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Total Synthesis of Alvaradoins E and F, Uveoside, and 10-epi-Uveoside

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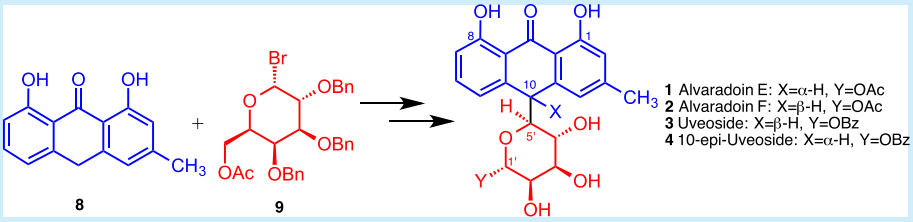
Supporting Information



ABSTRACT: Concise total syntheses of the anthracenone *C*-glycosides alvaradoins E and F, uveoside, and 10-epi-uveoside (**1**–**4**) have been accomplished from chrysophanic acid **8** and bromosugar **9**. Key steps in the syntheses include the DBU-induced coupling of **8** and **9** to produce *β*-*C*-glycoside **11**, and a $Pb(OAc)_4$-mediated Kochi reaction to introduce the C-1′ oxygen atom of the natural products. Isothermal titration calorimetry and fluorescence binding studies reveal that compounds **1** and **2** have good affinity for the plasma protein HSA.

The anthracenone *C*-glycosides alvaradoin E (**1**, Figure 1) and alvaradoin F (**2**, Figure 1) were isolated from the

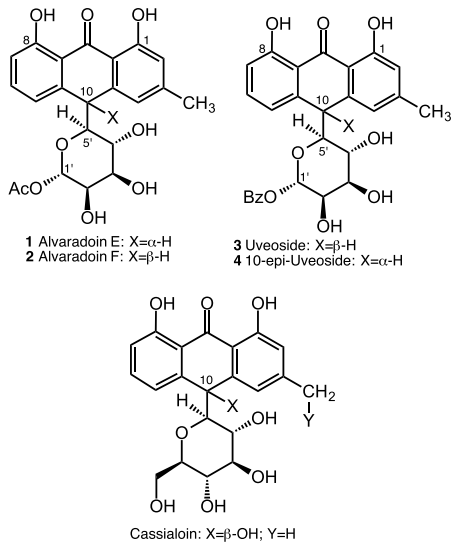


Figure 1. Chemical structures of alvaradoins E and F, uveoside, and 10-epi-uveoside.

leaves of the tropical tree *Alvaradoa haitiensis* in 2005 and 2007.[1] Both substances exhibited pronounced cytotoxicities toward human oral epidermoid carcinoma (KB) cell lines (EC_{50}(**1**) = 0.050 μM; EC_{50}(**2**) = 0.065 μM) among others; furthermore, alvaradoin E was demonstrated to induce apoptosis of cultured LNCaP cells. These results prompted the investigators to evaluate the *in vivo* activity of **1** and **2** in the P388 murine lymphocytic leukemia model. Alvaradoin E showed antileukemic activity (125% T/C) at a dose of 0.2 mg/kg per injection when administered intraperitoneally. Uveoside (**3**, Figure 1) was isolated in 1998 from the chloroform extract of the roots of *Picramnia antidesma* by Hernandez-Medel and co-workers;[2] further work on the root bark of *Picramnia antidesma* by the same research group resulted in the isolation (in 2002) of 10-epi-uveoside (**4**, Figure 1), a substance also displaying elevated cytotoxicity toward KB cells.[3] Given the heightened biological profile of this family of *C*-glycoside natural products, together with the fact that there is only a single previous total synthesis of a related anthrone *C*-glycoside,[4] we decided to undertake a synthetic study of compounds **1**–**4**.

