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Synthesis of a new structural analogue of (+)-porothramycin

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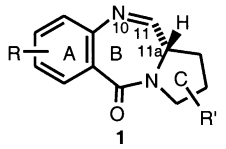
Abstract—A new phosphonate analogue of (+)-porothramycin has been synthesized from (*S*)-pyroglutaminol. Circular dichroism of diastereomeric intermediates **9** has been studied. The cytostatic activity of the new pyrrolo[2,1-*c*][1,4]benzodiazepine has been evaluated against human KB cells. © 2001 Elsevier Science Ltd. All rights reserved.

1. Introduction

Several natural pyrrolo[2,1-*c*][1,4]benzodiazepines (PBDs) of general formula **1**, isolated from various *Streptomyces* species, exhibit interesting antibiotic and antitumour activities. Among them, anthramycin **2** has been the subject of extensive investigations. These studies have shown a selective DNA binding of anthramycin, with a preference for PuGPu sequences and the formation of a covalent adduct in the minor groove of DNA.[1] Therefore, such compounds could be interesting as potential gene targeted drugs.[1,2]



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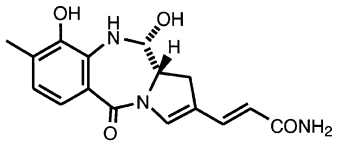
In these PBDs, two common structural features are essential to their biological properties: the *S* absolute configuration at C-11a and the presence of an imine or an equivalent carbinolamine function at N-10–C-11. Indeed, this absolute configuration provides these molecules with a twisted shape, allowing their fit within the minor groove of DNA and a nucleophilic attack by the N-2 amino group of a guanine residue to form an aminal linkage at C-11.[3] These two requirements are also the major problems encountered in the synthesis of PBDs, owing to frequently observed racemization at the C-11a position and to the great chemical sensitivity of the imine function.

Several syntheses of PBDs and structural analogues have already been described.[4] Most of the routes developed for this are based upon a cyclization of amino-aldehydes, prepared following different chronologies which require suitable protection and deprotection steps. Among them, the cyclization of amino thioacetals has significant advantages and was shown to be rather general, but its application to the synthesis of 9-demethoxyporothramycin **3** failed in our hands.[5] The other methods, including more recent reduction of azides,[6] as well as the cyclization through intramolecular aza-Wittig reactions,[7] have been mainly applied to simple PBDs such as DC 81 **4** or its *O*-benzyl derivative. Our introduction of Raney nickel as catalyst to reduce aromatic nitro group of PBD precursors[8] have already been extended to the enantioselective synthesis of (+)-9-demethoxyporothramycin **3** and (+)-porothramycin **5**, the double bond of the C-2 side chain being preserved under these conditions.[9]



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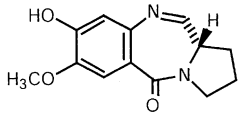

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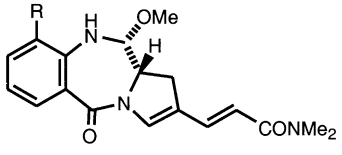
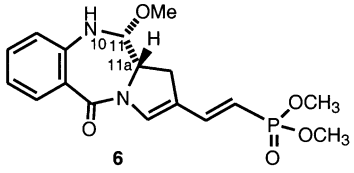


3 : R = H

5 : R = OCH_3



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Keywords: pyrrolo[2,1-*c*][1,4]benzodiazepines; phosphonate; Horner–Wadsworth–Emmons reaction; circular dichroism; cytotoxicity.

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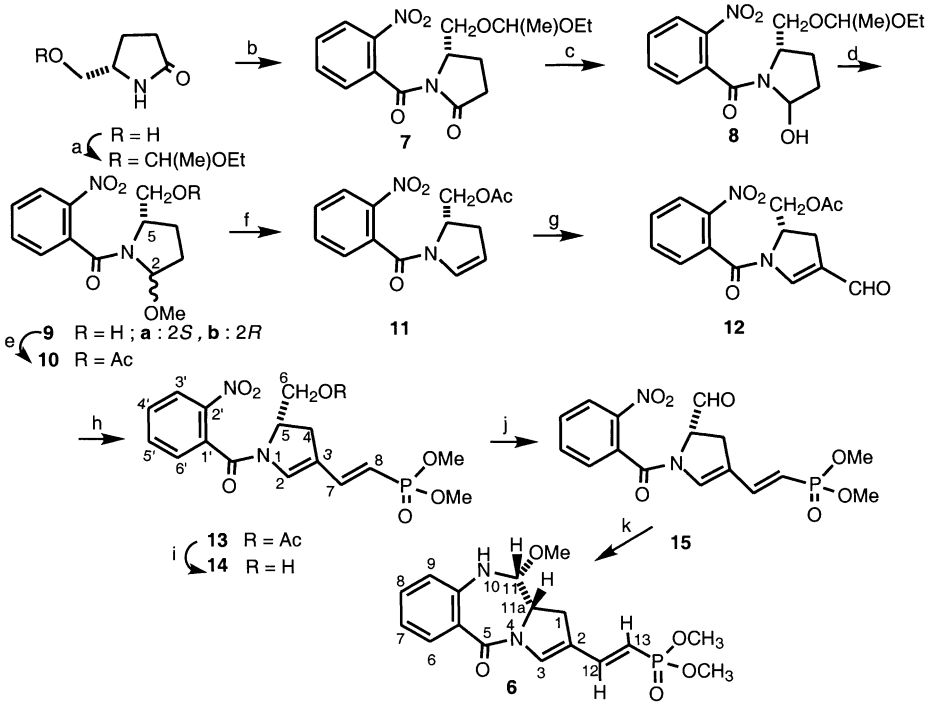

