Synthesis of 3,3′-Di-O-methyl Ardimerin and Exploration of Its DNA Binding Properties

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ABSTRACT: The 3,3′-di-O-methyl derivative (15) of the bis-C-aryl glycoside natural product ardimerin (1) has been synthesized in 11 steps from 2,3,4,6-tetrabenzylglucose (2) and 1,2,3-trimethoxybenzene (3). Key steps in the synthesis involve a Lewis acid mediated Friedel–Crafts type glycosylation and a Yamaguchi lactonization under Yonemitsu conditions. 3,3′-Di-O-methyl ardimerin aggregates in aqueous solutions at concentrations greater than 1 μM, and both UV and fluorescence binding studies indicate that 15 has a low affinity for duplex DNA.

Plants used in traditional Chinese medicine have yielded a wealth of chemical constituents with important biological activities.1 Ardimerin (1a, Figure 1), a dimeric lactone with radical scavenging activity, was isolated from Ardisia japonica by Ryu et al. in 2002.2 Subsequently, ardimerin digallate (1b) was isolated from the same species, along with the flavonoid quercitrin and the terpenoids friedelin, epifriedelinol, baurenol, and baurenyl acetate.3 The digallate derivative of ardimerin was shown to inhibit HIV-1 and HIV-2 RNase H in vitro with IC50 values of 1.5 and 1.1 μM, respectively.

C-Aryl glycosides are an important class of naturally occurring compounds endowed with remarkable stability toward acid and enzymatic hydrolysis;4 this affords them a sufficient intracellular lifetime to allow trafficking to the nucleus, where they bind DNA to form stable complexes.5 The bis-C-aryl glycoside altromycin B has been shown by NMR studies to associate with DNA via a helix-threading mode of binding, with carbohydrate moieties positioned in opposite grooves of the duplex.5e Given that ardimerin is a symmetrical bis-C-aryl glycoside, we envisioned that, despite the non-planarity of its aglycone,6 it might also be capable of the recognition of nucleic acids by a threading mode of intercalation, with the glucosyl substituents positioned in both the major and minor grooves of DNA. To assess this possibility, we decided to undertake its synthesis and investigate its DNA binding properties.

We envisioned (Figure 2) that the C–C linkage between carbohydrate and aromatic moieties could be fashioned by a Lewis acid mediated Friedel–Crafts type glycosylation reaction between protected glucose 2 and 1,2,3-trimethoxybenzene (3).5c Aromatic ring carbonylation and selective ortho methoxy group deprotection would then provide 7, a crucial substrate for esterification with the derived carboxylic acid monomer 8. Oxidation, macrolactonization, and protecting group removal would then provide the natural product.

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The carbohydrate coupling partner (2) required for the C-glycosylation reaction may be prepared in 72% overall yield from dextrose as previously described. Treatment of 2a with a 1:1 solution of trifluoroacetic anhydride and CH₂Cl₂ for 30 min, followed by evaporation and combination with commercially available 1,2,3-trimethoxybenzene (1.5 equiv) and BF₃·OEt₂ (1.1 equiv) in CH₂Cl₂ at room temperature for 30 min, afforded coupled product 4 (>20:1 β/α at C1) in 62% yield (Scheme 1). Interestingly, 2-O-benzyl-1,3-dimethoxybenzene

(Scheme 1. Synthesis of C-Glycoside Monomers 7 and 8)

attached to the aldehyde of 7 (corresponding to the C3/C3’ position of the natural product) by extended exposure to AlCl₃/NaI (80 °C) resulted in substrate decomposition.

With both 7 and 8 in hand, we set out to identify conditions for the construction of the eight-membered diolide (Scheme 2).

(Scheme 2. Model Study: Synthesis of Diolide 12)

Attempts to directly dimerize the model compound 2,3-dimethoxysalicylic acid (SOCl₂, dilute toluene, reflux; DCC or EDC, DCM, rt; TFAA, DCM, 0 °C) failed, producing only uncharacterized oligomers in low yields. In line with literature precedent, coupling of the known acid 9a and aldehyde 10a was accomplished via direct addition of sodium alkoxide 11 to reuxing thionyl chloride leads directly to eight-membered diolides of the type 12, arising from acid chloride formation, in situ benzyl ether cleavage, and macrocyclization of the hydroxy acid chloride; however, we observed that reuxing 11 in SOCl₂ for 3 h led only to the intermediate hydroxy acid chloride, which was sufficiently stable to survive aqueous reaction workup. Instead, the acid chloride intermediate was diluted in benzene or toluene (0.01 M) and treated with 3 equiv of DMAP and stirred at room temperature overnight. In this way, diolide 12 could be secured in 50–60% yield.

