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An Improved Synthesis of the *C*-Linked Glucuronide of *N*-(4-Hydroxyphenyl)retinamide

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Abstract—Retinoic acid analogues such as *N*-(4-hydroxyphenyl)retinamide (4-HPR) are effective chemopreventatives and chemotherapeutics for numerous types of cancer. The *C*-linked analogue of the *O*-glucuronide of 4-HPR (4-HPRCG) has been shown to be a more effective agent. The synthetic route to this molecule has been significantly improved by access to a key *C*-benzyl-glucuronide intermediate through employment of a Suzuki coupling reaction between an exoanomeric methylene sugar and an aryl bromide. Preliminary evidence shows 4-HPRCG has chemotherapeutic activity.

Retinol (**1**; Fig. 1) and its metabolites are involved in many biological processes including vision, cell differentiation, and growth. Besides being essential to normal cell function, the retinol metabolite retinoic acid (**2**) also shows antiproliferative action in skin disease and cancer.[1] Unfortunately, at pharmacological doses retinoic acid causes severe toxicity. Therefore, development of retinoid analogues possessing a higher therapeutic index is of interest. One of the most investigated synthetic retinoids is *N*-(4-hydroxyphenyl)retinamide (4-HPR; **3**), which has been shown to be effective in numerous types of tumor models and has been involved in phase III clinical trials.[2]

Glucuronidation of drugs and natural products is a common metabolic pathway that usually facilitates excretion.[3] An important metabolite of **3** is 4-HPR-*O*-glucuronide (**4**), in which the phenolic hydroxyl group is linked to the sugar. Subsequent to its discovery, **4** was synthesized and evaluated for bioactivity, and was shown to have excellent chemopreventative and chemotherapeutic activity in a rat mammary tumor model.[4] However, it was not determined if the glucuronide **4**, which was shown to be hydrolyzed to **3** via β-glucuronidase,[5] was advantageous due to improved bioavailability of **3** or had activity in its own right as intact **4**. To study this issue, an enzymatically stable glucuronide analogue was synthesized replacing the phenolic oxygen with a methylene group. As summarized in Table 1, the carbon-linked analogue 4-HPR-*C*-glucuronide (4-HPRCG; **5**) was shown to have chemopreventative qualities superior to the *O*-linked **4**.[6] It has not yet been shown whether **5** shares the chemotherapeutic activity of **4** in the

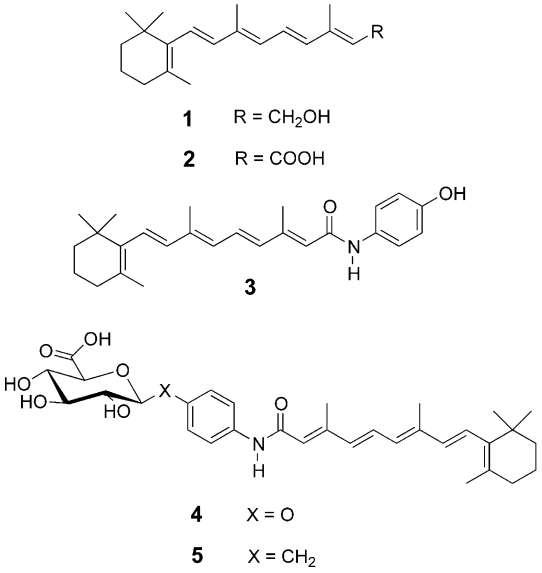

Figure 1. Natural and synthetic retinoids.

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Table 1. Effects of retinoid glucuronides on DMBA-induced rat mammary tumor development[a,b]

Dietary supplement[c]	Mean tumor latency (days)	Rats with tumors (%)	Mean number of tumors/rat
Control diet	40	79	1.43±0.29
4-HPROG (**4**)	48	57	0.71±0.19[e]
4-HPRCG (**5**)	55[d,e]	27	0.36±0.20[e]

[a]Adapted from ref 6.
[b]15 rats/group.
[c]Retinoid dose of 2 mmol/kg diet.
[d]Underestimate of mean latency since not all rats developed tumors.
[e]$P<0.05$ versus control.

