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N-LINKED GLYCOSIDE/GLUCURONIDE CONJUGATES OF RETINOIDS: ACITRETIN

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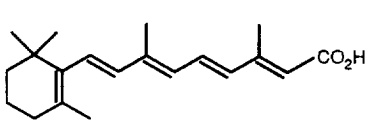
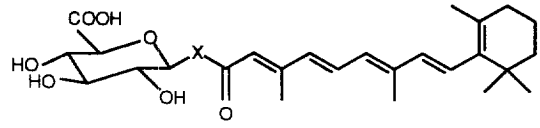
Abstract: Glucuronide conjugates of retinoids have been found to be active metabolites of the parent molecules. However, *O*-acylglucuronide retinoids are liable to hydrolysis and retinoid ring oxidation. We have prepared stable *N*-linked glycosides of acitretin in efforts to overcome these problems. The synthesis and preliminary biological studies of the analogs are reported. © 1997 Elsevier Science Ltd.

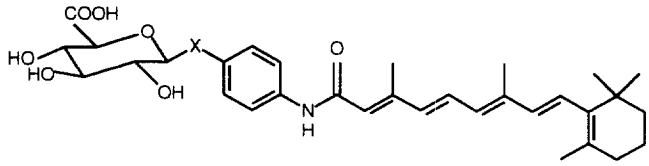
Retinoic acid (**1**) and its analogs (the retinoids) have emerged as important therapeutic agents for the treatment of various skin diseases (e.g., acne and psoriasis) and as potential cancer chemopreventive agents.[1] Certain metabolites of retinoic acid have also been observed to be active, in particular the glucuronide conjugate of retinoic acid (**2**).[2] However, the utility of this compound is limited by its lability toward chemical and enzymatic hydrolysis. Other retinoid glucuronides have also been synthesized and biologically evaluated, for example *N*-(4-hydroxyphenyl)retinamide-*O*-glucuronide (4-HPROG; **3**).[3,4]



1



2 X = O
6 X = CH_2
7 X = NH

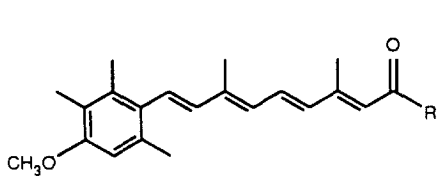


3 X = O
4 X = -
5 X = CH_2

It has been demonstrated that 4-HPROG, an *O*-glucuronide of the synthetic retinoid *N*-(4-hydroxyphenyl)retinamide, has advantages as a mammary tumor chemopreventive agent.[5] While 4-HPROG also has good potential in the treatment of ocular surface diseases,[6] it is still hydrolytically unstable in the solution vehicle used for administration (unpublished results). It has also been found that the cyclohexenyl ring of 4-HPROG suffers

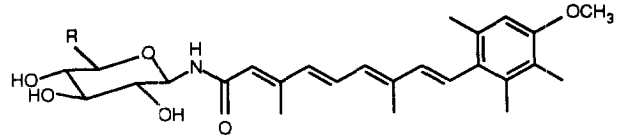
various oxidations in this solution (unpublished results). The natural retinoids are also susceptible to these oxidative processes that generally reduce activity.[7] Previous work in our lab was thus targeted toward more stable *C*-linked analogs of 4-HPROG (**4,5**)[8] and retinoyl-β-glucuronide (**6**).[9] The chemical and enzymatic stability of these compounds was offset by the difficulty and expense of the syntheses. In view of these observations, *N*-linked retinoyl-β-glucuronide analogs such as **7** were synthesized and were found to be both active and relatively stable to hydrolysis.[10] However, the possibility of oxidative inactivation in ophthalmic administration vehicles still remained.

Acitretin (**8**) is the active metabolite of etretinate (**9**), a retinoid introduced for the treatment of psoriasis.[11] Replacement of the labile trimethyl cyclohexenyl ring of retinoic acid by the trimethyl methoxyphenyl ring of etretinate gives this compound greatly increased oxidative stability,[12] but it is virtually insoluble in aqueous and physiological media. Therefore, the synthesis of the acitretinamido glycosides (**10** and **11**) to obtain compounds with increased solubility as well as stability toward hydrolysis and oxidation was targeted.



