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Short communication

In vitro cytostatic activity of 9-demethoxyporothramycin B

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Summary — (11a*S*)-9-Demethoxyporothramycin B was synthesized in an enantiomerically pure form from (*S*)-pyroglutamic acid. It exhibits marked *in vitro* cytostatic activity in several cancer cell lines. MDR+ cell lines are not significantly more resistant to this compound than their MDR- counterparts.

(*S*)-pyroglutamic acid / antitumor pyrrolo[2,1-*c*][1,4]benzodiazepine / cytostatic activity / MDR

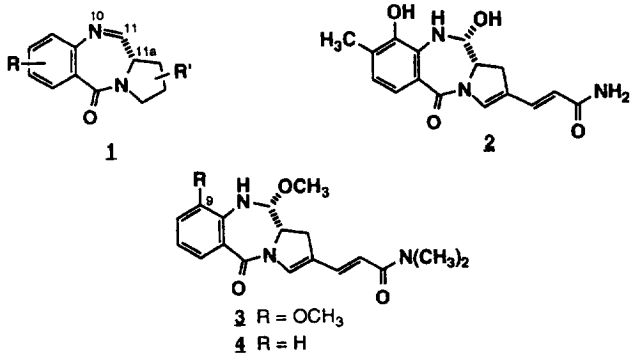
Introduction

The pyrrolo[2,1-*c*][1,4]benzodiazepine group of antitumor antibiotics of general formula **1** (N10-C11 imine or chemical equivalent) produced by various *Actinomycetes* includes several potent antitumor compounds, such as anthramycin **2** [1], tomaymycin [2], sibiromycin [3, 4], the neothramycins A and B [5] and porothramycin B **3** [6]. Their cytotoxic activities are attributed to their ability to bind covalently to cellular DNA by a unique mechanism [7–12].

Two common structural features of the naturally occurring pyrrolo[2,1-*c*][1,4]benzodiazepines are: a) the presence of an imine or an equivalent carbinolamine or carbinolamine ether function at the N10-C11 position; and b) the *S* configuration at C11a. This configuration provides the molecules with a twisted shape, which is essential for their fit into the minor groove of DNA, allowing nucleophilic attack from the N-2 amino group of guanine residues to form an aminal linkage to C11 [8–12].

The potential of these antibiotics as clinically effective anticancer agents is compromised by their cardiotoxicity [9, 10]. In order to avoid such a side effect, phenolic hydroxy groups at C7 and C9 should be avoided, since these may be tautomerized or oxidized to the *p*- and *o*-quinone imines, respectively, which are predicted to be cardiotoxic [9, 10].

We have previously developed synthetic routes to several members of this family and structural analogs,



starting from inexpensive (*S*)-pyroglutamic acid [13–16]. In order to minimize the cardiotoxic potential of these compounds, we chose to synthesize a new analog **4**, retaining the acrylamide side chain at C2 of porothramycin B **3** but devoid of hydroxy or alkoxy groups on the aromatic ring [16]. The present paper describes the *in vitro* cytostatic activity of this synthetic analog in various cancer cell lines.

Chemistry

(*S*)-Pyroglutamic acid has been recognized as an interesting chiral starting material to introduce the required (*S*)-configuration of the asymmetric center, as well as the *N,N*-dimethylacrylamide side chain of 9-demethoxy porothramycin **4**. The total synthesis of **4** from this amido-acid was achieved in 13 steps with excellent yields during the first 12 steps [16]. The

