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NOVEL SYNTHESIS AND ANTITUMOR ACTIVITY OF 14,14-DIFLUORO-4-DEMETHOXYDAUNORUBICIN

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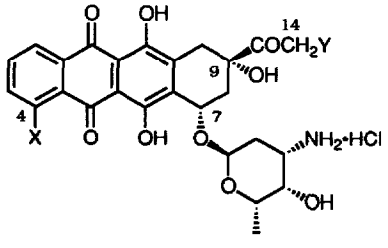
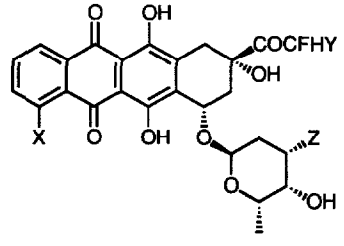
Abstract: The title anthracycline was synthesized *via* (-)-14,14-difluoro-4-demethoxy-7-deoxydaunomycinone by featuring the Reformatsky reaction of ethyl bromodifluoroacetate with an aldehyde as a key step. This novel anthracycline was found to exhibit prominent *in vitro* cytotoxicity and *in vivo* antitumor activity against P388 murine leukemia.

The anthracyclines, adriamycin (**1**) and daunorubicin (**2**), are important anticancer agents with clinical effectiveness against many types of human cancers.[2,3] However, their utilization for cancer chemotherapy are seriously restricted by their side effects, the most notable of which is dose-related cardiotoxicity. Thus, numerous synthetic studies have been devoted to overcome these disadvantages culminating in the development of unnatural 4-demethoxyadriamycin (**3**) and 4-demethoxydaunorubicin (**4**), which could show better therapeutic indices than natural **1** and **2**.[2-4]

In recent years, a great number of the fluorinated biologically active compounds have been synthesized to improve therapeutic property of the parent compounds or to explore novel pharmacological activity.[5] In the field of anthracyclines, some derivatives possessing fluorinated sugar or D-ring have been synthesized.[6,7] In connection with our program directed toward the development of novel synthetic anthracycline congeners as anticancer agents, we recently achieved the first synthesis of the 14-fluoroanthracyclines (**5-8**), which have a fluoroacetyl group as their C-9 side chain. Furthermore, these novel anthracyclines showed prominent antitumor activity against P388 murine leukemia *in vitro* and *in vivo*.[8] In the light of these results it appeared very interesting to evaluate the antitumor activity of 14,14-difluoro-4



