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TOTAL SYNTHESIS OF A NOVEL BENZ[A]ANTHRACYCLINE ANALOG OF THE ANTITUMOR AGENT 4-DEMETHOXYDAUNORUBICIN

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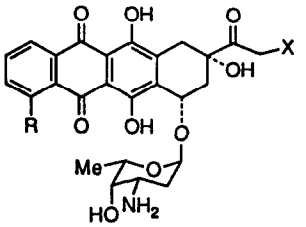
Abstract: The total synthesis of 2*S*, 4*S*-2-acetyl-4-[(3-amino-2,3,6-trideoxy-α-*L*-lyxo-hexopyranosyl)oxy]-1,2,3,4-tetrahydro-2,5,6-trihydroxy-benz[a]anthracene-7,12-dione (**5**) and its 2*R*, 4*R*-diastereomer (**23**) was accomplished in 11 steps (1.4% overall yield) from 5,6-dimethoxy-2-tetralone (**8**). These angular analogs of 4-demethoxydaunorubicin were inactive in tissue culture assays in comparison with doxorubicin.

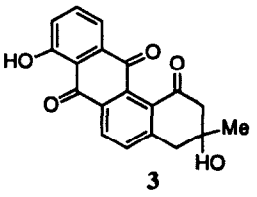
Since the isolation of daunorubicin (**1**) and doxorubicin (**2**), the anthracycline field has been the focus of intense research.[3] However, during recent years, the number of synthetic and biological studies has diminished, in part due to the number of routes now available and to the comparative lack of promising new biological findings to follow-up. Our objective was to design a novel and 'non-classical' analog of these potent antitumor agents which, if successful, would rekindle interest in the chemistry and biology of this therapeutic class.

Our attention turned to a growing number of natural products which contain a tetracyclic benz[a]anthracene framework collectively known as the angucyclines.[4] One of the first members of this class to be described was tetrangomycin[5] (**3**), and since then, over a hundred new angucyclines have been reported. A large number of angucyclines contain a benz[a]anthraquinone functionality as well as C- and/or O-glycosidic linkages with one or more carbohydrate units (*e.g.* **4**, saquayamycin A[6]). In addition to their novel architecture, the angucyclines display a range of biological activities such as antitumor and antibacterial properties.[4]

The majority of the anthracyclines and angucyclines share common structural features. Both classes of natural products contain an anthraquinone moiety and O-glycosidic linkages with deoxy sugars. Based on these structural similarities, we became interested in preparing a hybrid of these two families of biologically active compounds which would combine the angular features of the angucyclines with those known to be essential for the active anthracyclines. Our target became compound **5**, an analog of 4-demethoxydaunorubicin[7] (**6**) with a benz[a]anthraquinone framework bearing an O-glycosidic linkage with *L*-daunosamine. It was uncertain if such an analog would retain the DNA intercalative properties[8] of the parent anthracyclines. However, like the anthracyclines, **5** may in principle be able to undergo bioreductive activation,[9] leading to the formation of semi- or hydroquinone and quinone methide intermediates. In the anthracyclines, these reactive species[9,10] have been associated with macromolecule alkylation[11] and formation of oxygen radicals[12] which may be responsible for their cytotoxic properties. Interestingly, an angular chromone analog (**7**) of **6** has been reported to display moderate antitumor activity.[13]



1, R = OMe; X = H
2, R = OMe; X = OH
6, R = X = H



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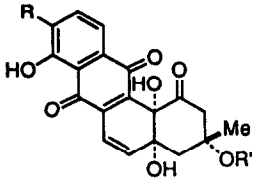

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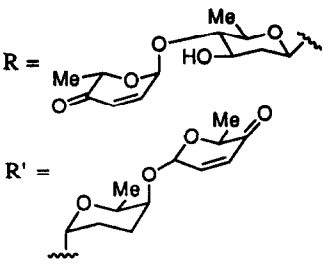