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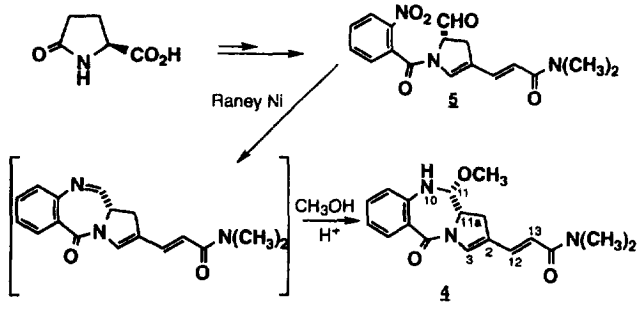
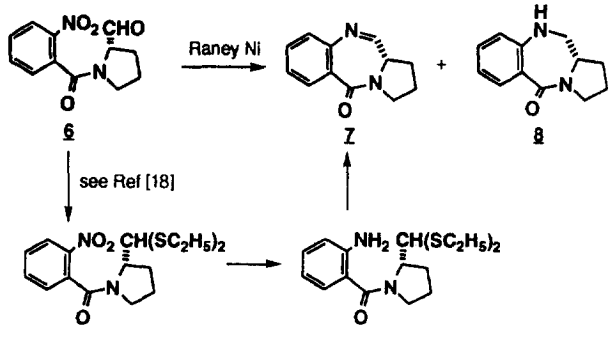
strategy involved at the last stage reductive cyclization of the *N*-(2-nitrobenzoyl)pyrrolidine carboxaldehyde **5** to give the desired compound **4** in 65% yield. This was achieved with Raney nickel, followed by treatment with anhydrous methanol in the presence of small amounts of trifluoroacetic acid (scheme 1). Alternatives were investigated in order to improve this yield, which was the lowest of our synthetic sequence. In general, the control of the reduction of *N*-(2-nitrobenzoyl)pyrrolidine carboxaldehydes is problematic, as has been recently reviewed [17]. We showed previously, with the simple model **6**, that Raney nickel gave better results when compared with hydrogenation with various palladium catalysts. It allowed us to convert the aldehyde **6** into the pyrrolo[2,1-*c*]-[1,4]benzodiazepine **7** in 75% yield, with minimum over-reduction to the secondary amine **8** (scheme 2).

The transient protection of the aldehyde function as a thioacetal [18] or dimethylacetal [19] has previously been used in the synthesis of simple compounds of this family, such as **7** (scheme 2). However, we did not succeed in efficient preparation of the diethylthioacetal derivative from **5**. This experiment gave rise to a mixture of products after about 50% conversion. Nevertheless, treatment of **5** with trimethylorthoformate afforded the dimethylacetal (90%)



Scheme 1.



Scheme 2.

(scheme 3). Subsequent reduction of the aromatic nitro group with either stannous chloride or samarium diiodide [20] led to only poor yields of the amine **10** (39 and 20%, respectively, scheme 3), because the chemoselective reduction of this functional group is complicated by the presence of the conjugated double bonds.

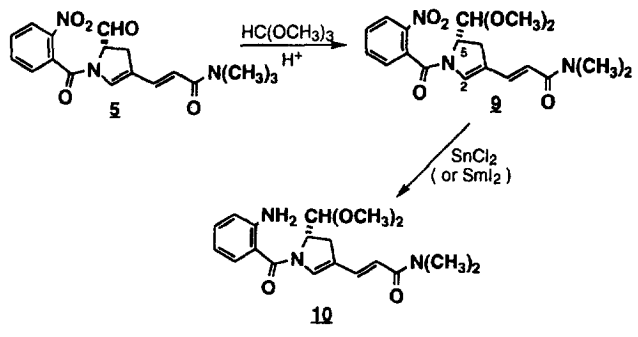
Therefore, we preferred to use the more efficient and direct Raney nickel route. This reagent was used as a slurry in water after being washed with water until pH 7.5 to retain the optical purity of the sensitive aldehyde **5**. 9-Demethoxyporothramycin **4** was purified by careful crystallization and rapid chromatography of the residue; its ^{1}H-NMR spectrum is very similar to that of the antitumor agent porothramycin B **3** [6].

Biological results

The *in vitro* cytostatic activities of the new analog **4** are summarized in the table I. They are expressed as the molar concentration of the compound which caused 50% inhibition of cell proliferation (IC_{50}) for each cell line tested. Mitomycin, vinblastine and doxorubicin were also tested for comparative purposes. As can be seen from the table I, compound **4** exerted strong cytostatic activities (at lower than micromolar concentrations) in all four tumor cell lines examined and on the nonmalignant but immortalized VERO monkey kidney cells.

Furthermore, this compound was equally active in cell lines endowed with the MDR$^+$ (multidrug-resistant) phenotype and on their MDR$^-$ counterparts. The reference anticancer drugs were tested in parallel, and were significantly less active in the resistant lines than in the normally sensitive ones.

Our *in vitro* data warrant further study of compound **4** in terms of its potential *in vivo* antitumor activity and possible lack of cardiotoxicity. They also encourage further investigations of other compounds in this family.



Scheme 3.

Table I. IC_{50} (M) of compound **4** in various cell lines.

