M+1) 566.5.

(2,3,4-Tri-*O*-acetyl-ß-D-xylopyranosyl)phenylmethane. 1,2,3,4-Tetra-*O*-acetyl-ß-D-xylopyranose (2.07 g, 6.5 mmol) was converted to **16** as previously described. Crude **16** was dissolved in 100 mL of ether and added to a refluxing solution of benzylmagnesium chloride prepared from 11.5 mL (0.10 mol) of benzyl chloride and 2.43 g of magnesium (0.1 g-atom) in 250 mL of ether. The mixture was kept at reflux for 4 h then poured into 400 mL of H_2O containing 20 mL of acetic acid. The aqueous layer was concentrated to dryness and the residue reacted with 75 mL each of acetic anhydride and pyridine for 8 h. The mixture was poured into 400 mL of H_2O and extracted with 3 x 100 mL of ether. The ether extracts were washed with 3 x 100 mL of saturated $NaHCO_3$, dried ($MgSO_4$), concentrated and recrystallized from 2-propanol to yield 989 mg (43%): mp 118-119 °C; IR (KBr) 3027, 2962, 2942, 2931, 2886, 2871, 1745, 1494, 1456, 1432, 1369, 1249, 1224, 1132, 1037, 993, 937, 750, 701; UV (MeOH) λ_{max} 258 nm (log ε 2.53), 220 nm (log ε 2.83); ^{1}H NMR: δ 1.96 (s, 3H), 2.02 (s, 6H, overlapping -OAc), 2.65-2.85 (m, 2H), 3.18 (t, 1H, J=10 Hz), 3.55-3.65 (m, 1H), 4.05 (dd, 1H, J=5.6 Hz, 10 Hz), 4.90 (t, 1H, J=9.2 Hz), 4.96 (m, 1H), 5.16 (t, 1H, J=9.2 Hz), 7.15-7.35 (m, 5H); ^{13}C NMR: δ 20.48, 20.52, 38.21, 66.71, 69.35, 72.45, 74.11, 78.94, 126.46, 128.23, 129.27, 137.20, 169.58, 170.19; HRMS calcd for $C_{18}H_{22}O_7$ 350.1365, found 350.1383.

(2,3,4-Tri-*O*-acetyl-ß-D-xylopyranosyl)-4-aminophenylmethane. The peracetylated xylosyl phenylmethane (989 mg, 2.82 mmol) was nitrated and reduced as described in the synthesis of **14** and **15** to yield 160 mg (16%): IR (KBr) 3450, 3387, 2927, 2870, 1747, 1625, 1519, 1432, 1367, 1247, 1222, 1135, 1095, 1060, 1031; UV (MeOH) λ 289 nm (log ε 3.35), λ 237 nm (log ε 4.01); ^{1}H NMR: δ 1.97 (s, 3H), 2.02 (s, 3H), 2.04 (s, 3H), 2.59 (dd, 1H, J=13.5 Hz, 8.1 Hz), 2.72 (dd, 1H, J=13.5 Hz, 2.7 Hz), 3.15 (t, 1H, J=9.7

Downloaded At: 09:43 23 January 2011

Hz), 3.55-3.65 (m, 1H), 3.6 (br. s, 2H), 4.04 (dd, 1H, J=5.6 Hz, 10 Hz), 4.86 (t, 1H, J=9.7 Hz), 4.9-5.0 (m, 1H), 5.15 (t, 1H, J=9.7 Hz), 6.6 (m, 2H), 6.95 (m, 2H).

(2,3,4-Tri-*O*-acetyl-ß-D-xylopyranosyl)-4-retinamidophenylmethane. Retinoylation of the xylosyl aminophenylmethane (160 mg, 0.44 mmol), was performed analogous to the corresponding transformation of **15** to yield 194.5 mg (70%): mp 88-90 °C; IR (KBr) 3322, 2925, 2863, 1752, 1670, 1652, 1600, 1581, 1515, 1438, 1411, 1365, 1311, 1247, 1222, 1182, 1157, 1097, 1062, 1031, 968, 935, 906, 883, 730; UV (MeOH) λ_{max} 357 nm (log ε 4.60); ^{1}H NMR: δ 1.00 (s, 6H), 1.4-1.7 (m, 4H), 1.98 (br s, 11H), 2.02 (s, 3H), 2.38 (s, 3H), 2.63 (dd, 1H, J=9 Hz, 15 Hz), 2.75 (dd, 1H, J=3 Hz, 15 Hz), 3.08 (t, 1H, J=9.3 Hz), 3.45-3.55 (m, 1H), 4.04 (dd, 1H, J=5.4 Hz, 10.2 Hz), 4.83 (t, 1H, J=9.3 Hz), 4.91 (dd, 1H, J=5.4 Hz, 9.6 Hz), 5.12 (t, 1H, J=9.3 Hz), 5.77 (s, 1H), 6.0-6.3 (m, 4H), 6.92 (dd, 1H, J=11.4 Hz, 15.0 Hz), 7.05-7.15 (m, 2H), 7.4-7.5 (m, 2H), 7.7 (br s, 1H); ^{13}C NMR: δ 12.73, 13.60, 19.15, 20.50, 20.80, 21.55, 28.85, 33.01, 34.18, 37.49, 39.62, 66.59, 69.30, 72.33, 74.04, 78.93, 119.81, 121.40, 128.35, 129.43, 129.71, 130.11, 135.34, 136.97, 137.16, 137.69, 138.96, 150.07, 165.14, 169.57, 170.10; FAB MS calcd for $C_{38}H_{49}NO_8$ 647.34, found (M+1) 648.44.