Table 2. Retinoid binding to RARs[a]

Retinoid	RAR (K_i, nM)		
	α	β	γ
Retinoic acid (**2**)	0.4	0.5	1.4
4-HPR (**3**)	>2200	>2800	>6000
4-HPROG (**4**)	>2200	>2800	>6000
4-HPRCG (**5**)	>2200	>2800	ND[b]

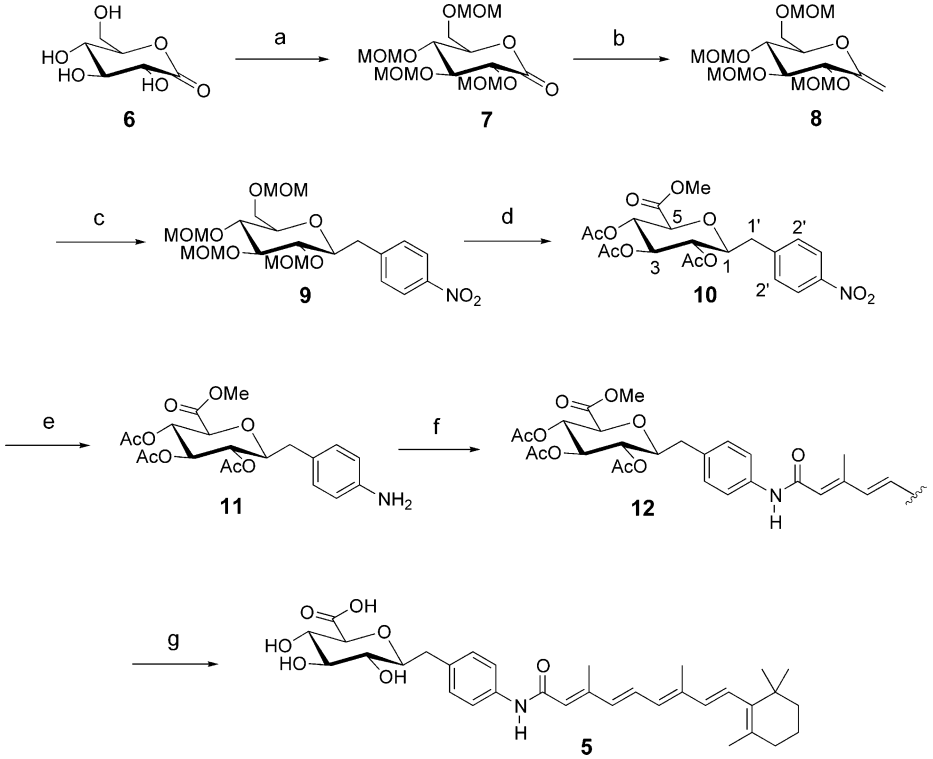
[a]Adapted from ref 6.
[b]ND, not determined.

carcinogen-induced rat mammary tumor model. Furthermore, much like **3** and **4**, **5** shows very low affinity for the nuclear retinoic acid receptors (RARs), thought to mediate most of the actions of retinoids (Table 2). This raises the question about the mode of action of these synthetic retinoids.

C-Glycosides are stable, conformationally similar analogues of the *O*-glycosides and are important compounds that can be used to study the function of carbohydrates in cellular processes or serve as stable metabolite mimics. Efficient synthetic routes to the 1-position *C*-glycosides have become of increasing interest and these usually involve a carbon–carbon bond forming reaction with the anomeric carbon. Many approaches have been utilized including electrophilic and nucleophilic substitution reactions, transition metal mediated glycosidations, anomeric radical reactions, cycloadditions and rearrangements.[7,8] One important electrophilic substitution reaction is the olefination of sugar lactones. The resultant exoanomeric methylene sugars can serve as *C*-glycoside precursors.[9]

The previous syntheses[5,10] of **5** did just allow the generation of modest quantities required for chemopreventative studies.[6] However, improvements in the synthetic route were needed to facilitate further studies. Starting with a fairly expensive sugar (~$20/g), the target was made in an overall yield of 2.2% over 13 steps. The key step involved reaction of a benzyl Grignard reagent with a bromosugar to form the anomeric carbon–carbon bond, which was followed by nitration of the aromatic ring, resulting in a mixture of nitro regioisomers. We now wish to report a much more efficient and less expensive synthesis of the title compound.