Scheme 1. Reagents: (a) see Ref. 11; (b) NaH, KI, *o*-NO_2PhCOCl (95%); (c) DIBAL–H, Tol (80%); (d) MeOH, TsOH (100%); (e) Ac_2O, Py (98%); (f) 15 mol% QCS, Tol (94%); (g) $POCl_3$–DMF (100%); (h) $CH_2[P(O)(OMe)_2]_2$, *n*BuLi (87%); (i) $Ba(OH)_2$, then CO_2 (86%); (j) DMSO, $COCl_2$, *i*Pr_2NEt (99%); (k) Raney-Ni, then MeOH, H^+ (36%).

We report here the synthesis of **6**, an analogue of **3** bearing an unsaturated phosphonate chain at C-2, according to this methodology. Indeed, only few modifications of C-2 side chain of synthetic PBDs have been achieved,[10] whereas we described some years ago a very efficient method to prepare an enamido-aldehyde as a good scaffold from which a variety of unsaturated C-2 side chains could be introduced.

2. Results and discussion

The new PBD **6** was synthesized as outlined in Scheme 1.

(5*S*)-5-(Ethoxy-ethoxymethyl)pyrrolidin-2-one was easily prepared from (*S*)-pyroglutaminol.[11] After deprotonation, this compound was treated with *o*-nitrobenzoyl chloride affording the imide **7** in 95% yield. The α-hydroxy-*ortho*-nitrobenzamides **8** were generated by partial and regioselective reduction of **7** with DIBAL–H at −78°C in toluene (80%), and were quantitatively converted into the more stable α-methoxy-*ortho*-nitrobenzamides **9**. The mixture of diastereomers (**9a/9b** ca. 9:1) was used in the next step but **9a** and **9b** have also been separated by column chromatography on silica gel for the purposes of characterization and checking the validity of the next elimination-step with each diastereomer. The coupling constants observed in ^{1}H NMR spectra allowed us to assign the 2,5-*cis* relative configuration to **9a**. The pyrrolidine-rings of these derivatives adopt a near-envelope conformation with C-3 pointing out of the plane, on the side opposite to the C-2 methoxy group. It is interesting to note that the CD curves of **9a** and **9b** are mirror images with five Cotton effects alternating in sign, between 225 and 335 nm, in spite of the same absolute configuration of the C-5 centre (Fig. 1). These results could be explained by the inherent chirality of the chromophore due to non-planarity of the *ortho*-nitrobenzamide group. X-Ray analysis of **9a** (2*S*) showed that the nitro group is practically coplanar with the aromatic ring, whereas this is almost perpendicular to the amide carbonyl group.[12] The aromatic ring adopts a conformation with the nitro group pointing on the side opposite to the methoxy group at C-2, giving an M-helicity for the chirality axis CO–nitroaryl (Fig. 2). Because of steric factors, the diastereomer **9b** (2*R*) is postulated to adopt a conformation with the nitro group pointing on the opposite side with a P

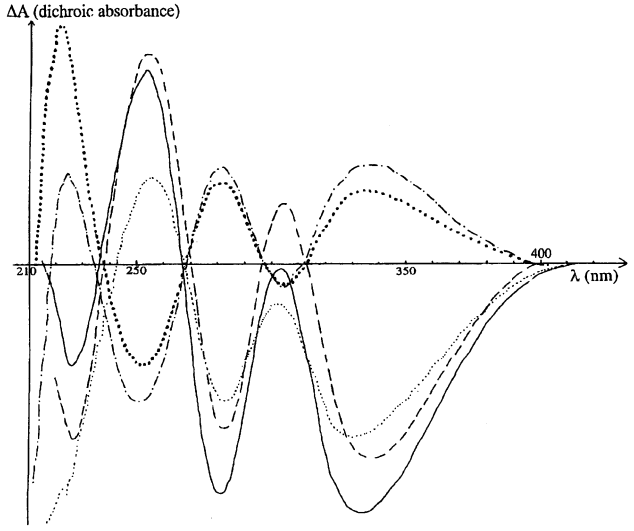

Figure 1. CD curves (MeOH) of **9a** (—), **9b** (–·–), **10a** (– –), **10b** (···) and (*S*)-*N*-*o*-nitrobenzoylprolinol [(···), λ nm (Δε)]: 255 (+1.0), 283 (−1.7), 301 (−0.5), 330 (−2.2).

