

## Synthesis and conformational preference of novel 8-fluoroanthracyclines\*

Paolo Lombardi\*\*, Fabio Animati, Amalia Cipollone, Giuseppe Giannini, Edith Monteagudo and Federico Arcamone

*Menarini Ricerche Sud, Via Tito Speri, 10, 00040 Pomezia, Italy*

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Analogues of daunorubicin possessing a fluorine atom at position C(8) of ring A have been synthesized with the aim of comparing their DNA-drug interaction and antitumour properties with those of the clinically useful anthracyclines doxorubicin and idarubicin. The synthesis of (8*S*)-8-fluoro-4-demethoxydaunorubicin, **1**, is reported and molecular mechanics and NMR studies which guided towards the synthesis of the epimeric (8*R*)-8-fluoro-4-demethoxydaunorubicin, **2**, are discussed. Both compounds were prepared by divergent routes starting from the common intermediate, **6**, obtained *via* the Diels-Alder cyclisation between quinizarin diquinone, **3**, and 2-(1-hydroxyethyl)-1,3-butadiene, **4**. The synthesis of the (8*S*)-fluoroepimer proceeded *via* epoxidation of the C(8)-C(9) olefinic bond of **6**, oxidation, oxirane cleavage by  $\text{BF}_3 \cdot \text{Et}_2\text{O}$  to give the fluorohydroxyketone, **9**, followed by the introduction of the hydroxyl moiety at C(7) and glycosylation. Conversely, the synthesis of the (8*R*)-fluoroepimer involved the fluorobromination of the C(8)-C(9) olefinic bond of **6**, formation of the C(9)-C(13) epoxide, **20** which, after regioselective hydrolysis and oxidation of the resulting fluorodiol to the epimeric fluorohydroxyketone, **21**, similarly gave the desired fluoroaglycone, **25** and, hence, the corresponding glycoside, **2**. The cytotoxic properties of the two 8-fluoroanthracycline analogues, **1** and **2**, were markedly affected by the stereochemistry of the fluorine substituent.

The anthracycline glycosides are of the greatest importance because of their spectrum of activity against a variety of haematological malignancies and of solid tumours in humans. Clinical effectiveness has been established for different compounds of the series and doxorubicin, together with related drugs daunorubicin, idarubicin (4-demethoxydaunorubi-

cin) and epirubicin (Fig. 1) are widely applied in the medical treatment of different tumour diseases. However, considerable room for improvement exists due to the limitation of the present chemotherapy, due mainly to development of resistance in patients that originally responded to treatment. In addition there exist limitations deriving from the natural resistance

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\*\*To whom correspondence should be addressed.

**Abbreviations:** AIBN, azobisisobutyronitrile; DMSO, dimethylsulphoxide; NBS, *N*-bromosuccinimide; NOE, nuclear Overhauser effect; TFA, trifluoroacetic acid; TMSOTf, trimethylsilyl triflate; r.t., room temperature.

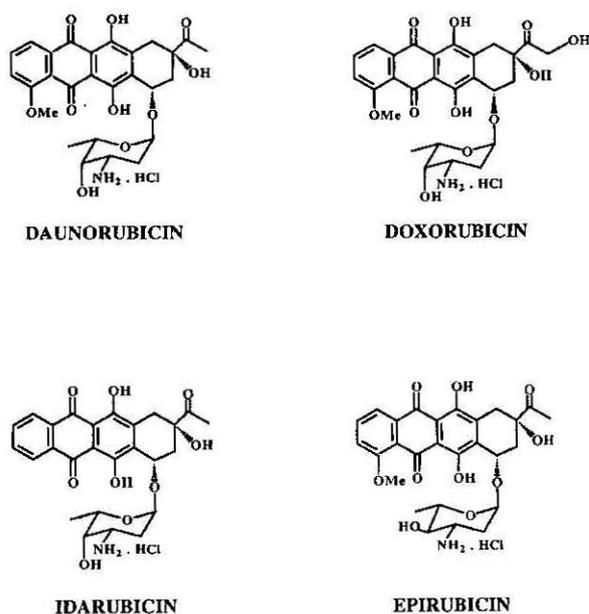


Fig. 1. Clinically useful anthracyclines.

of major cancers, including important ones such as colon carcinomas, most lung cancers, pancreatic tumour and malignant melanoma.

The modification and enhancement of the biological activity of drugs by fluorination represents one of the most fruitful recent developments in medicinal chemistry. Electron withdrawal by fluorine results in a strong polarization of the C-F bond and the pronounced electronic effects can have implications for reactions at adjacent centres, that is for drug-target interactions.

Evidence is available that cell DNA is the main biological target of antitumour anthracyclines, in agreement with the high-affinity constant of doxorubicin and its clinically useful congeners for native double-stranded DNA [1, 2]. Different X-ray diffraction studies of anthracycline-DNA complexes are now available. In particular, the crystal structure of the daunorubicin-d(CGTACG)<sub>2</sub> intercalation complex (Fig. 2) [3] shows that the C(9)-OH group of the aglycone is involved in hydrogen bonding with both the C(2)-NH<sub>2</sub> and the N(3) of the adjacent guanine base. The aminosugar lies in the minor groove without bonding to the DNA: the C(3')-NH<sub>2</sub> and C(4')-OH groups point away from the deoxyoligonucleotide and are possibly available for other interactions. This observation can be related to the established dependence of bioac-

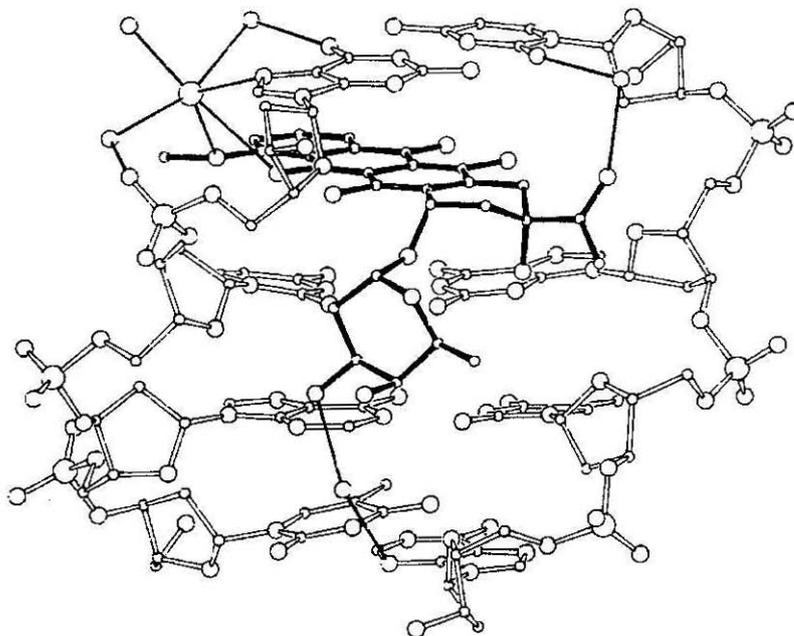


Fig. 2. The crystal structure of the daunorubicin-d(CGTACG)<sub>2</sub> complex solved by X-ray diffraction analysis [3].

