The Synthesis of Eurystatin A[1]

Ulrich Schmidt* and Steffen Weinbrenner

Institut für Organische Chemie und Isotopenforschung der Universität Stuttgart, Pfaffenwaldring 55, D-70569 Stuttgart, Germany

Eurystatin A, a new prolyl endopeptidase inhibitor isolated from *Streptomyces eurythermus* has now been synthesized.

The thrombin inhibitors cyclotheonamide A and B,[2] the prolyl endopeptidase inhibitor poststatin,[3] as well as the prolyl endopeptidase inhibitors eurystatin A and B (**1a** and **1b**)[4] all contain a β-amino-α-oxocarboxylic acid moiety, which appears to be the common active centre in these enzyme-inhibiting peptides.

During the synthesis of cyclotheonamide B,[5] the β-amino-α-oxocarboxylic acid unit was formed by oxidation of the corresponding β-amino-α-hydroxy compound. The latter was prepared by the reaction of an α-acylaminoaldehyde with tris(methylthio)methyllithium and subsequent Hg^{2+}-mediated hydrolysis of the trithioorthocarboxylate.

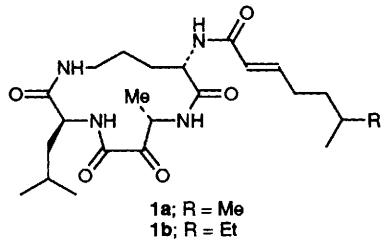
Here, we describe the first total synthesis of eurystatin A (**1a**) in which we have utilized the Passerini reaction for the construction of the diastereoisomeric β-amino-α-hydroxy compound **5**.

The synthesis of the β-amino-α-hydroxybutyric acid and its conversion into dipeptolide **4** can be achieved in one step by the Passerini reaction of (*S*)-Z-alaninal (**3**) with methyl (*S*)-2-isocyano-4-methylpentanoate (**2**). Compound **2** was obtained in an optically pure form by the triphosgene dehydration[6] of (*S*)-*N*-formylleucinate. No optical induction can be observed during the Passerini reaction and the product is a 1 : 1 mixture of the diastereoisomers.

Saponification of **4** and replacement of the benzyloxycarbonyl group by a *tert*-butyloxycarbonyl protecting function furnished the carboxylic acid **5**. Coupling with benzyloxycarbonyl ornithine methyl ester gave the diastereoisomeric products **6a** and **6b**, whose configurations could not be elucidated. In an attempt to avoid the difficulties associated with the reactions and the characterisation of diastereoisomeric mixtures in subsequent steps, the mixture of **6a**, **6b** was oxidized. However, saponification of the α-oxocarboxylate was not successful and only decomposition products were isolated. Thus, the diastereoisomers **6a**, **6b** were separated by medium pressure LC and subsequent reactions were carried out with the pure diastereoisomers.

For the peptide ring closure we introduced the pentafluorophenyl ester.[7] This procedure has been our method of choice for several years in the synthesis of natural biologically active cyclopeptides. The construction of the cyclopeptide alkaloids zizyphine A and B,[8] mucronine,[9] dihydrozizyphines A, B[10] and G[11] and frangulanine,[12] of the cytostatic cyclotetrapeptides chlamydocine[13] and WF-3161[14] and of the antibiotic glidobactin[15] have been mostly realized in 80–95% yields by catalytic hydrogenation of the corresponding linear ω-Z-amino pentafluorophenyl esters with palladium/charcoal. This cyclisation process takes place on the surface of the palladium where the free amino group is adsorbed after the Z-group has been cleaved.

In the cases of the synthesis of the thiazole-containing cyclopeptides—ulicyclamide,[16] ulithiacyclamide,[17] patellamide B[18] and the dolastatin[3] isomers[18]—where catalytic deprotection of the benzyloxycarbonyl group is not possible, ring closure has been achieved by reacting the ω-amino pentafluorophenyl esters (obtained from ω-Boc-amino pentafluorophenyl esters) with a base in dioxane. If this reaction is performed in the two-phase system chloroform–hydrogen carbonate–water without the use of high dilution conditions often the yield is so high[19] within a few minutes that we believe that the conformations of the transition state for the cyclisation is the same as those of the linear substrate.

