Serendipitous Discovery of a Cascade Approach to Perhydrodibenzofuranones Related to the Natural Product Incarviditone

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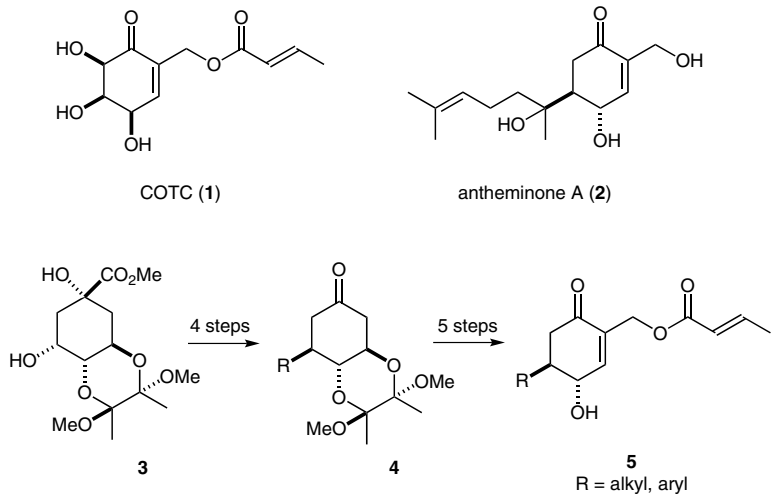
Abstract: The unexpected discovery of an eliminative deprotection and cyclodimerization cascade reaction sequence led to the preparation of a series of perhydrodibenzofuranones that bear a structural resemblance to the natural product incarviditone. One of the novel dimers was found to show significant antiproliferative activity towards a non-small-cell lung cancer cell line, and represents a useful lead compound for the discovery of more potent anti-cancer agents.

Key words: natural products, medicinal chemistry, cascade reactions, heterocycles, biomimetics

The α-oxyalkylcyclohex-2-en-1-one-containing natural products [(3*R*,4*R*,5*R*)-3,4,5-trihydroxy-6-oxocyclohex-1-en-1-yl]methyl (2*E*)-but-2-enoate (**1**; COTC)[1,2] and antheminone A (**2**)[3] both demonstrate notable toxicity towards a variety of cancer cell lines, and have therefore attracted significant attention from the scientific community. By using the butane–diacetal protected methylquinate **3** as the key building block, we have recently completed the synthesis of an array of hybrid analogues of **1** and **2** of general structure **5**, which display potent antitumor activities (Scheme 1).[4]

Encouraged by the promising biological activities of these new hybrid analogues, we are currently engaged in the development of a synthetic approach to the antipodal series of compounds (*ent*-**5**) by using the cyclohexylidene-protected lactone quinide **6** as the starting material.[5] The base-lability of several of the intermediates on the synthetic route has presented a significant challenge during this venture, one particularly problematic transformation being the eliminative deprotection of adducts **8**[6] to give cyclohex-2-en-1-ones **9** (Scheme 2).

In 1989, Danishefsky and co-workers reported a high-yielding conversion of the acetonide-protected dihydroxycyclohexanone **10a** into the enone **12** by using 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) at elevated temperatures in the presence of *tert*-butyldimethylsilyl chloride (TBSCl) as a trapping reagent for the incipient allylic alcohol **11** (Scheme 3).[7] These investigators also described the instability of alcohol **11** due, in part, to its propensity to undergo tautomeric decomposition to cyclohexane-1,4-dione. In our hands, cyclohexylidene-protected compound **10b** could also be converted into **12**, albeit in a slightly lower yield than that reported for the corresponding conversion of **10a**.[5] Unfortunately, however, exposure of aryl-substituted cyclohexanones **8** to identical reaction conditions did not meet with the same success, and little if any of the expected TBS-protected compounds



Scheme 1 α-Oxyalkylcyclohex-2-enone-containing natural products, and an approach to the synthesis of their analogues

