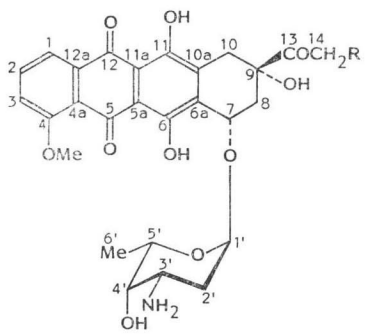
SUGAR-RING ANALOGS OF DAUNORUBICIN: 3′-EPIDAUNORUBICIN, 3′-HYDROXY-3′,5′-DIEPIDAUNORUBICIN AND 3′,6′-DIHYDROXY-3′,5′-DIEPIDAUNORUBICIN*

Sir:

Daunorubicin analogs having the sugar portion replaced by 3-amino-2,3,6-trideoxy-L-*xylo*-hexose, 2,6-dideoxy-D-*arabino*-hexose, and 2-deoxy-D-*arabino*-hexose have been synthesized and evaluated *in vivo* in the P-388 murine tumor screen. Coupling of the sugar to daunomycinone was effected by the glycal method with the amino sugar to give a separable 7: 1 mixture of anomers; the Koenigs-Knorr method was used with the deoxy sugars, affording mixtures difficult to resolve in the product from the dideoxyhexose.

The clinical usefulness of the anthracycline antibiotics daunorubicin (**1**) and doxorubicin (**2**) is well proven and documented[1], as well as such unfavorable side-effects as bone-marrow damage, stomatitis, alopecia, and especially a cumulative, dose-limiting cardiotoxicity. In a search for more-effective analogs and to understand structure-activity relationships, a program[2] in this laboratory has focused on structural modifications of daunorubicin incorporating sugars of varied functionality and stereochemistry, as well as modifications at C-14. This report described the synthesis and evaluation of several variants of daunorubicin incorporating functional and stereochemical modification in the sugar ring.



1 R = H

2 R = OH

An initial target was the 3′-epimer of daunorubicin. An earlier report from this laboratory[3] has already described the synthesis of the requisite sugar in the form of a glycoside, methyl 3-amino-2,3,6-trideoxy-β-L-*xylo*-hexopyranoside (**3**). For coupling to daunomycinone, the glycal method was selected as this procedure had earlier permitted satisfactory coupling of amino sugars to daunomycinone. Trifluoroacetylation of the glycoside **3** with trifluoroacetic anhydride in dichloromethane, evaporation with toluene at 50°C, and subsequent treatment with sodium hydrogencarbonate gave the glycal **4** crystalline and analytically pure in 45% yield. Physico-chemical properties: mp 119~120°C; $[\alpha]_D^{28}$ −85° (*c* 0.2, $CHCl_3$). A small proportion of the *N*-trifluoroacetylated glycoside (**5**) was isolated as a side-product.

p-Nitrobenzoylation of the glycal derivative **4** gave a quantitative yield of the amorphous 4-*p*-nitrobenzoate **6** ($[\alpha]_D^{26}$ −87° (*c* 0.2, $CHCl_3$)), which underwent coupling with daunomycinone in hot benzene in the presence of a trace of *p*-toluenesulfonic acid. Use of a two-fold excess of the sugar with respect to aglycon led to 87% of isolated coupled product (based on daunomycinone used) after 3 days at the boiling point; the reaction was terminated at this stage as the desired α-L anomer **7** (NSC 305992) initially produced was becoming progressively contaminated with the β-L anomer **8** (NSC 305993). Resolution of the product-mixture on a column of silica gel allowed isolation of the crystalline, levorotatory α-L anomer **7** in 76% yield. Physico-chemical properties: mp 164~170°C; $[\alpha]_D^{28}$ −96° (*c* 0.02, $CHCl_3$). The faster-migrating, dextrorotatory β-L anomer **8** was also obtained crystalline, in 11% yield. Physico-chemical properties: mp 150~153°C; $[\alpha]_D^{22}$ +267° (*c* 0.02, $CHCl_3$).

The foregoing reaction-sequence was judged a satisfactory, high-yielding synthetic route to the protected 3′-epidaunorubicin (**7**). Conventional deprotection by *N*,*O*-deacylation gave a product having the anticipated chromatographic characteristics of the free daunorubicin analog, but the procedure was not pursued to optimization and full characterization of the product following the appearance in the literature of a synthesis of 3′-epidaunorubicin with indication that it was inactive at dose-levels up to