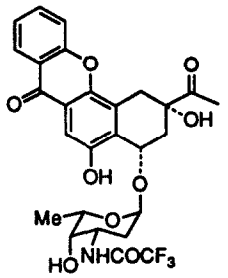

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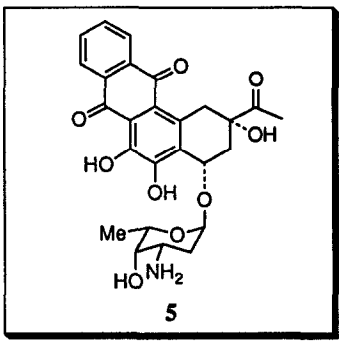

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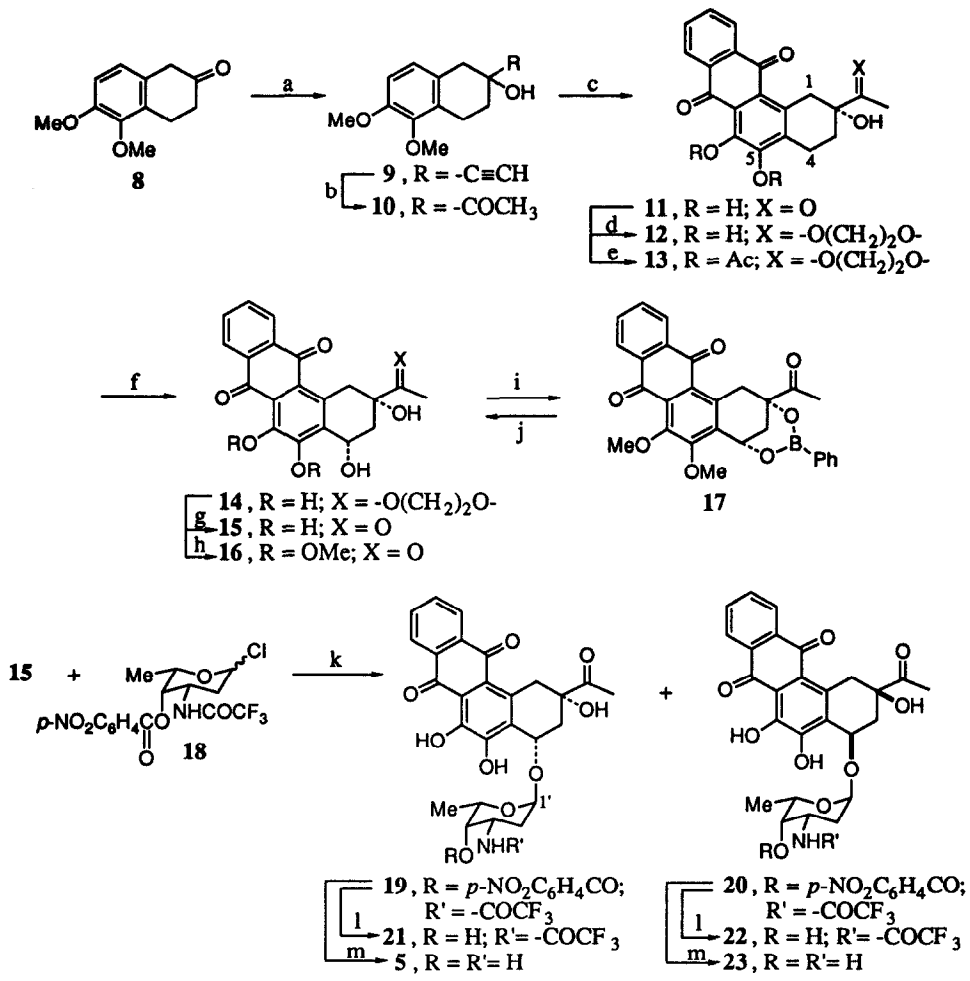
Treatment of 5,6-dimethoxy-2-tetralone[14] (**8**) with ethynyl magnesium bromide gave acetylenic alcohol **9** (40%, along with 42% of recovered **8**) which was converted to α-hydroxyketone **10** by reaction with yellow HgO in acetone/water[15] (80%). After significant experimentation, Friedel-Crafts diacylation of **10** with phthalic anhydride and $AlCl_3$ at high temperatures[16] (188-190 °C) gave tetracyclic catechol **11** in satisfactory isolated yield (52%). Work-up with strong acid (6N HCl) instead of saturated oxalic acid[16] was essential apparently due to the greater chelating strength of the catechol quinone system of **11** in comparison to the linear 6,11-dihydroxy-5,12-naphtacenedione system present in the anthracyclines. In addition, the stability of the tertiary hydroxyl group is notable.