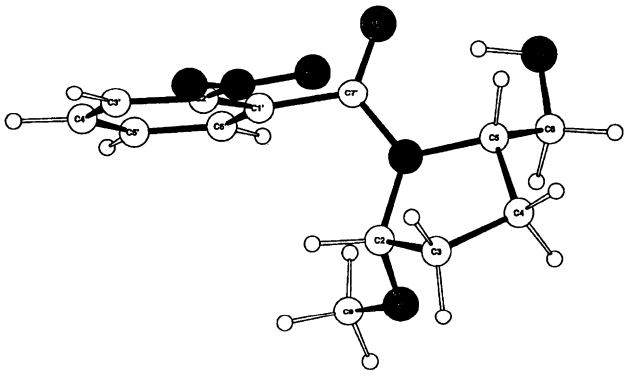

Figure 2. X-Ray, general view of **9a**.

chirality sense of the helix. Thus, the CD curves of **9a** and **9b** are virtually of enantiomorphous type since the stronger perturber of the nitrophenyl chromophore is the CO–N moiety, approximately perpendicular to the aromatic ring (N1–C7′–C1′–C2′=90.9° in **9a**). X-Ray structure showed a hydrogen bond between the hydroxy and the carbonyl groups in the crystal, and therefore an *s-trans* conformation of the amide bond. However, the hydrogen bond is not essential for this conformation since the CD curves were recorded in methanol as solvent able to break internal H-bridges, and the same curves, albeit of somewhat smaller amplitudes, were obtained, respectively, with the acetates **10a** and **10b**. It is also worthy of note that (*S*)-*N*-*o*-nitrobenzoylprolinol (devoid of the α-methoxy group) gives a CD curve qualitatively similar to that of **9a**, indicating a preferred M-helicity around the CO–nitroaryl bond.

The acetates **10** were prepared to protect efficiently the primary alcohol function of **9** during the following step.[13] The methoxy groups of the mixture of diastereomers **10a**+**10b** were eliminated to form the enamide **11** in high yield (94%), by heating in toluene in the presence of quinoleinium camphosulfonate as catalyst.[13–15] A Vilsmeier–Haack reaction converted quantitatively the compound **11** into the versatile unsaturated aldehyde **12**, provided a large excess of reagent was used to preclude the formation of dimeric compounds.

This key intermediate, despite its vinylogous imide structure, gave excellent results in Horner–Wadsworth–Emmons reactions. Thus, condensation of the anion obtained by deprotonation of tetramethyl methylenediphosphonate with one equivalent of *n*BuLi at 0°C provided the derivative **13** as a single stereoisomer (87%). The *E* geometry of the newly created double bond was confirmed by the coupling constants observed in ^{1}H NMR between the ethylenic protons H-7 and H-8 (17 Hz).[16] The acetate **13** was saponified with $Ba(OH)_2$ to afford **14** (86%).

Swern oxidation of the primary alcohol **14**, with *i*Pr_2NEt as base, led almost quantitatively to the aldehyde **15** (99%). The aldehyde **15**, isolated in a partially hydrated form, could be used without purification because it gave easily a methyl acetal when methanol was co-eluent in chromatographic separations and it could be sensitive to racemization. The stereogenic centre was preserved in these conditions as shown by the control of the enantiopurity of **15** through its reduction with $NaBH_4$. The optical rotation of the reduction product indicated that no racemization had occurred.

The reductive cyclization of **15** with an excess of Raney nickel, followed by a treatment with methanol and small amounts of trifluoroacetic acid, led to the new pyrrolo[2,1-*c*][1,4]benzodiazepine **6** together with two unidentified minor by-products. The compound **6** could not be efficiently crystallized and was therefore purified by preparative TLC on silica gel. However, the great reactivity of the carbinolamine ether functional group under these conditions explains the modest isolated yield (36%) which was not optimized owing to the relative cytostatic activity of **6**. The configuration 11*R* was assigned on the basis of absence of coupling between 11-H and 11a-H.

The cytostatic activity of the new phosphonate **6** was evaluated in vitro using the human KB cell line. The IC_{50} value (7.5×10^{-7} M), compared to the results obtained with 9-demethoxyporothramycin **3** ($IC_{50}=8.6\times10^{-8}$ M), indicated a weaker activity, the compound **6** being atoxic between 10^{-7} M and 10^{-9} M. Although the studies devoted to the mechanism of action of PBDs concerned principally anthramycin **2** (and, more recently, PBD dimers),[17] the biological activities of (+)-porothramycin **5** and 9-demethoxy analogue **3** indicate that the snug fit within the minor groove of DNA tolerates the substitution of the C-2 acrylamide group by two methyl groups.[5,18] The 3D structures of **3** and **6** could explain the difference of their cytostatic activities. The molecular modelling study shows an *s-trans* preferred conformation for the side-chain double bond in **3** (*E*=135.2 vs 138.3 kJ/mol), as well as in the phosphonate analogue **6** (*E*=113.8 vs 121.6 kJ/mol), as displayed by the parent anthramycin methyl ether itself, according to its X-ray structural analysis.[19] In the 3D structure of the most stable conformations of **3** and **6**, the carbons C-1 to C-13 are superimposable and the main difference lies in the tetrahedral character of the phosphorus atom with, as a consequence, the pointing of one of the phosphonate methoxy groups out the general plane of the C-2 side chain and this steric factor probably could be responsible for the difference in the fit to DNA.

3. Conclusions

The total synthesis of the new PBD **6**, the phosphonate analogue of 9-demethoxyporothramycin **3**, has been achieved from inexpensive (*S*)-pyroglutaminol through a particularly efficient Horner–Wadsworth–Emmons reaction with the enamido-aldehyde **12**, to elaborate the side chain. The compound **6** has been tested in vitro in the human KB cell line and shown to be almost 10-fold less cytostatic than **3**.