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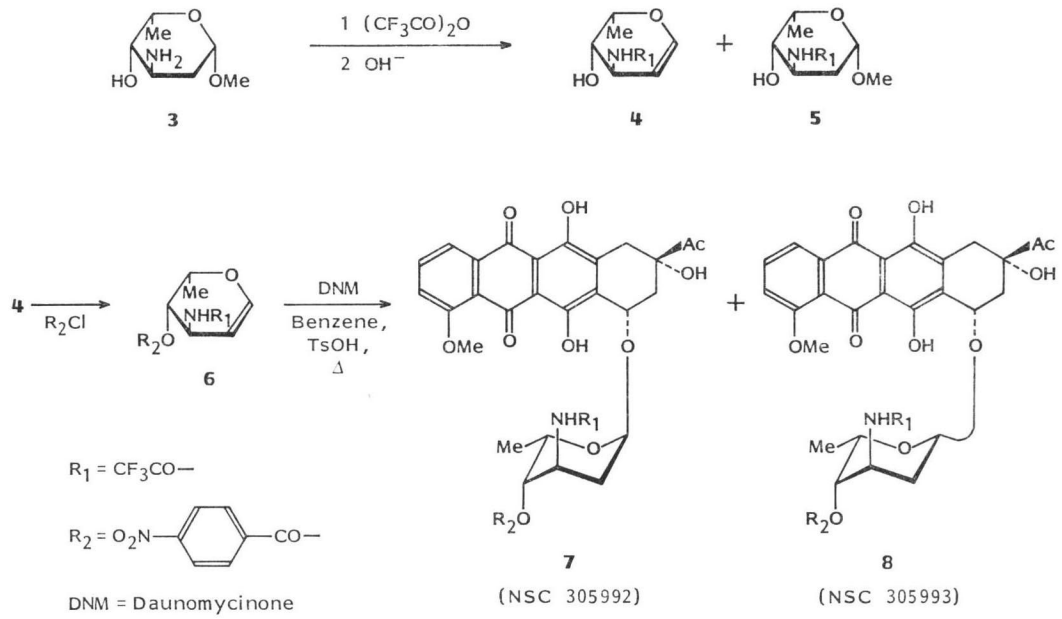
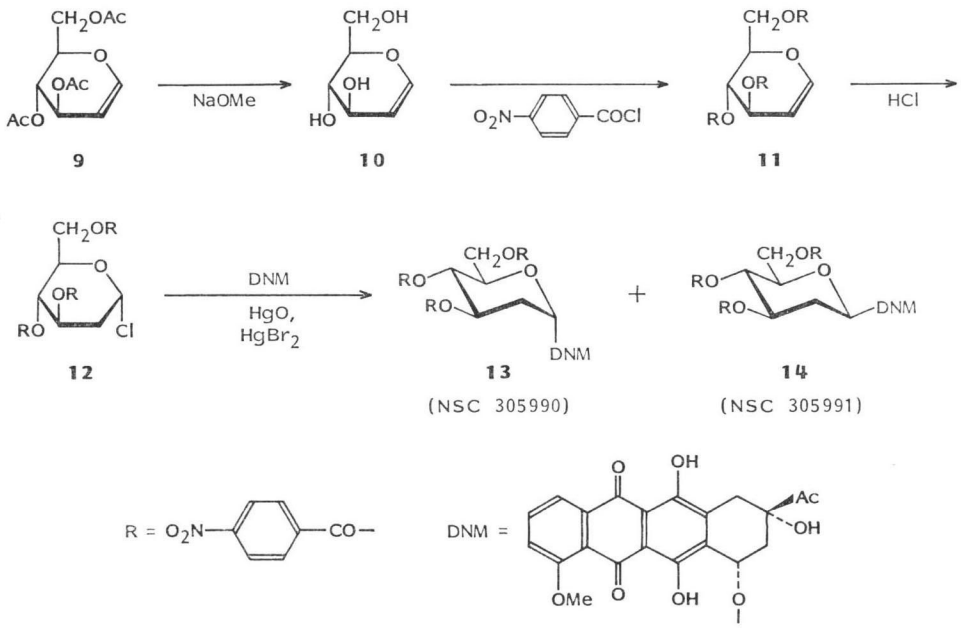


50 mg/kg in the *in vivo* murine P-388 leukemia screen[1].

The promising chemotherapeutic behavior of 3′-deamino-3′-hydroxydaunorubicin prompted the synthesis of daunorubicin analogs incorporating other, non-aminated deoxy sugars, first of all the readily available 2-deoxy-D-*arabino*-hexose, accessed by way of D-glucal triacetate **9**. The most satisfactory conditions for coupling this sugar to daunomycinone were found to be by Koenigs-Knorr coupling of a suitable glycosyl halide. D-Glucal triacetate (**9**) was *O*-deacylated with sodium methoxide to D-glucal (**10**) and the crystalline 2,4,6-tris(*p*-nitrobenzoate) (**11**) of **10** prepared in 88% yield by conventional acylation; the assigned structure of the glycal **11** (mp 94~95°C) was well supported by its 200 MHz NMR spectrum; ^1H NMR ($CDCl_3$) δ 6.67 (dd, 1H, $J_{1,2}$= 6.3, $J_{1,3}$=1.0 Hz, H-1), 5.12 (dd, 1H, $J_{2,3}$= 3.4 Hz, H-2). Addition of hydrogen chloride