In particular, the structure of the intercalation complex shows that the C(9)-OH group of the aglycone is involved in hydrogen bonding with both the C(2)-NH<sub>2</sub> and the N(3) of an adjacent guanine base. The aminosugar lies in the minor groove without bonding to the DNA: the C(3')-NH<sub>2</sub> and C(4')-OH groups are pointing away from the deoxyoligonucleotide. (Drawing kindly provided by Dr. G. Ughetto, Consiglio Nazionale delle Ricerche, Montelibretti, Roma).

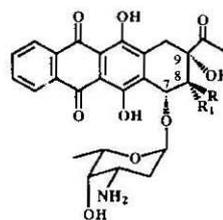
tivity on the orientation of the sugar substituents in the antitumour anthracycline glycoside [4]. The preference for the intercalation site (GC) would be due to the C(9)-OH group because of hydrogen bonding interactions, and that for the AT-pair flanking the intercalation site would be attributed to repulsion between the daunosamine amino group and a guanine residue in that position.

Conceivably, the introduction of a fluorine atom adjacent to the C(9)-OH function may alter the biorelevance of the hydroxyl group by affecting the strength and/or specificity of the hydrogen bond, providing pharmacologically interesting new compounds. We have therefore synthesized analogues of daunorubicin possessing a fluorine atom at position C(8) with the aim of comparing their DNA-drug interaction and antitumour properties with those of the clinically useful anthracyclines.

Ring A, the alicyclic moiety of the tetracyclic anthracycline chromophore, bears a two-carbon-atom side chain and a tertiary hydroxyl, both characteristic features of most anthracyclines. In addition, ring A also contains the site of sugar attachment (the benzylic carbon at C(7)), and therefore both asymmetric centres of the aglycone moiety of the antitumour glycosides. Moreover, derivatives modified at C(8), which is the position closest to both C(9) and C(7), and which invariably corresponds to a CH<sub>2</sub> group in all classical anthracyclines, are of interest for two reasons. First, this position appears with a methoxy group in the related biologically active, mold metabolite, steffimycin [5]. Second, the main metabolic reaction inactivating the antitumour anthracyclines *in vivo* is an enzymatically catalyzed reductive deglyco-

sylation leading to the corresponding C(7)-deoxyglycones [6], apparently devoid of antitumour properties. The presence of substituents at the nearby C(8) position would possibly affect the affinity of the substrate for the enzyme responsible for the transformation and therefore enhance the selectivity of action of the drugs.

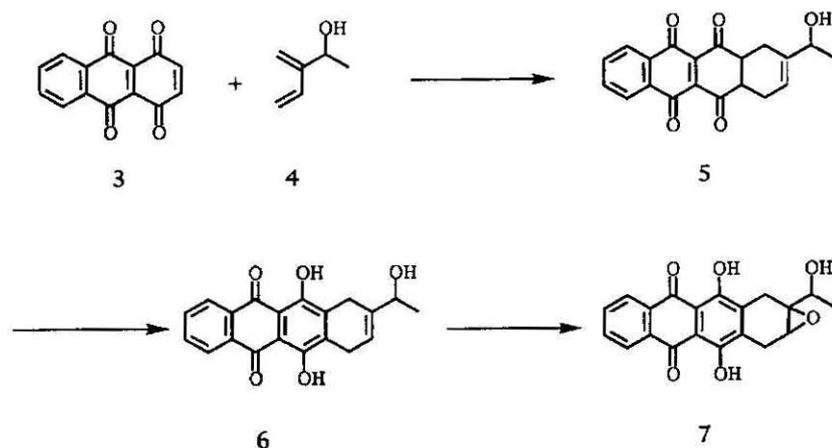
In this paper we present the synthesis of (8S)-8-fluoro-4-demethoxydaunorubicin (**1**) and discuss how the biological properties shown by this compound, compared to those of doxorubicin and idarubicin, suggested further molecular mechanics and NMR studies that guided towards the synthesis of its epimer (8R)-8-fluoro-4-demethoxydaunorubicin (**2**). The aglycones of the two glycosides differ by having a *trans* and a *cis* 8,9-fluorohydrin system, respectively. The new stereogenic centre generated in the aglycone results in a synthetic challenge for the correct construction of the three aligned stereogenic centres C(7), C(8) and C(9). A preliminary account of the synthesis of the *N*-trifluoroacetyl derivative of **1**, based on a different synthetic methodology, has already been reported by one of us [7].



**1**: R = F, R<sub>1</sub> = H

**2**: R = H, R<sub>1</sub> = F

The assemblage and the correct functionalisation of the naphthacenequinone ring system to



Scheme 1. Assemblage and functionalisation of the naphthacenequinone ring system [8].

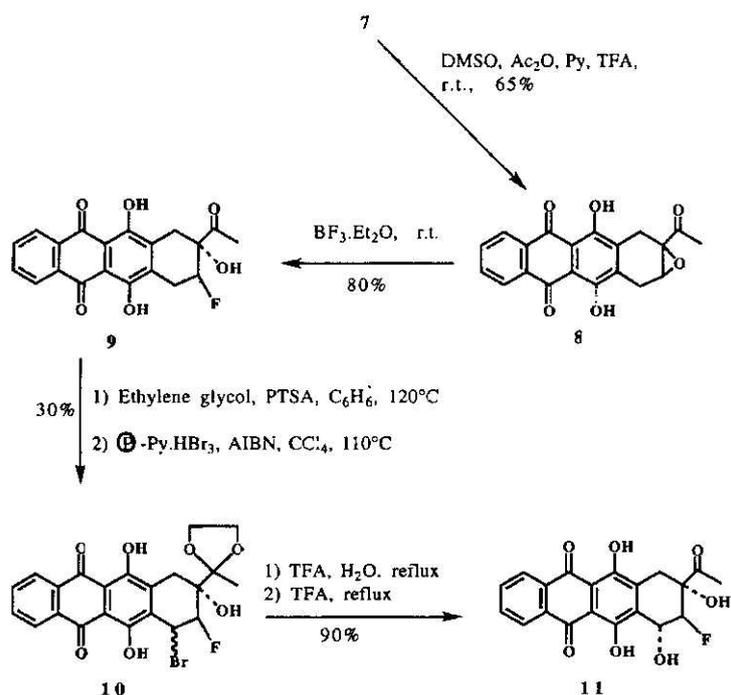
be subjected to the introduction of the fluorine atom at C(8) was accomplished by resorting to a Diels-Alder based synthetic methodology developed for synthesis of racemic 4-demethoxydaunomycinone (idarubicinone) (Scheme 1). Thus, as previously reported by one of us [8], quinizarin diquinone (3) was treated with an equimolecular amount of 2-(1-hydroxyethyl)-1,3-butadiene (4) to yield the adduct 5, which was tautomerized to the red anthraquinone derivative 6, from which the oxirane 7, as a mixture of diastereoisomers, was obtained.

