Studies toward Luzopeptins: Assembly of the Elusive Serine−PCA Dipeptide

Marco A. Ciufolini and Ning Xi

Department of Chemistry, MS 60, Rice University, 6100 Main Street, Houston, Texas 77005-1892

Received February 4, 1997

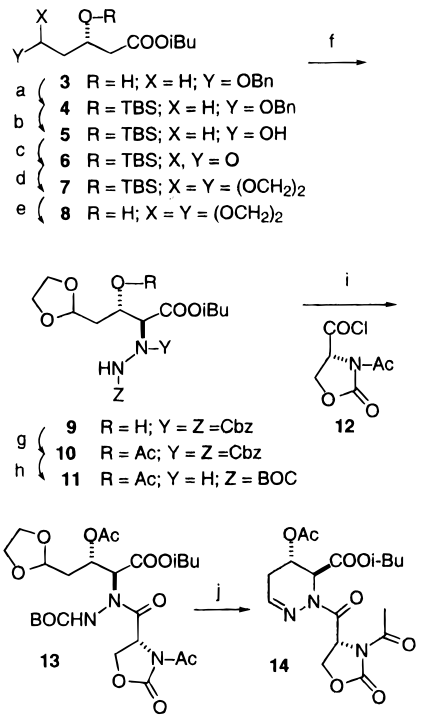
Luzopeptins A−C (BBM928, **1a**−**c**, Chart 1) are metabolites of *Actinomadura luzonensis*.[1] Interest in these substances, originally engendered by their extreme potency against tumors,[2] has been magnified by the discovery of their inhibitory action against reverse transcriptase (RT),[3] a crucial enzyme for retroviral replication, and consequently a target for intervention against HIV. It is noteworthy that while antitumor effectiveness decreases in the order A, B, C, antiretroviral activity follows the opposite trend, and it is most pronounced in the weakly cytotoxic C series at noncytopathic doses.[4]

No synthetic studies toward luzopeptins have been reported, though the preparation of individual components of **1** and of systems related to it have been investigated.[5] However, none of these model systems contained the hallmark tetrahydro-4-hydroxypyridazine-3-carboxylic acid **2** ("PCA").[5b] Herein, we describe the synthesis of a D-serine−PCA dipeptide,[6] a goal that has heretofore proved elusive[7] due to the instability and poor reactivity of **2**.[5b]

The known scalemic ester **3**[8] was advanced to compound **8** in a straightforward manner, as shown in Scheme 1.[9] Gennari−Evans−Vederas[10] reaction of **8** with dibenzyl azodicarboxylate furnished an 18:1 mixture of *anti* (**9**, major, desired) to *syn* diastereomers. Sequential O-acetylation and catalytic debenzylation of the emerging **10** in the presence of BOC_2O yielded mono-BOC hydrazine **11**.[11] No hydrogenolytic damage of the presumed free hydrazine intermediate, a fragile substance, was incurred during this step. The delicate mono-BOC hydrazine **11** condensed with the reactive, yet well-behaved, protected D-serinyl chloride **12**[12] in the presence of *sym*-collidine to afford **13**.[13] This material cyclized cleanly and in high yield to blocked serine−PCA dipeptide **14** under aqueous acidic conditions.

Deacetylation of **14** could be effected cleanly and quantitatively with hydrazine hydrate in acetonitrile.[14] Subsequent attempts to convert **15** to **20** by Kunieda cleavage[15] of the oxazolone were complicated by the extreme sensitivity of PCA esters related to **15** to

Scheme 1[a]



[a] (a) TBSCl, imidazole, DMF, rt, 16 h, 90%; (b) H_2, 10% Pd/C, cyclohexane, rt, 95%; (c) DMSO, $(COCl)_2$, −78 °C; Et_3N, CH_2Cl_2, 92%; (d) ethylene glycol, cat. PPTS, benzene, reflux, 3 h, 88%; (e) TBAF, THF, rt, 4 h, 85%; (f) Cbz-N=N-Cbz, LDA, THF, −78 °C, 61%; (g) Ac_2O, pyridine, rt, 16 h, 95%; (h) BOC_2O, H_2, 10% Pd/C, rt, 8 h, 97%; (i) *sym*-collidine, CH_2Cl_2, 0 °C, 0.5 h, 60%; (j) 9:1 TFA/H_2O, 0.5 h, 97%.

(1) (a) Ohkuma, H.; Sakai, F.; Nishiyama, Y.; Ohbayashi, M.; Imanishi, H.; Konishi, M.; Miyaki, T.; Koshiyama, H.; Kawaguchi, H. *J. Antibiot.* **1980**, *33*, 1087. (b) Tomita, K.; Hoshino, Y.; Sasahira, T.; Kawaguchi, H. *J. Antibiot.* **1980**, *33*, 1098. (c) Arnold, E.; Clardy, J. *J. Am. Chem. Soc.* **1981**, *103*, 1243.

(2) The most active member of the family, luzopeptin A, is reported to be over 100 times more potent than mitomycin C against P388 leukemia (ref 1a,b).