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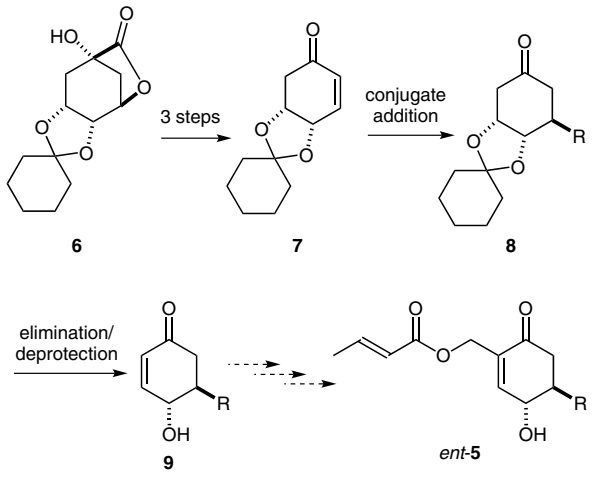
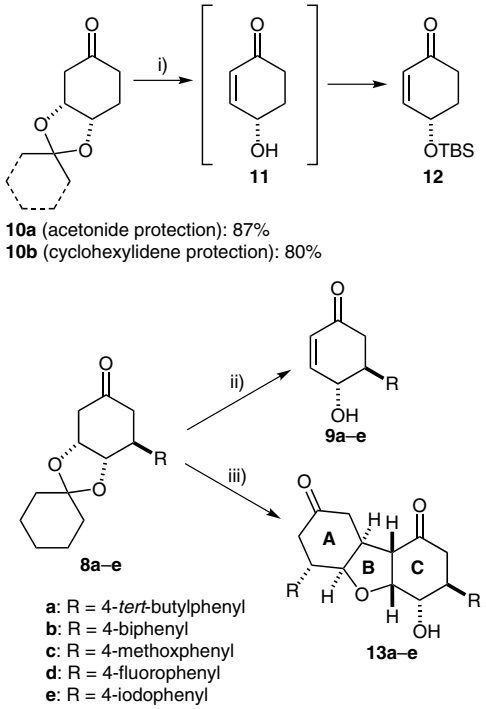


Scheme 2 Synthetic approach to hybrid analogues of antheminone A and COTC

could be isolated. After extensive investigations into this reaction in both the presence and absence of a silyl trapping agent, we discovered that conversion of aryl-substituted cyclohexanones **8a**–**e** into the allylic alcohols **9a**–**e** could be accomplished in variable, sometimes quite unpredictable, yields by using either triethylamine (two equivalents) in 1,4-dioxane–water (10:1) at room temperature or a catalytic quantity of aqueous sodium hydroxide (0.5 M) in tetrahydrofuran at room temperature.[8] The most generally applicable and reproducible protocol, however, involved the use of DBU in dichloromethane,



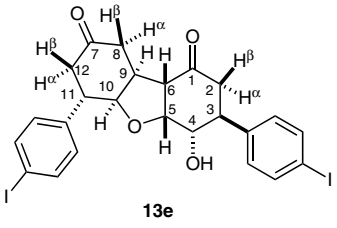
Scheme 3 Eliminative deprotection reactions of ketal-protected β,γ-dihydroxycyclohexanones **10a**, **10b**, and **8a**–**e**. *Reagents and conditions*: (i) DBU, TBSCl, benzene, reflux, 6 h; (ii) DBU, CH_2Cl_2, r.t., 2 h; (iii) 0.5 M aq NaOH (a few drops), THF, r.t., 18 h.

also at room temperature, which gave the desired alcohols in yields of 57–81% (Scheme 3).

It transpired that a major reason for the capricious nature of the sodium hydroxide-mediated reaction was the 'bipolar reactivity' of the products **9a**–**e** under the basic reaction conditions, which induced an unexpected dimerization process.[9] Indeed, when the conjugate adducts **8a**–**e** were exposed to 0.5 M aqueous sodium hydroxide in tetrahydrofuran for a prolonged reaction time (18 hours), the diasteroisomerically pure perhydrodibenzofuranones **13a**–**e** were the only isolable products, obtained in 55–86% yields.

In the first instance, the structures of the dimeric products were identified by means of extensive one- and two-dimensional NMR analyses (COSY, NOESY, and NOE); typical chemical-shift values, together with the associated geminal/vicinal coupling constants for the 4-iodophenyl-substituted compound **13e** are listed in Table 1. A significant difficulty encountered during the assignment of the data was the apparent absence of a three-bond coupling between C(9)H and C(6)H. A long-range COSY experiment in which cross-peaks arising from small coupling interactions were enhanced highlighted the appropriate signal, and a corresponding weak cross-peak was also identified in the NOESY spectrum. By using the MM2 force field, an energy-minimized structure for **13e** was generated; this predicted an internuclear distance of ~2.8 Å between C(6)H and C(9)H, in accord with the NOESY observation.

