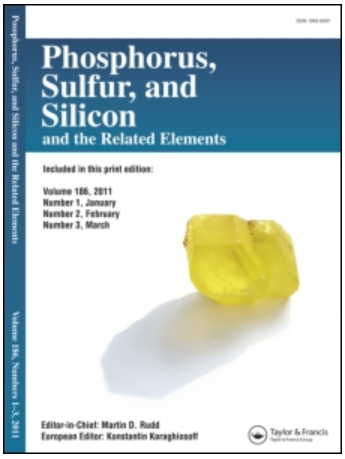

Phosphorus, Sulfur, and Silicon and the Related Elements
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713618290

SYNTHESIS OF GEM-BISPHOSPHONIC DOXORUBICIN CONJUGATES

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To cite this Article Fabulet, O. and Sturtz, G.(1995) 'SYNTHESIS OF GEM-BISPHOSPHONIC DOXORUBICIN CONJUGATES', Phosphorus, Sulfur, and Silicon and the Related Elements, 101: 1, 225 — 234
To link to this Article: DOI: 10.1080/10426509508042521
URL: http://dx.doi.org/10.1080/10426509508042521

Phosphorus, Sulfur, and Silicon, 1995, Vol. 101, pp. 225–234
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SYNTHESIS OF GEM-BISPHOSPHONIC DOXORUBICIN CONJUGATES

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(Received October 25, 1994; in final form November 30, 1994)

Four gem-bisphosphonic doxorubicin conjugates are prepared by coupling the amino function of doxorubicin with the activated carboxylic functions of 3,3-bis(diethylphosphono)propanoic acid **2**, 4,4-bis(diethylphosphono)butanoic acid **3**, and (R + S)-*N*-(9-fluorenylmethyloxycarbonyl)-2-amino-4,4-bis(diethylphosphono)butanoic acid **6**. The last compound provides two epimers which are separated by chromatography. Sodium salts are obtained. These original products participate in a biological study about a delivery-targeting concept of antineoplastic agents in bone cancer therapy, with the assistance of gem-bisphosphonic building blocks.

Key words: Gem-bisphosphonate, gem-bisphosphonic, doxorubicin, antineoplastic activity, delivery-targeting concept.

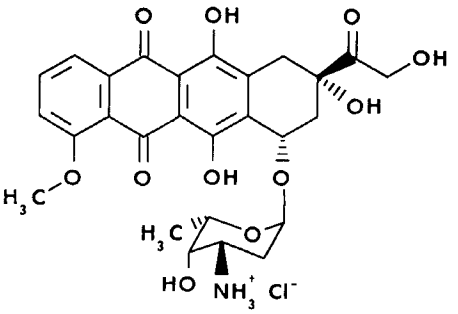
INTRODUCTION

In previous communications we reported that methotrexate gem-bisphosphonic derivatives showed interesting results with regard to the control of bone tumour development on human osteosarcoma models.[1,2]

The results seemed to confirm the hypothesis of drug targeting with the aid of bisphosphonates. So it appeared of interest to extend this study to other antineoplastic drugs.

In this respect, doxorubicin **1** (Figure 1) is usually used in the treatment of bone cancer, like methotrexate.

For this reason we have prepared gem-bisphosphonic doxorubicin conjugates.



1

FIGURE 1 [Doxorubicin].

RESULTS AND DISCUSSION

The doxorubicin is an antineoplastic antibiotic from the anthracycline family which was extracted from Streptomyces Peucetius Var. Caesius in 1975.[3]

Nowadays it presents the widest activity spectrum of all known chemotherapeutic agents, and it is used in the treatment of many human cancers such as bone cancer.

A lot of doxorubicin analogs have been synthesised.[4] They present different sites of modification.

In our case, we wanted to keep the same bond as in methotrexate gem-bisphosphonate derivatives, which contain an amid bond between the carboxylic acid function of methotrexate and the amino function of each gem-bisphosphonate derivative. Doxorubicin has no carboxylic acid function but an amino one located on the sugar part and which is easily involved in an amid function to provide N-substituted doxorubicin derivatives.[4,5,6,7] So we thought that we could build compounds containing an amid function. This required to use gem-bisphosphonate derivatives containing a carboxylic acid function.

Synthesis of the Gem-Bisphosphonate Structures

We chose two ordinary gem-bisphosphonate structures **2** and **3** (Figure 2), and a third one **4** with an amino group to provide a gem-bisphosphonate doxorubicin conjugate containing an amino function like the initial doxorubicin.

The preparation of gem-bisphosphonate derivatives **2** and **3** was carried out according to J. Guervenou.[8,9]

The amino-gem-bisphosphonate derivative **4** must be N-protected before its condensation with doxorubicin. Because of doxorubicin's glycosidic bond sensitivity to acid conditions, such conditions must be avoided during the deprotection step. So we chose 9-fluorenylmethyloxycarbonyl (FMOC) as protecting group. It can be removed in anhydrous basic conditions by means of a base such as piperidin,[10] morpholin,[11] ammoniac,[5] or a dialkylamin.[6]

The derivative **4** was prepared starting from ethyl 2-amino-4,4-bis(diethylphosphono) butanoate **5**, the synthesis of which was reported by us.[9]

The carboxylic acid was deprotected by saponification, and the zwitterion **4** was treated with 9-fluorenylmethyl-succinimidyl carbonate in a dioxane-water mixture