(3) (a) Inouye, Y.; Take, Y.; Nakamura, S. *J. Antibiot.* **1987**, *40*, 100. (b) Take, Y.; Inouye, Y.; Nakamura, S.; Allaudeen, H. S.; Kubo, A. *J. Antibiot.* **1989**, *44*, 107.

(4) Luzopeptin C completely suppressed replication of HIV-1 in infected MT-4 cells at around 2.5−5.0 μg/mL without significant cytopathic effects (ref 3).

(5) First synthesis of PCA: (a) Hughes, P.; Clardy. J. *J. Org. Chem.* **1989**, *54*, 3260. Synthesis and chemistry of PCA: (b) Ciufolini, M. A.; Xi, N. *J. Chem. Soc., Chem. Commun.* **1994**, 1867 and references cited therein. See also: Greck, C.; Bischoff, L.; Genet, J. P. *Tetrahedron: Asymmetry* **1995**, *8*, 1989. Recent work on piperazic acids: (c) Schmidt, U.; Braun, C.; Sutoris, H. *Synthesis* **1996**, 223. Synthesis of 3-hydoxyvalines: (d) Ciufolini, M. A.; Swaminathan, S. *Tetrahedron Lett.* **1989** *30*, 2037. (e) Shao, H; Goodman, M. *J. Org. Chem.* **1996**, *61*, 2582. Synthesis of the quinaldic acid unit: (f) Boger, D. L.; Chen J. H. *J. Org. Chem.* **1995**, *60*, 7369. Total synthesis of sandramycin, a congener of **1** lacking the PCA unit: (g) Boger, D. L.; Chen, J.-H. *J. Am. Chem. Soc.* **1993**, *115*, 11624. (h) Boger, D. L.; Chen, J.-H.; Saionz K. W. *J. Am. Chem. Soc.* **1996**, *118*, 1629. (i) Unnatural analogues of **1**: Olsen, R. K.; Apparao, S.; Bhat, K. L. *J. Org. Chem.* **1986**, *51*, 3079.

(6) Portions of this work dealing with the preparation of hydrazine **8** by the route shown here and the use of our serinyl chlorides (**12**) for the creation of PCA−serine dipeptides related to **14** were presented at the 210th National Meeting of the American Chemical Society, Chicago, IL, Aug 1995.

(7) Cf. (a) Rebert, N. W. Dissertation, Utah State University, Logan, UT, 1987. (b) Schmidt, U.; Riedl, B. *Synthesis* **1993**, 809. (c) Schmidt, U.; Riedl, B. *Synthesis* **1993**, 815.

(8) Brooks, D.; Kellogg, R. P.; Cooper, C. S. *J. Org. Chem.* **1987**, *52*, 192.

(9) A noteworthy aspect of this sequence is that hydrogenolytic debenzylation of **4** to **5** was best conducted in nonpolar cyclohexane. Polar solvents such as EtOAc favored cyclization of **5** to the lactone. Furthermore, we found that minimization of the number of steps after introduction of the sensitive hydrazine unit (cf. **8** → **9**) was crucial for maximum efficiency. Therefore, it was best to advance **3** to **8** prior to the Gennari−Evans−Vederas reaction.

(10) (a) Gennari, C.; Colombo, L.; Bertolini, G. *J. Am. Chem. Soc.* **1986**, *108*, 6394. (b) Evans, D. A.; Britton, T. C.; Dorow, R. L.; Dellaria, J. F. *J. Am. Chem. Soc.* **1986**, *108*, 6395. (c) Trimble, L. A.; Vederas, J. C. *J. Am. Chem. Soc.* **1986**, *108*, 6397. See also: (d) Guanti, G.; Banfi, L.; Narisano, E. *Tetrahedron* **1988**, *44*, 5553.

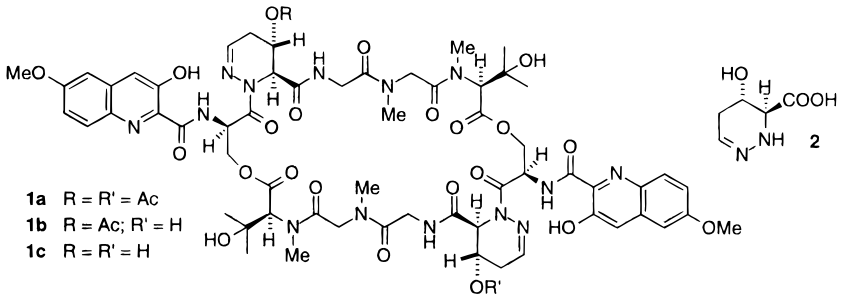
(11) The same transformation may also be achieved by BOC-derivatization of the terminal nitrogen in **10** (BOC_2O, 4-DMAP, Et_3N, CH_2Cl_2, rt, 96%) followed by hydrogenolysis (99%). The protocol described in the text removes the need for a separate *N*-BOC formation.

(12) Xi, N.; Ciufolini, M. A. *Tetrahedron Lett.* **1995**, *36*, 6595.