4. Experimental

4.1. General

Optical rotations were measured on a Perkin–Elmer 241; the concentrations in $CHCl_3$ solution (unless otherwise indicated) are given in g/100 mL. IR spectra (ν cm^{-1}, $CHCl_3$)

were recorded on a Nicolet 205 (FT). ^{1}H NMR spectra were obtained ($CDCl_3$ unless otherwise indicated, Me_4Si, δ=0 ppm) from Bruker AC200, AC250 or AM300; coupling constant J values are given in hertz (s, d, t, dd and m indicate singlet, doublet, triplet, doublet of doublets, and multiplet, respectively). ^{13}C NMR spectra were recorded on AC250 (62.5 MHz) or AM300 (75.0 MHz). CD curves were recorded on a Jobin–Yvon dichrographe V. Mass spectra and high resolution mass spectra were, respectively, measured on an AEI MS50 or on a Kratos MS80 spectrometer. Flash chromatography was performed on silica gel (SDS 230–400 mesh) and preparative thin layer chromatography on silica gel (Merck HF 254+366). Usual workup means that the organic layer was dried over magnesium sulfate, filtered and evaporated under vacuum.

4.1.1. (5*S*)-5-(1-Ethoxy-ethoxymethyl)-1-(2-nitro-benzoyl)-pyrrolidin-2-one 7. A solution of (5*S*)-5-(1-ethoxy-ethoxymethyl)-pyrrolidin-2-one (5.05 g, 27 mmol) in dry THF (32 mL) was added dropwise to a suspension of NaH (50% in oil, 1.30 g, 27 mmol) and potassium iodide (4.48 g, 27 mmol) in dry THF (32 mL) at 0°C under argon. The mixture was stirred for 1.5 h at room temperature and a solution of 2-nitrobenzoyl chloride (3.56 mL, 27 mmol) in dry THF (32 mL) was added dropwise. After being stirred for 10 min at the same temperature, the reaction medium was quenched with a saturated aqueous solution of ammonium chloride and extracted with ethyl acetate. After the usual treatment, the crude product (9.0 g) was purified by column chromatography (eluent: heptane–EtOAc 1:1) to give (5*S*)-5-(1-ethoxy-ethoxymethyl)-1-(2-nitro-benzoyl)-pyrrolidin-2-one **7** (8.65 g, 95%). MS: 290 ($(M^{+\cdot}-NO_2)$, 205, 150 (100%), 73. IR: 2985, 1750, 1682, 1530. ^{1}H NMR (200 MHz), 8.20 (d, 1H, $J_{3',4'}$=8 Hz) and 7.32 (m, 1H), 3′-H, 6′-H; 7.70 and 7.55 (2 dd, 2H, $J_{3',4'}=J_{4',5'}=J_{5',6'}$=8 Hz, 4′-H and 5′-H), 4.70 (m, C*H*–CH_3 and 5-H), 3.95 (m, 1H), 3.55 (m, 2H), 2.75 (m,1H), 2×CH_2O), 2.30 (m, 4H, 3-H_2 and 4-H_2), 1.25 (m, 6H, CHC*H*$_3$ and CH_2CH_3).

4.1.2. (5*S*)-5-(1-Ethoxy-ethoxymethyl)-1-(2-nitro-benzoyl)-pyrrolidin-2-ols 8. To a stirred solution of (5*S*)-5-(1-ethoxy-ethoxymethyl)1-(2-nitro-benzoyl)-pyrrolidin-2-one (3.50 g, 10.4 mmol) in dry toluene (100 mL), stirred at −78°C under argon, were added a solution of DIBAL–H in toluene (1.5 M, 12.5 mL, 18.75 mmol) and, after 10 min, a saturated aqueous solution of ammonium chloride. The mixture was extracted with CH_2Cl_2 and treated as usual to give the crude compound which was purified by column chromatography (eluent: heptane–ethyl acetate 1:3, 2.82 g, 80%). MS: 321 ($(M^{+\cdot}-OH)$, 249, 226, 150 (100%). IR: 3340, 2930, 1683, 1530. ^{1}H NMR (200 MHz) 8.12 (d, 1H, $J_{3',4'}$=7 Hz, Ar–H), 7.58 (m, 3H, Ar–H), 4.90 (m, 2H, attributed to 2-H and C*H*–CH_3), 4.7–3.5 (5H, 5-H and 2×CH_2O), 2.18 and 1.95 (2m, 4H, 4-H_2 and 3-H_2), 1.40 (dd, 3H, CHC*H*$_3$), 1.25 (2t, 3H, J=8 Hz, CH_2CH_3).

4.1.3. (5*S*)-5-Hydroxymethyl-2-methoxy-1-(2-nitro-benzoyl)-pyrrolidines 9. *p*-Toluenesulfonic acid (2.14 g, 12.44 mmol) was added slowly to a stirred solution of **8** (4.46 g, 13.2 mmol) in a CH_2Cl_2–MeOH 9:1 mixture (15 mL) at 0°C. The mixture was stirred for 15 min before the addition of aqueous Na_2CO_3 solution (10% w/v) followed by extraction with CH_2Cl_2. After usual workup, the 2-methoxy derivatives **9** were isolated in 100% yield.

The two diastereomers have been separated by chromatography on silica gel (eluent: CH_2Cl_2–MeOH 97:3) to be characterized.