One of the synthetic challenges anticipated en route to compounds **1**–**4** was the installation of the acid-labile anomeric C-1′ acetate and benzoate esters, a structural feature absent in the previously prepared anthrone glycoside cassialoin.[4] We envisioned that a Hunsdiecker-type reaction[5] on a carboxylic acid precursor would allow us to install the C-1′ oxygen atom in the form of a more stable acetal moiety, which could be transformed into the requisite C-1′ ester in the penultimate step of the synthesis. The realization of this plan is detailed in the present Letter.

Over the last two decades, there have been numerous advances in *C*-glycoside synthesis.[6] Transition-metal catalyzed cross-coupling[7] and metathesis[8] reactions, [3,3]-sigmatropic

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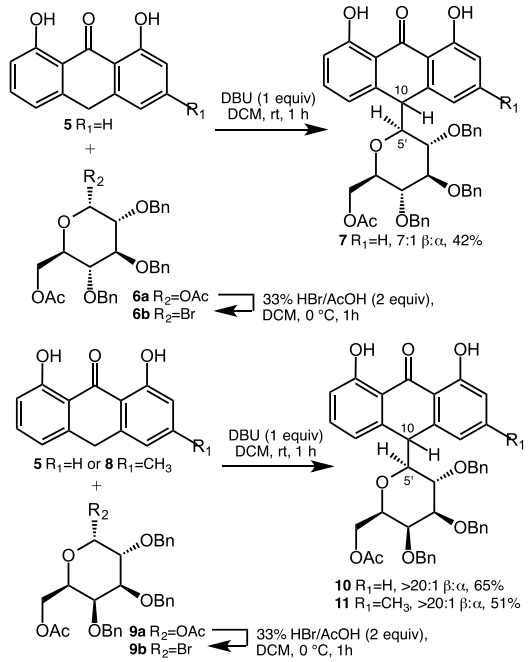
rearrangements,[9] and radical-olefin couplings[10] have been used to prepare arene- and C(sp^3)-linked *C*-glycosides. Since it is known that anthracenones undergo base-induced alkylation reactions at C-10,[11] we decided to attempt a direct *C*-glycoside synthesis by combining anthracenones with C-1 bromosugars under basic conditions. Our initial studies employed anthralin (**5**, Scheme 1) as the nucleophile and bromoglucoside **6b**[12] as

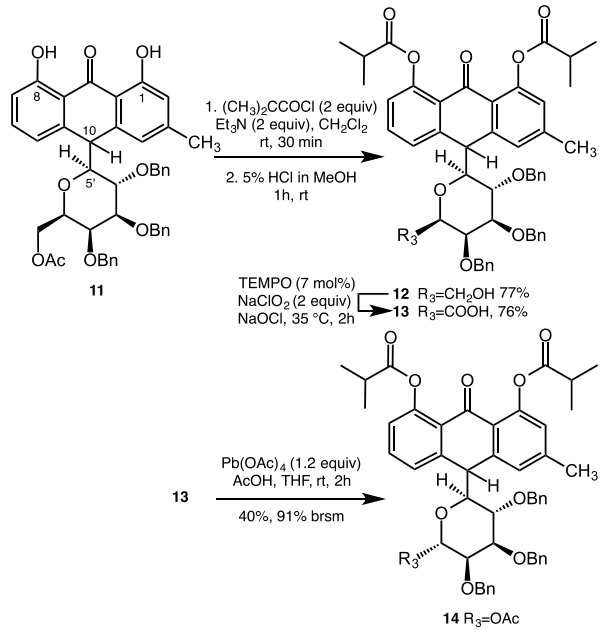
Scheme 1. *C*-Glycosylation of Anthralin (5) and Chrysophanol (8)



