

Total synthesis of (+)-belactosin A[†]

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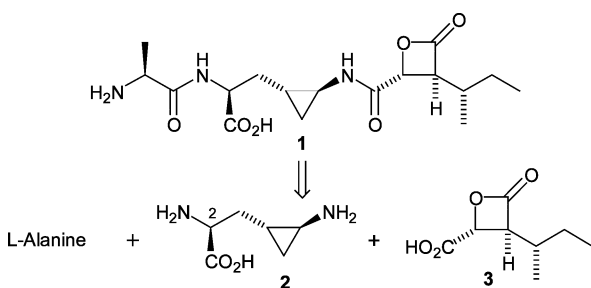
A concise first total synthesis of the antitumour antibiotic belactosin A is reported, involving coupling of β -lactone carboxylic acid **3** with *N*-Ala-aminocyclopropyl alanine **11**.

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 reports³ 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**.

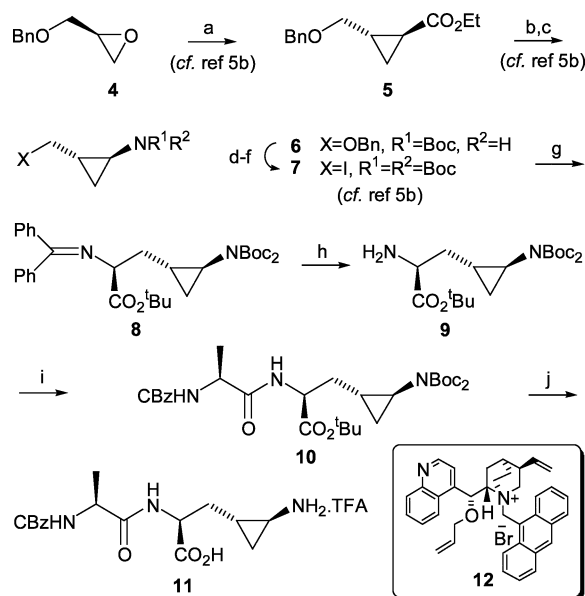
We first prepared 3-(*trans*-2-aminocyclopropyl)alanine **8** in 7 steps from (*R*)-glycidol benzyl ether **4** (Scheme 2). As in our reported synthesis of *ent*-**2**^{5b} the key steps included "Wadsworth–Emmons cyclopropanation" to convert the glycidol derivative **4** into the required enantiomerically 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/CH₂Cl₂ 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 CH₂Cl₂, 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/CH₂Cl₂ 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/H₂O₂ 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/NaHCO₃ 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. ¹H 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/CH₂Cl₂ in high yield, maintaining the integrity of the β -lactone ring.

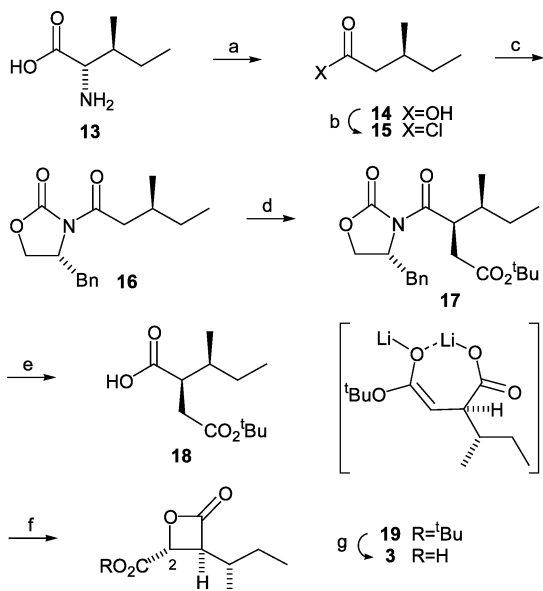


Scheme 1 Retrosynthetic analysis of belactosin A.



Scheme 2 (a) Triethyl phosphonoacetate, NaH, toluene, 110 °C, 14 h, 63%; (b) NaOH (aq), EtOH, 96%; (c) DPPA, ^tBuOH, NEt₃, reflux, 53%; (d) Boc₂O, MeCN, DMAP, 95%; (e) Pd/C, H₂, cat. AcOH, THF, 98%; (f) Bu₄NI, DDQ, PPh₃, CHCl₃, rt; (g) 2 eq *N*-(diphenylmethylene)glycine *tert*-butyl ester, 20 mol% **12**, 10 eq CsOH·H₂O (s), toluene/CH₂Cl₂ (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/CH₂Cl₂, 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/>



Scheme 3 (a) $\text{H}_2\text{NOSO}_3\text{H}/\text{KOH}$ (aq), 0°C to rt, 74%; (b) $(\text{COCl})_2$, CH_2Cl_2 , 0°C to rt, 80%; (c) $(4R)$ -benzyl-2-oxazolidinone, ${}^n\text{BuLi}$ -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%.

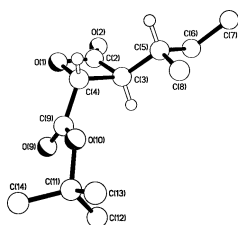
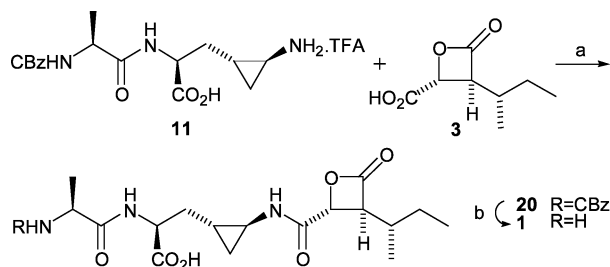


Fig. 1 X-Ray structure of β -lactone **19**.

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 $\text{Et}_3\text{N}/\text{Pr}_2/\text{DMF}$ 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 $\text{CH}_2\text{Cl}_2/\text{H}_2\text{O}$ at 0°C (Scheme 4),¹¹ followed by transfer of the organic phase to a cooled **11**/ $\text{Et}_3\text{N}/\text{Pr}_2/$



Scheme 4 (a) EDCI/HOBT in $\text{CH}_2\text{Cl}_2/\text{H}_2\text{O}$, 0°C ; then **11**/ $\text{Et}_3\text{N}/\text{Pr}_2/\text{DMF}$, 0°C , 50%; (b) Pd/C, H_2 , THF/ HCO_2H (3 : 2), 96%.

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.

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% $\text{NaHCO}_3/\text{D}_2\text{O}$), 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 $\text{p}K_a$ 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. $186\text{--}187^\circ\text{C}$, $[\alpha]_D^{21}+4.8$ (c 0.84, H_2O) (lit.^{2a} m.p. $184\text{--}185^\circ\text{C}$, $[\alpha]_D^{27}+4.8$ (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.

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