## RESULTS AND DISCUSSION

### Synthesis of racemic (7*R*\*, 8*S*\*, 9*R*\*)-8-fluoro-4-demethoxydaunomycinone (11)

The preparation of *trans* fluorohydrins by ring opening of oxiranes is one of the most common and most selective reactions for introducing a fluorine atom into biologically relevant molecules [9, 10]. Therefore, the epoxyketone 8 (Scheme 2), obtained from 7 by the Moffat oxidation, was considered a convenient precursor for the synthesis of the racemic (7*R*\*, 8*S*\*, 9*R*\*)-8-fluoroaglycone 11. Various fluorinating reagents exist with the capacity to add selectively to epoxides, including Olah's reagent (HF • pyridine) [11], which was previously used on the 6,11-dimethoxy derivative of 8 in the reported synthesis of 11 [7], and boron trifluoride eth-

erate [12]. Most satisfactorily, the adduct 8 underwent diaxial cleavage of the epoxide ring upon treatment with neat BF<sub>3</sub> • Et<sub>2</sub>O at room temperature to afford the expected *trans* 8,9-fluorohydrin 9. In accord with previous experience [7] indicating that the classical bromination procedure at benzylic C(7), an intermediate step for the introduction of the hydroxyl group, reported for the non-fluorinated compounds [13], lacked regioselectivity as a possible consequence of the deactivation of the benzylic position by the adjacent fluorine substituent, the introduction of bromine onto the ketal derivative of 9 was performed with polymer-supported pyridinium hydrobromide perbromide [14] in the presence of azobisisobutyronitrile (AIBN) as a radical initiator, to yield the bromide 10 as a mixture of epimers at C(7). This mixture was hydrolysed with aqueous trifluoroacetic acid at reflux and equilibrated with refluxing *anhydrous* trifluoroacetic acid to give racemic (7*R*\*, 8*S*\*, 9*R*\*)-8-fluoro-4-demethoxydaunomycinone 11 almost quantitatively [<sup>1</sup>H NMR (CDCl<sub>3</sub>) d<sub>H</sub>: 2.50 (3H, s, CH<sub>3</sub>-14), 3.20 (2H, AB system, CH<sub>2</sub>-10), 3.75 (1H, d, OH-7), 4.60 (1H, s, OH-9), 4.82 (1H, dd, H-8), 5.20 (1H, m, H-7), 7.82 (2H, m, H-2, 3), 8.28 (2H, m, H-1, 4), 13.28 (1H, s, phenolic proton), 13.60 (1H, s, phenolic proton)]. The relative orientation of the fluorine atom with respect to the hydroxyl groups at C(7) and C(9) was determined using Nuclear Overhauser Effect (NOE) experi-



Scheme 2. Synthesis of racemic (7*R*\*, 8*S*\*, 9*R*\*)-8-fluoro-4-demethoxydaunomycinone (11).

ments. Irradiation of H-8 resulted in an enhancement of the signals for OH-7 and OH-9, indicating that all three of these protons are on the same face of the ring A.

#### Synthesis and cytotoxicity of (8S)-8-fluoro-4-demethoxydaunorubicin (1)

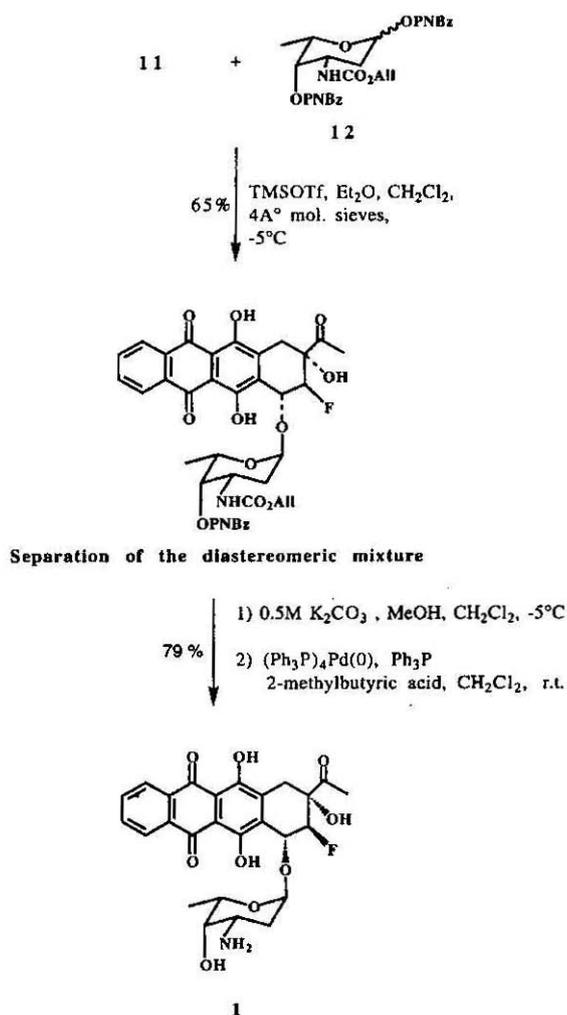
The aglycone **11** was glycosylated with *N*-allyloxycarbonyl-1,4-bis(*O*-*p*-nitrobenzoyl)-*L*-daunosamine (**12**) and trimethylsilyl triflate (TMSOTf) as a condensing agent [15] (Scheme 3), and the resulting diastereomeric glycosides were separated by flash column chromatography. The C(4')-OH and the C(3')-NH<sub>2</sub> were deprotected with K<sub>2</sub>CO<sub>3</sub> in the cold and with tetrakis triphenylphosphine palladium (0) and added Ph<sub>3</sub>P [16], respectively. The desired diastereoisomer **1** was singled out on the basis of the similarity of its circular dichroism spectrum

to that of natural daunorubicin. The *N*-allyloxycarbonyl-1,4-bis(*O*-*p*-nitrobenzoyl)-*L*-daunosamine (**12**) was prepared by *p*-nitrobenzoylation of *N*-allyloxycarbonyl-*L*-daunosamine obtained from the acidic hydrolysis of *N*-allyloxycarbonyldaunorubicin (A. Cipollone, unpublished results from this laboratory). The choice of this protecting group made possible the preparation of the desired aminoglycoside because of the mild conditions used in the de-blocking step.

The novel fluoroanthracycline **1** was then examined in preclinical models for its biological activity in comparison with doxorubicin and idarubicin. *In vitro* studies on three relevant human tumour cell lines (A431, A2780 and H460) were performed (Table 1) and the cytotoxic effects were studied following one hour exposure. The results, expressed as IC<sub>50</sub>, showed that **1** exhibits a cytotoxic potency comparable to that of doxorubicin and much lower than that of the structurally more related idarubicin. Thus, the introduction of fluorine into the hitherto largely untouched C(8) position of anthracyclines seemed disappointing and apparently fruitless. Nevertheless, we suspected that, in spite of its radius being only slightly greater than that of hydrogen, the pronounced electronic effects exerted by the fluorine atom might cause unpredicted steric consequences which affected the interaction of the anthracycline with its biological target(s).

