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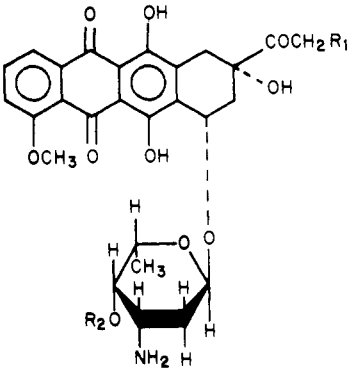
Communications to the Editor

Synthesis and Antitumor Activity of 4′-*O*-Methyldaunorubicin, 4′-*O*-Methyladriamycin, and Their 4′-Epi Analogues

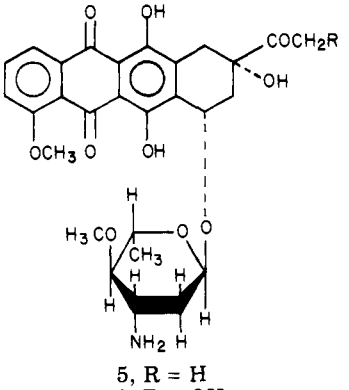
Sir:

The synthesis of analogues of daunorubicin (**1**) and



1, R_1 = H; R_2 = H
2, R_1 = OH; R_2 = H
3, R_1 = H; R_2 = CH_3
4, R_1 = OH; R_2 = CH_3

doxorubicin (**2**) in which the amino sugar moiety is functionally and/or configurationally modified is of interest in relation to structure–activity relationships, which, in turn, may lead to new and improved drugs.[1,2] In our continuing concern with new anthracyclines, we now report the synthesis and preliminary biological data of 4′-*O*-methyldaunorubicin (**3**), 4′-*O*-methyladriamycin (**4**), 4′-*epi*-4′-*O*-methyldaunorubicin (**5**), and 4′-*epi*-4′-*O*-methyladriamycin (**6**). The new glycosides **3** and **4** have



5, R = H
6, R = OH

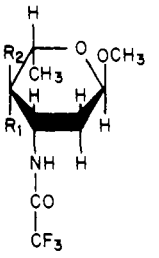
the natural amino sugar, daunosamine (3-amino-2,3,6-trideoxy-L-*lyxo*-hexose), replaced by the corresponding

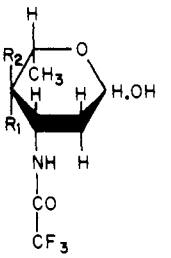
Table I. Activity of 4′-*O*-Methyldaunorubicin (**3**), 4′-*O*-Methyladriamycin (**4**), and 4′-*epi*-4′-*O*-Methyldaunorubicin (**5**) on P 388 Leukemia in Mice

compd	optimal dose[b]	T/C[c]
1	2.9	169
2	4.4	254
3	2.9	156
4	6.6	270
5	20	174

[a] Tumor inoculum 10^5 cells, ip. [b] Treatment ip on day 1 (mg/kg of body weight). No toxic deaths were observed at optimal doses. [c] Average survival time expressed as percent of untreated controls.

4-*O*-methyl analogue which was prepared from methyl *N*-(trifluoroacetyl)-α-L-daunosaminide (**7**). Compounds





7, R_1 = OH; R_2 = H
8, R_1 = OCH_3; R_2 = H
12, R_1 = H; R_2 = OH
13, R_1 = H; R_2 = OCH_3

9, R_1 = OCH_3; R_2 = H
14, R_1 = H; R_2 = OCH_3

5 and **6** are new anthracycline glycosides containing actinosamine (3-amino-2,3,6-trideoxy-4-*O*-methyl-L-*arabino*-hexose), an amino sugar constituent of the antibiotic actinoidin.[3] Whereas the synthesis of derivatives of *N*-acetylactinosamine has been previously described,[4] we have prepared its *N*-trifluoroacetyl derivatives starting from methyl *N*-(trifluoroacetyl)-α-L-acosaminide [methyl 2,3,6-trideoxy-3-(trifluoroacetamido)-α-L-*arabino*-hexopyranoside, **12**].

