COMMUNICATION

ChemComm
www.rsc.org/chemcomm

Total synthesis of (+)-belactosin A†

Alan Armstrong* and James N. Scutt
Department of Chemistry, Imperial College London, South Kensington, London, UK SW7 2AZ.
E-mail: A.Armstrong@imperial.ac.uk; Fax: +44 (0) 20 75945804

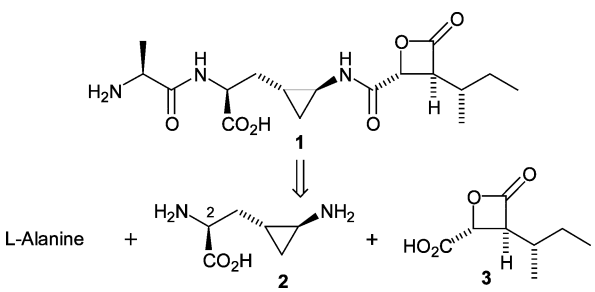
Received (in Cambridge, UK) 11th December 2003, Accepted 14th January 2004
First published as an Advance Article on the web 27th January 2004

A concise first total synthesis of the antitumour antibiotic belactosin A is reported, involving coupling of β-lactone carboxylic acid 3 with *N*-Ala-aminocyclopropyl alanine 11.

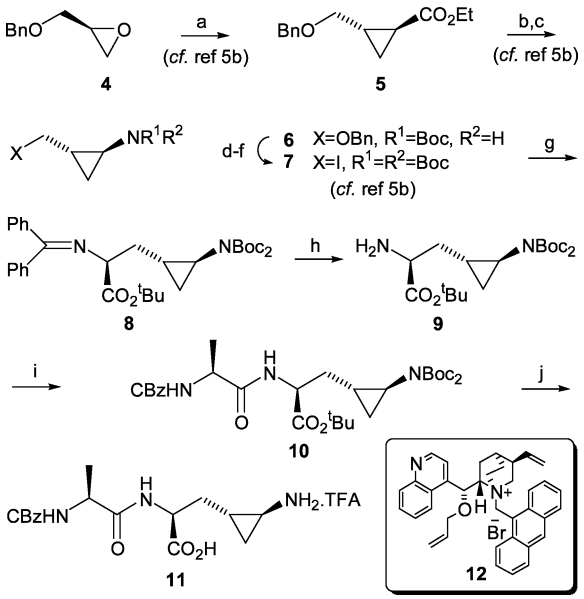
Small molecules which modulate the cell cycle offer promise for the control of proliferative diseases.[1] The recently isolated natural product belactosin A **1** arrests cell-cycle progression at the G2/M phase,[2] and recent reports[3] indicate that this compound and analogues effect 20S proteasome inhibition, an important target for the control of cancer and auto-immune diseases.[4] We have embarked upon a programme aimed at the total synthesis of **1** allowing access to appropriate derivatives for probing its precise role in various cellular processes. We envisaged that coupling of L-alanine and the β-lactone carboxylic acid **3** to the intriguing and unique central (2*S*,1′*R*,2′*S*)-3-(*trans*-2-aminocyclopropyl)alanine core **2**[5] would constitute an effective and flexible synthetic strategy (Scheme 1). Recently, we reported a novel stereocontrolled route to *ent*-**2**[5b] (*vide infra*). In this paper, we report application of this chemistry to protected **2** itself, stereocontrolled synthesis of the β-lactone **3**, and coupling of these fragments to complete the first total synthesis of **1**.