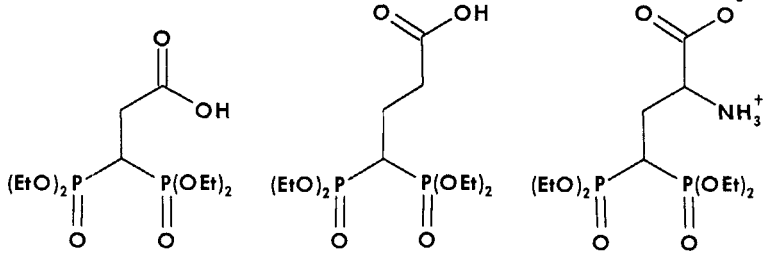
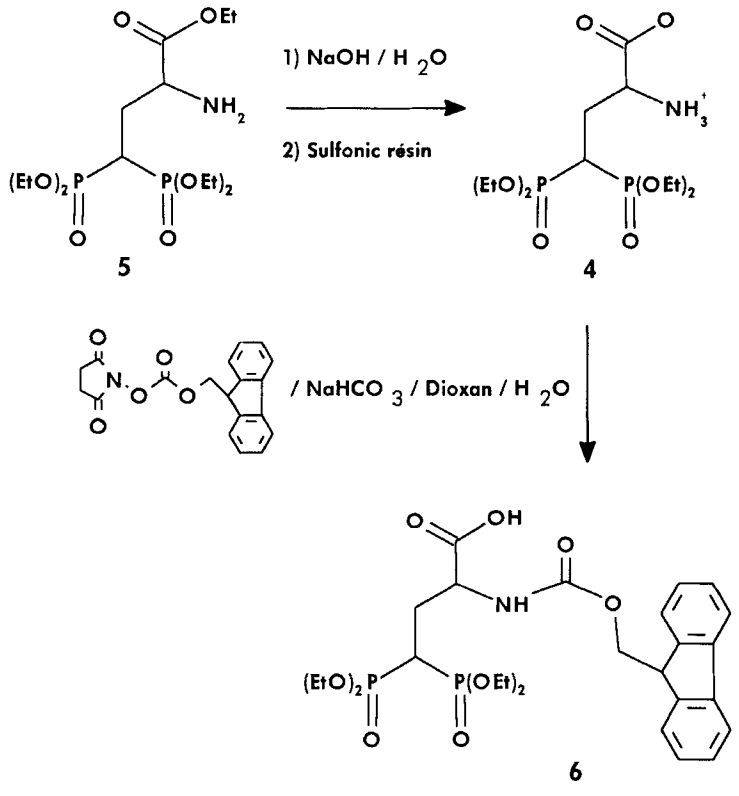


FIGURE 2



SCHEME I

in the presence of sodium hydrogen carbonate[6] to give the N-protected amino gem-bisphosphonate **6** in the form of a racemate (Scheme I).

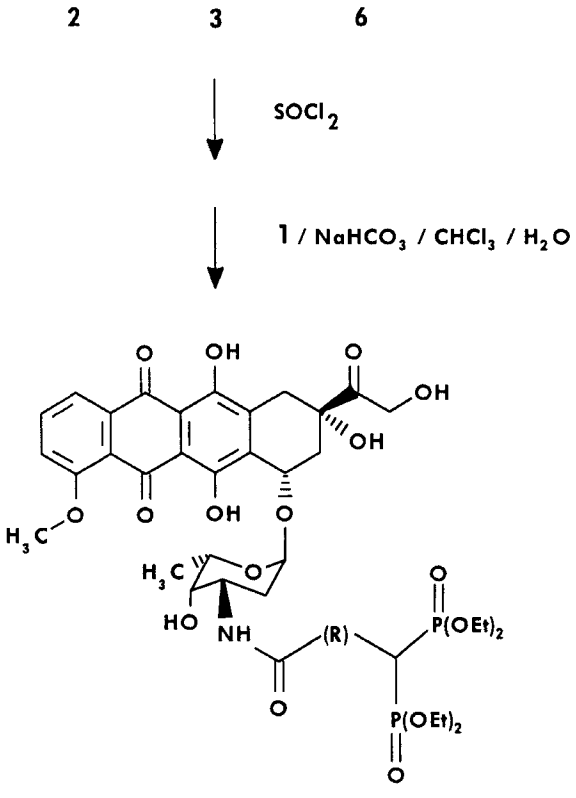
Coupling Reactions

A bibliographical study about the synthesis of doxorubicin derivatives containing an amid function,[5,6,7,12,13,14] and a preliminary approach, using cyclohexylamin as a pattern instead of doxorubicin, lead us to activate the carboxylic acid as an acid chloride, with the aid of thionyl chloride, and to carry out the condensation in Schotten-Bauman conditions, using potassium hydrogen sulphate in chloroform-water according to L. O. Rosik and F. Sweet.[14]

Gem-bisphosphonate doxorubicin conjugates **7**, **8** and **9** (Scheme II) were obtained in good yields (80 to 99%) after purification by chromatography on a silica-gel column.