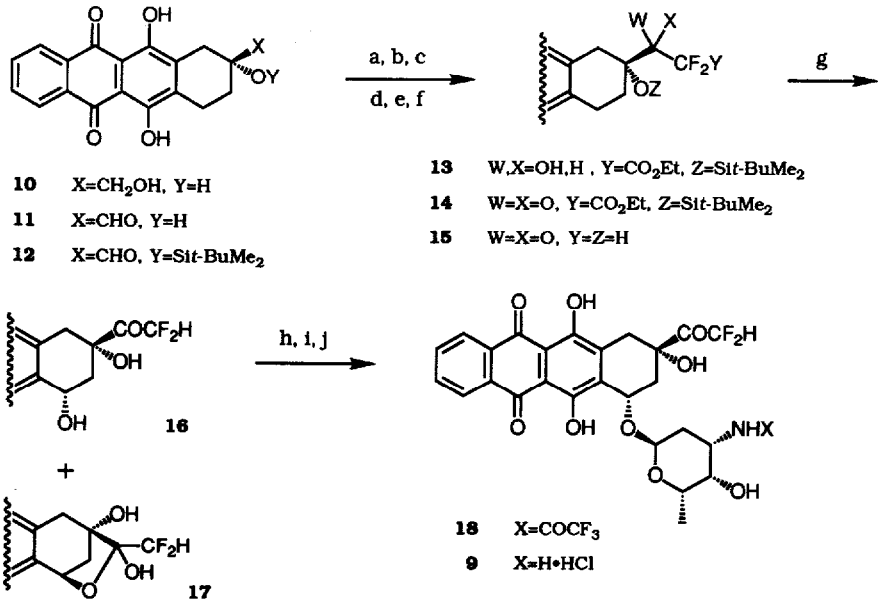


1 X=OMe, Y=OH
2 X=OMe, Y=H
3 X=H, Y=OH
4 X=Y=H

5 X =OMe, Y=H, Z=NH_2•HCl
6 X=Y=H, Z=NH_2•HCl
7 X=OMe, Y=H, Z=OH
8 X=Y=H, Z=OH
9 X=H, Y=F, Z=NH_2•HCl

demethoxydaunorubicin (**9**), the most representative member of 14,14-difluoroanthracyclines. In this communication we wish to report the first synthesis of **9** accomplished by employing the Reformatsky reaction as a key step. The novel anthracycline (**9**) was found to exhibit prominent *in vitro* cytotoxicity and *in vivo* antitumor activity against P388 murine leukemia.

After numerous preliminary experimentations carried out using racemic anthracyclinone derivatives to construct a difluoroacetyl side chain at C-9 position,[9] an efficient synthetic method for 14,14-difluoro-4-demethoxy-7-deoxydaunomycinone (**15**) was eventually explored employing the Reformatsky reaction of ethyl bromodifluoroacetate[10] with the siloxyaldehyde (**12**) as a key step. The optically pure (-)-aldehyde (**12**), mp 251-252 C°, $[\alpha]_D^{20}$ -76.9° (chloroform), was derived from the readily available diol (**10**)[13] by sequential oxidation and silylation of the resulting (-)-hydroxyaldehyde (**11**), mp 248-249 C°, $[\alpha]_D^{20}$ -54.5° (dioxane). The Reformatsky reaction of ethyl bromodifluoroacetate with **12** took place cleanly under the usual conditions[10] giving rise to the difluoro ß-hydroxyester (**13**) as an epimeric mixture. The ratio of two epimers roughly estimated by the NMR spectra of the mixture was 2:1. Without separation of the epimers, **13** was immediately oxidized to the (-)-ß-ketoester (**14**), mp 119-121 C°, $[\alpha]_D^{20}$ -91.8° (chloroform), using the Dess-Martin periodinane as an oxidant.[15] Sequential hydrolysis of the ethyl ester and decarboxylation of the formed β-ketoacid afforded (-)-14,14-difluoro-4-demethoxy-7-deoxydaunomycinone (**15**).[17] mp 225-228 °C. $[\alpha]_D^{20}$ -26.1° (dioxane).



a) SO_3•Py, Et_3N, DMSO, rt, 10 min, 79% b) TBDMSOTf, 2,6-Lu, CH_2Cl_2, 0 °C ~ rt, 5.5 h, 86% c) $BrCF_2CO_2Et$, Zn, THF, reflux, 30 min, 42% d) Dess-Martin reagent, CH_2Cl_2, 0 °C ~ rt, 20 min, 45% e) 1) 0.5N-KOH, THF-MeOH, 0 °C, 30 min 2)1N-HCl f) DMF, 60 °C, 35 min, 73% (2 steps) g) 1) Br_2, hv, CCl_4-$CHCl_3$-H_2O, rt, 2.5 h 2) 2.5N-NaOH, 0 °C, 15 min, 26% (**16**), 31% (**17**) h) L-Daunosamine derivative, TMSOTf, 4A-MS, CH_2Cl_2-Et_2O-THF, -10 °C, 5.5 h i) 0.1N-NaOH, MeOH, rt, 20 min, 89% (2 steps) j) 1) 0.05N-NaOH, THF, rt, 50 min 2) 0.25N-HCl, MeOH, 40% (2 steps).