#### Molecular mechanics and NMR studies

The results of different studies, dealing with the conformation of antitumour anthracyclines in the solid state, gave indications that the cyclohexene ring (ring A) was in the "half-chair" conformation (Fig. 2), as also shown by the <sup>1</sup>H NMR spectra of different daunorubicin derivatives, with the sugar moiety nearly perpendicular to the plane of the chromophore [1]. The conformation of ring A can be important for the interaction of these drugs with DNA and it is believed to be a relevant structural property for antitumour activity [17]. The existence of the preferred  $\alpha$  half-chair conformation of the ring A in daunorubicin (Fig. 3) and doxorubicin in solution has been proven by <sup>1</sup>H NMR [18, 19]. Two interactions were thought to favour the  $\alpha$  half-chair conformation: the possibility of hydrogen bonding between C(9)-OH and C(7)-O, and the longer distance between phenolic C(6)-



Scheme 3. Synthesis of (8S)-8-fluoro-4-demethoxydaunorubicin (**1**).

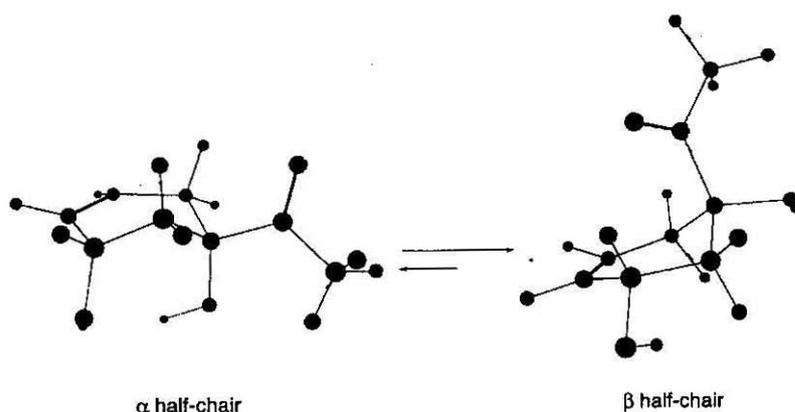


Fig. 3. Conformational flexibility of ring A in aglycones of anthracyclines.

OH and C(7)-O, which decreases the repulsive interaction between the two corresponding dipoles. In this respect, semisynthetic 6-deoxyanalogues, which were shown to assume preferentially the  $\beta$  half-chair conformation for ring A by  $^1\text{H}$  NMR studies in polar solvents, have a lower affinity for native DNA than daunorubicin [17].

Consequently, molecular mechanics calculations were performed to determine the relevant conformational forms of ring A of 4-demethoxydaunomycinone, of the 8-fluoroanalogue **11** and of its corresponding 8-epimer. Previous work demonstrated that the results can be extrapolated to the corresponding glycosides [20].

The conformation of a six-membered ring with a double bond can be described, using the Truncated Fourier Formalism [21], with only two coordinates, the phase angle  $P2$  and the puckering amplitude  $\phi_2$ . In order to map the whole conformational space, we have calculated for ring A of the aglycones all the endocyclic torsions for ten values of  $P2$  (from  $P2 = 0^\circ$  to  $P2 = 360^\circ$ ) and  $\phi_2 = 25^\circ$ .  $\phi_2$  Represents the degree of puckering of the ring, and is equal to half the maximum possible internal dihedral angle. For example, a half-chair with  $\phi_2 = 25^\circ$  will present a dihedral angle opposite to the double bond of  $50^\circ$ .

With this set of internal dihedral angles we have calculated with Discover Insight software the energy of the corresponding conformations using a modified force-field in which we introduced an extra potential to account for the attractive gauche effect (Fig. 4). The curves plotted indicate a preference for the  $\alpha$  half-chair conformation for 4-demethoxydaunomycinone and a preference for the  $\beta$  half-chair conformation for the (8S)-8-fluoro-4-demethoxy-

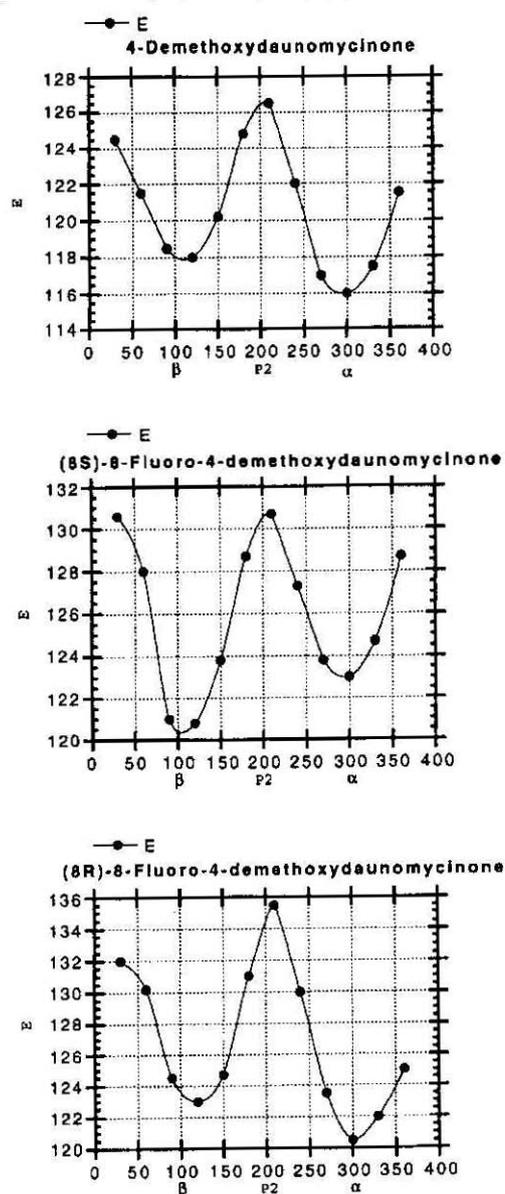


Fig. 4. Calculated energies corresponding to the  $\alpha$  half-chair and  $\beta$  half-chair conformations for the 4-demethoxydaunomycinone and the two 8-fluoroaglycones.

daunomycinone (**11**). They predict as well a propensity for the  $\alpha$  half-chair conformation for the epimer of **11**, that is the (8*R*)-8-fluoro-4-demethoxydaunomycinone, suggesting that the inversion of configuration at the carbon atom bearing the fluorine substituent would make the fluoroanthracycline **2** assume the conformational preference of the antitumour anthracyclines.