We first prepared 3-(*trans*-2-aminocyclopropyl)alanine **8** in 7 steps from (*R*)-glycidol benzyl ether **4** (Scheme 2). As in our reported synthesis of *ent*-**2**[5b] the key steps included "Wadsworth–Emmons cyclopropanation" to convert the glycidol derivative **4** into the required enantiomerically pure cyclopropane **5**, followed by Curtius rearrangement to generate the cyclopropylamine **6**. Conversion to **7** and alkylation with a glycine enolate derivative in the presence of the cinchonidinium phase-transfer catalyst **12** (*O*(9)-allyl-*N*-(9-anthracenylmethyl)cinchonidinium bromide),[6] then allowed installation of the C2-stereocentre, affording **8** in 67% yield and 93 : 7 diastereomeric ratio. Recrystallisation to diastereomeric purity was followed by selective deprotection of the imine using 15% citric acid, setting the scene for coupling to *N*-CBz-alanine. Under standard DCC/HOBt/CH_2Cl_2 conditions, **10** was obtained in 88% yield, but this was accompanied by partial epimerisation at the alanine stereocentre (89 : 11 d.r.). We attributed this to the limited solubility of HOBt in CH_2Cl_2, and indeed after switching to DMF as the reaction solvent epimerisation was completely suppressed, to yield **10** in 47% yield (> 95 : 5 d.r.), or 100% yield (> 95 : 5 d.r.) when 2 eq of *N*-CBz-Ala were employed. All remaining acid-labile protecting groups were then removed with TFA/CH_2Cl_2 to furnish the amine-TFA salt **11** in high yield.

Encouraged by literature precedent, we hoped that synthesis of β-lactone **3** would be possible *via* stereoselective chlorination of the monosubstituted succinate **18**[7,8] followed by cyclisation. Synthesis of **18** commenced with hydrodeamination of L-isoleucine with a slight modification to the literature procedure (Scheme 3),[9] followed by conversion to the oxazolidinone derivative **16** and a highly diastereoselective alkylation using *tert*-butyl bromoacetate to afford **17** in 82% yield and 93 : 7 d.r.[7] Recrystallisation followed by hydrolysis using LiOH/H_2O_2 then furnished the desired acid **18** in 92% yield and > 95 : 5 d.r. The key chlorination step was achieved by prior conversion to the dianion using LiHMDS followed by treatment with 1 equivalent of carbon tetrachloride.[8] Lactonisation then proceeded as described by Barlaam through exposure to a biphasic ether/$NaHCO_3$ mixture,[8] required in order to remove chloride from the ether phase and prevent S_N2 ring opening at C2.[10] This two-step sequence was further optimised to a one-pot protocol providing β-lactone **19** in 55% yield and > 95 : 5 d.r. from **18**. The exceptional diastereoselectivity presumably arises *via* a cyclic transition state presenting the less-hindered face of the enolate to the chlorinating agent. ^{1}H NMR and crystallographic analysis of *tert*-butyl ester **19** confirmed the relative stereochemistry to be in agreement with that found for the natural product (Fig. 1). Lactone **19** was then converted to its acid **3** with anhydrous TFA/CH_2Cl_2 in high yield, maintaining the integrity of the β-lactone ring.



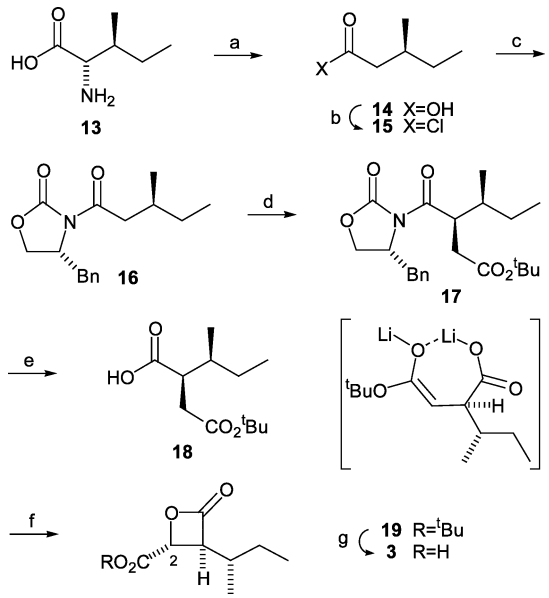
Scheme 1 Retrosynthetic analysis of belactosin A.



Scheme 2 (a) Triethyl phosphonoacetate, NaH, toluene, 110 °C, 14 h, 63%; (b) NaOH (aq), EtOH, 96%; (c) DPPA, ^{t}BuOH, NEt_3, reflux, 53%; (d) Boc_2O, MeCN, DMAP, 95%; (e) Pd/C, H_2, cat. AcOH, THF, 98%; (f) Bu_4NI, DDQ, PPh_3, $CHCl_3$, rt; (g) 2 eq *N*-(diphenylmethylene)glycine *tert*-butyl ester, 20 mol% **12**, 10 eq $CsOH·H_2O$ (s), toluene/CH_2Cl_2 (1 : 1), −40 °C, 40 h; 67%; (h) 15% citric acid/THF, 84%; (i) 2 eq *N*-CBz-Ala, DCC/HOBt/DMF, 100%; (j) TFA/CH_2Cl_2, 15 °C, 20 h, 90%.