the carbohydrate electrophile for the substitution reaction. Treatment of an equimolar CH_2Cl_2 solution of **6b** and **5** with 1 equiv of DBU resulted in a rapid disappearance of **6b**, along with the formation of *C*-glycoside **7** on TLC. Upon isolation, it was found that compound **7** was formed in 42% yield as a 7:1 mixture of β and α stereoisomers at C-5′. Encouraged by this result, we prepared the bromogalactoside **9b**[13] by HBr/AcOH treatment of 1,6-di-*O*-acetyl-2,3,4-tribenzyl-galactose **9a**. Combination of **9b** with **5** in the presence of one equivalent of DBU gave rise to a 65% yield (from **6a**) of *C*-glycoside **10** as a single β-stereoisomer (>20:1 β:α) at C-5′. Analogously, treatment of a mixture of **9b** and chrysophanol (**8**) with DBU provided a 51% yield (from **9a**) of *C*-glycoside **11**, again with the selective production (>20:1 β:α) of the C-5′ β stereoisomer in excess; in addition, a 1:1 mixture of diastereomers was formed at C-10.[14] TLC also showed the formation of oxidized aromatics (anthraquinones) as well as hydrolyzed carbohydrates (**6** or **9**, R_2 = OH), accounting for the balance of the material in these reactions. All attempts to thwart their production by performing the reaction under vigorously anhydrous and anaerobic conditions led to no significant improvement in the yields of **7**, **10**, or **11** obtained.

In order to manipulate the hydroxymethyl group of the carbohydrate moiety to achieve installation of the C-1′ oxygen atom, it was necessary to protect the C-1 and C-8 hydroxyl groups as sterically bulky esters that would be resistant to conditions for hydrolysis of the acetate ester of **11**. While the bis-pivaloate derivative initially showed promise in this direction, late-stage removal of these esters under acidic conditions proved to be problematic (*vide infra*) and ultimately motivated a switch to the less bulky isobutyrate esters, which were installed by treatment of **11** with isobutyryl chloride and triethylamine in CH_2Cl_2 (Scheme 2). Meth-

Scheme 2. Transformation of Glycoside 11 into Acetate 14



analysis of the primary acetate was then achieved by treatment with 5% HCl in methanol, affording the primary alcohol **12** in 77% overall yield. TEMPO-catalyzed oxidation to the carboxylic acid in 76% yield was then achieved under Zhao's conditions, affording **13**.[15]

After an extensive survey of conditions for achieving a Hunsdiecker-type conversion[5] of acid **13** to the corresponding glycosyl halide, we discovered that Kochi's protocol[16a] involving treatment of **13** with lead tetraacetate in acetic acid and THF leads to a clean and stereoselective conversion to α-acetate ester **14**,[16b] albeit in moderate yields (40%). However, the starting material could be efficiently recovered in good yields (51%) from this reaction and recycled to increase overall throughput to compound **14**.

With acetate **14** in hand, we next attempted hydrolysis of the isobutyrate and acetate esters. Exposure of **14** to basic conditions (NaOMe in MeOH; cat. NaCN, MeOH; RNH_2, MeOH) resulted in substrate decomposition with the production of anthraquinone byproducts; in addition, the recovered *C*-glycosides possessed a mixture of stereoisomers at C-5′ (Scheme 3). Exposure of **14** to acidic conditions (5−20% HCl in MeOH) instead resulted in elimination of the C-1′ acetate group and the formation of alkene-containing byproducts. After extensive experimentation, it was found that solvolysis of the acetate with allyl alcohol could be achieved in the presence of $BF_3{\cdot}OEt_2$; subsequent treatment of the resulting allyl glycoside with 20% HCl in MeOH at 40 °C gave a 73% overall yield of diol **15**. Exposure of **15** to 10 mol % $PdCl_2$ in MeOH and THF then resulted in the clean formation of α-configured hemiacetal **16** in 86% yield.[17]

For the preparation of alvaradoins E and F, installation of the acetate moiety at C-1′ was required. Compound **16** was