Based on energetic considerations, it was decided to describe the system with a two-state model. This assumption implies that only the  $\alpha$  and  $\beta$  half-chair conformations will be in fast equilibrium in solution. Each of these conformers represents a whole set of conformations, including the related boats and half-boats. However, it is known that the process of "pseudolibration" [22, 23] (the interconversion between conformations that are not separated by an energy barrier), does not alter significantly the value of the expected coupling constant,  $J_{H-H}$ . In other words, our model will consist of two "dense" conformations, but when we refer to the  $\alpha$  half-chair, we will consider it as the central position of a distribution of conformers.

The vicinal NMR coupling constants,  $^3J_{H7-H8}$ , were calculated in the two conformers of our model, using the Karplus equation as modified by Altona [24]. The values obtained,  $^3J_{H7-H8} = 2.5$  Hz (100%  $\alpha$  half-chair) and  $^3J_{H7-H8} = 6.0$  Hz (100%  $\beta$  half-chair) were compared with the

experimental data  $J_{exp}$  for the 4-demethoxydaunomycinone and for the 8-fluoroaglycone **11**, considering that:

$$J_{exp} = x\alpha J\alpha + (1-x\alpha) J\beta$$

where  $x\alpha$  represents the molar fraction of the  $\alpha$  half-chair conformer,  $J\alpha = 2.5$  Hz and  $J\beta = 6.0$  Hz (calculated values). The experimental  $^3J_{H7-H8}$ , (corrected for possible non-first-order effects, using the LAOCOON routine [25]) of 4.0 Hz for the compound **11** in dimethylsulphoxide, showed that the  $\beta$  half-chair conformation is as important as the  $\alpha$ . This is an interesting result since the only example in the literature of a preferred  $\beta$  half-chair conformation for the ring A of anthracyclines was that of the 6-deoxy derivatives in dimethylsulphoxide [17].

The good correlation of the molecular mechanics calculated results with those obtained experimentally for the 4-demethoxydaunomycinone [19, 20] and for the 8-fluoroaglycone **11** allowed us to use the results for the 8-fluoroepimer of **11** with a high level of confidence. In the fluorinated anthracycline analogue **1** the importance of the  $\beta$  half-chair conformation in the equilibrium could be due to a stereoelectronic attractive gauche effect between C(7)-O...C(8)-F and C(9)-OH...C(8)-F. Conversely, the same effect would dictate a propensity for the  $\alpha$  half-chair conformation for the epimer **2** (Fig. 5).

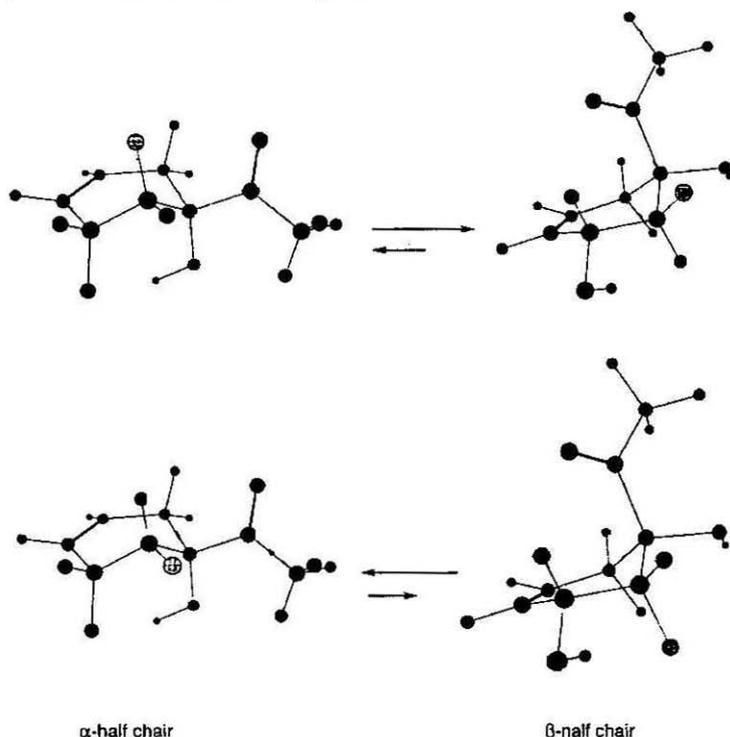
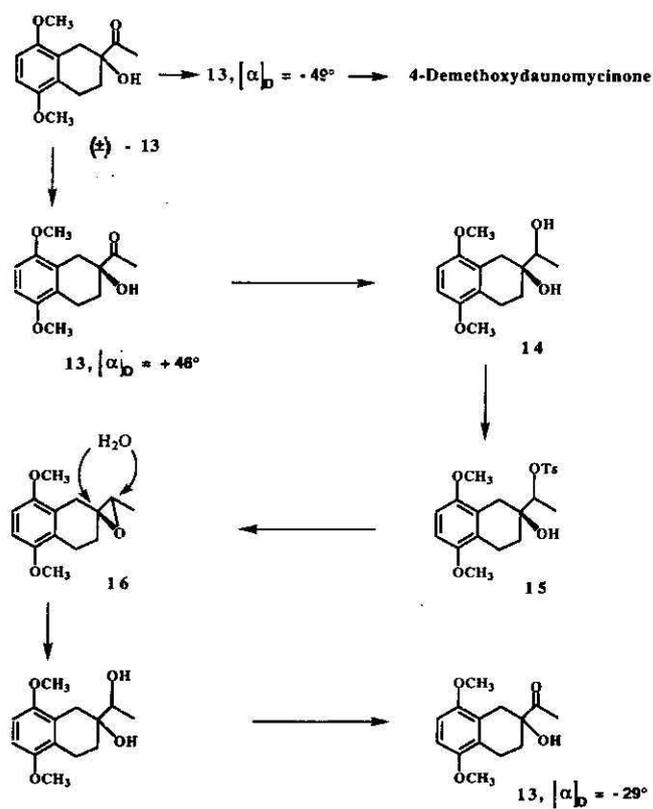


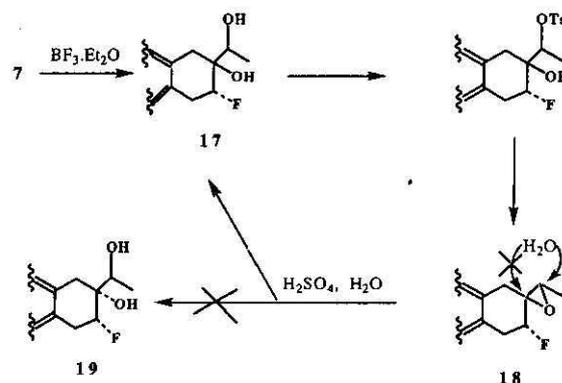
Fig. 5. Conformational flexibility of ring A in fluorinated aglycones of anthracyclines **1** (top) and **2** (bottom).

