Simple access to the nonproteinogenic peptide fragments of lysobactin from azetidin-2-one frameworks

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A convenient route to the β-hydroxy α-aminoacid-derived tripeptides found in the macrocyclic peptide lactone antibiotic lysobactin from azetidin-2-one frameworks is provided for the first time.

Lysobactin, Fig. 1, is a macrocyclic peptide lactone antibiotic isolated from the fermentation of Lysobacter sp.ATCC 53042.[1] The mode of antibacterial action of this antibiotic has been shown to be comparable with the observed selectivity and potency of the well known clinically useful antibiotic vancomycin. Since infectious strains of vancomycin-resistant bacteria have been isolated,[2] lysobactin might be the alternative antibacterial agent of choice in spite of its relative toxicity. Consequently, the development of new semisynthetic peptides of lysobactin with improved therapeutic index is of considerable interest and, thereby, a concise approach to the key structural elements, the α-amino β-hydroxy acids, present in this antibiotic is essential. Although a number of suitable methods for the stereoselective construction of α-amino β-hydroxy acids[3,4] exists, it would be desirable and conceptually new to develop a synthesis of non-proteinogenic α-amino acid derivatives ready for direct use in peptide coupling reactions. Here we report that α-hydroxy(alkoxy)β-lactams fulfil this criterion and provide a direct way for the construction of the tripeptides **A** and **B** of lysobactin.

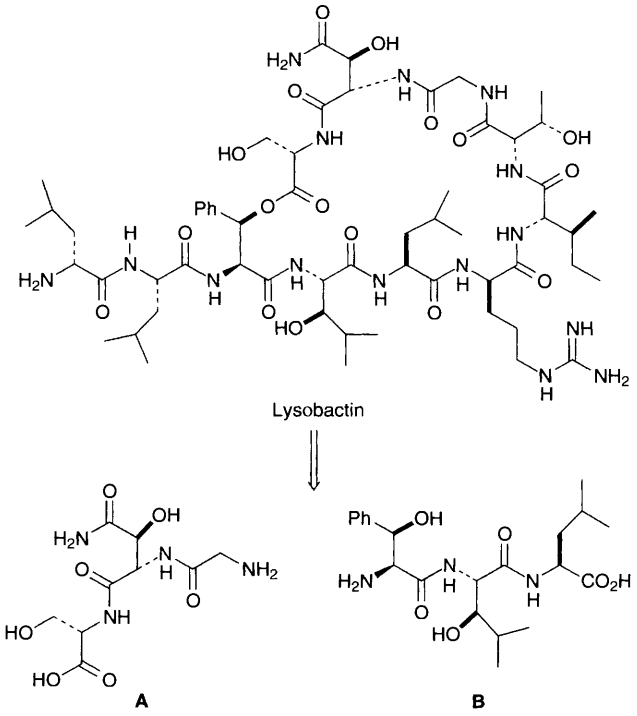
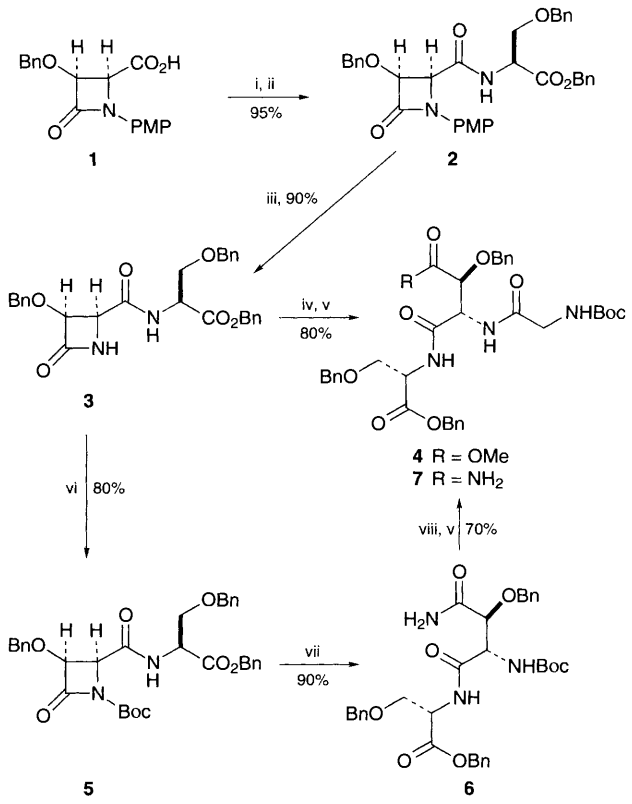