Deprotection Reaction

The amino function of the conjugate **9** was deprotected by diethylamin in dimethylformamide (DMF) according to D. H. King.[6] The rection was almost instanta-



	7	8	9
(R)	CH_2	CH_2-CH_2	CH_2-CHNH(FMOC)
Yield	99 %	80 %	99 %

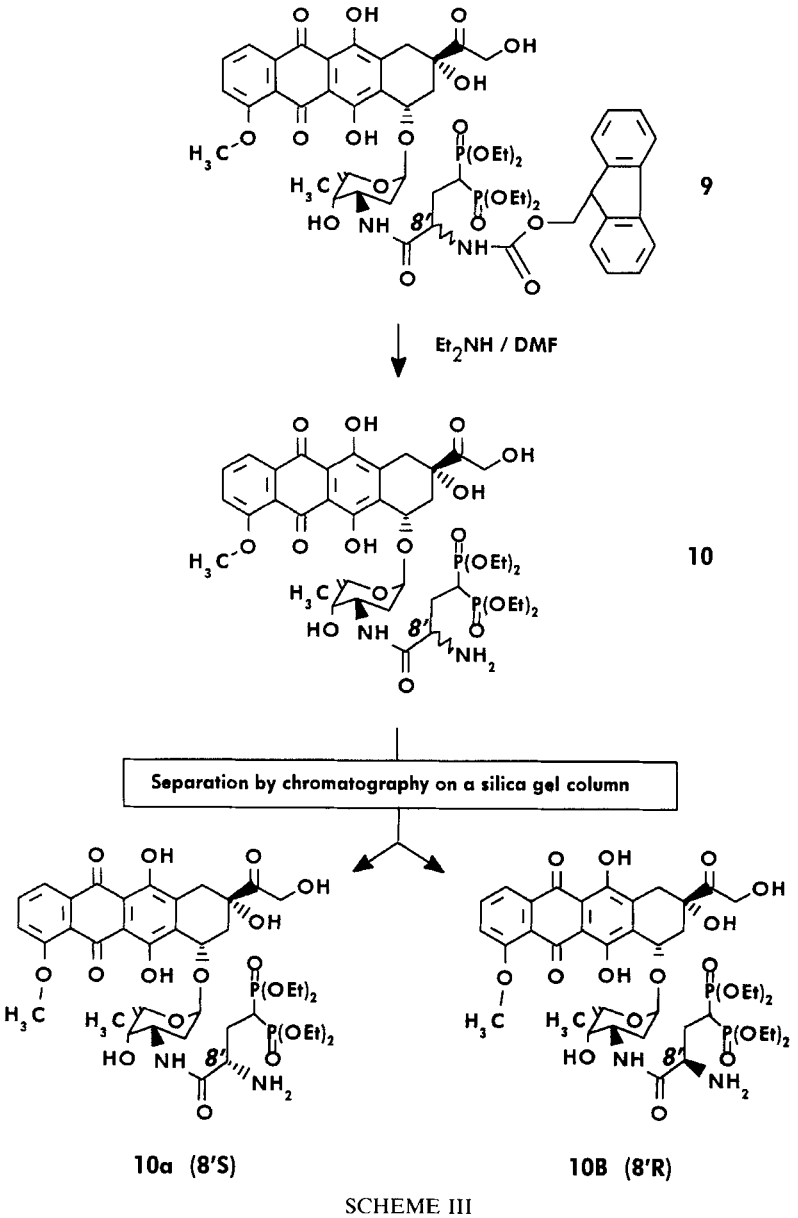
SCHEME II

neous, and two diasteroisomers **10a** and **10b** were obtained. They were separated by chromatography on a silica-gel column with 20% and 28% yield, respectively (Scheme III).

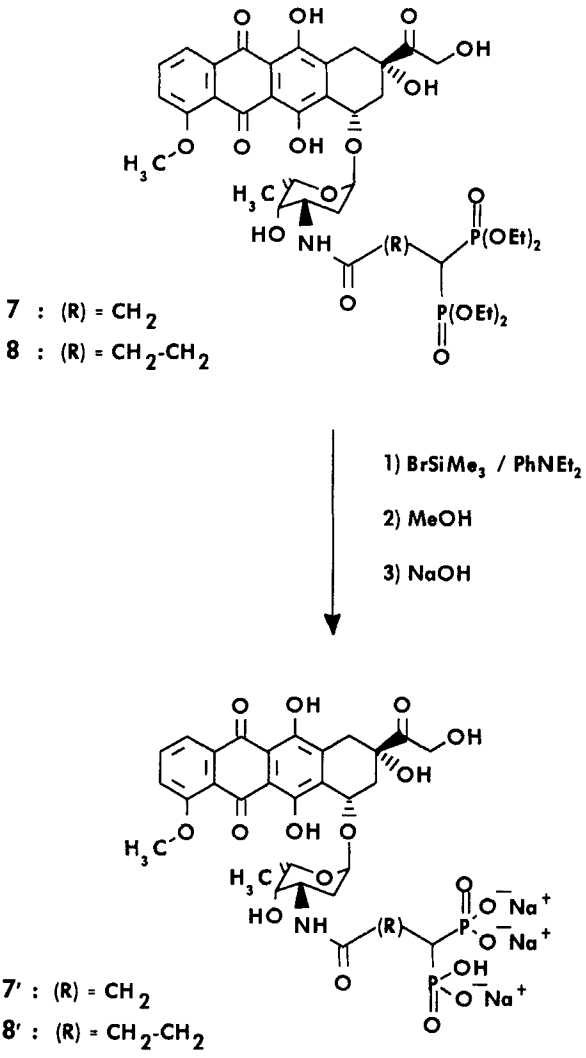
Access to the Sodium Salts

The two first gem-bisphosphonate doxorubicin derivatives **7** and **8** were salified to carry out preliminary biological experiments.