### Synthetic studies for the construction of the *cis* 8,9-fluorohydrin

A first synthetic strategy aimed at obtaining the 8-fluoroepimer of the aglycone **11** tried to take advantage of a finding two of us described in reporting an alternative synthesis of 1,4-dimethoxy-6-acetyl-6-hydroxytetralin (**13**), a key intermediate in the industrial synthesis of 4-demethoxydaunomycinone, the aglycone of idarubicin (Scheme 4) [26]. Resolution of racemic **13** yielded, together with the intermediate of correct stereochemistry, the unwanted (+)-antipode. By studying a possible recovery of this material, it was transformed into an epoxide **16** *via* reduction to the diol **14** and ring closure of the corresponding monotosylate **15**. Upon acidic hydrolysis, the epoxide **16** gave a diol which, after oxidation, afforded acetylhydroxytetralin partially inverted at C(6), indicating either that attack of water at the protonated oxirane occurred at both sides but with preference at the tertiary carbon, or that a tertiary carbenium ion was solvolitically generated with one face more exposed to nucleophilic attack, or that both mechanisms were concomitant.



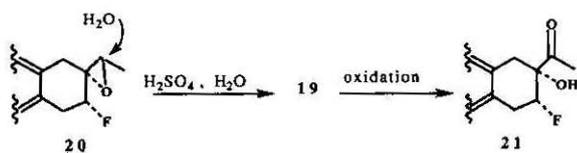
It was hoped, therefore, that the 8,9-fluorohydrin with the required *cis* geometry could be obtained to some extent by repeating the above procedure on the *trans* 8,9-fluorohydrin system of the 8-fluoroaglycone **11** (Scheme 5). The starting 8-fluoro-9,13-diol **17** was directly obtained from the epoxyalcohol **7** with  $BF_3 \cdot Et_2O$  and it was converted into the epoxide **18** by tosylation, which occurred solely at the more reactive C(13) secondary alcohol, followed by basic treatment. The latter compound, subjected to acidic hydrolysis, disappointingly gave starting fluorodiol **17** and no trace of the target fluorodiol **19** was detected.



Scheme 5. Attempted access to the *cis* 8,9-fluorohydrin system **19**.

In the light of this results, it was thought that the strong electronic effect exerted by the adjacent fluorine atom on the regioselectivity of the nucleophilic cleavage of the protonated oxirane ring could be advantageously exploited, provided that a fluoroepoxide of the correct geometry such as **20** could be built (Scheme 6). This, on exposure to acidic aqueous conditions, would yield the sole fluorodiol **19**, which would be oxidized to the fluorohydroxyketone **21**.

Scheme 4. Resolution of racemic 1,4-dimethoxy-6-acetyl-6-hydroxytetralin (**13**), an intermediate for the industrial synthesis of idarubicin, and obtaining of a partially inverted material from the unwanted antipode [27].

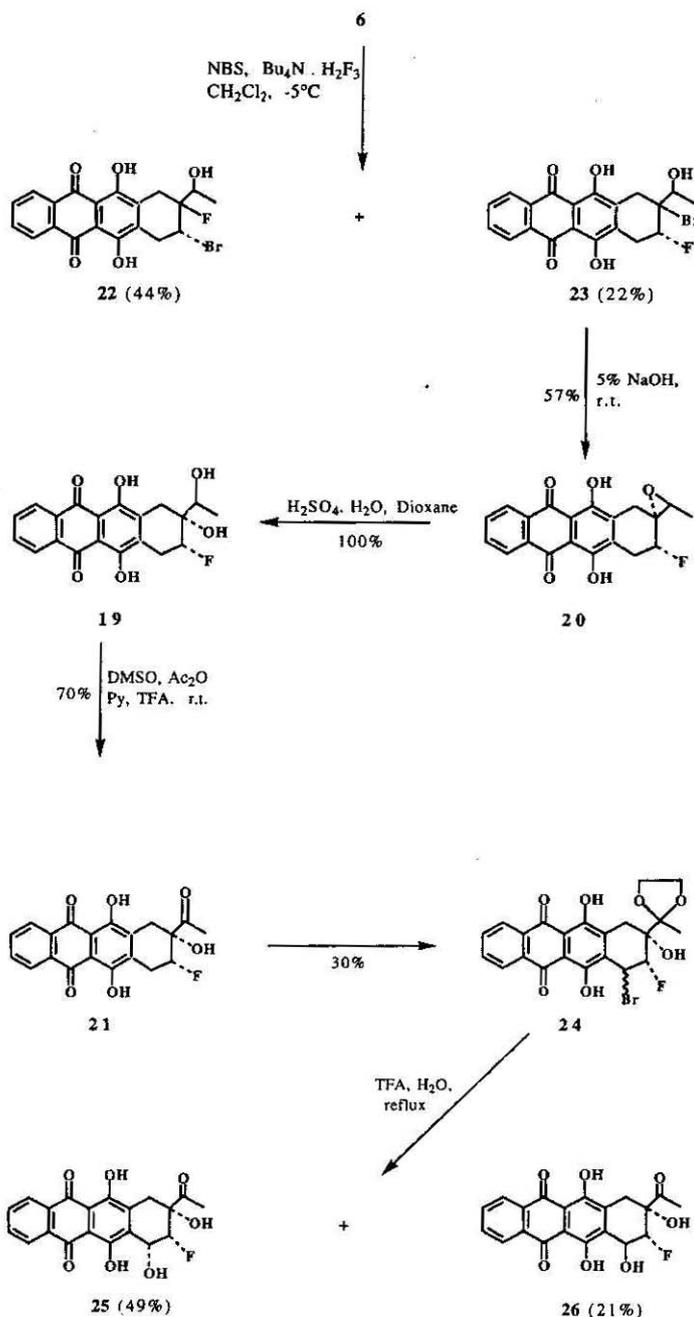


Scheme 6. Proposed access to the *cis* 8,9-fluorohydroxy system 21.

#### Synthesis of racemic (7*R*\*, 8*R*\*, 9*R*\*)-8-fluoro-4-demethoxydaunomycinone (25)

The allylic alcohol **6** from the Diels-Alder cycloaddition was then reacted with *N*-bromosuccinimide and tetrabutylammonium dihydrogen trifluoride [27] to give the two regioisomeric *trans* fluorobromo compounds **22** and **23** in a 2:1 ratio, respectively (Scheme 7). Although unfavourable regiochemistry was obtained, enough **23** could be easily prepared and separated to allow the continuation of the synthesis. Thus, basic treatment of the 8-fluoro-9-bromo-13-hydroxy derivative **23** gave the required *syn* fluoroepoxide **20** in acceptable yield, together with aromatized by-products. As expected, the compound **20** underwent regioselective oxirane ring opening in aqueous acidic medium to afford solely fluorodiol **19** which was oxidized to the *cis* fluorohydroxyketone **21** by the Moffat methodology. Bromination at C(7), performed as described for the *trans* isomer **9**, furnished the bromoketal **24** as a mixture of epimers which was hydrolysed with aqueous trifluoroacetic acid to give the desired racemic (7*R*\*, 8*R*\*, 9*R*\*)-8-fluoro-4-demethoxydaunomycinone **25** [<sup>1</sup>H NMR (CDCl<sub>3</sub>) d<sub>H</sub>: 2.50 (3H, s, CH<sub>3</sub>-14), 3.24 (2H, ABX system, H-10), 3.82 (1H, d, OH-7), 4.80 (1H, s, OH-9), 5.15 (1H, dd, H-8), 5.50 (1H, m, H-7), 7.82 (2H, m, H-2, 3), 8.29 (2H, m, H-1, 4), 13.25 (1H, s, phenolic proton), 13.60 (1H, s, phenolic proton)] and its C(7) epimer **26** [<sup>1</sup>H NMR (CDCl<sub>3</sub>) d<sub>H</sub>: 2.50 (3H, s, CH<sub>3</sub>-14), 3.20 (2H, AA' system, H-10), 5.25 (1H, dd, H-8), 5.45 (1H, dd, H-7), 7.92 (2H, m, H-2, 3), 8.20 (2H, m, H-1, 4), 13.20 (1H, s, phenolic proton), 13.92 (1H, s, phenolic proton)] in a 7:3 ratio. In the case of compound **25**,