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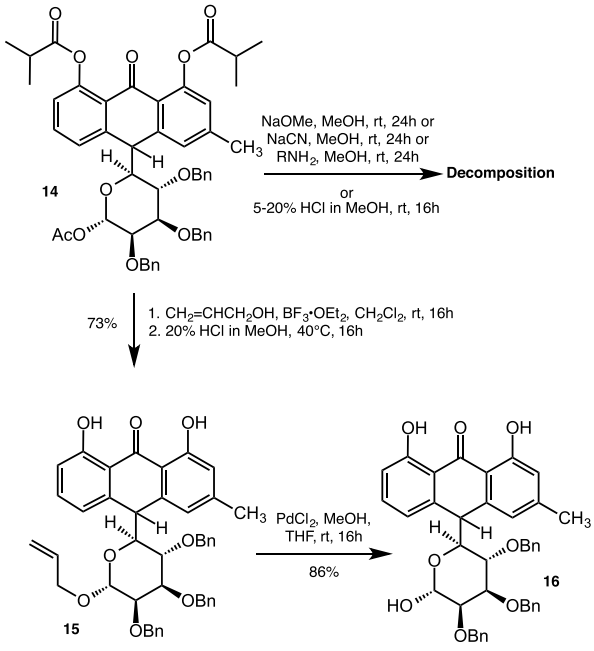
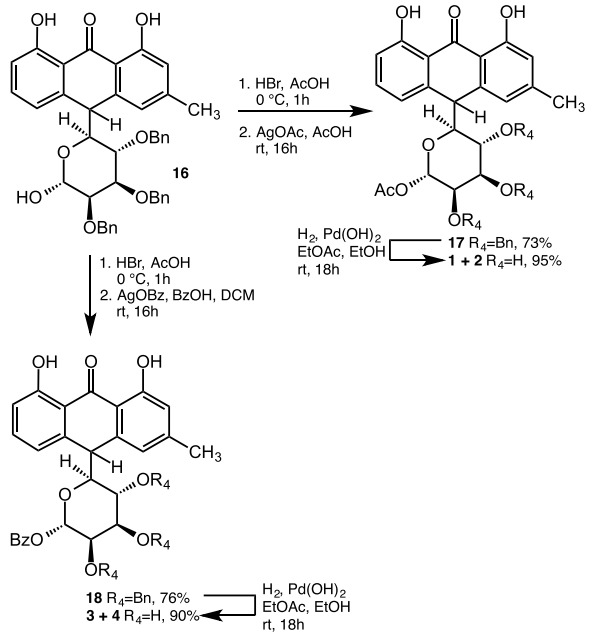
Scheme 3. Preparation of Hemiacetal 16



exposed to HBr in acetic acid to form the C-1′ glycosyl bromide, which was immediately combined with silver acetate in acetic acid to provide α-acetate ester **17** in 73% yield (Scheme 4).[18] Hydrogenation of **17** over Pearlman's catalyst[19]

Scheme 4. Completion of the Syntheses of 1−4



then afforded a 95% yield of alvaradoins E and F as a 1:1 mixture of diastereomers, which could be separated by careful and repeated column chromatography (SiO_2, 98:2 → 94:6 $CHCl_3$/MeOH). NMR (^1H and ^{13}C),[29] mass spectroscopy (MS), and optical rotation data recorded for synthetic alvaradoins E and F were in accord with those reported for the natural compounds. Similarly, exposure of **16** to HBr in acetic acid, followed by treatment with silver benzoate and benzoic acid in CH_2Cl_2, gave rise to α-benzoate **18** in 76% yield. Hydrogenation then provided uveoside and 10-epi-uveoside in 90% yield as a 1:1 mixture of diastereomers, which could be separated by radial chromatography. Once again, NMR (^1H and ^{13}C), MS, and optical rotation data recorded for synthetic uveoside and 10-epi-uveoside were in accord with those reported for the natural compounds.[29]