8 R = OH

9 R = OCH_2CH_3



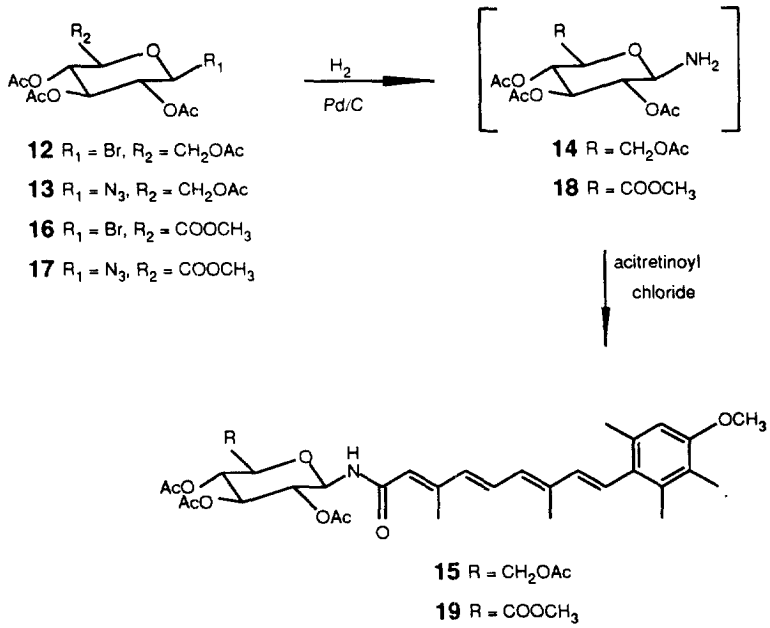
10 R = CH_2OH

11 R = COOH

The strategy for the synthesis of **10** is shown in Scheme 1. The known azido sugar **13** was prepared from the known acetobromoglucose **12** by treatment with two equivalents of sodium azide. Reduction of **13** by catalytic hydrogenation at -15 °C to form the protected aminoglucose **14** was immediately followed by reaction with acitretinoyl chloride prepared in situ to give the acitretinamide **15**. The reaction was routinely conducted at -78 °C over a period of 65 h in THF and was followed by treatment of crude product mixture with diazomethane to facilitate chromatographic separation of the acitretinamide from unreacted methyl acitretin. Chromatographic purification (SiO_2; EtOAc:hexanes,1:1) gave acitretinamide **15** in 62% overall yield. Deprotection of the hydroxyl groups (K_2CO_3/MeOH) gave a 92% yield of **10** which was further purified by chromatography (RP-18; MeOH:H_2 O,4:1).[13]

The synthesis of **11** follows a similar route and is shown in Scheme 1. Displacement of bromide from the known compound **16** with two equivalents of sodium azide gave methyl(2,3,4-tri-O-acetyl-β-glucopyranuronosyl)azide **17** in 76% yield. Reduction of **17** at -15 °C by catalytic hydrogenation gave the amine **18**, which was then reacted with acitretinoyl chloride at -78 °C followed by treatment with diazomethane and chromatography to give the protected glucuronamido acitretin **11** in 60% overall yield. Amide **11** obtained by saponification of **19** (K_2CO_3/MeOH followed by KOH/MeOH/H_2O), was formed at best as an inseparable 5:1 mixture of β:α anomers in 65% yield followed by further RP-18 chromatography as for **10**.[14] This observation of anomer formation in the glucuronamide preparation is consistent with our earlier results with **7** and its glucoside analog.[10] Further reduction of hydrogenation temperature in order to improve the anomeric ratio resulted in failure of the

reduction to proceed.



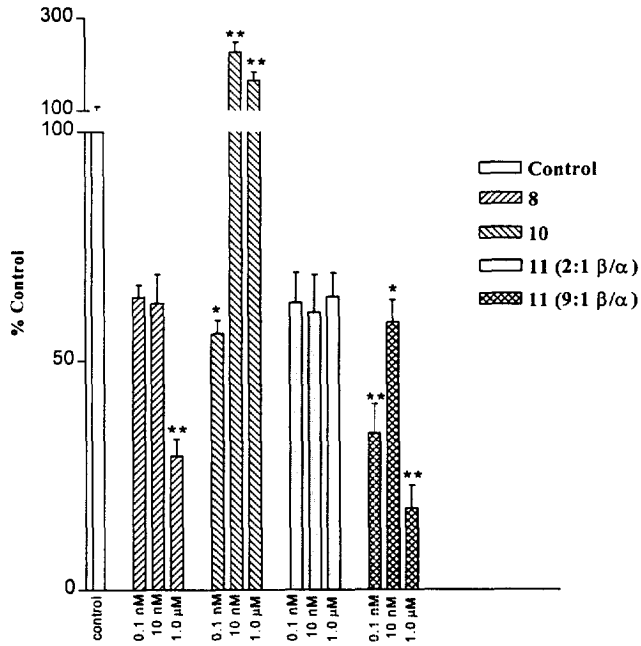
Scheme 1. Synthesis of acitretinamido glycosides.

While we found amidoglucuronides are not β-glucuronidase substrates,[10] to gauge the relative hydrolytic stability of these conjugates samples of 4-HPROG and **11** were separately dissolved in 1:1 methanol:0.01 N HCl and aliquots of these solutions were removed periodically and analyzed by high performance liquid chromatography for hydrolysis or degradation of the retinoid. After 8 h of sampling, approximately 47% of the *O*-glucuronide had degraded while only 20% of the *N*-glucuronide had, suggesting these amides have the desired prolonged hydrolytic stability. As expected, **11** also is substantially more soluble than acitretin in this medium.