Fig. 1

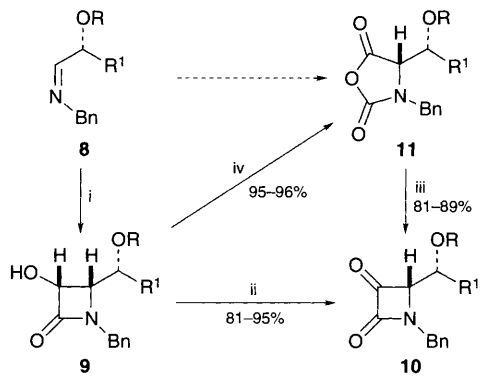
The synthesis of tripeptide **A** started from the 4-carboxy β-lactam **1**[5] [mp 207–209 °C; $[\alpha]_D^{25}$ = −115.8° (*c* = 1.0, Me_2CO)] as a β-hydroxy aspartic acid form possessing the β-carboxyl group and the α-amino moiety simultaneously protected. The dipeptide unit **2** [mp 197–199 °C, $[\alpha]_D^{25}$ = −25.0° (*c* = 0.5, CH_2Cl_2)] was obtained in 95% overall yield after activation of the carboxyl group with cyanuric fluoride and subsequent coupling with *O*-benzyl-L-serine benzyl ester according to Carpino's procedure.[6] To achieve this, **2** was first *N*-deprotected in the usual way[7] with the addition of methylene chloride as cosolvent to give **3** as a pale yellow solid [90%, mp 142–144 °C, $[\alpha]_D^{25}$ = −10.5° (*c* = 0.8, CH_2Cl_2)]. Formation of tripeptide **4** was accomplished in 80% yield by ring opening of **3** and subsequent acylation of the resulting free β-amino ester intermediate with BocGlyF and *N*-methylmorpholine (NMM). Alternatively, to facilitate the β-lactam cleavage by means of other nucleophiles,[8] **3** was treated with $(Boc)_2O$ and DMAP to give **5** with complete chemoselectivity [80% yield, mp



Scheme 1 *Reagents and conditions*: i, cyanuric fluoride, pyridine, CH_2Cl_2, 6 h, room temp.; ii, S–$NH_2CH(CH_2OBn)CO_2Bn$, CH_2Cl_2, NMM, 3 h, room temp.; iii, $(NH_4)_2Ce(NO_3)_6$, MeCN–H_2O–CH_2Cl_2, 1 h, 0 °C; iv, $ClSiMe_3$, MeOH, 1 h, 0 °C, room temp.; v, BocGlyF, NMM, CH_2Cl_2, 2 h, 0 °C; vi, $(Boc)_2O$ (2 equiv.), DMAP, MeCN, room temp., 16 h; vii, NH_4OH, DMF; viii, CF_3CO_2H, CH_2Cl_2, room temp.

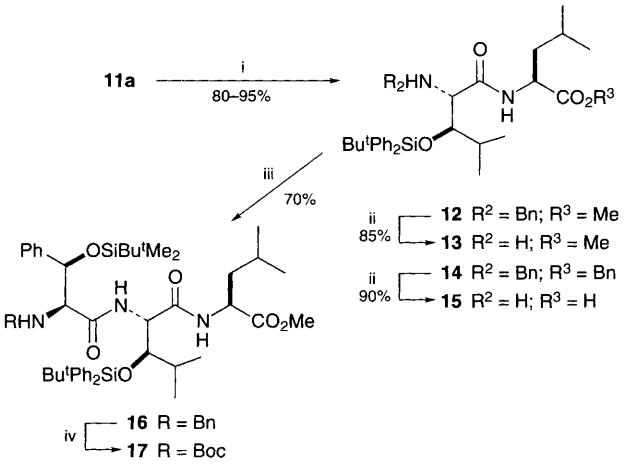
138–140 °C]. The opening of **5** with aq. NH_4OH (25%) (15–20 equiv.) in DMF proceeded cleanly within about 3 h to furnish **6** [mp 86–88 °C, $[\alpha]_D^{25}$ = +2.3° (*c* = 0.5, CH_2Cl_2)] in 90% isolated yield. Dipeptide **6** was next *N*-Boc deprotected and transformed into the tripeptide **7** as above [mp 106–108 °C $[\alpha]_D^{25}$ = +8.4° (*c* = 1.1, CH_2Cl_2)].