Major diastereomer ***9a***. $[\alpha]_D$=−230 (c=2.15). MS: 249 ($(M^{+\cdot}-OCH_3)$, 150 (100%). IR: 3500, 2950, 1640. ^{1}H NMR (400 MHz C_6D_6), 7.82 (d, 1H, J=8 Hz), 7.29 (d, 1H, J=8 Hz), 3′-H and 6′-H; 7.09 (dd, 1H, J~8 Hz), 6.92 (dd, 1H, J=8 Hz), 4′-H and 5′-H; 4.55 (m, 1H, 5-H), 4.22 (1H, 2-H), 4.24 (bd, 1H, 6-Ha), 4.05 (dd, 1H, $J_{6a,6b}$=11.5 Hz, $J_{5,6b}$=6.5 Hz, 6-Hb), 2.64 (s, 3H, OCH_3), 1.87 (m, 1H, 4-Ha), 1.80 (m, 1H, 4-Hb), 1.62 (dd, 1H, $J_{3a,3b}$=13 Hz, $J_{3,4a}$=7 Hz, 3-Ha), 1.44 (m, 1H, 3-Hb). ^{13}C NMR (50 MHz, C_6D_6), 134.0, 130.0, 129.1, 124.5 (CH, Ar), 91.7 (C-2), 65.4 (C-6), 61.4 (C-5), 54.4 (OCH_3), 29.8, 24.9 (C-3, C-4). CD [MeOH, c=0.81 mM, λ nm ($\Delta\epsilon$)], 225 (−2.3), 253 (+4.5), 282 (−5.3), 305 (−0.2), 335 (−5.8). Anal.: $C_{13}H_{16}N_2O_5$; Calcd: C, 55.71; H, 5.75; N, 10.00. Found: C, 55.59; H, 5.63; N, 9.98.

Minor diastereomer ***9b***. $[\alpha]_D$=+82 (c=1.15). MS: 249 ($(M^{+\cdot}-OCH_3)$, 150 (100%). IR: 3500, 2930, 1640. ^{1}H NMR (400 MHz, C_6D_6), 7.87 (d, 1H, J=8 Hz), 7.42 (d, 1H, J=8 Hz), 3′-H, 6′-H; 7.09 (dd, 1H, J=8 Hz), 6.89 (d, 1H, J=8 Hz), 4′-H, 5′-H; 4.64 (m, 1H, 5-H), 4.14 (1H, 6-Ha), 4.12 (d, 1H, J=3.5 Hz, 2-H), 4.07 (dd, 1H, $J_{6a,6b}$=11 Hz, $J_{5,6b}$=4.5 Hz, 6-Hb), 3.62 (OH), 2.46 (s, 3H, OCH_3), 2.11 (m, 1H, 4-Ha), 1.73 (m, 2H, 4-Hb, 3-Ha), 1.50 (dd, 1H, $J_{3a,3b}$=11.5 Hz, $J_{3b,4a}$=7 Hz, 3-Hb). ^{13}C NMR (50 MHz, C_6D_6), 168.7 (CO), 146.5, 133.1 (C-1′, C-2′), 133.7, 129.9, 129.4, 124.1 (CH, Ar), 91.0 (C-2), 64.8 (C-6), 61.0 (C-5), 54.1 (OCH_3), 29.4, 25.8 (C-3, C-4). CD [MeOH, c=0.84 mM, λ nm ($\Delta\epsilon$)], 225 (+4.3), 252 (−5.9), 282 (+4.4), 305 (−0.9), 335 (+4.4).

4.1.4. (5*S*)-5-Acetoxymethyl-2-methoxy-1-(2-nitro-benzoyl)-pyrrolidines 10. To a solution of (5*S*)-5-hydroxymethyl-2-methoxy-1-(2-nitro-benzoyl)-pyrrolidines **9** (2.34 g, 8.35 mmol) in anhydrous pyridine (55 mL) was added dropwise acetic anhydride (14 mL). After being stirred at room temperature for 1 h, the mixture was cooled to 0°C, before the addition of methanol. After additional stirring for 30 min, the solvents were eliminated under reduced pressure. Extraction of the product with EtOAc, washing of the organic phases with aqueous $NaHCO_3$ (5%) followed by usual treatment gave (5*S*)-5-acetoxymethyl-2-methoxy-1-(2-nitro-benzoyl)-pyrrolidines **10** (2.63 g, 98%).

Major diastereomer ***10a***. MS: 291 ($(M^{+\cdot}-OCH_3)$, 249, 216, 172, 150 (100%). IR: 3530, 2950, 1745, 1650, 1530, 1405, 1350. ^{1}H NMR (250 MHz), 8.13, 7.65 (2d, 2H, 3′-H, 6′-H), 7.50 (m, 2H, 5′-H, 4′-H), 4.50 and 4.30 (2m, 5-H and 6-H_2), 2.92 (s, 2-OCH_3), 2.18 and 1.95 (2m, 3-H_2 and 4-H_2), 2.07 (s, $COCH_3$). CD [MeOH, c=1.24 mM, λ nm ($\Delta\epsilon$)], 227 (−2.7), 255 (+3.2), 283 (−2.5), 305 (+0.9), 335 (−2.9).

