Synthesis and Chemical Properties of PCA, an Unusual Amino Acid in Luzopeptins

Marco A. Ciufolini* and Ning Xi

Department of Chemistry, Rice University, PO Box 1892, Houston, Texas 77251 USA

We describe a practical five-step synthesis of the unusual hydrazonoacid found in luzopeptins, together with observations concerning its chemical behaviour.

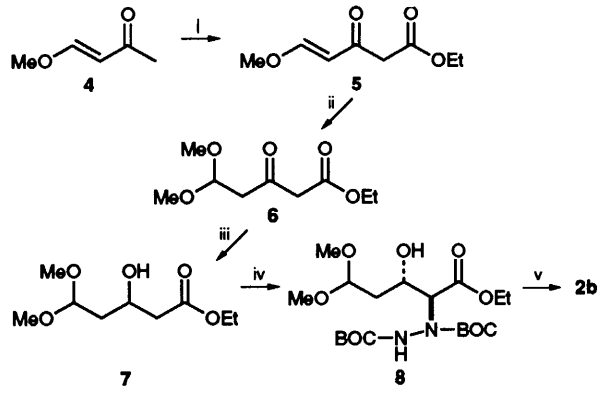
Luzopeptins **1a–c**[1] are dimeric antitumour[2] cyclodecadepsipeptide antibiotics isolated from *Actinomadura luzonensis*. Luzopeptin C also exhibits pronounced inhibitory action towards reverse transcriptase,[3] a key retroviral enzyme required solely for viral proliferation and a prime target for AIDS treatment. More significantly, non-cytotoxic doses of **1c** effectively arrest replication of HIV in infected T cells *in vitro*.[3] Important questions exist concerning the precise mechanism of action of **1c**, but because luzopeptins are very rare natural products, synthetic material will be necessary to address such issues.

A synthesis of **1** is intimately dependent on the availability of a practical method for the preparation of the unusual aminoacid **2**, which we term PCA (pyridazine carboxylic acid). A ten-step synthesis of optically active **2a** has been reported,[4] and indeed, similar piperazic acids, **3**, have attracted much synthetic interest in recent times.[5] Herein, we describe a five-step preparation of (±)-**2b** through a procedure amenable to modification to furnish scalemic end product. Moreover, we report on the chemical reactivity of PCA and on the implications of our findings on a total synthesis of luzopeptins themselves.

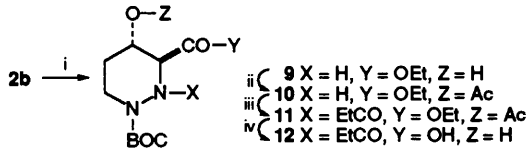
Condensation (Bu^tOK)[6] of 4-methoxybut-3-en-2-one **4** with the Mander reagent furnished cleanly ketoester **5** (Scheme 1). Conjugate addition of MeOH[7] and $NaBH_4$ reduction gave **7**. The dianion[8] of **7** reacted with *tert*-butyl azodicarboxylate[9] to furnish an 18 : 1 mixture (500 MHz ^1H NMR) of *anti* (major, **8**) and *syn* (minor) diastereoisomers of the adduct. Exposure of **8** to trifluoroacetic acid in CH_2Cl_2 induced rapid and quantitative conversion to PCA ethyl ester **2b**. The latter substance is delicate and very difficult to purify, but, fortunately, it emerges in a state of high purity (^{13}C, ^1H NMR) if analytically pure, crystalline **8**, mp 90–91 °C, is used in the cyclization step.† We note that because enantioselective reduction of ketoesters of the type **6** may be readily accomplished,[10] it should be possible to achieve an enantioselective synthesis of **2** by the present procedure.