As the next step of the synthesis, conversion of **15** into 14,14-difluoro-4-demethoxydaunomycinone (**16**) was studied. All the attempts to protect the ketonic function of **15** in a form of acetal turned out to be fruitless probably due to the adjacent two fluorine atoms. However, direct bromination of **15** with bromine under irradiation followed by direct treatment of the resulting unstable C-7 bromide with aqueous alkali to introduce C-7 hydroxyl group was found to give (+)-14,14-difluoro-4-demethoxydaunomycinone (**16**),[17] mp 210-213 °C, $[\alpha]_D^{20}$ +44.2° (dioxane), along with its C-7β epimer in a form of the (-)-hemiacetal (**17**),[18] mp 253-255 °C, $[\alpha]_D^{20}$ -153° (dioxane). The mixture of **16** and **17** could be separated with silica gel TLC.

With **16** in hand, glycosidation with L-daunosamine derivative was next attempted according to the procedure previously reported by us.[19] Thus, when **16** was allowed to react with 3-*N*-trifluoroacetyl-1,4-bis(*O*-*p*-nitrobenzoyl)-L-daunosamine in the presence of trimethylsilyl trifluoromethanesufonate and the formed glycoside was treated with dilute alkali to effect hydrolysis of the 4'-*O*-*p*-nitrobenzoyl group, (+)-3'-*N*-trifluoroacetyl-14,14-difluoro-4-demethoxydaunorubicin (**18**),[17] $[\alpha]_D^{20}$ +117° (dioxane), was obtained as a sole product. Further alkaline hydrolysis of the 3'-*N*-trifluoroacetyl group followed by salt formation furnished (+)-14,14-difluoro-4-demethoxydaunorubicin hydrochloride (**9**),[20] mp 168-171 °C, $[\alpha]_D^{20}$ +111° (methanol).

These 14,14-difluoroanthracyclines (**18** and **9**) were subjected to P388 murine leukemia *in vitro* assay. While **18** exhibited comparable cytotoxicity (IC_{50} = 1.1 x 10^{-3} μg/ml) to that of **1** (IC_{50} = 2.5 x 10^{-3} μg/ml), **9** was at least fifty times more active (IC_{50} = 4.6 x 10^{-5} μg/ml) than **1**. Since cytotoxicity of 14-fluoro-4-demethoxydaunorubicin (**6**) has already been disclosed to compare well with that of **1**,[8] it appears evident that **9** is obviously more cytotoxic than **6**. Additionally, in P388 *in vivo* test, **9** were found to show significant inhibitory activity [T/C = 161% (1.0 mg/kg)]. Further studies aimed at characterizing antitumor activity of **9** are in progress and will be reported shortly.

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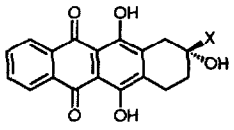
9) At the outset of this work, introduction of fluorine atom into the C-14 position of 14-fluoro-4-demethoxy-7-deoxydaunomycinone (**i**) was examined according to the fluorination method previously explored for 7-deoxydaunomycinones.[8] However, attempted bromination of **i** always produced complex mixtures of reaction products. Accordingly, the Reformatsky reaction of ethyl bromodifluoroacetate[10] was next examined, which had been frequently used to introduce a fluorinated carbon chain.[11] While the Reformatsky reaction with **11** or the acylimidazole derivative generated from the hydroxyacid (**ii**) resulted in decomposition or recovery of the starting material, it was finally revealed that the reaction with **12** successfully produced **13**. However, the addition product (**13**) could not be obtained from **12** when methyl difluoroiodoacetate[12] was employed instead of ethyl bromodifluoroacetate.



i X=$COCFH_2$

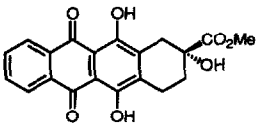
ii X=CO_2H

10) E.A. Hallinan and J. Fried, Tetrahedron Lett., **25**, 2301 (1984).

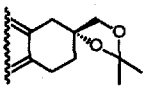
11) R.W. Lang and B. Schaub, *Tetrahedron Lett.*, **29**, 2943 (1988) and references cited therein.