It is generally believed that the actions of retinoids are mediated by association with a family of nuclear receptor proteins which regulate critical gene expression.[15] However, we have previously found that none of our retinoid glucuronides have significant affinity for retinoid receptors (unpublished results for **6** and see refs. 8 and 10). Furthermore, acitretin itself is known not to bind to the retinoic acid receptors.[11] Fortunately, antiproliferative activity toward the growth of the human mammary tumor cell line MCF-7 does appear to uncover the biological potential of these compounds.[4,8] Thus compounds **10** and **11** were compared versus acitretin (**8**) for their antiproliferative activity in MCF-7 cells using previously described protocols.[16] As shown in Figure 1, glycosamides **10** and **11** show surprisingly different effects on MCF-7 cell growth. Parent **8** shows measurable, dose-dependent inhibition of cell growth while glucosamide **10** shows some antiproliferative effects at the low dose evaluated (0.1 nM) but at higher doses it is strongly proliferative, an effect we have not previously seen for retinoids. On the other

hand, glucuronamide **11**, when employed as a 2:1, β:α anomer mixture (obtained by conducting reduction and acitretinoylation at near room temperature), shows moderate antiproliferative effect at all doses while a 9:1, β:α mixture (obtained by partial purification of the 5:1 product) shows concentration-dependent antiproliferative effects comparable to or exceeding those of **8**. Previously, simple ethylamide analogs of **8** have shown activity as inhibitors of carcinogen-induced skin papilloma formation[17,18] and thus the antiproliferative effects of **11** are not surprising.

Figure 1. Effects of Acitretin (**8**) and the Acitretinamido Glycosides (**10** and **11**) on MCF-7 Cell Growth.*



*MCF-7 cells (3 x 10^4 cells/well) were incubated for 4 days at 37 °C with vehicle (□), acitretin (□), **10** (□), **11** (2:1,β:α)(□), **11** (9:1,β:α)(□) at concentrations of 0.1 nM, 10 nM, and 1.0 μM; DNA synthesis as an index of growth was determined by measuring [^{3}H]-thymidine incorporation; n=6, *$p<0.05$, **$p<0.001$.

At present, we have no additional information with which to rationalize the surprising proliferative effect of **10** although these same results were obtained in a second extensive set of experiments with **10**. Nonetheless, we have successfully developed methods for the synthesis of glycosamides **10** and **11**. Glucuronamide **11** shows improved hydrolytic stability relative to 4-HPROG and shows good antiproliferative activity toward MCF-7 cells. Moreover, the enhanced activity of a mixture of **11** containing a much larger fraction of β-anomer implies a structurally specific effect inspite of the fact that we find that retinoid glucuronides do not bind to retinoid receptors. It remains to be demonstrated whether the desirable properties of **11** translate into the necessary stability for application in ocular surface diseases.

Acknowledgment. Partial Financial support of this work by Alcon Laboratories, Fort Worth, TX is gratefully acknowledged.

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13. Selected properties for **10**: ^{1}H NMR ($(CD_3)_2CO$) δ 2.14 (s, 3, CH_3), 2.18 (s, 3, CH_3), 2.28 (s, 3, CH_3), 2.32 (s, 3, CH_3), 2.43 (s, 3, CH_3), 3.3-3.7 (brm, 5, pyranose), 3.82 (s, 3, OCH_3), 4.2 (brt, 1, pyranose), 5.06 (t, 1, pyranose), 5.96 (s, 1, retinoid H-14), 6.35 (brm, 3, vinyls), 6.71 (s,1, Ar), 6.77 (d, 1, vinyl), 7.16 (dd,1, retinoid H-11), 7.68 (brd, 1, NH); RP-HPLC (CH_3OH:H_2O, 82:18, flow rate 0.75 mL/min) t_R = 12.2 min; FABMS, *m/e* (relative intensity) 510 (M+Na, 52.8), 488 (M+H, 39.1), 217 (100).

14. Selected properties for **11**: ^{1}H NMR ($(CD_3)_2CO$) δ 2.15 (s, 3, CH_3), 2.18 (s, 3, CH_3), 2.26 (s, 3, CH_3), 2.34 (s, 3, CH_3), 2.44 (s, 3, CH_3), 3.3-3.87 (m, 4, pyranose), 3.84 (s, CH_3, OCH_3), 5.14 (t, 1, pyranose), 5.94 (s, 1, retinoid H-14), 6.44 (brm, 3, vinyls), 6.71 (s, 1, Ar), 6.76 (d, 1, vinyl), 7.14 (dd, 1, retinoid H-11), 7.64 (brd, 0.17, α-NH), 7.78 (brd, 0.83, β-NH); RP-HPLC(CH_3OH:H_2O, 77:23, both with 10 mM NH_4OAc, flow rate 0.75 mL/min) t_R = 10 min; FABMS *m/e* (relative intensity) 524 (M+Na, 22.3), 502 (M+1, 25.7), 163 (100).

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