The phosphonic esters cleavage took place via trimethylsilylbromide[15] followed by methanolysis. The first step requires the addition of diethylanilin to neutralize



SCHEME III



SCHEME IV

the hydrogen bromide formed during the alcohol functions silylation. Basic extraction, purification by filtration on SEPHADEX G-10 gel and alcalinisation to pH = 8 with sodium hydroxyde gave sodium trisalts **7'** and **8'** (Scheme IV).

CONCLUSION

Our work showed that it is possible to obtain gem-bisphosphonic doxorubicin conjugates via an amid bond between the amino function of the doxorubicin and the carboxylic function of gem-bisphosphonate derivatives.

Two doxorubicine conjugates **7'** and **8'** have been tested against human tumour xenografts, but no effect was observed.

EXPERIMENTAL

Analytical thin-layer chromatography (TLC) was performed on silica gel sheets $60F_{254}$ Merck and RP-$18F_{254S}$, which were scanned under ultraviolet light (ν = 254 nm).

Preparative chromatography was carried out on silica-gel columns, Merck 60 (70-230 mesh).

The melting points were determined on a Kofler apparatus.

^1H, ^{13}C and ^{31}P NMR spectra were recorded on a Brucker AC300 spectrometer. For ^1H and ^{13}C NMR spectra, the chemical shifts are reported in ppm using 3-trimethylsilylperdeuteropropanoic acid sodium salt (TMPS) as reference in D_2O and tetramethylsilane (TMS) in organic solvents. For ^{31}P NMR spectra, the reference is phosphoric acid in water solution (85%). We numbered atoms as reported in Scheme V. Microanalysis were carried out at the "Service Central d'Analyse du C.N.R.S."

3,3-bis(diethylphosphono)propanoic acid **2**

This compound was prepared according to the described procedure.[8]

4,4-bis(diethylphosphono)butanoic acid **3**

This compound was prepared according to the described procedure.[9]

2-amino-4,4-bis(diethylphosphono)butanoic acid ethyl ester **5**

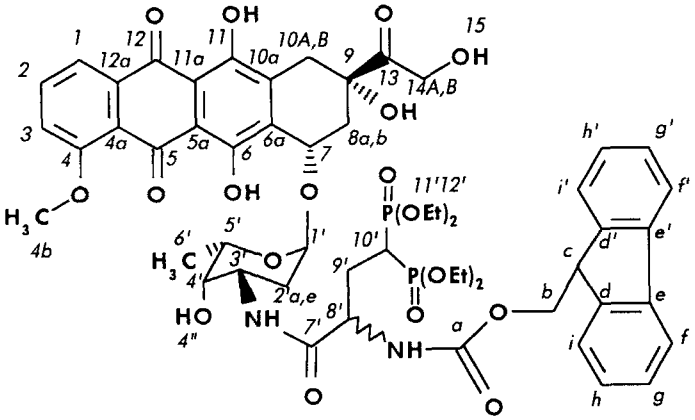
This compound was prepared according to the described procedure.[9]

N-(9-fluorenylmethyloxycarbonyl)-2-amino-4,4-bis(diethylphosphono)-butanoic acid **6**

3.6 g (8.9 mmol) of 2-amino-4,4-bis(diethylphosphono)butanoic acid ethyl ester **5** were diluted in 4 mL of distilled water. 8.9 mL (8.9 mmol) of 1.0 N NaOH were then added. After 1 h of stirring at room temperature, the aqueous phase was extracted twice with chloroform, and water was removed under reduced pressure to give 3.1 g (89%) of amino-acid in the form of a sodium salt.

The salt was dissolved in distilled water and filtered over a sulfonic resin (DOWEX 50 W × 8, 50-100 Mesh, 8.6 mL). Water was then removed under reduced pressure and the product was precipitated in acetone. Filtration, and washings with acetone and diethylether afforded 11.9 g (38%) of zwitterion **4** as a white powder; Rf ($CHCl_3$/MeOH, 2:1) = 0.38.

2.5 g (6.7 mmol) of **4** and 2.7 g (8 mmol) of 9-fluorenylmethylsuccinimidyl carbonate dissolved in 30 mL of dioxane/water (2/3) were successively added to 7 mL of a sodium hydrogen carbonate solution (0.56 g-6.7 mmol). Agitation was maintained for 3 h at room temperature, then the mixture was poured into 400 mL of a sodium hydrogen carbonate aqueous solution (2%). After three extractions with diethylether, the aqueous phase was cooled at 0°C, then acidified to pH = 1 with concentrated hydrochloric acid. The solution was extracted twice with ethylacetate, then the organic phases were combined, dried on $MgSO_4$, and concentrated to make the product crystallize. Filtration, washings with cold ethylacetate and diethylether, and drying, gave 3.2 g (80%) of **6** in the form of a white powder;