† Electronic Supplementary Information (ESI) available: Data/procedures for **1, 3, 8–11, 19, 20**; spectra for **1, 19**; X-ray data for **19** (CCDC 226865; cif format). See http://www.rsc.org/suppdata/cc/b3/b316142k/

DOI: 10.1039/b316142k



Scheme 3 (a) H_2NOSO_3H/KOH (aq), 0 °C to rt, 74%; (b) $(COCl)_2$, CH_2Cl_2, 0 °C to rt, 80%; (c) (4*R*)-benzyl-2-oxazolidinone, ^nBuLi −78 °C, 79%; (d) *tert*-butyl bromoacetate, NaHMDS, −78 °C, 82%; (e) LiOH (aq), H_2O_2, 0 °C to rt, 92%; (f) 2 eq LiHMDS, CCl_4, −78 °C to rt; then ether/$NaHCO_3$, 55%; (g) TFA/CH_2Cl_2, 0 °C, 20 h, 90%.

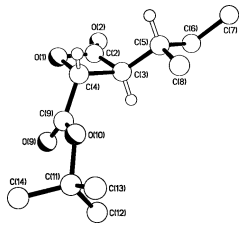
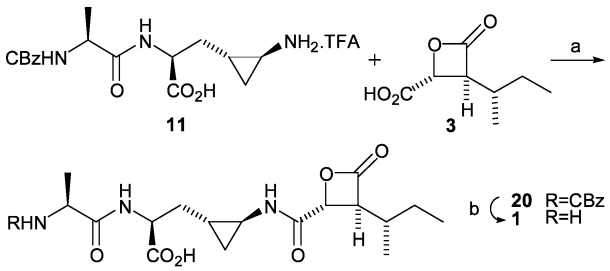


Fig. 1 X-Ray structure of β-lactone **19**.

With both fragments **11** and **3** in hand we were in a position to attempt the crucial coupling, with the ambitious aim of avoiding additional protection steps by selective activation of the β-lactone carboxylic acid **3** for direct coupling to the amino acid **11**. However, preliminary experiments in which **3** was mixed with 1 eq DCC/2 eq HOBt/DMF prior to addition to a solution of **11** in EtN^iPr_2/DMF gave only 25% of the coupling product. We suspected that conversion of **3** to its active ester was incomplete, but in this case use of excess DCC was not possible since any unreacted activating agent was likely to consume amino acid **11**. This problem was solved by exploiting biphasic conditions in which **3** was converted to the active ester through brief exposure to 2 eq EDCI/4 eq HOBt in CH_2Cl_2/H_2O at 0 °C (Scheme 4),[11] followed by transfer of the organic phase to a cooled **11**/EtN^iPr_2/DMF mixture. Under these conditions any unreacted EDCI/HOBt remains in the aqueous phase, thus allowing the use of excess activating agent without incompatibility with the coupling partner **11**. Amide **20** was obtained in 50% yield as a single diastereoisomer after purification.



Scheme 4 (a) EDCI/HOBt in CH_2Cl_2/H_2O, 0 °C; then **11**/EtN^iPr_2/DMF, 0 °C, 50%; (b) Pd/C, H_2, THF/HCO_2H (3 : 2), 96%.

After some experimentation, final deprotection of **20** was achieved by hydrogenation in the presence of Pd/C in THF under TFA activation, yielding the TFA salt of belactosin A. Although this material could be taken to its isoelectric pH (as determined by ^1H NMR spectroscopy using 5% $NaHCO_3/D_2O$), subsequent purification to remove sodium trifluoroacetate proved troublesome. Further consideration suggested that the free amino acid could be generated directly if we used a volatile acid catalyst with a higher pK_a than that of the carboxylate group in **20**. Pleasingly, when the reaction was carried out using H_2 and Pd/C in a 3 : 2 THF/HCO_2H solvent mixture the desired amino acid **1** was produced in 96% yield. The synthetic sample (m.p. 186–187 °C, $[\alpha]_D^{21}$+4.8 (*c* 0.84, H_2O) (lit.[2a] m.p 184–185 °C, $[\alpha]_D^{27}$+4.8 (*c* 0.37, H_2O)) displayed satisfactory HRMS data, and its TLC R_f value (0.5, butanol : acetic acid : water (71 : 14 : 15 v/v/v)), ^1H and ^{13}C NMR spectra were identical to those reported for the natural product.[2a]