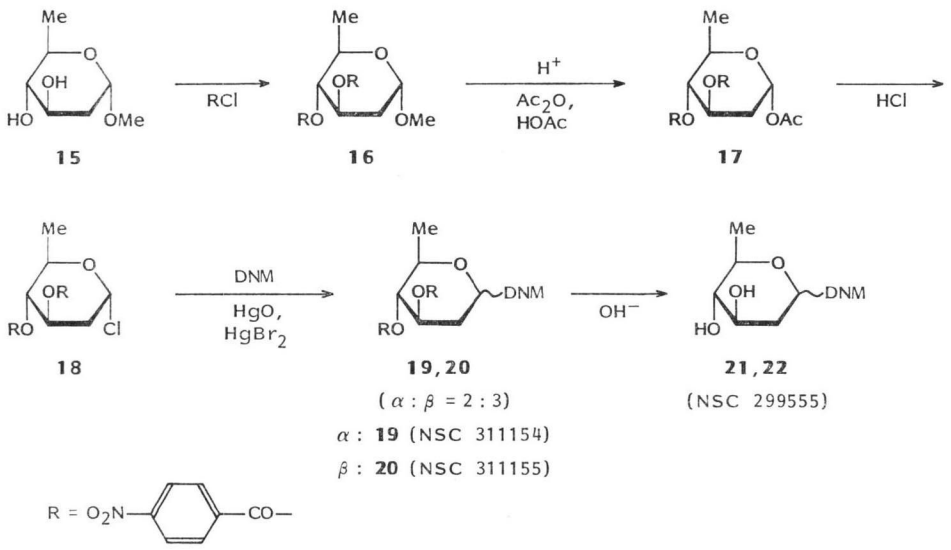


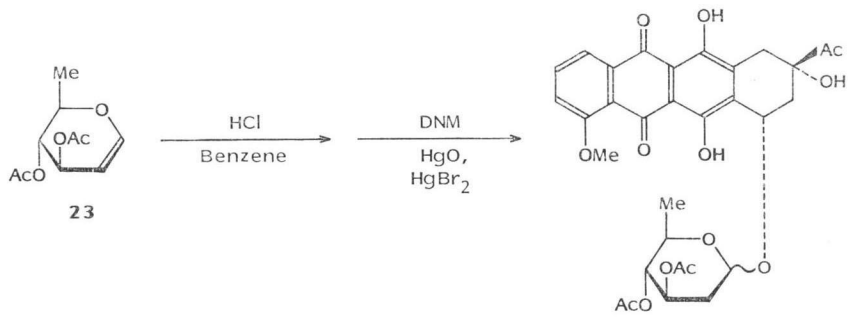
to **11** gave in 87% yield the crystalline glycosyl chloride **12** (mp 130~135°C); the latter was used without delay in the coupling reaction with daunomycinone.

A two-fold excess of the deoxy sugar derivative **12** was used in the coupling reaction, conducted in dichloromethane in the presence of mercuric bromide, yellow mercuric oxide, and 3Å Molecular sieves under conditions similar to those used in this laboratory for the synthesis of other daunorubicin analogs containing non-amino sugars. The coupled product was obtained as an anomeric mixture which was resolved on a column of silica gel to afford 51% of the crystalline, weakly dextrorotatory β-D anomer **14** (NSC 305991). Physico-chemical properties: mp 188~190°C; $[\alpha]_D^{23}$ +34° (*c* 0.02, $CHCl_3$); ^{13}C NMR (50 MHz, $CDCl_3$) δ 212.1 (C=O), 101.0 (C-1′), 56.7 (OMe) and 33.3 (C-2′). The crystalline, strongly dextrorotatory α-D anomer **13** was isolated in 11% yield. Physico-chemical properties: mp 240~244°C; $[\alpha]_D^{23}$ +272° (*c* 0.02, $CHCl_3$); ^{13}C NMR (50 MHz, $CDCl_3$) δ 211.0 (C=O), 93.7 (C-1′), 56.8 (OMe) and 30.4 (C-2′).

Parallel work to the foregoing sequence was conducted with the 6-deoxy analog, based on a hypothesis that a higher probability of biological activity existed with analogs having a methyl group at C-5′ than those bearing a hydroxymethyl group at this position[1]. The satisfactory coupling and anomeric-product resolution encountered with 2-deoxy-D-*arabino*-hexose gave reasonable expectation that similar coupling could be effected with the (less accessible) 2,6-dideoxy-D-*arabino*-hexose.