No literature record exists regarding the chemical properties of free PCA, despite the obvious relevance of such knowledge to a possible synthesis of the luzopeptins. We observed that compound **2b** exhibits a disconcerting fragility under diverse hydrolytic or acylating conditions.‡ This raises serious doubts about the possibility of incorporating PCA directly into a peptide chain.[11] A modestly successful protocol to circumvent the instability of PCA was developed as followed. Reduction of **2b** with $NaBH_3CN$ in aqueous acetic acid furnished a presumed hydrazine, which was intercepted *in situ* with BOC_2O to selectively yield monocarbamate **9** (Scheme 2). The OH group of this molecule reacts selectively over the NH group with acylating agents. For instance, acetylation cleanly furnished **10**. Reaction of **10** with acid chlorides, *e.g.*, propionyl chloride, proceeded normally to give **11**. Thus, the three nucleophilic sites of the intermediate hydrazine may be readily differentiated.[12] Furthermore, the ester group in **11** underwent hydrolysis to acid **12** with aq. LiOH without incident.

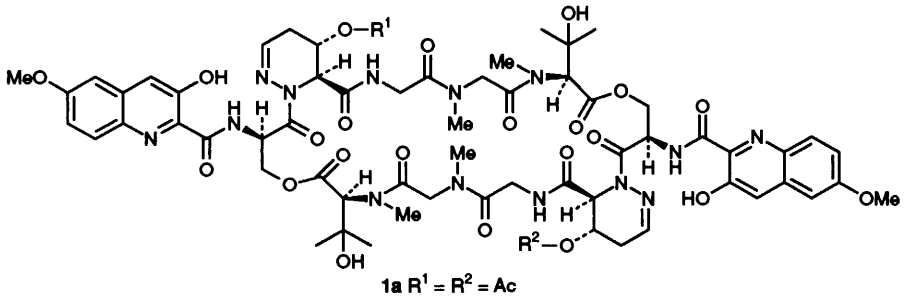
Methods for incorporation of piperazic acids into peptide chains and for reoxidation of monoacyl derivative of **12** back to hydrazones are known,[13] so that a total synthesis of

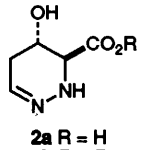


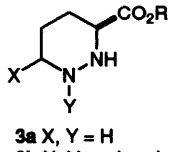
Scheme 1 *Reagents and conditions*: i, Bu^tOK, EtO_2CCN, THF, −78 °C 72% (90%); ii, Triton B, MeOH, room temp., 88%; iii, $NaBH_4$, EtOH, −78 °C, 90%; iv, 4 equiv. LDA, THF, −78 °C, then $(Bu^tO_2C–N=)_2$, 55% (78%), de = 89%; v, 30% TFA in CH_2Cl_2, 15 min, room temp., 95–100%. Yields refer to chromatographed products, except for **2b** (see text). Yields in parentheses are based on recovered starting material.



Scheme 2 *Reagents and conditions*: i, $NaBH_3CN$, H_2O, AcOH, then 2 equiv. BOC_2O, K_2CO_3, 10–20% ii, Ac_2O, py, room temp., 1 h, 45%; iii, EtCOCl, CH_2Cl_2, *N*-methylmorpholine, 0 °C → room temp., 76%; iv, LiOH, MeOH–H_2O (1 : 1), 80%







luzopeptins using the present approach may be possible. At the moment, however, a much better strategy towards **1** appears to be one involving cyclization of a suitable acyclic PCA precursor within a preformed peptide chain. Further ramifications of these ideas will be described in due course.

We are grateful to the National Institutes of Health (CA-55268), the National Science Foundation (CHE 91-16820), and the Robert A. Welch Foundation (C-1007) for support of our research program. M. A. C. is an Alfred P. Sloan Foundation Fellow, 1994–1996.

Received, 25th April 1994; Com. 4/02426E

Footnotes

† All compounds described herein were characterized by a combination of ^1H and ^{13}C NMR (including DEPT and HETCOR for **2b**) MS, IR, elemental analysis (**8**). Most compounds were purified by silica gel column chromatography. Purity was assessed by HPLC, TLC, and by the appearance of NMR spectra.