(13) Other coupling methods based on DCC, DCC−HOBt, BOP-Cl, mixed anhydrides, active esters, etc., failed completely to deliver a serinyl derivative of **11**. This may be attributed not only to the innate lack of nucleophilicity of the N-2-atom (ref 5b), exacerbated in the present case by the presence of an electron-withdrawing acyl group on N-1, or to steric congestion in its surroundings, but also to the ease of β-elimination of the OH functionality (or a protected variant thereof) in serine derivatives wherein strong activation has been provided to the COOH terminus.

S0022-3263(97)00195-3 CCC: $14.00

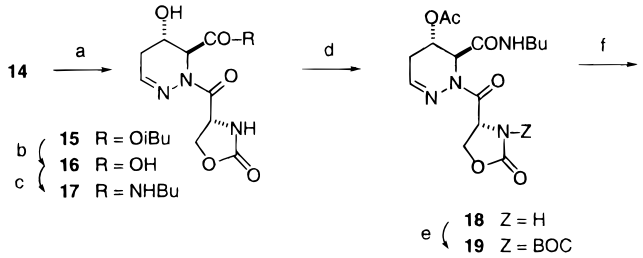
Chart 1

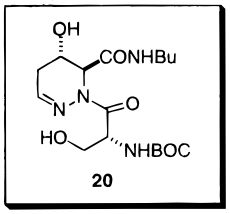


β-elimination of the oxygen functionality, regardless of the type of protection provided to the OH and of the basic agent employed.[16] This problem was corrected by creation of the considerably less α-C−H-acidic secondary amide **17**.[17] To that end, ester **15** was carefully hydrolyzed to acid **16**, which in turn delivered **17** upon reaction with $BuNH_2$ and *O*-benzotriazol-1-yl-*N*,*N*,*N*′,*N*′-tetramethylisouronium hexafluorophosphate ("HBTU") in DMF. Interestingly, hydrolysis of **15** proceeded well with NaOH, but not with the milder LiOH (much β-elimination).[18] O-Acetylation of **17** served to ensure high regioselectivity in favor of the oxazolone nitrogen upon reaction of **18** with BOC_2O.[19] Exposure of the resultant **19** to methanolic Cs_2CO_3 prompted clean, rapid decarboxylative opening of the oxazolone ring to give **20** (Scheme 2).

In light of the above observations, it is likely that a suitable glycinyl derivative of **16** should be amenable to conversion into a serine−PCA−glycine tripeptide suitable for incorporation into advanced luzopeptin intermediates. This suggests a plausible strategy for the construction of the challenging framework of **1a**−**c**. Additional developments in this area will be reported in due course.

Scheme 2[a]





[a] (a) NH_2NH_2, CH_3CN, rt, 80%; (b) NaOH, THF/H_2O, rt, 90%; (c) $BuNH_2$, HBTU, DMF, rt, 70%; (d) Ac_2O, pyridine, rt, 3h, 95%; (e) BOC_2O, cat. DMAP, Et_3N, CH_2Cl_2, 0 °C, 15min, 100%; (f) Cs_2CO_3, MeOH, rt, 15min, 90%.

Acknowledgment. We acknowledge the NIH (CA-55268), the NSF (CHE 95-26183), and the Robert A. Welch Foundation (C-1007) for their support of our research program. M.A.C. is a Fellow of the Alfred P. Sloan Foundation, 1994−1998.

Supporting Information Available: Experimental procedure and spectral data for selected compounds (12 pages).

JO9701952

(14) Attempted deacetylation with other nitrogen (morpholine, piperidine, *n*-butylamine, diethylamine), carbon (KCN/MeOH or MeCN), or oxygen (LiOH; NaOH; $MeOH/K_2CO_3$ or Cs_2CO_3) nucleophiles promoted β-elimination of the oxygenated functionality in PCA and subsequent decomposition through aromatization to a pyridazine. Reaction with pyrrolidine (ref 12) resulted not only in deacetylation but also in formal substitution of the OH group by pyrrolidine, probably by Michael addition of the amine into an intermediate α,β-unsaturated ester.

(15) Ishizuka, T.; Kunieda, T. *Tetrahedron Lett.* **1987**, *28*, 4185.

(16) Hydroxyl protecting groups examined included acetate, Et_3Si, and $tBuMe_2Si$; bases included LiOH, NaOH, $MeOH/K_2CO_3$, or Cs_2CO_3.

(17) For an important literature analogy see: Askin, D.; Verhoeven, T. R.; Liu, T. M.-H.; Shinkai, I. *J. Org. Chem.* **1991**, *56*, 4929.

(18) We attribute this to coordination of Li^+ by the β-OH group, an event that would facilitate departure of the oxygen functionality in an elimination reaction.

(19) Reaction of unprotected **17** with BOC_2O resulted in preferential OH derivatization; moreover, variable amounts of the *N*-BOC derivative of the *N*-butylamide were formed. This problem was no longer apparent with acetate **18**.