The methyl α-glycopyranoside (**15**) of 2,6-dideoxy-D-*arabino*-hexose, prepared by the route earlier described, was converted in 93% yield into its crystalline 3,4-bis(*p*-nitrobenzoate) **16**, which was converted by acetolysis into 1-*O*-acetyl-2,6-dideoxy-3,4-di-*O*-*p*-nitrobenzoyl-α-D-*arabino*-hexopyranose (**17**), obtained crystalline in 90% yield. Physico-chemical properties: mp 91~92°C; ^1H NMR (100 MHz, 3:1 benzene-d_6 - acetone-d_6) 6.28 (dd, 1H, $J_{1,2eq}$=2.0, $J_{1,2ax}$= 4.0 Hz, H-1). Treatment of this product with hydrogen chloride in dichloromethane gave the corresponding, crystalline α-chloride **18** (mp 133~134°C) in essentially quantitative yield; this chloride was coupled without delay with daunomycinone by essentially the same adaptation of the Koenigs-Knorr procedure as used in the preceding example. In this instance, the coupled product was obtained in almost quantitative yield, but as a 2:3 mixture of the α-D (**19**, NSC 311154; mp 149~152°C) and β-D (**20**, NSC 311155; mp 155~159°C) anomers that proved quite difficult to separate by column chromatography on silica gel, although each product was isolated, pure and crystalline, after tedious chromatography that yielded only small amounts of the isolated products. For this reason, *O*-deacylation of the coupled product was performed with the anomeric mixture **19**+**20** by use of sodium hydroxide in oxolane-water,





23

24(α), 25(β)
(NSC 333862)
(α : β = 4 : 1)

leading to a 2:3 mixture of the deprotected products **21** and **22**, obtained in 43% yield as a cocrystallized mixture.

In an alternative approach seeking a preparative route to the separate anomers **21** and **22**, use of the corresponding *O*-acetylated sugar was evaluated, employing the acetylated glycal (**23**) for access to the glycosyl chloride precursor. Thus, 3,4-di-*O*-acetyl-1,5-anhydro-2,6-dideoxy-D-*arabino*-hex-1-enitol (**23**) was treated with hydrogen chloride in benzene for 0.5 hour at 0°C and the resultant, unstable glycosyl chloride brought directly into reaction with daunomycinone under the established Koenigs-Knorr conditions. The coupled product was, however, obtained in only 40% yield by this procedure, and again as an anomeric mixture. In this instance the α-D anomer (**24**) preponderated over the β-D anomer (**25**) by a factor of 4:1 as established by detailed ^{1}H and ^{13}C NMR spectral analysis of the products in admixture, after chromatographic isolation. ^{1}H NMR (200 MHz, $CDCl_3$) for the α anomer **24**: δ 14.05, 13.26 (s, 1H, 6,11-OH), 5.32 (d, 1H, $J_{1',2'ax}$=2.9 Hz, H-1'), 5.11 (ddd, 1H, $J_{2'eq,3'}$=5.0, $J_{2'ax,3'}$=11.4, $J_{3',4'}$=9.5 Hz, H-3'); ^{13}C NMR (50 MHz, $CDCl_3$) δ 211.7 (C=O), 93.6 (C-1'), 17.4 (C-6'). ^{1}H NMR (200 MHz, $CDCl_3$) for the β anomer **25**: δ 13.96, 13.20 (s, 1H, 6,11-OH), 4.08 (s, 3H, OMe), 2.43 (s, 3H, H-14), 1.28 (d, 3H, $J_{5',6'}$=5.9 Hz, H-6'); ^{13}C NMR (50 MHz, $CDCl_3$) δ 101.1 (C-1'), 17.7 (C-6'). The anomers in this instance proved even more difficult to resolve by chromatography than in the case of the 3',4'-bis(*p*-nitrobenzoyl) analogs **19** and **20**.

Biological evaluation showed compounds **7** (NSC 305992), **13** (NSC 305990), **14** (NSC 305991), **19** (NSC 311154), **20** (NSC 311155), **21**+**22** (NSC 299555) and **24**+**25** (NSC 333862) to be inactive *in vivo* up to 50 mg/kg in the murine P-388 lymphocytic leukemia assay (**24**+**25** were tested up to 80 mg/kg). Up to the maximum dose-levels tested, none of the compounds exhibited toxicity.

Although the foregoing syntheses did not lead to products having significant antitumor activity, the synthetic work described established a range of useful intermediates and comparative routes. The results serve to demonstrate the difficulty of making predictive generalizations in regard to glycosidic coupling reactions between complex aglycons and sugars of varied constitution, even when the structural differences may appear small.

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