Treatment of **7**[2] (5.14 g, 0.02 mol) in dry methylene chloride at 0 °C with an excess of diazomethane in the same solvent and in the presence of a catalytic amount of boron trifluoride etherate[5] gave 4.6 g (86%) of the corresponding 4-*O*-methyl derivative **8**: mp 137–138 °C; $[\alpha]_D$ −150° (*c* 1, $CHCl_3$); MS *m/e* 271 (M^+); ^1H NMR ($CDCl_3$) δ 1.23 (d, *J* = 6.5 Hz, C_5-CH_3), 3.23 (s, C_1-CH_3O), 3.40 (s, C_4-CH_3O), 4.70 (m, W_H = 6.5 Hz, C_1-H). Anal. ($C_{10}H_{16}F_3NO_4$) C, H, F, N. Acid hydrolysis of **8** (3.5 N AcOH for 1 h at 100 °C) gave 2,3,6-trideoxy-4-*O*-methyl-3-(trifluoroacetamido)-α-L-*lyxo*-hexopyranose (**9**) in 97% yield: mp 193–194 °C; $[\alpha]_D$ −130° (*c* 0.97, $CHCl_3$); MS *m/e* 257

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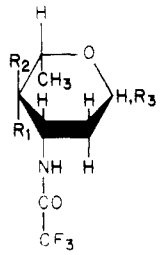
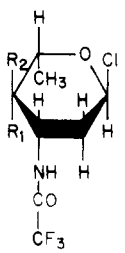
Table II. Comparison of 4′-*O*-Methyladriamycin (4) and 4′-*epi*-4′-*O*-Methyladriamycin (6) with Doxorubicin (2) on L 1210 Leukemia in Mice[a,b]

compd	dose, mg/kg	T/C[b] 1st expt	T/C[b] 2nd expt	no. of toxic deaths/ total
2	4.4	169		
	6.6	175	175	
	10.0	187	187	3/20
4	4.4	287	312	
	6.6	231	275	1/20
	10.0	75	62	17/18
6	6.6	169		
	10.0	187		
	15.0	181		2/10
	22.5	87		9/10

[a] Tumor inoculum 10^6 cells, ip. [b] As in Table I.

(M^+); ^1H NMR (Me_2SO-d_6) δ 1.13 (d, J = 6.0 Hz, C_5-CH_3), 3.53 (s, C_4-CH_3O), 5.11 (m, W_H = 8.0 Hz, C_1-H). Anal. ($C_9H_{14}F_3NO_4$) C, H, F, N. Paranitrobenzoylation of **9** gave the corresponding 1-*O*-*p*-nitrobenzoate (mixture of α and β anomers, **10**) in 92% yield: mp 168–170 °C; $[\alpha]_D$ −39°





10, R_1 = OCH_3; R_2 = H; R_3 = *O*-*p*-nitrobenzoyl
15, R_1 = H; R_2 = OCH_3; R_3 = *O*-*p*-nitrobenzoyl
11, R_1 = OCH_3; R_2 = H
16, R_1 = H; R_2 = OCH_3

(*c* 0.45, $CHCl_3$). Anal. ($C_{16}H_{17}F_3N_2O_7$) C, H, F, N. A solution of **10** in dry methylene chloride was saturated at 0 °C with anhydrous hydrogen chloride. After filtration of the precipitated *p*-nitrobenzoic acid, the 1-α-chloro derivative **11** was obtained in quantitative yield after evaporation of the filtrate. Compound **11** [^1H NMR ($CDCl_3$) δ 1.33 (d, J = 6.5 Hz, C_5-CH_3), 3.58 (s, CH_3O), 6.32 (m, W_H = 6.5 Hz, C_1-H)] was used for the coupling reaction without further purification.

The synthesis of glycoside **3** was performed by condensation of daunomycinone (3 mmol) with **11** (2 mmol) in methylene chloride (120 mL) at room temperature for 30 min, using silver trifluoromethanesulfonate as catalyst,[6] in the presence of molecular sieves (4 Å, Merck), to give stereoselectively the *N*-trifluoroacetyl derivative of 4′-*O*-methyldaunorubicin in 70% yield based on **11**: mp 151–152 °C; $[\alpha]_D$ +250° (*c* 0.06, methanol); ^1H NMR ($CDCl_3$) δ 1.33 (d, J = 6.5 Hz, C_5-CH_3), 2.40 (s, CH_3CO), 3.53 (s, C_4-CH_3O), 4.03 (C_4-CH_3O), 5.20 (br s, C_7-H), 5.50 (br s, C_1-H), 6.43 (NH), 7.16–8.06 (m, aromatic protons), 13.10 and 13.85 (2 s, phenolic OH). The α configuration of the glycosidic linkage was assigned on the basis of the $C_{1'}$-H NMR signal, which is a broad singlet (W_H = 6.5 Hz) at δ 5.50 ($CDCl_3$), characteristic of the α-glycosides of anthracyclinones.[2]