SCHEME V

mp 143°C; Rf ($CHCl_3$/MeOH, 2:1) = 0.68; ^1H NMR ($CDCl_3$): 1.3–1.4 (m, 12H, $H_{12'}$); 2.2–2.6 (m, 2H, $H_{8'}$); 2.7–3.0 (m, 1H, $H_{10'}$); 4.1–4.5 (m, 11H, $H_{b+c+11'}$); 4.5–4.7 (m, 1H, $H_{7'}$); 6.10, 6.12 (d, 1H, H_{NH}); 7.3 (d false t, 2H, J^3_{H-H} = 7 Hz, J_{H-H} = 1 Hz) + 7.4 (td, 2H, J^3_{H-H} = 7 Hz, J_{H-H} = 1 Hz) + 7.6 (false t, 2H, J^3_{H-H} = 7 Hz) + 7.75 (d, 2H, J^3_{H-H} = 7 Hz) = $H_{f+f'+g+g'+h+h'+i+i'}$; ^{13}C NMR ($CDCl_3$): 16.1 (d, $J^3_{C12'-P}$ = 6 Hz, $C_{12'}$); 27.9 ($C_{9'}$); 32.75 (t, $J^1_{C10'-P}$ = 135 Hz, $C_{10'}$); 46.8 (C_e); 53.0 ($C_{8'}$); 63.1 (dd, $J^2_{C11'-P}$ = 15 Hz, $J^4_{C11'-P}$ = 7 Hz, $C_{11'}$); 67.0 (C_b); 119.7, 125.0, 125.1, 126.9, 127.48, 127.50 ($C_{f+f'+g+g'+h+h'+i+i'}$); 140.99, 141.01, 143.5, 143.8 ($C_{d+d'+e+e'}$); 156.3 (C_a); 172.9 ($C_{7'}$); ^{31}P NMR ($CDCl_3$): 23.0, 23.3 (AB, J^2_{P-P} = 2 Hz).

3'-N-[4,4-bis(diethylphosphono)butanoyl] doxorubicin **8**

0.10 mL (1.38 mmol) of thionylchloride was added to 248 mg (690 μmol) of 4,4-bis(diethylphosphono)butanoic acid **3** dissolved in 8 mL of toluene. The stirring was maintained for 45 min at 90°C and the solvent was removed under reduced pressure. After an addition-evaporation of toluene, the acid chloride was ready for use.

200 mg (345 μmol) of doxorubicin hydrochloride were dissolved in 9 mL of distilled water. 9 mL of chloroform and 690 mg (6.8 mmol) of potassium hydrogen carbonate dissolved in 18 mL of distilled water were added to this solution. A vigorous stirring was maintained for about 10 min at room temperature, then the freshly prepared acid chloride dissolved in 13 mL of chloroform, was added.

The stirring was maintained for 1 h, then 0.5 equivalent of more freshly prepared acid chloride was added. After one more hour of stirring, the reaction was finished. The mixture was decanted and the aqueous phase was extracted four times with chloroform. Organic phases were combined, washed, and the chloroform removed under reduced pressure. The residue was purified by chromatography on a silica-gel column (chloroform/methanol, 20:1 then 11:1) and triturated with hexane to give 246 mg (80%) of product **8** in the form of an orange-red powder; Rf ($CHCl_3$/MeOH, 11:1) = 0.54; IR (KBr) cm^{-1}): ν_{O-H}, ν_{N-H}: 2700-3800; $\nu_{C=O}$: 1735, 1650, 1620; δ_{N-H}: 1580; $\nu_{P=O}$: 1210, 1240; ν_{P-O}: 1070–1120; UV (methanol) (nm): 233, 250, 289, 499, 535, 578; ^1H NMR ($CDCl_3$): 1.25–1.35 (m, 15H, $H_{a'+12'}$); 1.7 (dd, $J^1_{H2'a-H2'e}$ = 14 Hz, $J^3_{H2'a-H1'}$ = 4 Hz, 1H, $H_{2'a}$); 1.9 (td, $J^1_{H2'e-H2'a}$ = 4 Hz, $J^3_{H2'e-H1'}$ = $J^1_{H2'e-H1'}$ = 14 Hz, 1H, $H_{2'e}$); 2–2.6 (m, 7H, $H_{8B+8A+8'+9'+10'}$); 2.95 (d, $J^2_{H10B-H10A}$ = 18 Hz, 1H, H_{10B}); 3.05 (broad t, $J^3_{H15-H14}$ = 4 Hz, 1H, H_{15}); 3.2 (d, $J^2_{H10A-H10B}$ = 18 Hz, 1H, H_{10A}); 3.7 (s, 1H, $H_{4'}$); 3.8 (broad s, 1H, $H_{4'}$); 4.05 (s, 3H, H_{4b}); 4–4.2 (m, 10H, $H_{3'+5'+11'}$), 4.6 (s, 1H, H_9); 4.75 (d, $J^3_{H14-H15}$ = 4 Hz, 2H, H_{14}); 5.2 (s, 1H, H_7); 5.5 (d, $J^3_{H1'-H2'e}$ = 4Hz, 1H, $H_{1'}$); 6.25, 6.3 (d, 1H, $H_{7'}$); 7.35 (d, $J^3_{H3'-H2}$ = 8 Hz, 1H, H_3); 7.7 (t, J^3_{H2-H3} = 8 Hz, J^3_{H2-H1} = 8 Hz, 1H, H_2); 7.9 (d, J^3_{H1-H2} = 8 Hz, 1H, H_1); 13.2, 13.9 (2s, 2H, H_{6+11}); ^{13}C NMR ($CDCl_3$): 16.5 (d, J^3_{P-C} = 6 Hz, $C_{12'}$); 17.2 ($C_{6'}$); 21.7 (C_9); 29.5 ($C_{2'}$), 33.8 (C_8); 34.5 ($C_{8'}$); 35.1 (t, J^1_{P-C} = 134 Hz, $C_{10'}$); 35.8 (C_{10}); 46.2 ($C_{3'}$); 56.7 (C_{4b}); 63.1 (m, $C_{11'}$); 65.7, 67.8, 68.9, 69.5 ($C_{3'+5'+7+9}$); 76.7 (C_{14}); 101.1 ($C_{1'}$); 111.1, 111.3 (C_{5a+11a}), 118.6, 119.8, 120.5 (C_{1+3+4a}); 133.8, 134.2, 135.2, 135.9 ($C_{2+6a+10a+12a}$); 155.5, 156.4 (C_{6+11}); 161.0 (C_4); 172.0 ($C_{7'}$); 186.2, 186.6 (C_{5+12}); 214.2 (C_{13}); NMR ^{31}P ($CDCl_3$); 22.8, 22.9 (AB, J^2_{P-P} = 4 Hz); Anal. Calc. for $C_{39}H_{53}NO_{18}P_2$: C, 52.88; H, 6.03; N, 1.58; P, 6.99; Found: C, 52.75; H, 6.10; N, 1.50; P, 7.15.