‡ aq. LiOH; N-BOC–serine, DCC or *N*-methyl-2-chloropyridinium iodide; *N*-acetylimidazole, PPTS; 4-nitrophenyl octanoate, HOBt; Ac_2O–py; MeO_2CCl, aq. $NaHCO_3$; AcCl or octanoyl chloride, CH_2Cl_2, *N*-methylmorpholine.

References

1 M. Konishi, H. Ohkuma, F. Sakai, T. Tsuno, H. Koshiyama, T. Naito and H. Kawaguchi, *J. Am. Chem. Soc.*, 1981, **103**, 1241; E. Arnold and J. Clardy, *J. Am. Chem. Soc.*, 1981, **103**, 1243.

2 M. Konishi, H. Ohkuma, F. Sakai, T. Tsuno, H. Koshiyama, T. Naito and H. Kawaguchi, *J. Antibiot.*, 1981, **34**, 148.

3 Y. Inouye, Y. Take, K. Oogose, A. Kubo and S. Nakamura, *J. Antibiot.*, 1987, **40**, 105.

4 P. Hughes and J. Clardy, *J. Org. Chem.*, 1989, **54**, 3260.

5 *Cf.* I. H. Aspinall, P. M. Cowley, G. Mitchell and R. J. Stoodley, *J. Chem. Soc., Chem. Commun.*, 1993, 1179; Y. Nakamura and C.-G. Shin, *Chem. Lett.*, 1991, 1953; U. Schmidt and B. Reidl, *J. Chem. Soc., Chem. Commun.*, 1992, 1186; K. J. Hale, V. M. Delisser and S. Manaviazar, *Tetrahedron Lett.*, 1992, **33**, 7613.

6 In the present case, Bu^{t}OK (B. Ganem and T. A. Morgan, *Tetrahedron Lett.*, 1980, **21**, 2773) seemed more satisfactory than LDA or bis(trimethylsilyl)amide bases (*cf.* M. Koreeda and H. Akagi, *Tetrahedron Lett.*, 1980, **21**, 1197).

7 *Cf.* L. Dasaradhi, N. W. Fadnavis and U. T. Bhalerao, *J. Chem. Soc., Chem. Commun.*, 1990, 729.

8 G. Guanti, L. Banfi and E. Narisano, *Tetrahedron*, 1988, **44**, 5553.

9 C. Gennari, L. Colombo and G. Bertolini, *J. Am. Chem. Soc.*, 1986, **108**, 6394; D. A. Evans, T. C. Britton, R. L. Dorow and J. F. Dellaria, *J. Am. Chem. Soc.*, 1986, **108**, 6395; L. A. Trimble and J. C. Vederas, *J. Am. Chem. Soc.*, 1986, **108**, 6397.

10 *Cf., e.g.* M. Kitamura, M. Tokunaga, T. Ohkuma and R. Noyori, *Org. Synth.*, 1992, **71**, 1 (asymmetric hydrogenation); D. Seebach, M. A. Sutter, R. H. Weber and M. F. Züger, *Org. Synth.* 1984, **63**, 1 (biocatalytic reduction).

11 Analogous problems with similar hydrazonoacids have been observed by U. Schmidt and B. Riedl, *Synthesis*, 1993, 809.

12 As expected (*cf.* M. A. Ciufolini, C. W. Hermann, K. H. Whitmire and N. E. Byrne, *J. Am. Chem. Soc.*, 1989, **111**, 3473 and refs. cited therein) introduction of the second *N*-acyl group forced the molecule into a chair conformation in which the CO_2Et group occupies the axial position, while other PCA intermediates containing a free NH group, including those with an imino linkage, favour a chair conformation with an equatorial CO_2Et.

13 M. G. Bock, R. M. DiPardo, P. D. Williams, R. D. Tung, J. M.Erb, N. P. Gould, W. L. Whitter, D. S. Perlow, G. F. Lundell, *et al., Colloq. INSERM*, 1991, **208** (Vasopressin), 349: *Chem. Abstr.*, 1991, **115**, 115072.