The strategy for tripeptide **B** is based on our recent reported method for the construction of α-amino acid *N*-carboxy anhydrides (NCAs) *via* Baeyer–Villiger rearrangement of α-keto β-lactams.[9] As shown in Scheme 2, the [2 + 2] cycloaddition of benzyloxyketene, generated *in situ* from benzyloxyacetyl chloride and triethylamine,[10] with imines **8a** and **8b** and subsequent removal of the benzyloxy protective group afforded the α-hydroxy β-lactams **9a** and **9b** in 84 and 92% yield, respectively. Oxidation of both **9a** and **9b** using P_2O_5 in Me_2SO gave **10a** and **10b** which on Baeyer–Villiger rearrangement provided the respective NCAs **11a** [81%, mp 168–170 °C, $[\alpha]_D^{25}$ = +31.8° (*c* = 1.0, CH_2Cl_2)] and **11b** [89%, mp 120 °C, $[\alpha]_D^{25}$ = −70.1° (*c* = 1.0, CH_2Cl_2)]. Nevertheless, we found that the enantiopure NCAs **11a** and **11b** could directly be obtained in 95 and 96% yield respectively in a single pot operation from the corresponding α-hydroxy β-lactams **9a** and **9b** using a phosphate buffer (pH = 6.9) of commercial bleach and a catalytic amount of 2,2,6,6-tetramethylpiperidinyl-1-oxy (TEMPO),[11] thus making this method inexpensive and practical for the large-scale production of NCAs.† Completion of the synthesis of the tripeptide B of lysobactin was accomplished as shown in Scheme 3. First, **11a** was coupled with *S*-leucine methyl ester in methylene chloride as solvent to give the dipeptide **12** in 95% yield. Further hydrogenolysis of **12** with H_2 over 10% Pd on charcoal led to **13** in 85% yield. In a similar way the coupling reaction of **11a** with *S*-leucine benzyl ester and subsequent *N*-debenzylation of **14** gave the dipeptide **15** in 90% yield {$[\alpha]_D^{25}$ = −29.0° (*c* = 1.0, CH_2Cl_2)}. Surprisingly, the NCA **11b** proved resistant to ring opening by dipeptide **13** but with the addition of sodium azide in DMF as solvent gave the tripeptide product **16** {$[\alpha]_D^{25}$ = −26.9° (*c* = 1.0, CH_2Cl_2)} which was then converted into **17** under standard conditions.



a R = Bu^tPh_2Si; R^1 = Pr^i **b** R = Bu^tMe_2Si; R^1 = Ph

Scheme 2 *Reagents and conditions*: i, $BnOCH_2COCl$ (2 equiv.), NEt_3, CH_2Cl_2, −78 °C → room temp.; 20 h then NH_4HCO_2, Pd/C, Pr^iOH, reflux, 1 h, 70% overall for **9a**; and H_2, Pd/C, EtOH, room temp. 15 h, 78% overall for **9b**; ii, Me_2SO, P_2O_5, 20 h; iii, MCPBA, CH_2Cl_2, −40 °C, 1 h; iv, 1 mol dm^{-3} NaOCl, TEMPO(cat), $NaHCO_3$, KH_2PO_4–K_2HPO_4 (pH: 6.9), CH_2Cl_2



Scheme 3 *Reagents and conditions*: i, *S*-leuOMe or *S*-leuOBn, CH_2Cl_2, room temp., 15 h; ii, H_2, Pd/C, EtOH, room temp., 15 h; iii, **11b**, DMF, NaN_3 (1 equiv.), room temp. 15 h; iv, H_2, Pd/C, $(Boc)_2O$ (2 equiv.), EtOH, room temp.

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Footnote

† To ensure the optical purity of these NCAs both **11a** and **11b** were opened by methanol under reflux followed by *N*-debenzylation and subsequent acylation of the resulting α-amino esters with Mosher acid chloride and triethylamine. In each case a single set of signals were obtained in their ^1H, ^{13}C and ^{19}F NMR spectra.

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