Table 1 ^{1}H NMR Spectroscopic Data for Dimer **13e**



	δ (ppm)	Coupling constant (Hz)	
C(2)H$^{\alpha}$	2.38	$J_{2^{\alpha},2^{\beta}}$ = 13.7	$J_{2^{\alpha},3}$ = 3.9
C(2)H$^{\beta}$	2.88		$J_{2^{\beta},3}$ = 13.7
C(3)H	3.19		
C(4)H	4.39	$J_{3,4}$ = 10.4	
C(5)H	4.74	$J_{4,5}$ = 2.9	
C(6)H	3.05	$J_{5,6}$ = 4.0	
C(8)H$^{\alpha}$	2.43	$J_{8^{\alpha},8^{\beta}}$ = 16.2	$J_{8^{\alpha},9}$ = 5.8
C(8)H$^{\beta}$	2.78		$J_{8^{\beta},9}$ = 13.6
C(9)H	3.41	$J_{9,10}$ = 7.7	
C(10)H	4.32	$J_{10,11}$ = 9.8	
C(11)H	3.32		
C(12)H$^{\alpha}$	2.64	$J_{12^{\alpha},12^{\beta}}$ = 17.8	$J_{11,12^{\alpha}}$ = 13.9
C(12)H$^{\beta}$	2.37		$J_{11,12^{\beta}}$ = 3.4

Unfortunately, most of the novel dimers were isolated as amorphous powders; however extensive recrystallization

efforts eventually generated a sample of the *tert*-butylphenyl compound **13a** that was suitable for X-ray crystallographic analysis, and this confirmed the structural identity of the compound (Figure 1).[10]

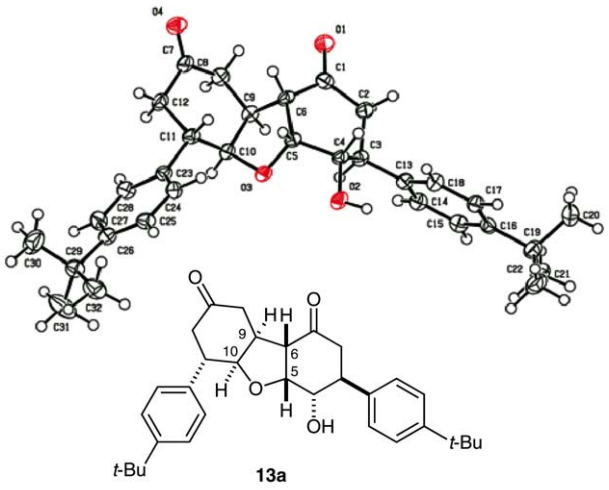


Figure 1 Crystal structure of dimer **13a** with ellipsoids at 50% probability

The *syn–anti–syn* arrangement of C(5)H/C(6)H, C(6)H/C(9)H, and C(9)H/C(10)H is consistent with a tandem 'suprafacial–suprafacial' oxa-Michael/carba-Michael cyclodimerization sequence (Scheme 4). This is initiated by conjugate addition of an oxyanion derived from one molecule of the starting material to the C3 position of another molecule, *anti* to the C5 aromatic substituent of the latter. A related cyclodimerization sequence, albeit under more strongly basic conditions, was recently used to great effect by Wu and co-workers[11] during their biosynthetically inspired synthesis of the natural product incarviditone (**15**).[12] They found that treatment of a racemic sample of rengyolone (**14**)[13] with one equivalent of sodium hydride in dichloromethane gave **15** in 40% isolated yield, together with a 38% yield of incarvileatone (**16**). They concluded that 'homocyclodimerization' of **14** [(+) with (+) / (–) with (–)] gave rise to **15**, whereas 'heterocyclodimerization' [(+)-**14** with (–)-**14**] gave **16**, possibly through intramolecular aldol cyclization of an initially formed perhydrodibenzofuranone **17**. The structures of the novel compounds **13a–e** reported here, which are similar to that of **15** and which arise from a 'homocyclodimerization' process, are in accord with this conclusion.