The current approach (Scheme 1) to the *C*-glucuronide of interest was adapted from the method of Johnson



Scheme 1. Reagents and conditions: (a) MOMCl, $(i\text{-Pr})_2NEt$, Bu_4NI, CH_2Cl_2, 48 h, 83%; (b) $Cp_2Ti(CH_3)_2$, $PhCH_3$, 70 °C, 18 h, 87%; (c) (i) 9-BBN-H, THF, reflux, 6 h; (ii) $PdCl_2$ (dppf), 3 M K_3PO_4, DMF, 1-bromo-4-nitrobenzene, 18 h, 54%; (d) (i) 6 N HCl, MeOH, 18 h; (ii) TEMPO, NaOCl, KBr, $NaHCO_3$, 2 h; (iii) HCl (g), MeOH, 40 °C, 5 h; (iv) Ac_2O, pyridine, DMAP, 18 h, 84%; (e) Pd/C, H_2, 40 psi, EtOAc, 4 h, 98%; (f) retinoic acid, $SOCl_2$, pyridine, 4 h, 86%; (g) (i) K_2CO_3, MeOH, 18 h; (ii) 5 N KOH, MeOH, 18 h, 86%.

and co-workers.[11,12] Starting with readily available δ-D-gluconolactone (~$0.70/g), hydroxyl group protection was accomplished with chloromethyl methyl ether and diisopropylethylamine to give **7**. Petasis reagent (Cp_2TiMe_2)[13,14] was prepared and used to olefinate the lactone to give the enol ether **8** in good yield. The exocyclic olefin was then hydroborated with 9-BBN-H and followed by a Suzuki coupling reaction with 1-bromo-4-nitrobenzene to give the β-arylmethyl-*C*-glycoside **9** in modest yield. Efforts were made to improve this coupling yield including changes in reaction temperature, reagent stoichiometry, and the bases employed; however, the original conditions[12] were found to be optimal. In order to obtain the glucuronide, the MOM groups were cleaved by acid and then the primary alcohol at the 6-position was selectively oxidized to the carboxylic acid using 2,2,6,6-tetramethyl-1-piperidinyloxy free radical (TEMPO).[10,15] Methylation of the carboxylic acid using HCl gas in methanol and acetylation of the remaining alcohols afforded the novel key intermediate, the protected *C*-benzyl-glucuronide **10**, in good yield over four steps.[16] The stereochemical outcome of the hydroboration has previously been shown to proceed to give the β-isomer exclusively.[9,12] This was further confirmed to also be the case here with intermediate **10** via NOE experiments.[17] Reduction of the nitro group was facile with hydrogen over palladium catalyst to give aniline **11**. To couple the retinoid to the sugar derivative, retinoyl chloride was generated, from the treatment of retinoic acid with thionyl chloride, and reacted with the aniline **11** to yield the protected retinoid conjugate **12** in good yield. Mild cleavage of the acetates was followed by saponification of the methyl ester to give **5** in 24% yield over 13 steps. Characterization of the final product gave data identical in all respects to the previously made material.[5]

To further justify a full chemotherapeutic study of **5**, a pilot study of the relative antitumor activity was undertaken using previously described methods.[4,18] Female rats treated ca. 50 days earlier with dimethylbenz[*a*]anthracene (DMBA) were fed for 10 days with the treatment retinoid. Each treatment group consisted of three tumor-bearing rats, which were killed after the 10 days of feeding. Each tumor on each rat was measured to estimate the mean tumor volume per group. Table 3 shows the results of the pilot study, which indicate that **5** is effective at reducing tumor volume and therefore warrants chemotherapeutic study of longer duration and with a larger treatment group size.

Table 3. Effect of retinoid treatment on DMBA-induced rat mammary tumor volume[a]

Dietary supplement[b]	Initial tumor volume[c]	Final tumor volume[c]	% Change
Control	0.25±0.12	0.56±0.28	+224[d]
Retinoic acid (**2**)	0.94±0.23	0.56±0.13	−40[e]
4-HPR (**3**)	0.16±0.05	0.12±0.03	−25[f]
4-HPRCG (**5**)	0.29±0.17	0.23±0.12	−21[g]

[a]At 10 days of feeding diet to three rats.
[b]Retinoid dose of 2 mmol/kg diet.
[c]Values = mean ±SE (cm^3).
[d]Two new tumors formed during the 10 days, no regression in 14/14 tumors.
[e]No new tumors, partial regression in 10/10 tumors.
[f]Two new tumors, partial regression in 10/14 tumors.
[g]No new tumors, partial regression in 6/8 tumors.