Cell line	*Nature*	*IC_{50} (M)*	*Comparison (IC_{50})*
KB (human)	Standard line	8.6×10^{-8}	Mitomycin C (5×10^{-7})[a]
KB-3-1 (MDR^-)	Sensitive clone	8.0×10^{-8}	Vinblastine (10^{-8})
KB-V1 (MDR^+)	Resistant clone	10^{-7}	Vinblastine (5×10^{-5})
VERO	Monkey kidney	7.5×10^{-8}	Doxorubicin (5×10^{-6})
K562 (MDR^-)	Sensitive clone	8.5×10^{-8}	Doxorubicin (5×10^{-8})
K562R (MDR^+)	Resistant clone	1.2×10^{-7}	Doxorubicin (7×10^{-6})
L-1210 (murine)	Murine leukemia	4.6×10^{-8}	Doxorubicin (3.6×10^{-8})[b]
HCT 116 (human)	Human colon carcinoma	5×10^{-8}	Doxorubicin (10^{-7})

[a]Doxorubicin $IC_{50} = 10^{-7}$; [b]mitomycin $IC_{50} = 3.8 \times 10^{-8}$.

Experimental protocols

Chemistry

Optical rotations were measured on a Perkin-Elmer 241; the concentrations in $CHCl_3$ solution were measured in g/100 ml. IR spectra (ν cm^{-1}, $CHCl_3$) were recorded on a Nicolet 205 (FT). ^{1}H-NMR spectra were obtained ($CDCl_3$, Me_4Si, δ = 0 ppm) from Bruker AC250, AM300 or AM400 spectrometers; coupling constants (*J* values) are given in Hertz (s, d, t, dd and m indicate singlet, doublet, triplet, doublet of doublets and multiplet respectively). Mass spectra were measured on AEI MS50 or Kratos MS80 spectrometer. Flash chromatography was performed on SDS 230-400 mesh silica gel and preparative thin-layer chromatography on Merck HF 254 + 366 silica gel. Usual workup means that the organic layer was dried over magnesium sulfate, filtered and evaporated under vacuum.

(S)-N-2'-Nitrobenzoyl-3-(N,N-dimethyl)acrylamide-5-dimethoxymethyl-2-pyrrolin ***9***

Trimethylorthoformate (3.6 ml) and *p*-toluenesulfonic acid (5 mg, 0.03 mmol) were successively added under argon to the aldehyde **5** (124 mg, 0.36 mmol). The mixture was stirred at 80°C for 1 h and cooled before addition of an aqueous solution of sodium carbonate (10% w/v, 10 ml) and extraction with dichloromethane (2 x 40 ml, 2 x 25 ml). The crude dimethylacetal **9** was purified by preparative TLC on silica gel (eluent: dichloromethane/methanol 9:1) to afford **9** as a yellow powder (127 mg, 90%). $[\alpha]_D^{23} = -144°$ (c = 0.45). HRMS: calcd for $C_{19}H_{23}N_3O_6$: 389.1587, found 389.1586. MS: 389 ($M^{+\bullet}$), 312, 269, 208, 150 (100%), 120, 75. IR: 3000, 1635, 1602, 1531, 1480, 1422, 1345. ^{1}H-NMR (250 MHz): 8.25 (dd, 1H, J = 7.5, J' = 1.5), 7.75 and 7.66 (2dd, 2H, J = J' = 7.5), 7.43 (dd, 1H, J = 7.5, J' = 1.5, ArH), 7.23 (d, 1H, J = 15, C-CH=), 6.22 (s, 1H, C2-H), 6.16 (d, 1H, J = 15, CO-CH=), 5.10 (d, 1H, J = 2, $CH(OCH_3)_2$), 4.83 (m, 1H, C5-H), 3.60 (s, 6H, OCH_3), 3.07 and 3.01 (2s + masked m, 7H, $N(CH_3)_2$ + C4-Ha), 2.86 (m, 1H, C4-Hb). ^{13}C-NMR (75 MHz): 166.49 (CO), 163.84 (CO), 145.61 (qC), 135.15 (C-CH=), 134.56, 130.86, 128.60 and 125.09 (CH, Ar), 132.16 (C2), 131.62 and 124.74 (qC), 117.19 (COCH=), 103.41 ($CH(OCH_3)_2$), 60.28 (NCH), 58.00 (OCH_3), 56.80 (OCH_3), 37.32 (NCH_3), 35.85 (NCH_3), 28.05 (C4).

Reduction of the nitro group of ***9***

Stannous dichloride. Stannous dichloride ($SnCl_2$, $2H_2O$, 116 mg, 0.51 mmol) was added under argon to a stirred solution of the dimethylacetal **9** (39.8 mg, 0.102 mmol) in methanol (0.31 ml) at room temperature. The mixture was stirred for 2 h, a saturated aqueous solution of sodium carbonate added to pH 8, and the mixture extracted with ethyl acetate to afford the crude product which was purified by preparative TLC (eluent: ethyl acetate/methanol 9:1). The compound **10** was obtained as a yellow powder (14.3 mg, 39%).