With a method to prepare the diolide core of arderimerin in hand, we proceeded to explore the similar union of aldehyde 7 and carboxylic acid 8 (Scheme 3). Treatment of compound 8 with oxalyl chloride in the presence of catalytic quantities of DMF gave rise to the corresponding acid chloride, which was added to the potassium salt of 7 in THF at 0 °C; the resultant crude aldehyde 13a was immediately oxidized under Pinnick–Lindgren–Kraus conditions to afford the stable carboxylic acid 13b. Refluxing 13b in SOCl₂ for 3 h gave rise to the corresponding benzyloxy acid chloride and not the desired hydroxyl acid chloride; further heating in SOCl₂ overnight led only to extensive substrate decomposition. To effect removal of the benzylic ether before conversion to the acid chloride, compound 13b was treated with a 1:1 mixture of TFA and toluene at room temperature for 5 min. The intermediate

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hydroxy acid was then treated with oxalyl chloride (cat. DMF, CH₂Cl₂), and a dilute solution (0.1 M) of the resulting acid chloride was then added dropwise to a refluxing solution of DMAP (3 equiv) in benzene. However, this condition gave rise to the hydroxycac acid monomer resulting from DMAP-induced cleavage of the ester linkage. Standard Yamaguchi lactonization conditions, involving slow addition of the mixed anhydride (secoc acid, 2,4,6-trichlorobenzoyl chloride, Et₃N, THF, rt) to a refluxing solution of DMAP in toluene, gave the same result. Gratifyingly, attempted macrocyclization under Yonemitsu conditions (seco acid, 2,4,6-trichlorobenzoyl chloride, Et₃N, DMAP, and the secoc acid in benzene, 1 × 10⁻³ M) at room temperature gave rise to the desired diolide 14 in 50% yield. Hydrogenation of 14 over Pearlman’s catalyst gave 3,3′-di-O-methyl aridimerin 15 in 90% yield. Interestingly, attempted acylation of 15 (Ac₂O, Pyr, rt, 16 h or Ac₂O, i-Pr₂NEt, DMAP, DMAP) led to none of the desired peracetate and the production of numerous side products. Ultimately, it was found that stirring 15 in neat acetyl chloride overnight led to formation of peracetate 16 in 85% yield. The β-stereochemistry of the glucosyl moieties was indicated by the 8.5 Hz coupling constant of the C.1 proton, and the connectivity of the molecule was verified by 1H−1H COSY and NOESY experiments (see Supporting Information).

In our attempts to access aridimerin by selective cleavage of the C.3 and C.3′ methyl ethers, treatment of 14 with BCl₃/CH₂Cl₂ (rt, overnight), AlCl₃/Nal (80 °C, CH₃CN, 3 h), or MgI₂ (50–80 °C, toluene) initially led to no starting material conversion, but after a prolonged reaction and an increase in the number of equivalents of Lewis acid, extensive decomposition products, arising from diolide cleavage, were formed. Similarly, use of sodium ethanethiolate in DMF (100 °C, 2 h) also led to dissolution of the bis lactone moiety. These data indicate that removal of the requisite methyl ethers is likely to be successful only on substrates prior to formation of the diolide core of the natural product.

The binding of 15 to duplex DNA was explored by UV and fluorescence spectroscopies. A concentration-dependent red shift in the absorption at λmax = 214 nm in the ultraviolet spectrum of 15 suggested that self-association/aggregation was occurring in an aqueous buffer solution (10 mM Tris-EDTA) at concentrations >1 μM (Figure 3). Thermal denaturation studies showed no significant shift in the Tm (68 °C) of salmon testes DNA in the presence of 15 at low ligand/DNA ratios.

Furthermore, compound 15 displayed relatively limited ability to displace bound ethidium bromide from calf thymus DNA as compared to control compound daunorubicin over the same concentration range (1 × 10⁻⁹ M to 4 × 10⁻⁷ M; see Supporting Information). These data suggest that 15 has a lower affinity for duplex DNA, perhaps indicative of the difficulty in accommodating the bulky chromophore-linked C-glycosyl moieties in the narrow minor groove, the initial site of small-molecule binding to DNA.

In summary, we have developed an 11-step synthesis of the 3,3′-di-O-methyl derivative of the natural product aridimerin and have shown that this substance readily aggregates in aqueous solution and has a low apparent affinity for duplex DNA. Current efforts toward the completion of the synthesis of aridimerin are centered around deprotection of the C.3 methyl ether of aldehyde 7.

**REFERENCES**

8. (h) Pavlopoulos, S.; Bicknell, W.; Craik, D. J.
(6) The recent characterization of the natural product leinamycin as a DNA intercalator despite the absence of a typical intercalating moiety (i.e., polycyclic aromatic unit) in the molecule raises the possibility that structures with relatively limited planar \( \pi \)-surfaces (a \( Z,E \)-penta-2,4-dienone moiety in the case of leinamycin) can bind nucleic acids by intercalation: Fekry, M. I.; Szekely, J.; Dutta, S.; Breydo, L.; Zang, H.; Gates, K. S. J. Am. Chem. Soc. 2011, 133, 17641 and references therein.


(10) Snieckus, V. Chem. Rev. 1990, 90, 879.


(25) Due to the self-association/aggregation of 15, low ligand/DNA ratios (0.05–0.01) were used in the thermal denaturation experiment: Shi, X.; Chaires, J. B. Nucleic Acids Res. 2006, 34, e14.