In summary, the improved synthesis uses considerably less expensive starting material, results in a ten times higher yield of **5**, and avoids the tedious separation of nitration regioisomers. Preliminary results from the pilot study suggest that **5** does show chemotherapeutic activity. The current synthetic route will facilitate the generation of quantities sufficient for further extensive chemotherapeutic animal studies and for gaining insight into the mechanism of action of this important retinoid analogue. Also, it appears that the exoanomeric methylene sugar **8** is a good precursor that can provide ready access to *C*-glycosides and *C*-glucuronides of other drugs or natural products.

Acknowledgements

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16. **(Methyl 2,3,4-tri-*O*-acetyl-β-D-glucopyranosyluronate)-4-nitrophenylmethane (10)**. Oil **9** (4.14 g, 8.71 mmol) was dissolved in 120 mL of methanol and placed in an rt water bath. HCl (6 N, 80 mL) was added and the solution stirred for 18 h. The mixture was concentrated to a paste. Saturated $NaHCO_3$ solution (150 mL) was added along with KBr (200 mg) and TEMPO (200 mg). Clorox® (150 mL) was then added dropwise while monitoring the pH of the reaction. NaOH (aq) was used to maintain the pH between 9 and 10. After the addition of the Clorox® was complete, the reaction was stirred for 2 h. The contents were washed (3×) with CH_2Cl_2, the aqueous layer acidified to pH 2 and concentrated to dryness. The remaining solid was triturated with methanol, which was then transferred to a two-neck flask. Next, HCl gas was bubbled through the solution warmed to 40 °C for 5 h. The reaction mixture was concentrated and placed in an rt water bath. Acetic anhydride (100 mL), pyridine (100 mL), and DMAP (75 mg) were added and the mixture stirred for 18 h. The contents were diluted with water and extracted (3×) with ethyl acetate. The organic layers were washed with water, brine, and dried ($MgSO_4$). This suspension was filtered and concentrated to a brown gum, dissolved in hot ethanol, and upon cooling gave 3.33 g (84%) of white crystals, mp 133–134 °C. IR (cm^{-1}) 3078 (w), 2952 (w), 2850 (w), 1758 (s), 1603 (m), 1521 (s), 1346 (s), 1219 (s), 1036 (m); ^1H NMR (400 MHz, DMK-d_6) δ 1.94 (s, 3H), 1.96 (s, 3H), 2.00 (s, 3H), 2.94 (dd, 1H, $J = 14.7$, 8.6 Hz), 3.12 (dd, 1H, $J = 14.7$, 3.1 Hz), 3.65 (s, 3H), 4.07 (dt, 1H, $J = 8.6$, 3.1 Hz), 4.21 (d, 1H, $J = 9.8$ Hz), 4.92 (t, 1H, $J = 9.8$ Hz), 5.06 (t, 1H, $J = 9.8$ Hz), 5.31 (t, 1H, $J = 9.8$ Hz), 7.56 (d, 2H, $J = 8.7$ Hz), 8.15 (d, 2H, $J = 8.7$ Hz); ^{13}C NMR (100 MHz, DMK-d_6) δ 20.38, 20.47, 20.62, 37.93, 52.72, 70.47, 72.33, 73.92, 76.30, 77.91, 123.92, 131.55, 146.37, 147.74, 168.26, 169.89, 170.29; HRMS (ES) calcd for $C_{20}H_{23}NO_{11}$ (M + Na) 476.1169, found 476.1175.

17. Three steady-state NOE experiments were performed on intermediate **10**. Irradiation at H1 (see Scheme 1 for numbering) resulted in enhancement of H5 (12%), H3 (9%), H2′ (5%) and H1′ (4%) at 3.12 ppm. Irradiation at H3 resulted in enhancement of H5 (7%) and H1 (6%). Irradiation at H5 resulted in enhancement of H1 (10%) and H3 (8%).

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