Removal of the protecting group with 0.1 N aqueous sodium hydroxide afforded in 70% yield 4′-*O*-methyldaunomycin (**3**), which was isolated as the hydrochloride: mp 173 °C dec; $[\alpha]_D$ +210° (*c* 0.042, methanol). Anal. ($C_{28}H_{32}ClNO_{10}$) C, H, N. The corresponding doxorubicin analogue **4**·HCl [mp 177 °C dec; $[\alpha]_D$ +259° (*c* 0.046, methanol). Anal. ($C_{28}H_{32}ClNO_{11}$·0.5H_2O) C, H, N] was obtained from **3** in 65% yield via the 14-bromo derivative, following an already described procedure for the chemical transformation of daunorubicin to doxorubicin.[7]

Treatment of **12**[2] (2.57 g, 0.01 mol) in methylene chloride with diazomethane–boron trifluoride etherate, as described for compound **7**, gave 1.7 g (63%) of methyl *N*-(trifluoroacetyl)-α-L-actinosaminide (**13**): mp 185 °C; $[\alpha]_D$ −101° (*c* 1, $CHCl_3$); MS *m/e* 271 (M^+); ^1H NMR ($CDCl_3$) δ 1.31 (d, J = 6.5 Hz, C_5-CH_3), 3.30 (s, C_1-CH_3O), 3.43 (s, C_4-CH_3O), 4.70 (m, W_H = 7.0 Hz, C_1-H). Anal. ($C_{10}H_{16}F_3NO_4$) C, H, N. Acid hydrolysis of **13** (3.5 N AcOH for 1 h at 100 °C) gave *N*-(trifluoroacetyl)actinosamine (**14**) in 98% yield: mp 201 °C; at equilibrium $[\alpha]_D$ −12.7° (*c* 0.48, $CHCl_3$); MS *m/e* 257 (M^+). Anal. ($C_9H_{14}F_3NO_4$) C, H, N.

Paranitrobenzoylation of **14** gave a mixture of α- and β-1-*O*-*p*-nitrobenzoates **15** in 80% yield: mp 159–160 °C; $[\alpha]_D$ −33.5° (*c* 0.47, $CHCl_3$). Anal. ($C_{14}H_{17}F_3N_2O_7$) C, H, N. Treatment of **15** in methylene chloride with hydrogen chloride gave in quantitative yield the 1-α-chloro derivative **16**: ^1H NMR ($CDCl_3$) 1.34 (d, J = 6.5 Hz, C_5-CH_3), 3.44 (s, CH_3O), 6.17 (m, W_H = 6.5 Hz, C_1-H).

Compound **16** was condensed with daunomycinone as previously described for the lyxo analogue **11**, to give the *N*-trifluoroacetyl derivative of 4′-*epi*-4′-*O*-methyldaunorubicin. Removal of the protecting group with 0.1 N aqueous sodium hydroxide gave in 20% yield based on **16** 4′-*epi*-4′-*O*-methyldaunorubicin (**5**), which was isolated as the hydrochloride: mp 192 °C dec; $[\alpha]_D$ + 275° (*c* 0.047, methanol). Anal. ($C_{28}H_{32}ClNO_{10}$·0.5H_2O) C, H, N. The configuration of the glycosidic linkage was assigned on the basis of the $C_{1'}$-H NMR signal [br s ($W_H \approx 7$ Hz) at δ 5.20 (Me_2SO-d_6)].

Compound **5** was transformed into the corresponding doxorubicin analogue **6** in 65% yield and isolated as the hydrochloride: mp 170 °C dec; $[\alpha]_D$ +252° (*c* 0.054, methanol). Anal. ($C_{28}H_{32}ClNO_{11}$·0.5H_2O) C, H, N.

Comparison of biological activity of the new analogues with the parent antibiotics on P-388 or L-1210 leukemias in mice is reported in Tables I and II. The new compounds **3**–**6** retain the antitumor activity of the parent drugs, compound **4** appearing of particular interest because of the high efficacy shown.[8]

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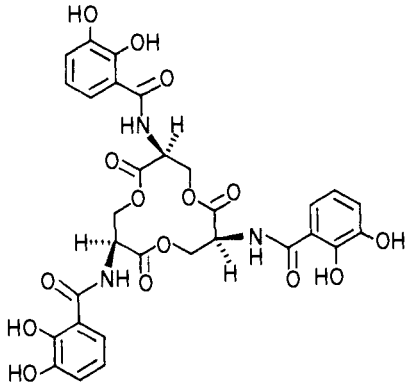
1,3,5-Tris(*N*,*N*′,*N*″-2,3-dihydroxybenzoyl)aminomethylbenzene, a Synthetic Iron Chelator Related to Enterobactin

Sir:

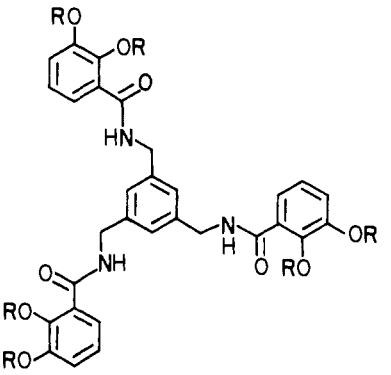
Secondary hemochromatosis, the iron-overload disease induced by a prolonged regimen of transfusion therapy for the genetic disease β-thalassemia major (Cooley's anemia), accounts for the eventual death of most patients by early adulthood. The search for an effective replacement for desferrioxamine, an iron chelator which has found limited use, is currently the subject of intense effort.[1] Of the many compounds tested as iron-chelating agents, those containing the 2,3-dihydroxybenzoyl (DHB) moiety, the active chelation site of the bacterial siderophore enterobactin, 1,[2] have shown greatest potential as orally effective drugs.[3]



1

While the natural product 1 has been demonstrated to be one of the most powerful iron chelators known ($K_s \simeq 10^{51}$)[4] and has undergone preliminary studies in test animals,[5] there now exists no plentiful source of this compound.[6] As part of a program directed toward the total synthesis of 1 and closely related, possibly useful analogues, we have designed and prepared the title compound **2**.

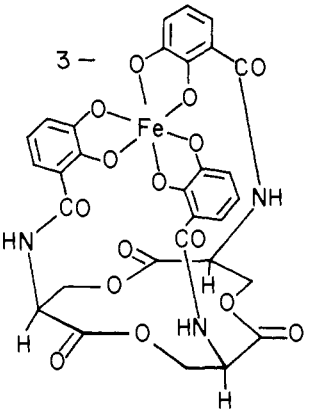
The synthesis of **2** proceeds straightforwardly and in high yield from known materials. Catalytic reduction of 1,3,5-benzenetrialdoxime[7] in ethanol–THF over 10% Pd



2, R = H
3, R = $CH_2C_6H_5$

on C in the presence of dry HCl gave 1,3,5-tris(aminomethyl)benzene trihydrochloride, mp >350 °C (EtOH–H_2O), in 97% yield. Acylation with 2,3-dibenzyloxybenzoyl chloride[8] under Schotten–Baumann conditions (aqueous NaOH) afforded protected **3** in 81% yield after silica gel chromatography. Removal of the benzyl protection by catalytic hydrogenation in ethanol–acetic acid (20:1) gave **2** as a white powder in 90–95% yield.[9]

The design of **2** as an isosteric equivalent of enterobactin (1) was governed by the geometrical feature of 1 responsible for its high affinity for iron(III), i.e., the incorporation of three DHB units into one ligand providing a chelation site of proper size. Comparison of the Dreiding models of the iron complexes of enterobactin, **4**, and the synthetic analogue, **5**, clearly indicates that the rigidity



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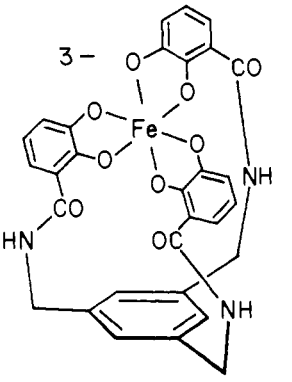

5

conferred upon **5** by the planar 1,3,5-benzylic carbon system effectively mimics that imparted to 4 by the 12-membered cyclic triester. Moreover, the hydrolytically

Table I. Growth Response Displayed by Ferric Catecholates in Enterobactin Requiring and Ferric Enterobactin Utilization Deficient Strains of Enteric Bacteria

	Diameter of Exhibition of Growth (mm)[a]					
	S. typhimurium[b]			*E. coli*[c]		
siderophore	concn, μM	*enb-1*	*enb-7*	concn, μM	RW193	RWB18
ferric enterobactin	30	26	26	3	18	8
				15	22	12
complex 5	5	20	20	2.5	10	7
				5	13	7
	50	28	29	10	17	11
dihydroxybenzoic acid	100	9	18			

[a] In each case, a 10-μL volume was pipetted onto a 6-mm filter disk; e.g., a response of 7-mm means an observed growth of 0.5 mm in radius. [b] Assays for siderophore activity were carried out according to ref 11b. [c] Assays for receptor site activity were carried out according to ref 12a; the "Tris" medium described in ref 12b was used, supplemented with 40 μg/mL each of L-Leu, L-Pro, and L-Trp, and 25 μg/mL thiamin chloride hydrochloride.

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