Samarium diiodide. A solution of the dimethylacetal **9** (28.0 mg, 0.07 mmol) in a mixture of methanol/THF (2:1, 0.45 ml) was added under argon to a stirred solution of samarium diiodide (0.1 M in THF, 4.3 ml, 0.43 mmol) at room temperature. After stirring for 24 h at room temperature, an aqueous solution of sodium thiosulfate (10%, 3 ml) was added. The crude product was obtained by extraction with ethyl acetate followed by the usual workup. The compounds were separated by preparative TLC (eluent: dichloromethane/methanol 96:4) affording the starting dimethylacetal **9** (13.1 mg, 47%) and the reduced derivative **10** (5.0 mg, 20%). $[\alpha]_D^{26} = -70°$ (c = 0.19). HRMS (CI): calcd for $C_{19}H_{25}N_3O_4$: 359.1845, found 359.1864. MS (FAB): 360 (M + 1), 328 (M-OCH_3), 283, 251, 120 (100%). IR: 3680, 3400, 3000, 1640, 1600, 1493, 1409. ^{1}H-NMR (250 MHz): 7.35 (d, 1H, J = 15, C-CH=), 7.22 and 7.19 (2H, ArH), 6.80, 6.73, 6.70 (3H, ArH + C2-H), 6.13 (d, 1H, J = 15, CO-CH=), 5.00 (bd, 1H, J = 2, $CH(OCH_3)_2$), 4.80 (m, 1H, C5-H), 4.61 (bs, 2H, NH_2), 3.54 and 3.48 (2s, 6H, $CH(OCH_3)_2$), 3.06 and 3.02 (2s + masked m, 7H, $CON(CH_3)_2$ + C4-Ha), 2.78 (m, 1H, C4-Hb).

9-Demethoxyporothramycin B ***4***

A solution of the aldehyde **5** (342 mg, 1.0 mmol) in ethyl acetate/methanol 85:15 (15 ml) was added to an excess of Raney nickel (50% slurry in water, washed with water until pH 7.5) stirred under argon. After completion of reaction as monitored by TLC, the mixture was filtered through silica gel (70-230 mesh) which was washed with ethyl acetate/methanol 85:15. The solvents were evaporated under reduced pressure.

The residue was dissolved in dichloromethane (devoid of ethanol)/methanol 88:12 (18 ml). To this stirred solution was added a solution of trifluoroacetic acid in dichloromethane (0.015%, 3.6 ml) under argon. The mixture was stirred for 16 h at room temperature before evaporation of the solvents under reduced pressure. The residue was crystallized in methanol to give 9-demethoxyporothramycin B **4** (179 mg, 55%). The residue was purified by preparative TLC followed by crystallization in methanol to afford a second crop of **4** (32.8 mg, 10%). Mp dec: 228°C (Kofler apparatus), $[\alpha]_D^{25} = +805°$ ($c = 0.23$). HRMS: calcd for $C_{17}H_{17}N_3O_2$ ($M^{+\bullet} - CH_3OH$): 295.1321, found: 295.1321. MS: 327 ($M^{+\bullet}$ very weak), 295 (100%), 251, 249 (100%), 223, 120. IR: 3320, 2924, 2850, 1620. ^{1}H-NMR (400 MHz): 8.03 and 6.65 (2d, 2H, $J_{6,7} = J_{8,9} = 7.5$, C6-H and C9-H), 7.30 and 6.87 (2dd, 2H, $J_{6,7} = J_{7,8} = J_{8,9} = 7.5$, C7-H and C8-H), 7.53 (s, 1H, C3-H), 7.52 (d, 1H, $J_{12,13} = 15$, C12-H), 6.06 (d, 1H, $J_{12,13} = 15$, C13-H), 5.34 (d, 1H, $J_{10,11} = 5.6$, N10-H), 4.61 (d, 1H, $J_{10,11} = 5.6$, C11-H), 4.30 (dd, 1H, $J_{1a,11a} = 10.5$, $J_{1b,11a} = 4.5$, C11a-H), 3.38 (s, 3H, OCH_3), 3.16 (masked m, 1H, C1-Ha), 3.11 (s, 3H, NCH_3), 3.05 (s, 3H, NCH_3), 2.87 (dd, 1H, $J_{1a,1b} = 15.5$ and $J_{1b,11a} = 4.5$, C1-Hb).