12) O. Kitagawa, T. Taguchi, and Y. Kobayashi, *Tetrahedron Lett.*, **29**, 1803 (1988). We are grateful to Profs. Y. Kobayashi and T. Taguchi for providing us with sample of methyl difluoroiodoacetate.

13) The diol (**10**) was readily prepared by reduction of the optically pure ester (**iii**),[14] mp 214-215 °C, $[\alpha]_D^{20}$ -63.6° (chloroform), with sodium borohydride in the presence of cerium(III) chloride heptahydrate ($CHCl_3$-MeOH, rt, 3 h). The reduction product sparingly soluble to usual organic solvents was purified with silica gel chromatography in a form of the acetonide (**iv**), mp 260-263 °C, $[\alpha]_D^{20}$ -50.0° (dioxane) [CSA, $Me_2C(OMe)_2$-acetone-THF, rt, 4 h, 78%, from **iii**]. According to this procedure, **iv** could be obtained in higher yield than that previously reported for the reduction of **iii** with lithium tri-*tert*-butoxyaluminumhydride (55%, isolated in a form of **iv**).[14] Acidic hydrolysis of **iv** yielded a pure sample of **10** (12N-HCl, THF, 0 °C, 4 h, 90%).



iii



iv

14) M. Suzuki, T. Matsumoto, M. Ohsaki, Y. Kimura, and S. Terashima, *Chem. Pharm. Bull.*, **35**, 3658 (1987).

15) The oxidation of **13** appeared to be exceptionally difficult. Oxidizing reagents other than the Dess-Martin periodinane[16] resulted in complete recovery of **13**.

16) a) R.J. Linderman and D.M. Graves, *Tetrahedron Lett.*, **28**, 4259 (1987). b) *Idem., J. Org. Chem.*, **54**, 661 (1989).

17) The NMR spectra ($CDCl_3$) measured with samples of **15**, **16** and **18**, purified with silica gel chromatography revealed that these compounds consist of a mixture of the ketonic and the hydrate form in which the former predominate (*ca.* 10:1). Signals due to the hydrate form disappeared, after azeotropic removal of water using toluene. Formation of the hydrate form may be rationalized by electron-withdrawing property of the two fluorine atoms. Representative NMR spectral data are as follows, **15**: ^{1}H-NMR δ 6.01 (t, J=53 Hz, CHF_2, hydrate), 6.40 (t, J=54 Hz, CHF_2, ketone); ^{19}F-NMR δ -127.1 (d, J=54 Hz, CF_2H, ketone). **16**: ^{1}H-NMR δ 5.97 (t, J=55 Hz, CHF_2, hydrate), 6.60 (t, J=53 Hz, CF_2H, ketone); ^{19}F-HMR δ -129.5, -130.0 (each, dd, J=315 and 53 Hz, CF_2H, ketone), -133.2 (d, J=55 Hz, CF_2H, hydrate). **18**: ^{1}H-NMR δ 6.00 (t, J=55 Hz, CHF_2, hydrate), 6.60 (t, J=55 Hz, CHF_2, ketone).

18) In the ^{1}H-NMR spectrum ($CDCl_3$) of **17**, a single triplet assignable to the C-14 methine proton appeared at δ 5.58 (J=54 Hz). Thus, it become evident that this compound consists of a single hemiacetal whose stereostructure could not be determined. All the attempts to epimerize **17** under acidic conditions resulted in a simple recovery of the starting material. This is probably due to the electron-withdrawing effect accumulated by the two fluorine atoms, which makes the hemiacetal form highly stable.

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20) It was disclosed by the NMR spectra (CD_3SOCD_3-D_2O) of **9** that this compound consists of a mixture of the ketonic and the hydrate form (3:1). Representative NMR spectral data are as follows, **9**: ^{1}H-NMR δ 6.04 (t, J=56 Hz, CHF_2, hydrate), 6.85 (t, J=53 Hz, CHF_2, ketone); ^{19}F-NMR δ -128.0 (d, J=53 Hz, CF_2H, ketone), -131.2 (d, J=56 Hz, CF_2H, hydrate).

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