3'-N-[3,3-bis(diethylphosphono)propanoyl] doxorubicin **7**

The compound **7** was synthesised according to the procedure described above with the following modifications:

The reaction was started with three equivalents of acid chloride. After 50 min of stirring, one more equivalent was added. After 30 more minutes of stirring, a last equivalent was added with further 10 min stirring.

The product **7** was isolated by chromatography on a silica gel column (chloroform/methanol, 40:1, then 11:1) with nearly a 100% yield; Rf ($CHCl_3$/MeOH, 11:1) = 0.36; ^1H NMR ($CDCl_3$): 1.25–1.4 (m, 15H, $H_{a'+12'}$); 1.7 (dd, $J^1_{H2'a-H2'e}$ = 14 Hz, $J^3_{H2'a-H1'}$ = 4 Hz, 1H, $H_{2'a}$); 1.9 (td, $J^1_{H2'e-H2'a}$ = 4 Hz, $J^3_{H2'e-H1'}$ = $J^1_{H2'e-H1'}$ = 14 Hz, 1H, $H_{2'e}$); 2.15 (dd, $J^2_{H8B-H8A}$ = 14 Hz, J^3_{H8B-H7} = 4 Hz, 1H, H_{8B}); 2.35 (d, $J^2_{H8A-H8B}$ = 14 Hz, 1H, H_{8A}); 2.45–2.8 (m, 2H, $H_{8'}$); 3.0 (d, $J^2_{H10B-H10A}$ = 14 Hz, 1H, H_{10B}); 2.95–3.2 (m, 2H, $H_{10'+15}$); 3.25 (d, $J^2_{H10A-H10B}$ = 14 Hz, 1H, H_{10A}); 3.85 (s, 1H, $H_{4'}$); 4.0 (broad s, 1H, $H_{4'}$); 4.05 (s, 3H, H_{4b}); 4.05–4.2 (m, 10H, $H_{3'+5'+11'}$); 4.7 (s, 1H, H_9); 4.75 (d, $J^3_{H14-H15}$ = 3Hz, 2H, H_{14}); 5.3 (s, 1H, H_7); 5.55 (d, $J^3_{H1'-H2'}$ = 4 Hz, 1H, $H_{1'}$); 6.15, 6.2, (d, 1H, $H_{7'}$); 7.4 (d, $J^3_{H3'-H2}$) = 8 Hz, 1H, H_3); 7.8 (t, J^3_{H2-H3} = J^3_{H2-H1} = 8 Hz, 1H, H_2); 8.0 (d, J^3_{H1-H2} = 8 Hz, 1H, H_1); 13.2, 13.9 (2s, 2H, H_{6+11}); ^{31}P NMR ($CDCl_3$): 22.45 (s), 22.5 (s); Anal. Calc. for $C_{38}H_{51}NO_{18}P_2$: C, 52.35; H, 5.90; N, 1.61; P, 7.11; Found: C, 52.25; H, 5.80; N, 1.50; P, 7.20.

3'-N-[9-fluorenylmethyloxycarbonyl)-2-amino-4,4-bis(diethylphosphono)butanoyl] doxorubicin **9**

The compound **9** was synthesised according to the procedure described for **8**, with the following modifications:

The reaction was started with two equivalents of acid chloride. After 1 h of stirring, two more equivalents were added and stirring was continued for 1 h.