Cytostatic activity assays

The *in vitro* cytostatic activities of compound **4** were evaluated in the following cell lines: VERO (African green monkey kidney); KB (human epidermoid carcinoma of the mouth); K-562 (human erythroleukemia); and HCT-116 (human colon carcinoma). Tests were also made on two sublines of KB and K-562 cells, which had been selected for increased resistance to vinblastine and doxorubicin, respectively, and are thus endowed with the MDR$^+$ phenotype: KB-V1 and K-562R.

VERO and standard KB cells were from our own collection. K-562 cell lines were provided by Dr H Tapiero (Institut de cancérologie et d'immunogénétique, Villejuif, France), the KB-3-1 and KB-V1 clones were provided by Dr MM Gottesman (National Cancer Institute, Bethesda, MD, USA) and the HCT-116 cell line was provided by Dr MC Bissery (Rhône-Poulenc Rorer, Vitry-sur-Seine, France).

Standard KB cells and VERO cells were grown in medium 199 + 10% newborn calf serum, K562 cells in RPMI-1640 medium +5% fetal calf serum (FCS), L-1210 cells in RPMI-1640 + 10% FCS, HCT-116 cells in Fischer medium 50% and CMRL 1066 medium 50% + 10% FCS, cloned KB cells in Dulbecco minimal essential medium + 10% FCS (in the presence of 1 μg/ml vinblastine for the resistant subline).

KB and VERO cells were grown as monolayers in Costar 12-well plastic plates (12×10^4 to 15×10^4 cells seeded in 2 ml medium); K562 and L-1210 cells were grown in suspension in Leighton tubes (10×10^4 cells seeded in 1 ml medium). For the tests, serial dilutions in the media of a stock solution of the compound in DMSO were added to the cultures at the time of cell seeding (the final concentration of DMSO was less than 1%). These cultures were incubated at 37°C in a 95% air/5% CO_2 incubator. After 48 h incubation, cell viability in the monolayers was determined by further 18 h incubation in a medium containing neutral red, followed by lysis with sodium dodecylsulfate and photometric quantification of the extracted dye at 540 nm. For the cells growing in suspension, cell viability was determined after 72 h incubation using the MTT assay as described by Mossman [21].

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References

1 Leimgruber W, Stefanovic V, Schenker F, Karr A, Berger J (1965) *J Am Chem Soc* 87, 5791–5793
2 Arima K, Kohsaka M, Tamura G, Imanaka H, Sakai H (1972) *J Antibiotics* 25, 437–444
3 Brazhnikova MG, Konstantinova NV, Mesentev AS (1972) *J Antibiotics* 25, 668–673
4 Leber JD, Hoover JRE, Holden KG, Johnson RK, Hecht SM (1988) *J Am Chem Soc* 110, 2992–2993
5 Takeuchi T, Miyamoto M, Ishizuka M *et al* (1976) *J Antibiotics* 29, 93–96
6 Tsunakawa M, Kamei H, Konishi M, Miyaki T, Oki T, Kawaguchi H (1988) *J Antibiotics* 41, 1366–1373
7 Glaubiger D, Kohn KW, Charney E (1974) *Biochim Biophys Acta* 361, 303–311
8 Petrusek RL, Anderson GL, Garner TF *et al* (1981) *Biochemistry* 20, 1111–1119
9 Thurston DE, Hurley LH (1983) *Drugs of the Future* 8, 957–971
10 Hurley LH, Thurston DE (1984) *Pharm Res* 1, 52–59
11 Graves DE, Pattaroni C, Krishnan BS, Ostrander JM, Hurley LH, Krugh TR (1984) *J Biol Chem* 259, 8202–8209
12 Hurley LH, Reck T, Thurston DE *et al* (1988) *Chem Res Toxicol* 1, 258–268
13 Andriamialisoa RZ, Langlois N (1986) *Tetrahedron Lett* 27, 1149–1152
14 Langlois N, Andriamialisoa RZ (1989) *Heterocycles* 29, 1529–1536
15 Langlois N, Favre F (1991) *Tetrahedron Lett* 32, 2233–2236
16 Langlois N, Favre F, Rojas A (1993) *Tetrahedron Lett* 34, 4635-4638
17 Thurston DE, Bose DS (1994) *Chem Rev* 94, 433–465
18 Langley DR, Thurston DE (1987) *J Org Chem* 52, 91–97
19 Mori S, Ohno T, Harada H, Aoyama T, Shioiri T (1991) *Tetrahedron* 47, 5051–5070
20 Molander GA (1992) *Chem Rev* 92, 29–68
21 Mossmann T (1983) *J Immunol Methods* 65, 55–63