In conclusion, we have completed the first total synthesis of belactosin A **1**. The synthetic strategy, particularly the knowledge gained from the synthesis of the central 3-(*trans*-2-aminocyclopropyl)alanine core **8** and the conditions for coupling **11** to the sensitive β-lactone unit **3**, will now facilitate the preparation of a wide range of synthetic probes of some significant biological pathways. Studies along these lines are currently underway.

We thank the EPSRC (studentship to JNS) for support of this work. We are grateful to Pfizer, Merck Sharp and Dohme, and Bristol-Myers Squibb for unrestricted support of our research programmes. We thank Dr. A. Asai of Kyowa Hakko Kogyo Co. Ltd. for supplying the ^1H NMR spectrum of **1** and Dr. A. J. P. White for X-ray structure determination.

Notes and references

1 C. M. Crews and R. Mohan, *Curr. Opin. Chem. Biol.*, 2000, **4**, 47.
2 (*a*) T. Mizukami, A. Asai, Y. Yamashita, R. Katahira, A. Hasegawa, K. Ochiai and S. Akinaga, *Eur. Patent* 768317, 1997 (*Chem. Abstr.*, 1997, **126**, 338840) (*b*) A. Asai, A. Hasegawa, K. Ochiai, Y. Yamashita and T. Mizukami, *J. Antibiot.*, 2000, **53**, 81; (*c*) K. Yasuki, I. Atsushi, T. Yoshiichi, S. Shiro and A. Shiro, *Jpn. Patent* 2002047202, 2002 (*Chem. Abstr.*, 2002, **136**, 172760).
3 A. Asai, T. Tsujita, S. V. Sharma, Y. Yamashita, S. Akinaga, M. Funakoshi, H. Kobayashi and T. Mizukami, *Biochem. Pharmacol.*, 2004, **67**, 227.
4 C. Garcia-Echeverria, *Mini-Rev. Med. Chem.*, 2002, **2**, 247.
5 For synthetic studies, see: (*a*) M. Brandl, S. I. Kozhushkov, K. Loscha, O. V. Kokoreva, D. S. Yufit, J. A. K. Howard and A. de Meijere, *Synlett*, 2000, 1741; (*b*) A. Armstrong and J. N. Scutt, *Org. Lett.*, 2003, **5**, 2331; (*c*) D. Diez, P. Garcia, I. S. Marcos, N. M. Garrido, P. Basabe, H. B. Broughton and J. G. Urones, *Org. Lett.*, 2003, **5**, 3687; (*d*) R. P. Jain and J. C. Vederas, *Org. Lett.*, 2003, **5**, 4669; (*e*) O. V. Larionov, S. I. Kozhushkov, M. Brandl and A. de Meijere, *Mendeleev Commun.*, 2003, 199.
6 (*a*) E. J. Corey, F. Xu and M. C. Noe, *J. Am. Chem. Soc.*, 1997, **119**, 12414; (*b*) B. Lygo and P. G. Wainwright, *Tetrahedron Lett.*, 1997, **38**, 8595.
7 R. P. Beckett, M. J. Crimmin, M. H. Davis and Z. Spavold, *Synlett*, 1993, 137.
8 B. Barlaam, G. T. Bird, C. Lambert-van der Brempt, D. Campbell, S. J. Foster and R. Maciewicz, *J. Med. Chem.*, 1999, **42**, 4890.
9 G. A. Doldouras and J. Kollonitsch, *J. Am. Chem. Soc.*, 1978, **1**, 341.
10 J. S. Bajwa and M. J. Miller, *J. Org. Chem.*, 1983, **48**, 1114.
11 This two-phase system has previously been used for direct amide formation without isolation of the active ester. G-J. Ho, K. M. Emerson, D. J. Mathre, R. F. Shuman and E. J. J. Grabowski, *J. Org. Chem.*, 1995, **60**, 3569.