In order to introduce the benzylic functionality at carbon 4 (C-4), quinone **11** was converted to ethylene ketal **12** (94%) and then to diacetyl ester **13** (95%). The ketal functionality was anticipated to create steric bulk near the benzylic position at C-1, favoring functionalization at C-4. Acetylation of the catechol moiety was essential for the success of the subsequent benzylic halogenation. Otherwise, only starting **12** was recovered, presumably due to reaction of the benzylic free radical with the non-hydrogen bonded phenolic OH at C-5. Bromination of **13** with N-bromosuccinimide in the presence of azaisobutyronitrile[17] (AIBN) proceeded smoothly to give a mixture of bromides. Due to their instability, the latter were reacted directly with AgOAc in acetic acid[17] followed by treatment with K_2CO_3 in acetone/water to give *cis*-dihydroxy derivative **14** . After three steps, a regio- and stereoselective benzylic hydroxylation as well as deacetylation of the catechol moiety was accomplished in 40% overall yield. Stereoselective introduction of the benzylic functionality is in contrast to such functionalization in the tetrahydronaphtacenedione series in which mixtures of *cis* and *trans* dihydroxy derivatives are usually obtained.[15,17] Careful removal of the ketal functionality with aqueous trifluoroacetic acid[18] at -5 °C for 20 min afforded angular aglycone **15** in 66% yield. Longer reaction time or warmer temperatures resulted in partial epimerization (7%) to the undesired *trans* aglycone. Confirmation of the *cis* stereochemistry was accomplished by chemical transformations. Methylation (MeI, K_2CO_3) of **15** gave dimethoxy derivative **16** (48%) which reacted smoothly with phenylboric acid in the presence of catalytic *p*-TsOH[19] to afford cyclic phenylboronate **17** in 93% yield. Compound **17** was then subjected to an exchange reaction with 1,3-propanediol in acetone to give a diol identical by ^{1}H-NMR to aglycone **16**, confirming the *cis* nature of this series.

Glycosidation of racemic aglycone **15** was accomplished by treatment with freshly prepared *L*-daunosamyl chloride derivative **18**[20] and $AgOSO_2CF_3$ in the presence of 4Å molecular sieves[21] to afford glycoside **19** and its diastereomer **20** (47 and 36% yields, respectively). Both glycosides possessed the desired α configuration at the anomeric center (C-1' H, br s). Removal of the *p*-nitrobenzoyl protecting group in the sugar moiety was accomplished by treatment with K_2CO_3 in cold (-5 °C) methanol/water[16] to afford glycosides **21** and **22**. Attempts to hydrolyze the N-trifluoroacetyl group with a number of aqueous bases[7,16] only resulted in recovery of the corresponding aglycone. After some experimentation, it was found that glycosides **19** and **20** could be converted directly to final compounds **5** and **23**,[22] isolated as their hydrochloride salts, by treatment with a saturated solution of NH_3/MeOH followed by work up with ethereal HCl (39 and 46%, respectively). The absolute configuration of glycosides **5**, **21-23** was established by comparison of their circular dichroism (CD) curves with that of **1**.[23] Glycosides **5** and **21** displayed negative CD curves at 280 and 282 nm in similarity to **1**, suggesting that they possessed the natural *S*, *S* configuration. Glycosides **22** and **23** displayed positive CD curves at 276 nm.