Minor diastereomer ***10b***. IR: 3475, 2950, 1745, 1660, 1575, 1530, 1485. ^{1}H NMR (250 MHz): 8.20, 7.70 (2d, 2H, 3′-H, 6′-H), 7.60 (m, 2H, 5′-H, 4′-H), 4.56 (m, 6-H_2), 4.30 (m,

1H, 5-H), 4.00 (dd, $J_{2,3a}$=6 Hz, $J_{2,3b}$=2 Hz, 2-H), 2.71 (s, 2-OCH_3), 2.17 and 1.88 (2m, 3-H_2 and 4-H_2), 2.07 (s, $COCH_3$). CD (MeOH, c=1.54 mM, λ nm ($\Delta\epsilon$)], 222 (+2.9), 254 (−1.2), 283 (+1.0), 305 (−0.3), 335 (+0.9).

4.1.5. (*S*)-5-Acetoxymethyl-1-(2-nitro-benzoyl)-2-pyrroline 11. To a solution of (5*S*)-5-acetoxymethyl-2-methoxy-1-(2-nitro-benzoyl)-pyrrolidines **10** (1.45 g, 4.5 mmol) in anhydrous toluene (11 mL) was added quinoleinium camphosulfonate (0.245 g, 0.675 mmol). After being stirred under argon at 110°C for 2 h, the solvent was evaporated under vacuum. The crude product was purified by flash column chromatography (eluent: pentane–Et_2O 1:3) to afford (*S*)-5-acetoxymethyl-1-(2-nitro-benzoyl)-2-pyrroline **11** (1.23 g, 94%). $[\alpha]_D$=−170 (c=0.26). MS: 290 ($M^{+\cdot}$), 249, 230, 150 (100%), 104, 80, 76. IR: 3100, 2900, 2825, 1730, 1640, 1615, 1520. ^{1}H NMR (200 MHz), 8.14 (dd, 1H, J=7.5 Hz, J'=1.5 Hz, Ar–H), 7.70–7.37 (m, 3H, Ar–H), 5.83 (m, 1H, 2-H), 5.09 (m, 1H, 3-H), 4,88 (m, 1H, 5-H), 4.39 (m, 2H, 6-H_2), 2.98 (m, 1H, $J_{4a,4b}$=17 Hz, $J_{4a,5}$=10 Hz, 4-Ha), 2.50 (bd, 1H, $J_{4a,4b}$=17 Hz, 4-Hb), 2.06 (s, 3H, $COCH_3$). Anal.: $C_{14}H_{14}N_2O_5$; Calcd: C, 57.93; H, 4.86; N, 9.65. Found: C, 57.68; H, 5.01; N, 9.47.

4.1.6. (*S*)-5-Acetoxymethyl-3-formyl-1-(2-nitro-benzoyl)-2-pyrroline 12. Phosphorus oxychloride (2.05 mL, 22 mmol) was added at 0°C under a nitrogen atmosphere to DMF (1.7 mL, 22 mmol) and the mixture was stirred at room temperature for 20 min. Excess of $POCl_3$ was removed by evaporation in the presence of toluene. To a stirred solution of this reagent in dry dichloromethane (6 mL) was added at 0°C under argon a solution of (5*S*)-5-acetoxymethyl-1-(2-nitro-benzoyl)-2-pyrroline **11** (0.481 g, 1.66 mmol) in CH_2Cl_2 (6 mL). After being stirred for 3.5 h at room temperature, sodium carbonate was added until pH 14, and the mixture was extracted with CH_2Cl_2. The crude product was purified by column chromatography (ether) to afford (*S*)-5-acetoxymethyl-3-formyl-1-(2-nitro-benzoyl)-2-pyrroline **12** (0.529 g, 100%). Mp=94–96°C (EtOAc–Et_2O). $[\alpha]_D$=−219 (c=1.0). MS: 318 ($M^{+\cdot}$), 258, 150 (100%). IR: 2900, 2825, 1740, 1655, 1605, 1530. ^{1}H NMR (200 MHz), 9.37 (s, 1H, CHO), 8.21, 7.49 (2d, 2H, J=8 Hz, 3′-H, 6′-H), 7.79, 7.67 (2 dd, 2H, $J_{3',4'}$=$J_{4',5'}$=$J_{5',6'}$=8 Hz, 4′-H, 5′-H), 6.83 (s, 1H, 2-H), 5.00 (m, 1H, 5-H), 4.62 (dd, 1H, $J_{6a,6b}$=11 Hz and $J_{5,6a}$=5 Hz, 6-Ha), 4.27 (dd, 1H, $J_{6a,6b}$=11 Hz, $J_{5,6b}$<3 Hz, 6-Hb), 3.08 (dd, 1H, $J_{4a,4b}$=16 Hz and $J_{4a,5}$=10 Hz, 4-Ha), 2.79 (dd, 1H, $J_{4a,4b}$=16 Hz, $J_{4b,5}$<3 Hz, 4-Hb), 2.07 (s, 3H, $COCH_3$). ^{13}C NMR: 185.7 (CHO), 170.8 (OCO), 164.8 (NCO), 145.4 (C-2′), 144.5 (C-2), 134.9, 131.3, 128.6 and 125.0 (CH, Ar), 130.5 (C-1′), 126.9 (C-3), 63.4 (C-6), 58.1 (C-5), 28.7 (C-4), 20.5 ($COCH_3$). Anal.: $C_{15}H_{14}N_2O_6$; Calcd: C, 56.60; H, 4.43; N, 8.80. Found: C, 56.52; H, 4.60; N, 8.68.