Given that numerous *C*-aryl glycosides are known to form strong complexes with duplex nucleic acids,[20] we undertook DNA binding studies with synthetic compounds **1**−**4** (see Supporting Information). Thermal denaturation studies[21] with CT DNA were precluded by the fact that compounds **1**−**4** underwent decomposition in aqueous phosphate buffer solution (pH = 7.21) at elevated temperatures, as evidenced by UV spectroscopy. Therefore, utilizing fluorescence spectroscopy, we investigated the displacement of ethidium bromide (10 μM) from CT DNA (10 μM) by increasing concentrations of synthetic alvaradoins E and F, and found only a small effect on ethidium fluorescence emission intensity (at 590 nm) over the 0.01−100 μM concentration range of **1** and **2** (pH = 7.21, NaH_2PO_4/Na_2HPO_4 buffer); indeed, 85% of the initial fluorescence intensity of ethidium was measured at 50 μM of the ligands, and extrapolation of the data gave a C_{50} value of 240 μM. Similar results were obtained in ethidium displacement studies employing uveoside and 10-epi-uveoside (extrapolated C_{50} = 430 μM).[22] Under otherwise identical experimental conditions, positive control netropsin produced a significant decrease in ethidium emission fluorescence intensity with a directly measurable C_{50} value of 10 ± 2.5 μM. These results indicate that, despite structural similarities to the *C*-aryl glycoside family of natural products, the anthracenone *C*-glycosides associate only weakly with duplex nucleic acids.

In light of these data, we explored the possibility that this family of *C*-glycosides may form complexes with proteins; indeed, synthetic *C*-glycosides have been previously shown to associate with plant-derived carbohydrate binding proteins.[23] Titration of the abundantly available plasma protein human serum albumin[24] (HSA; N form at pH 7) with alvaradoins E and F showed a marked quenching of the Trp 214 fluorescence emission at 338 nm (λ_{ex} = 280 nm; K_{sv} = 2.79 ± 0.33 × 10^4 M^{-1}; K_b = 6.60 ± 1.42 × 10^4 M^{-1}; see Supporting Information).[25,26] A similar experiment performed with uveoside and 10-epi-uveoside revealed significantly less quenching of the Trp 214 fluorescence emission as compared to the alvaradoins and a notably weaker binding (K_{sv} = 6.11 ± 0.55 × 10^3 M^{-1}; K_b = 0.88 ± 0.36 × 10^3 M^{-1}). Isothermal titration calorimetry[27] (ITC) of HSA (57 μM) with alvaradoins E and F (500 μM solution, 25 °C, pH = 7.21, NaH_2PO_4/Na_2HPO_4 buffer) gave a binding constant K_b of 3.01 ± 0.58 × 10^4 M^{-1}, along with an enthalpy of binding, ΔH, of −11.7 ± 0.5 kcal/mol and an entropy of binding, ΔS, of −18.8 ± 2.2 cal/mol·K; similarly, ITC experiments with the uveosides showed only weak binding (K_b < 1 × 10^3 M^{-1}). HSA competition binding experiments employing warfarin (site I marker) or ibuprofen (site II marker) and the alvaradoins are currently underway to clarify which binding site (Sudlow site I or Sudlow site 2) on the protein is preferred by the anthacenone-*C*-glycosides.[28]

In summary, we have developed short total syntheses of the anthracenone-*C*-glycosides alvaradoins E and F, uveoside, and 10-epi-uveoside. Although these compounds display poor binding to duplex DNA, alvaradoins E and F show significant affinity for the plasma protein human serum albumin.

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■ ASSOCIATED CONTENT

Supporting Information

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Detailed experimental procedures including spectroscopic and analytical data (PDF)

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Notes

The authors declare no competing financial interest.

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(14) (a) The C-5′ proton resonance in the ^{1}H NMR spectrum of compound **10** ($CDCl_3$) at 3.50 ppm (doublet of doublets) displayed coupling constants of 1.6 and 9.4 Hz; the C-10 proton resonance of **10** at 3.69 ppm (doublet) displayed a coupling constant of 2.0 Hz. These data are indicative of the β-configuration at C-5′; see ref 16b and Procko, K. J.; Li, H.; Martin, S. F. *Org. Lett.* **2010**, *12*, 5632. (b) We attempted chromatographic separation (SiO_2) of the C-10 diastereomers of compound **11** (as well as later compounds **17** and **18**) but were unsuccessful. Since the diastereomers lead to different natural products, we proceeded with the mixtures until adequate chromatographic resolution could be achieved at the natural product stage, as indicated in the original isolation papers (ref 1).

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