(ß-D-Xylopyranosyl)-4-retinamidophenylmethane (9). The acetylated retinamide (180 mg, 0.286 mmol) was deacetylated by analogy to the preparation of **5** to yield 82 mg (55%) of **9**: mp 110-112 °C; IR (KBr) 3374, 2927, 2865, 1662, 1604, 1519, 1442, 1411, 1365, 1311, 1257, 1160, 1095, 1064, 1022, 971, 829, 644; UV (MeOH) λ_{max} 357 nm (log ε 4.18); ^{1}H NMR (acetone-d_6): δ 1.00 (s, 6H), 1.4-1.7 (m, 4H), 1.72 (s, 3H), 2.02 (br s, 5H), 2.32 (dd, 1H, J=3 Hz, 14 Hz), 2.40 (s, 3H), 2.60 (dd, 1H, J=8 Hz, 14 Hz), 3.0-3.7 (m, 8H), 3.7-3.9 (m, 1H), 4.3-4.4 (m, 1H), 6.06 (s, 1H), 6.1-6.4 (m, 4H), 7.04 (dd, 1H, J=11.4, 15.0 Hz), 7.1-7.2 (m, 2H), 7.5-7.6 (m, 2H), 9.3 (br s, 1H); ^{13}C NMR (acetone-d_6): δ 12.91, 13.72, 19.95, 21.90, 33.64, 34.92, 38.20, 40.47, 70.61, 71.32, 74.55, 80.08, 82.05, 119.96, 123.62, 128.66, 130.16, 130.42, 130.62, 130.92, 135.10, 137.06, 138.46, 138.65, 139.14, 149.63, 165.76; FAB MS calcd for $C_{32}H_{43}NO_5$ 521.31, found (M + 1) 522.27.

Downloaded At: 09:43 23 January 2011

ACKNOWLEDGMENTS

Partial financial support of this work by a grant from the National Cancer Institute (CA49837) and a Training Grant Postdoctoral Fellowship to

M.J.P. (T 32 CA09498-07) is gratefully acknowledged. The assistance of The Ohio State University Campus Chemical Instrument Center in obtaining mass spectra is acknowledged. We thank Ms. Kimberly Risch and Ms. Joan Dandrea for the preparation of this manuscript.

REFERENCES AND NOTES

1. R.G. Mehta, A.B. Barua, J.A. Olson, and R.C. Moon, *Oncology*, **48**, 505 (1991).
2. D.L. Hill and C.J. Grubbs, *Ann. Rev. Nutr.*, **12**, 161 (1992).
3. R.C. Moon, H.J. Thompson, P.J. Becci, C.J. Grubbs, R.J. Gander, D.L. Newton, J.M. Smith, S.L. Phillips, W.R. Henderson, L.T. Mullen, C.C. Brown, and M.B. Sporn, *Cancer Res.*, **39**, 1339 (1979).
4. T.A. Hultin, C.M. May, and R.C. Moon, *Drug Metab. Disp.* **14**, 7 1 4 (1986).
5. H.M. Abou-Issa, V.A. Duruibe, J.P. Minton, S. Larroya, C. Dwivedi, and T.E. Webb, *Proc. Natl. Acad. Sci. USA*, **85**, 4184 (1988).
6. R. Bhatnagar, H. Abou-Issa, R.W. Curley, Jr., A. Koolemans-Beynan, M.L. Moeschberger, and T.E. Webb, *Biochem. Pharmacol.*, **41**, 1471 (1991).
7. A.B. Barua and J.A. Olson, *Biochem. J.*, **244**, 231 (1987).
8. See accompanying manuscript.
9. C.D. Hurd and W.A. Bonner, *J. Am. Chem. Soc.*, **67**, 1972 (1945).
10. K. Heyns and H. Paulsen, *Adv. Carbohydr. Chem.*, **17**, 169 (1962).
11. J.M. Craig and W.A. Bonner, *J. Am. Chem. Soc.*, **72**, 4808 (1950).
12. M.I. Dawson and P.J. Hobbs, *Carbohydr. Res.*, **85**, 121 (1980).
13. W. Korytnyk and J.A. Mills, *J. Chem. Soc.*, 636 (1959).
14. a) T. Ishikawa and H.G. Fletcher, Jr., *J. Org. Chem.*, **34**, 563 (1969); b) C.P.J. Glaudemans and H.G. Fletcher, Jr., *Methods in Carbohydrate Chemistry*, R.L. Whistler, Ed. Academic Press: New York, 1972, Vol. 6, p 375.
15. R. Noyori, S. Suzuki, M. Okayama, K. Sakurai, S. Kamohara, and J. Ueno, U.S. Patent 4 454 123; *Chem. Abstr.*, **101**, 152261C (1984).
16. R.R. Schmidt and H. Dietrich, *Angew. Chem. Int. Ed. Engl.*, **30**, 1328 (1991).
17. R.S.T. Loeffler, M.L. Sinnott, and M.C. Whiting, *J. Chem. Soc., Chem. Commun.*, 984 (1974).
18. J.L. Webb, *Enzyme and Metabolic Inhibitors* Vol. II; Academic Press: New York, 1966, pp 423-429.
19. R.C.Bernotas and B. Ganem, *Tetrahedron Lett.*, **26**, 4981 (1985).
20. M. Petkovich, *Ann. Rev. Nutr.*, **12**, 443 (1992).

Downloaded At: 09:43 23 January 2011