4.1.7. (*S*)-5-Acetoxymethyl-3-[2-(dimethylphosphono)-ethenyl]-1-(2-nitro-benzoyl)-2-pyrroline 13. To a solution of tetramethyl methylenediphosphonate (264 mg, 1.13 mmol) in dry THF (5.0 mL) stirred at 0°C under argon was added *n*BuLi (1.5 M in hexane, 0.76 mL, 1.13 mmol). After being stirred for 0.5 h at 0°C a solution of (*S*)-5-acetoxymethyl-3-formyl-1-(2-nitro-benzoyl)-2-pyrroline **12** (329 mg, 1.03 mmol) in dry THF (10 mL) was added. After complete reaction, a saturated aqueous solution of NH_4Cl (10 mL) was added and the mixture was extracted with ethyl acetate. Usual workup afforded the crude product which was purified by preparative TLC on silica gel (eluent: CH_2Cl_2–MeOH 93:7) to give the (*S*)-5-acetoxymethyl-3-[2-(dimethylphosphono)-ethenyl]-1-(2-nitro-benzoyl)-2-pyrroline **13** (380 mg, 87%). $[\alpha]_D$=−142 (c=0.88). MS: 424 ($M^{+\cdot}$), 351, 347, 317, 230, 214 (100%), 150, 104. IR: 3003, 2950, 1748, 1669, 1661, 1529, 1416, 1350. ^{1}H NMR (200 MHz): 8.24, 7.49 (2d, 2H, J∼8 Hz, 3′-H, 6′-H), 7.80, 7.68 (2dd, 2H, J=7 Hz, H-4′, H-5′), 7.07 (dd, 1H, $J_{P,H}$=21 Hz, $J_{7,8}$=17 Hz, 7-H), 6.21 (s, 1H, 2-H), 5.43 (dd, 1H, $J_{P,H}$=$J_{7,8}$=17 Hz, 8-H), 5.01 (m, 1H, 5-H), 4.63 (dd, 1H, $J_{6a,6b}$=11 Hz, $J_{6a,5}$=5 Hz, 6-Ha), 4.34 (dd, 1H, $J_{6a,6b}$=11 Hz, $J_{6b,5}$=4 Hz, 6-Hb), 3.69 (d, 6H, 2×P(O)OCH_3), 3.08 (dd, 1H, $J_{4a,4b}$=16 Hz, $J_{4a,5}$=11 Hz, 4-Ha), 2.66 (dd, 1H, $J_{4a,4b}$=16 Hz, $J_{4b,5}$=4 Hz, 4-Hb), 2.09 (s, 3H, $COCH_3$). ^{13}C NMR (62.5 MHz), 170.8 ($COCH_3$), 163.9 (CON), 145.3 (C-2′), 141.9 (C-7), 134.7 (C-4′), 133.2 (C-2), 131.1 (C-5′), 130.9 (C-1′), 128.6 (C-6′), 124.9 (C-3′), 123.1, 122.7 (C-3), 113.2, 110.2 (C-8), 63.5 (C-6), 57.0 (C-5), 52.3 (OCH_3), 31.0 (C-4), 20.7 ($COCH_3$).

4.1.8. (*S*)-3-[2-(Dimethylphosphono)-ethenyl]-5-hydroxymethyl-1-(2-nitro-benzoyl)-2-pyrroline 14. A saturated aqueous solution of $Ba(OH)_2$ (2.6 mL) was added under inert atmosphere to a solution of the acetate **13** (170 mg, 0.40 mmol) in dioxan (5.8 mL). The mixture was stirred at room temperature until complete reaction and neutralized by addition of CO_2. After filtration, the product was extracted with dichloromethane to give the primary alcohol **14** (131.4 mg, 86%) after usual workup. $[\alpha]_D$=−114 (c=0.84). MS: 382 (weak, $M^{+\cdot}$), 317 (100%), 285, 261, 202, 170, 150. HRMS Calcd for $C_{16}H_{19}N_2O_7P$: 382.0930; Found: 382.0943. IR: 3402, 1649, 1616, 1529, 1416, 1343. ^{1}H NMR (200 MHz): 8.26, 7.49 (2d, 2H, J=8 Hz, 3′-H, 6′-H), 7.80, 7.72 (2dd, 2H, 4′-H, 5′-H), 7.03 (dd, 1H, $J_{P,H}$=21 Hz, $J_{7,8}$=17 Hz, 7-H), 6.20 (s, 1H, 2-H), 5.44 (dd, 1H, $J_{P,H}$=$J_{7,8}$=17 Hz, 8-H), 4.84 (m, 1H, 5-H), 4.03 (bd, 1H, $J_{6a,6b}$=12, 6-Ha), 3.93 (dd, 1H, $J_{6a,6b}$=12 Hz, $J_{6b,5}$=5 Hz, 6-Hb), 3.68 (d, 6H, 2×P(O)OCH_3), 3.07 (dd, 1H, $J_{4a,4b}$=16 Hz, $J_{4a,5}$=11 Hz, 4-Ha), 2.65 (dd, 1H, $J_{4a,4b=}$16 Hz, $J_{4b,5}$=4 Hz, 4-Hb). ^{13}C NMR (50 MHz), 164.9 (CO), 145.4 (C-2′), 142.1 (C-7), 134.8 (C-4′), 133.2 (C-2), 131.3 (C-1′), 131.0 (C-5′), 128.7 (C-6′), 125.0 (C-3′), 124.0, 123.5 (C-3), 113.8, 110.0 (C-8), 63.8 (C-6), 61.4 (C-5), 52.4 (OCH_3), 31.2 (C-4).