irradiation of H-8 did *not* result in an enhancement of the signals for the hydroxyl hydrogens (OH-7 and OH-9), indicating that in this compound the proton H-8 was on the opposite side of ring A to the two hydroxyl groups; in the case of compound **26**, an enhancement of the resonance of OH-7, but not of OH-9, was obtained by irradiation of H-8, indicating that H-8 was on the same side as OH-7 and on the opposite side



Scheme 7. Synthesis of racemic (7*R*\*, 8*R*\*, 9*R*\*)-8-fluoro-4-demethoxydaunomycinone (**25**).

to OH-9. Differently from the 8S\* analogue, additional treatment with anhydrous trifluoroacetic acid failed to epimerise **26** to **25**, indicating that even the stereochemistry of the fluorine substituent at C(8) had some implications at C(7) adjacent centre.

#### Synthesis and cytotoxicity of (8R)-8-fluoro-4-demethoxydaunorubicin (**2**)

The aglycone **25** was glycosylated with the L-daunosamine derivative **12** in a manner identical to that used for the fluoroepimer **11** (Scheme 8). After separation of these resulting diastereomeric glycosides and deprotection of the relevant protecting groups on the sugar moiety, the fluoroanthracycline **2** was singled out on the basis of the similarity of its circular dichroism spectrum to that of natural daunorubicin.

The results of the *in vitro* studies on the three relevant human tumour cell lines A431, A2780 and H460 showed that the (8R)-8-fluoro-4-demethoxydaunorubicin (**2**) displayed a cyto-

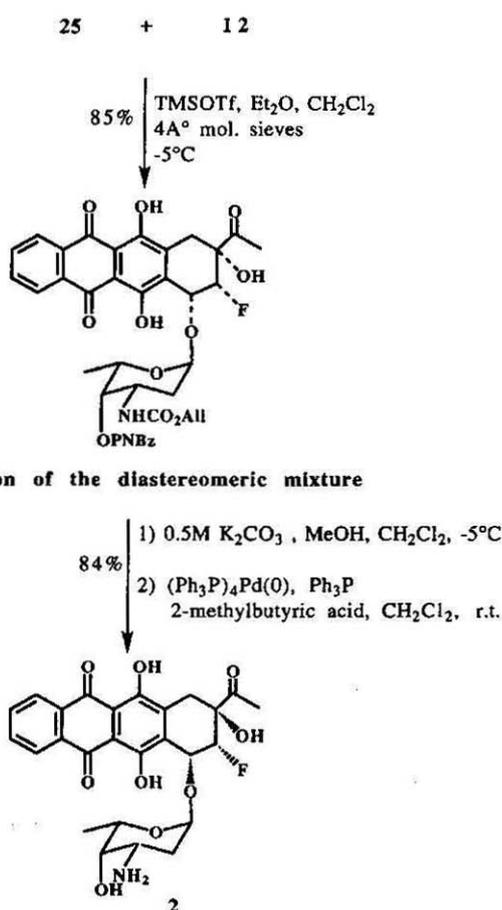
toxic activity higher than that of doxorubicin and of the (8S)-8-fluoro-4-demethoxydaunorubicin (**1**) and comparable to that of idarubicin (Table 1). The different biological behaviours

Table 1

Comparative cytotoxic activity of doxorubicin (DXR), idarubicin (IDA), (8S)-8-fluoro-4-demethoxydaunorubicin (**1**) and (8R)-8-fluoro-4-demethoxydaunorubicin (**2**) on human tumour cell lines following 1 h exposure.

The results are expressed as IC<sub>50</sub> (µg/ml) and are the mean of at least three experiments. (Data from Istituto Nazionale Tumori, Milano).

Cell line	DXR	IDA	(1)	(2)
A-431 (cervix cancer)	0.26	0.01	0.22	0.03
A-2780 (ovarian cancer)	0.20	0.008	0.20	0.002
H-460 (lung cancer)	0.06	0.001	0.08	0.025



Scheme 8. Synthesis of (8R)-8-fluoro-4-demethoxydaunorubicin (**2**).

were ascribed to the stereochemistry of the fluorine substituent, which modifies the conformational preference of the ring A. This fact can be predicted by using molecular mechanics calculations and agrees with the experimental data obtained by NMR studies.

#### CONCLUSIONS

We have synthesized the anthracycline analogues **1** and **2** bearing a fluorine atom at the same C(8) of ring A of the aglycone residue, but with the different stereochemistry 8S and 8R, respectively, with the aim of comparing their DNA-drug interaction and antitumour properties with those of the clinically useful anthracyclines doxorubicin and idarubicin.

It is generally accepted that the presence of fluorine in a molecule has minor steric effects (due to its radius being only slightly greater than that of hydrogen), but potentially major electronic consequences [28]. However, the pronounced electronic effects exerted by the introduction of the fluorine atom in the C(8) prostereogenic centre of anthracyclines resulted in modifications to a conformational preference of ring A, as could be predicted theoretically and confirmed by NMR experi-

ments, resulting in dramatically different biological behaviour. The (8*S*)-8-fluoro-4-demethoxydaunorubicin (**1**) showed a cytotoxicity comparable to that of doxorubicin and much lower than that of the structurally related idarubicin. Conversely, the (8*R*)-8-fluoro-4-demethoxydaunorubicin (**2**) showed a cytotoxic activity higher than that of doxorubicin and of **1** and comparable to that of idarubicin. Thus, the cytotoxic properties of the two 8-fluoroanthracycline analogues, **1** and **2**, were markedly affected by the stereochemistry of the fluorine substituent, suggesting that the conformational preference of ring A should be considered in planning modifications in anthracyclines.