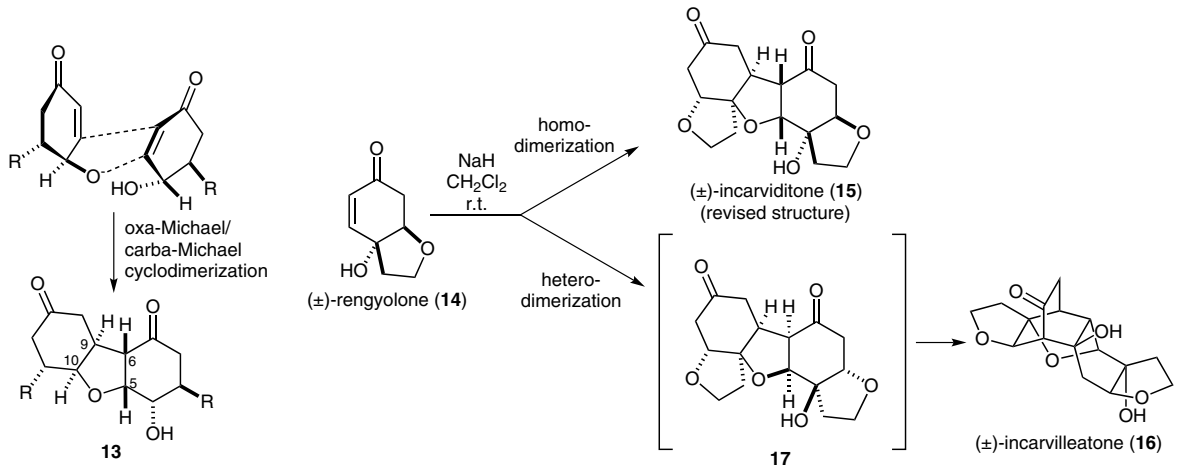
Both rengyolone (**14**) and incarviditone (**15**) have been reported to demonstrate toxicity towards cancer cell lines, so, prompted by this finding, we assessed the toxicity of a selection of the novel dimers, as well as one of their immediate precursors (enone **9a**), towards the A549 non-small-cell lung cancer cell line. The cells were exposed to varying concentrations of the compounds for 96 hours, and toxicity was assessed by using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (Table 2).[14]

Table 2 Assessment of the Toxicities of Analogues of Incarviditone and Rengyolone toward the Non-Small-Cell Lung Cancer Cell Line A549

Compound	IC_{50}[a] (μM)
9a	1.96 ± 0.38
13a	12.01 ± 1.6
13c	nontoxic at 100 μM
13d	nontoxic at 100 μM
13e	102.61 ± 41.62

[a] IC_{50} = concentration required to reduce proliferation by 50%. Toxicity experiments were repeated in triplicate and data within individual experiments were derived from four separate observations; average values are given.

Perhaps unsurprisingly, enone **9a** proved to be the most potent of the compounds tested. Being a Michael acceptor, this compound would be expected to alkylate intracellular nucleophiles, such as protein thiols, leading to cell damage. Of the dimers that we assayed, only the *tert*-



Scheme 4 Cyclodimerization reactions to give perhydrodibenzofuranones

butylphenyl analogue **13a** demonstrated significant antiproliferative activity. As in the case of incarviditone, the biological mechanism of action of **13a** is unknown; however, this compound represents a useful lead compound for the discovery of more potent antiproliferative agents, and investigations to achieve this goal are now underway.

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Supporting Information for this article is available online at http://www.thieme-connect.com/ejournals/toc/synlett.

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(10) Crystal data for **13a**: $C_{32}H_{40}O_4$; M = 488.64, space group $P2_1$, a = 7.1087(4), b = 9.6046(9), c = 19.1101(16) Å, β = 96.131(7)°, U = 1297.30(18) Å^3, d_{calcd} = 1.251 Mg/m^3. Intensity data were collected by using an Agilent Supernova diffractometer; 7390 reflections were collected, of which 3995 were unique, R_{int} = 0.0532. Data processing was carried out by using *CrysAlis PRO*, and the structure was solved by direct methods using SIR92. All nonhydrogen atoms were refined anisotropically and hydrogen atoms were refined isotropically. Refinement on F^2 was carried out by using *SHELXL97*: Final $R1$ = 0.0553, $wR2$ = 0.1155 for data with; $I > 2\sigma(I)$. Crystallographic data for compound **13a** have been deposited with the accession number CCDC 983059, and can be obtained free of charge from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; Fax: +44(1223)336033; E-mail: deposit@ccdc.cam.ac.uk; Web site: www.ccdc.cam.ac.uk/conts/retrieving.html.
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