4.1.9. (*S*)-3-[2-(Dimethylphosphono)-ethenyl]-5-formyl-1-(2-nitro-benzoyl)-2-pyrroline 15. To dichloromethane (2.5 mL) cooled to −30°C under argon was added dropwise oxalyl chloride (0.11 mL, 1.26 mmol) and a solution of DMSO (0.18 mL, 2.54 mmol) in CH_2Cl_2 (2.5mL). After being stirred at −30°C for 0.3 h, a solution of primary alcohol **14** (251 mg, 0.657 mmol) in CH_2Cl_2 (5.0 mL) was added dropwise. The mixture was stirred at −30°C for 1.5 h, then iPr$_2$NEt (0.63 mL) was added and the mixture was stirred for 10 min and at 0°C for 20 min before the addition of citrate–phosphate buffer (pH 5.6), followed by extraction three times with EtOAc. Each organic phase was washed three times with small amounts of H_2O. Usual treatments afforded the crude aldehyde **15** (247.1 mg, 99%), pure

enough for the next step. A purification by preparative TLC (eluent: EtOAc–MeOH 9:1) give **15** (80% yield) containing some methylacetal form. MS: 380 ($M^{+\cdot}$), 351, 317, 285, 230, 202, 170, 150 (100%), 94. HRMS: Calcd for $C_{16}H_{17}N_2O_7P$ ($M^{+\cdot}$): 380.0773; Found: 380.0794. IR: 1735, 1655, 1616, 1529, 1416, 1357. ^{1}H NMR (250 MHz): 9.87 (s, CHO), 8.27, 8.25 (2dd, 1H, J=8 Hz, J'=1 Hz, Ar–H), 7.83, 7.80, 7.73, 7.70 (2 double dd, 4′-H, 5′-H), 7.57, 7.52 (1H, Ar–H), 7.05 (2dd, 1H, $J_{P,H}$=21 Hz, $J_{7,8}$=17 Hz, 7-H), 6.35 (bs, 2-H), 6.23 (bs, 2-H, acetal), 5.46 (2dd, 1H, $J_{P,H}=J_{7,8}$=17 Hz, 8-H), 5.23 (dd, J=11 Hz, J'=5 Hz, 5-H), 4.86 (m, H-5, acetal), 3.70 (d, 6H, 2×P(O)OCH$_3$), 3.50 (2s, OCH$_3$, acetal), 3.2–2.6 (4-H$_2$).

4.1.10. (11*R*,11a*S*)-2-[2-(Dimethylphosphono)-ethenyl]-1,10,11,11a-tetrahydro-11-methoxy-5*H*-pyrrolo[2,1-*c*]-[1,4]benzodiazepine-5-one 6. A solution of aldehyde **15** (76.2 mg, 0.20 mmol) in EtOH–MeOH 85:15 (3.8 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 the reaction (ca. 8 min), the mixture was filtered through silica gel (70–230 mesh) and silica gel was washed with EtOAc–MeOH 8:2. The solvents were evaporated under reduced pressure to give crude PBD (66.5 mg) which was dissolved in a mixture CH_2Cl_2 (devoid of stabilizing EtOH)– MeOH 85:15. To this stirred solution was added a solution of CF_3CO_2H in CH_2Cl_2 (0.015%, 0.9 mL) under argon. The mixture was stirred for 16 h at room temperature. The solvents were evaporated under reduced pressure and the residue was purified by preparative TLC (eluent: CH_2Cl_2–MeOH 96:4) to give **6** (26.0 mg, 36%). $[\alpha]_D$=+404 (c=0.55). MS: 332 [(M−CH_3OH), 100%], 330, 235, 220. HRMS: Calcd for $C_{16}H_{17}N_2O_4P$: 332.0926; Found: 332.0910. ^{1}H NMR (300 MHz): 8.01 (d, 1H, 6-H), 7.52 (bs, 1H, 3-H), 7.35 (1H, masked, 12-H), 7.30 (masked, 8-H), 6.87 (dd, 1H, 7-H), 6.63 (d, 1H, 9-H), 5.45 (d, 1H, $J_{10,11}$=6 Hz, N_{10}-H, exch. with D_2O), 5.34 (dd, 1H, 13-H), 4.59 [d, 1H, $J_{10,11}$=6 Hz, (singlet after exch. with D_2O)], 11-H), 4.30 (dd, 1H, J=11 Hz, J'=5.2 Hz, 11a-H), 3.77, 3.74 (d, 6H, 2×P(O)OCH$_3$), 3.35 (OCH$_3$), 3.14, 2.81 (2m, 2H, 1-H$_2$).

4.2. Computational procedure

Ten thousand conformations of each compound, i.e. the 9-demethoxyporothramycin **3** and its phosphonate analogue **6**, were generated by random search Monte-Carlo method[20] and optimized by TNGG Truncated Newton molecular mechanics minimization method[21] using the Macromodel (version 5.5) program[22] with the MM2 force field. The search was carried out on blocks of 1000 Monte-Carlo steps until no additional conformation was found to be of lower energy than the current minimum. Duplicated conformations as well as those that had chirality changes were discarded. From these conformational searches, all the possible conformations within 3 kcal/mol from the global minimum were analyzed.

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