After chromatography on a silica-gel column (chloroform/methanol, 20:1, then 1:1), the product **9** was isolated as a mixture of two epimers with nearly a 100% yield; Rf ($CHCl_3$/MeOH, 11:1) = 0.55 and 0.59; ^1H NMR ($CDCl_3$): 1.25–1.4 (m, 15H, $H_{6'+12'}$); 1.7 (dd, $J^1_{H2'a-H2'e}$ = 14 Hz, $J^3_{H2'a-H1'}$ = 4 Hz, 1H, $H_{2'a}$); 1.9 (td, $J^1_{H2'e-H2'a}$ = 4 Hz, $J^3_{H2'e-H1'}$ = $J^3_{H2'e-H1'}$ = 14 Hz, 1H, $H_{2'e}$); 2.1–2.4 (m, 4H, $H_{8B+8A+9'}$); 2.6–2.8 (m, 1H, $H_{10'}$); 3.0 (d, 1H, $J^2_{H10B-H10A}$ = 18 Hz, H_{10B}); 3.15 (t, 1H, $J^3_{H15-H14}$ = 4 Hz, H_{15}); 3.3 (d, 1H, $J^2_{H10A-H10B}$ = 18 Hz, H_{10A}); 3.7 (s, 1H, $H_{4'}$); 4.05 (s, 3H, H_{4b}); 4.05–4.3 (m, 11H, $H_{5'+4''+5''+11'}$); 4.3–4.5 (m, 4H, $H_{8''+9+1''}$); 4.65 (s, 1H, H_9); 4.75 (d, $J^3_{H14-H15}$ = 4 Hz, 2H, H_{14}); 5.3 (s, 1H, H_7); 5.5 (d, $J^3_{H1'-H2'e}$ = 4 Hz, 1H, $H_{1'}$); 6.15, 6.3 (2d, 1H, $H_{7'}$); 7.1–8.0 (m, 11H, $H_{1+2+3+1'+2'+3'+4'+NH3+}$); 13.16, 13.18, 13.90, 13.93 (4s, 2H, H_{6+11}); ^{13}C NMR ($CDCl_3$): 16.0 ($C_{12'}$); 16.6 ($C_{6'}$); 27.0 ($C_{9'}$); 28.9 ($C_{2'}$); 32.5 (t, J^1_{P-C} = 134 Hz, $C_{10'}$); 33.2 (C_8); 35.3 (C_{10}); 45.8, 46.5, 46.6, 46.7 ($C_{1',3'}$); 53.0 ($C_{8'}$); 56.0 (C_{4b}); 62.7, (m, $C_{11'}$); 65.1, 66.8, 66.9, 68.0, 69.1 ($C_{4',5',7,9,b}$); 76.1 (C_{14}); 100.5 (C1'); 110.6 (4s, C_{5a+11a}); 118.1, 119.2, 119.5, 119.6, 120.0, 124.8, 124.9, 125.0, 126.7, 126.8, 127.3, 127.4, ($C_{1+3+4a+1+2+3+1'+2'+3'+4'}$); 133.1, 133.2, 133.4, 133.5, 134.7, 135.2 ($C_{2+6a+10a+12a}$); 140.7, 140.8, 140.9, 143.2, 143.4, 143.6, 143.7 ($C_{4+6+4'+6'}$); 154.8, 154.9, 155.8, 156.0, 156.2 (C_{6+11}); 160.4 (C_4); 169.7, 170.9 ($C_{7'}$); 186.0, 185.8 (C_{5+12}); 213.7 (C_{13}); ^{31}P NMR ($CDCl_3$): 22.5–23.2 (m); Anal. Calc. for $C_{54}H_{64}N_2O_{20}P_2$: C, 57.75; H, 5.74; N, 2.49; P, 5.52; Found: C, 57.62; H, 5.82; N, 2.41; P, 5.60.

3'-N-[2-amino-4,4-bis(diethylphosphono)butanoyl] doxorubicin **10**

84 mg (74 mmol) of **9** (mixture of two epimers) were dissolved in 8.4 mL of dimethylformamid. 0.84 mL (4.8 mmol) of diethylamine was added under nitrogen atmosphere. The stirring was maintained for 15 min at room temperature, and the solvent was removed under reduced pressure.

The residue was purified by chromatography on a silica-gel column (eluant: $CHCl_3$/MeOH, 14:1, 11:1, then 8:1) to give 19 mg (28%) of the first diastereoisomer, Rf ($CHCl_3$/MeOH, 11:1) = 0.85, and 14 mg (20%) of the second one, Rf ($CHCl_3$/MeOH, 11:1) = 0.19. The NMR data are about the same for the two compounds: ^1H NMR ($CDCl_3$): 1.25–1.35 (m, 15H, $H_{6'+12'}$); 1.75 (dd, $J^1_{H2'a-H2'e}$ = 14 Hz, $J^3_{H2'a-H1'}$ = 4 Hz, 1H, $H_{2'a}$); 1.85–2.45 (m, 5H, $H_{2'e+8B+8A+9'+NH3+}$); 2.7 (tt, $J^3_{H10'-H9'}$ = 7 Hz, $J^2_{P-H10'}$ = 24 Hz, 1H, $H_{10'}$); 3.0 (d, $J^2_{H10B-H10A}$ = 18 Hz, 1H, H_{10B}); 3.25 (d, $J^2_{H10A-H10B}$ = 18 Hz, 1H, H_{10A}); 3.6 (t, $J^3_{H8'-H9'}$ = 7 Hz, 1H, $H_{8'}$); 3.7 (s, 1H, $H_{4'}$); 4.05 (s, 3H, H_{4b}); 4.05–4.3 (m, 11H, $H_{5'+4''+5''+11'}$); 4.6 (broad s, 1H, H_9); 4.7 (d, $J^3_{H14-H15}$ = 3 Hz, 2H, H_{14}); 5.3 (s, 1H, H_7); 5.5 (d, $J^3_{H1'-H2'e}$ = 3 Hz, 1H, $H_{1'}$); 7.25, 7.4 (d, 1H, $H_{7'}$); 7.4 (d, J^3_{H3-H2} = 8 Hz, 1H, H_3); 7.8 (t, J^3_{H2-H3} = 8 Hz, J^3_{H2-H1} = 8 Hz, 1H, H_2); 8.0 (d, J^3_{H1-H2} = 8 Hz, 1H, H_1); 13.95 (broad s; 1H, $H_{6\ or\ 11}$); ^{13}C NMR ($CDCl_3$): 16.3 (d, J^3_{P-C} = 6 Hz, $C_{12'}$); 16.9 ($C_{6'}$); 29.4 ($C_{2'}$); 31.4 ($C_{9'}$); 33.0 (t, J^1_{P-C} = 134 Hz, $C_{10'}$); 33.8 (C_8); 35.6 (C_{10}); 45.57 ($C_{3'}$); 53.7 ($C_{8'}$); 56.6 (C_{4b}); 63.0 (m, $C_{11'}$); 65.5, 67.3, 68.7, 69.5 ($C_{4',5',7,9}$); 77.2 (C_{14}); 100.6 (C1'); 111.2, 111.4 (C_{5a+11a}); 116.4, 119.8, 120.6 (C_{1+3+4a}); 133.6, 133.8, 135.4, 135.7 ($C_{2+6a+10a+12a}$); 155.6, 156.2 (C_{6+11}); 161.0 (C_4); 173.9 ($C_{7'}$); 186.5, 186.9 (C_{5+12}); 214.0 (C_{13}); ^{31}P NMR ($CDCl_3$): 22.35–22.9 (AB, J^2_{P-P} = 4 Hz); Anal. Calc. for $C_{39}H_{54}N_2O_{18}P_2$: C, 52.00; H, 6.04; N, 3.11; P, 6.88; Found **10a**: C, 51.81; H, 6.15; N, 3.09; P, 6.95; Found **10b**: C, 51.90; H, 5.95; N, 3.05; P, 7.11.