The novel glycosides **5** and **23** were tested for cytotoxicity *in vitro* over the concentration range of 0.0001 - 1 μM against the human T-lymphoid leukemic cell line CEM and its doxorubicin-resistant counterpart CEM/VLB.[24] After incubation for 4 days in the CEM cell line assay, the isomers showed an IC_{50} level of 1 μM whereas doxorubicin showed an IC_{50} of 0.02 μM. Doxorubicin was thus 50-fold more potent in this test than either analog. In the resistant cell line (CEM/VLB) both glycosides and doxorubicin were inactive at 1 μM. In



Reagents: (a) HC≡CMgBr, THF, 0→25°C, 16h, 40% plus 42% recovered s. m.; (b) HgO (yellow), 1.5N H_2SO_4, acetone, 60h, 80%; (c) 2:1 $AlCl_3$/NaCl, phthalic anhydride, 188-190°C, 10 min, 6N HCl work up, 52%; (d) $(HOCH_2)_2$, *p*-TsOH, PhH, reflux, Dean-Stark trap, 6h, 94%; (e) Ac_2O, pyr, 0.5h, 95%; (f) i. NBS, AIBN, CCl_4, reflux, 1.5h; ii. AgOAc, H_2O, HOAc, 15h; iii. 0.5M K_2CO_3, acetone, 5°C, 8h, 40% overall; (g) 90% aq. TFA, -5°C, 18 min, 66%; (h) MeI, K_2CO_3, acetone, reflux, 9h, 48%; (i) $PhB(OH)_2$, *p*-TsOH, PhMe, 4h, 93%; (j) 1,3-Propanediol, acetone, 4 days, 55%; (k) $AgOSO_2CF_3$, 4Å MS, THF, -60→ -20°C, 2h, **19**, 47% and **20**, 36%; (l) 0.5M K_2CO_3, MeOH, 0°C, 1h, **21**, 75% and **22**, 77%; (m) NH_3/MeOH, 14h, HCl/Et_2O work up, **5**, 39% and **23** 46%.

similar tests utilizing P388 (murine leukemia) cell lines, both sensitive and resistant to doxorubicin, similar results were obtained.

In summary, a novel hybrid from the angucycline and anthracycline series, benz[a]anthracycline **5**, was prepared from 5,6-dimethoxy-2-tetralone in 11 steps. Key steps involved the regio- and stereoselective introduction of the C-4 hydroxyl group and removal of the N-trifluoroacetyl group from the sugar unit (*L*-daunosamine) under anhydrous conditions. Unfortunately, this compound did not display significant antitumor activity, suggesting that the angular-shaped chromophore of **5** cannot interact with macromolecules (*i.e.* DNA) through pathways that may lead to cytotoxicity. Once again it would seem that preparation of a hybrid series has resulted in progeny with all of the defects of their progenitors and none of their virtues.

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22. Compound **5**: Orange solid; mp 162-163°C; ^{1}H-NMR (DMSO-d_6) δ 13.51 (s, 1H, ArOH), 10.74 (s, 1H, ArOH), 8.22 (d, 1H, J = 6.9 Hz), 8.17 (d, 1H, J = 6.9 Hz), 7.90-7.98 (m, 2H), 7.88 (br s, 3H, NH_3^+), 5.64 (s, 1H, C-2 OH), 5.38 (br s, 2H, C-4 H and C-4' OH), 5.24 (s, 1H, C-1' H), 4.16 (d, 1H, J = 16.9 Hz, C-1 H_{eq}), 3.90-3.95 (m, 2H, C-5' H), 3.50 (br s, 1H, C-4' H), 3.34-3.41 (m, 1H, C-3' H), 3.23 (d, 1H, J = 16.9 Hz, C-1 H_{ax}), 2.10-2.25 (m, 2H), 2.16 (s, 3H), 1.86-1.94 (m, 1H), 1.67-1.71 (m, 1H), 1.01 (d, 3H, J = 6.1 Hz); IR (KBr) 3400, 2978, 1713, 1657, 1634, 1592 cm^{-1}; FABMS (magic bullet) *m/z* (relative intensity) 498 ([M + 1]$^+$, 55), 309 (100); CD (EtOH) $[\theta]_{282} = -0.49 \times 10^4$; $[\theta]_{300} = 0.69 \times 10^4$. Compound **23**: Orange solid, mp 179-180°C; FABMS (magic bullet) *m/z* (relative intensity) 498 ([M + 1]$^+$, 12), 279 (100); CD (EtOH) $[\theta]_{276} = 1.21 \times 10^4$.

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