## REFERENCES

- Arcamone, F. (1981) *Doxorubicin, Medicinal Chemistry Series* (Stevens, G., ed.) vol. 17, Academic Press, New York.
- Arcamone, F. (1984) Antitumor anthracyclines: Recent developments. *Med. Res. Rev.* **4**, 153–188.
- Wang, A.H.-J., Ughetto, G., Quigley, G.J. & Rich, A. (1987) Interactions between an anthracycline antibiotic and DNA: Molecular structure of daunomycin complex to d(CpGpTpApCpG) at 1.2 Å resolution. *Biochemistry* **26**, 1152–1163.
- Arcamone, F. & Penco, S. (1987) Chemical derivatives of anticancer antibiotics with different DNA binding properties; in *Molecular Mechanism of Carcinogenic and Antitumor Activity* (Chagas, C. & Pullman, B., eds.) pp. 225–241, Pontificia Academia Scietiarum, Citta del Vaticano.
- Kelly, R.C., Schletter, I., Koert, J.M., MacKellar, F.A. & Wiley, P.F. (1977) Structures of steffimycin and steffimycin B. *J. Org. Chem.* **42**, 3591–3596.
- Bachur, N.R. & Craddock, J.C. (1970) Daunomycin metabolism in rat tissue slices. *J. Pharmacol. Exp. Ther.* **175**, 331–337.
- Giolitti, A., Guidi, A., Pasqui, F., Pestellini, V. & Arcamone, F. (1992) The synthesis of 8-(*S*)-fluoro-*N*-trifluoroacetylidarubicin. *Tetrahedron Lett.* **33**, 1637–1640.
- Del Nero, S. & Lombardi, P. (1984) A Diels-Alder synthetic approach to racemic 4-demethoxydaunomycinone. *Gazz. Chim. Ital.* **114**, 517–520.
- Sharts, C.M. & Sheppard, W.A. (1974) Modern methods to prepare monofluoroaliphatic compounds. *Org. React.* **21**, 125–406.
- Shimizu, M. & Yoshioka, H. (1988) Highly selective ring opening of epoxides with silicon tetrafluoride: preparation of fluorohydrins. *Tetrahedron Lett.* **29**, 4101–4104.
- Olah, G.A., Welch, J.T., Vankar, Y.D., Nojima, M., Kerekes, I. & Olah, J.A. (1979) Synthetic methods and reactions. 63. Pyridinium poly(hydrogen fluoride) (30% pyridine-70% hydrogen fluoride): a convenient reagent for organic fluorination reactions. *J. Org. Chem.* **44**, 3872–3881.
- Bowers, A., Ibanez, L.C. & Ringold, H.J. (1959) Steroids. CXXVIII. Synthesis of halogenated steroid hormones: 6 $\alpha$ -fluoro-17 $\alpha$ -acetoxyprogesterone and some saturated analogs. A new class of highly active oral progestational hormones. *J. Am. Chem. Soc.* **81**, 5991–5993.
- Arcamone, F., Bernardi, L., Patelli, B., Giardino, P., Di Marco, A., Casazza, A.M., Soranzo, C. & Pratesi, G. (1978) Synthesis and antitumor activity of new daunorubicin and adriamycin analogues. *Experientia* **34**, 1255–1256.
- Sket, B. & Zupan, M. (1986) Polymers as reagents and catalysts. 12. Side chain bromination of aromatic molecules with a bromine complex of poly(styrene-co-4-vinylpyridine). *J. Org. Chem.* **51**, 929–931.
- Kimura, Y., Suzuki, M., Matsumoto, T., Abe, R. & Terashima, S. (1986) Novel glycosidation of 4-demethoxyanthracyclines by the use of trimethylsilyl triflate. Synthesis of optically active 4-demethoxydaunorubicin and 4-demethoxyadriamycin. *Bull. Chem. Soc. Jpn.* **59**, 423–431.
- Jeffrey, P.D. & McCombie, S.W. (1982) Homogenous, palladium(0)-catalyzed exchange deprotection of allylic esters, carbonates, and carbamates. *J. Org. Chem.* **47**, 587–590.
- Penco, S., Vigevani, A., Tosi, C., Fusco, R., Borghi, D. & Arcamone, F. (1986) Conformational flexibility of ring A in a series of substituted anthracyclines: <sup>1</sup>H-n.m.r. and quantum mechanical studies. *Anti Cancer Drug Design* **1**, 161–165.
- Arcamone, F., Cassinelli, G., Franceschi, G., Mondelli, R., Orezzi, P. & Penco, S. (1970) Struttura e stereochimica della daunomicina. *Gazz. Chim. Ital.* **100**, 949–989.
- Ragg, E., Ulbright, C., Mondelli, R., Fronza, G. & Penco, S. (1990) Conformational analysis of doxorubicin and analogues in aqueous solution. Determination of the glycosidic linkage geometry by <sup>1</sup>H 2D-inversion recovery difference NOE spectroscopy. *Gazz. Chim. Ital.* **120**, 501–509.
- Penco, S., Angelucci, F., Ballabio, M., Barchielli, G., Suarato, A., Vanotti, E., Vigevani, A. & Arcamone, F. (1984) Synthesis and ring A con-

- formation of new anthracyclines. *Heterocycles* **21**, 21–27.
21. Haasnoot, C.A.G. (1992) The conformation of six-membered rings described by puckering coordinates derived from endocyclic torsion angles. *J. Am. Chem. Soc.* **114**, 882–887.
  22. Haasnoot, C.A.G., de Leeuw, F.A.A.M., de Leeuw, H.P.M. & Altona, C. (1981) The relationship between proton-proton NMR coupling constants and substituent electronegativities. Part II. Conformational analysis of the sugar ring in nucleosides in solution using a generalized Karplus equation. *Org. Magn. Reson.* **15**, 43–52.
  23. de Leeuw, F.A.A.M., Altona, C., Kessler, H., Bernel, W., Friedrich, A., Krack, G. & Hull, W.E. (1983) Conformational analysis of proline ring from proton spin-spin coupling constants and force-field calculations: application to three cyclic tripeptides. *J. Am. Chem. Soc.* **105**, 2237–2246.
  24. Haasnoot, C.A.G., de Leeuw, F.A.A.M. & Altona, C. (1980) The relation between proton-proton NMR coupling constants and substituent electronegativities. I. An empirical generalization of the Karplus equation. *Tetrahedron* **36**, 2783–2792.
  25. Castellano, S. & Bothner-By, A.A. (1964) Analysis of NMR spectra by least squares. *J. Chem. Phys.* **41**, 3863–3869.
  26. Del Nero, S., Gandolfi, C., Lombardi, P. & Arcamone, F. (1981) Preparation of (-)-1,4-dimethoxy-6-acetyl-6-hydroxytetralin: intermediate for anthracyclines synthesis. *Chem. Ind. (London)*, 810–811.
  27. Albanese, D., Landini, D., Penso, M. & Pratelli, M. (1991) Tetrabutylammonium dihydrogen-trifluoride: an effective source of fluoride ion for halofluorination of alkenes. *Gazz. Chim. Ital.* **121**, 537–541.
  28. Harper, D.B. & O'Hagan, D. (1994) The fluorinated natural products. *Nat. Prod. Rep.* **11**, 123–133.