3'-N-[4,4-bis(diethylphosphono)butanoyl] doxorubicin sodium trisalt **8'**

1.76 mL (13.8 mmol) of N,N-diethylaniline and 0.55 mL (4.2 mmol) of trimethylsilylbromide were successively added to a solution of 246 mg (277 μmol) of **8** in 10 mL of methylene chloride. The mixture was stirred at room temperature under nitrogen atmosphere for 24 h. 0.07 mL (554 μmol) of trimethylsilylbromide was added and stirring continued for 12 h. The methylene chloride was then removed under reduced pressure and the solid residue was dissolved in 10 mL of methanol and stirred at room temperature for 1 h. After the removal of the methanol, the residue was dissolved in about 20 mL of distilled water, and the solution was basified (pH = 8.6) by adding sodium hydroxide (0.1 N). After ten extractions with diethylether, the aqueous phase was lyophilised to give a mixture of product **8'** and sodium bromide (500 mg).

300 mg of this mixture were dissolved in 1 mL of water and the solution was filtered on a SEPHADEX-gel (G-10) column (15 cm × 7 mm—Mobile phase:distilled water). Red coloured fractions were put together and the pH was adjusted to pH = 8 by adding a sodium hydroxide solution.

The water was removed under reduced pressure, at room temperature, to give 62 mg (73 μmol = 44%) of salt **8'** in the form of a red solid. IR (KBr) (cm^{-1}): $\nu_{O-H,\ N-H}$: 2700–3800; $\nu_{C=O}$: 1730, 1640, 1620; δ_{N-H}: 1585; ν_{P-O}: 1210; ν_{P-O}: 1080–1120; UV (methanol) (nm): 233, 253, 286, 497; ^{1}H NMR (D_2O): 1.3 (d, $J^3_{H6'\ H5'}$ = 6Hz, 3H, $H_{6'}$), 1.7–2.8 (m, 10H, $H_{2'a+2'e+8B+8A+8'+9'+10'+10B}$); 3.0 (d, $J^2_{H10A\ H10B}$ = 18 Hz, 1H, H_{10A}); 3.8 (s, 1H, $H_{4'}$); 3.9 (s, 3H, H_{4b}); 4.2 (broad d, $J^3_{H3'-H2'e}$ = 12 Hz, 1H, $H_{3'}$); 4.3 (broad q, $J^3_{H5'\ H6'}$ = 6 Hz, 1H, $H_{5'}$); 4.9 (broad s, 1H, H_7); 5.5 (s, 1H, $H_{1'}$); 7.3 (m, 2H, $H_{2,3}$); 7.6 (d, $J^3_{H1\ H2}$ = 7 Hz, 1H, H_1); ^{13}C NMR (D_2O): 18.9 ($C_{6'}$); 25.7 ($C_{9'}$); 31.2 ($C_{2'}$); 35.0, 38.0, 39.1 ($C_{8,8',10}$); 41.6 (t, $J^1_{P\ C}$ = 112 Hz, $C_{10'}$); 48.4 ($C_{3'}$); 59.1 (C_{4b}); 67.2, 70.5, 70.8, 71.2 ($C_{4',5',7',9}$); 78.7 (C_{14}); 102.7 ($C_{1'}$); 113.1 ($C_{5a,11a}$); 121.3, 122.2 ($C_{1,3+4a}$); 136.3, 136.5, 139.3 ($C_{2',6a,10a,12a}$); 156.9, 158.4 ($C_{6,11}$); 163.0 (C_4); 178.8 (C_7); 188.1, 188.4 (C_5+C_{12}); 217.3 (C_{13}).

3'-N-[3,3-bis(diethylphosphono)propanoyl] doxorubicin sodium trisalt **7'**

We synthesised product **7'** according to the procedure described above for **8'** (55% in yield).

ACKNOWLEDGEMENTS

We wish to thank the "Ligue Nationale contre le Cancer—Comité du Finistère" and the "Langlois Foundation" for their financial support.

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