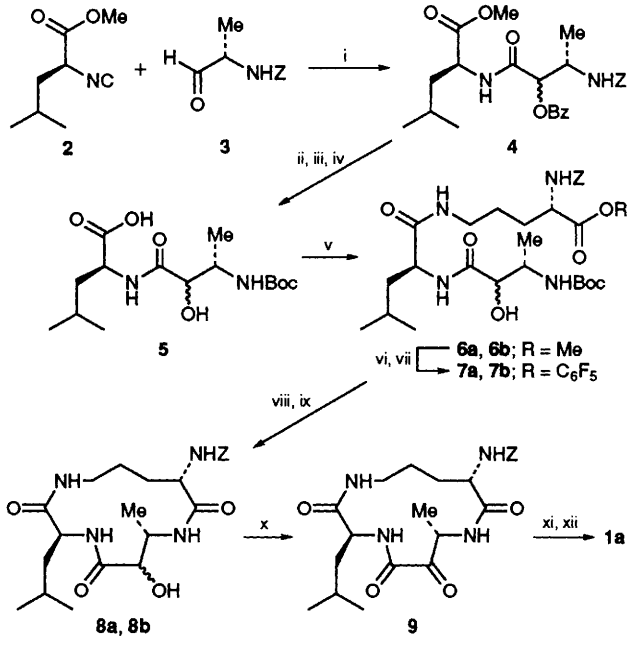


1a; R = Me
1b; R = Et

The synthesis of the cytostatic didemnins,[20] the antibiotics biphenomycin A[21] and B,[22] the encyminhibitor OF-4949[23] and of the ACE inhibitors lyciumin A and B[24] have been realized in high yields by this procedure.

Saponification of **6a** and **6b** and esterification with pentafluorophenol afforded the esters **7a** and **7b**, the starting materials for the ring closure step. The Boc protection was removed to leave the hydrochloride and ring closure was realized in good yields (72% for **8a**, 63% for **8b**, each over four steps) in the previously described two-phase system composed of chloroform and aqueous sodium hydrogen carbonate.

Swern oxidation, which has been used with success for linear β-acylamino-α-hydroxycarboxylic acid derivates (such



Scheme 1 *Reagents and conditions*: i, benzoic acid, CH_2Cl_2, room temp., 48 h, 85%; ii, MeOH, Cs_2CO_3, 15 min, room temp., 98%; iii, MeOH, Boc_2O, Pd/C, H_2, 2 h, 96%; iv, LiOH, H_2O–dioxane, room temp., 96%; v, Z-(H)-Orn*HCl, 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU), CH_2Cl_2, 35% **6a**, 25% **6b**; vi, LiOH, H_2O–dioxane, room temp.; vii, C_6F_5OH, *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (EDC), −15 to 20 °C, 16 h; viii, 6 mol dm^{-3} HCl, dioxane, 2 h, 20 °C; ix, $CHCl_3$–$NaHCO_3$, room temp., 6 h, vi–ix, **6a** to **8a** 72%, **6b** to **8b** 63%; x, pyridinium dichromate, DMF, room temp., 48 h, **8a** to **9** 75%, **8b** to **9** 30%; xi, MeOH–CH_2Cl_2, Pd/C, H_2, 2 h; xii,DMF–CH_2Cl_2, (*E*) 6-methylhept-2-enoyl chloride, pyridine, room temp., 3 h, xi + xii, 50%

as **6**), failed in the cases of the cyclic compounds **8a** and **8b**. However, the required transformation was achieved with pyridinium dichromate and it was found that the diastereoisomer **8a** reacted to give a higher yield (75%) than **8b** (30%).

Subsequent hydrogenolytic deprotection of **9**† and acylation with (*E*)-6-methylhept-2-enoyl chloride then completed the synthesis of eurystatin A (**1a**).‡ The product thus synthesized was found to be identical in all respects (NMR, optical rotation, melting point and MS) with the naturally occurring compound.

Support of this research work by the Fonds der chemischen Industrie and the Deutsche Forschungsgemeinschaft is gratefully acknowledged.

Received, 14th January 1994; Com. 4/00241E

Footnotes

† δ_H (250 MHz, $(CD_3)_2SO$) 8.83 (d, *J* 7.7, 1H), 8.23 (d, *J* 8.1, 1H), 7.45–7.15 (m, 7H), 5.05 (d, *J* 12.5, 1H), 4.98 (d, *J* 12.5, 1H), 4.69–4.64 (m, 1H), 4.12–4.10 (m, 2H), 3.04 (br, 2H), 1.61–1.48 (m, 7H), 1.16 (d, *J* 6.7, 3H), 0.89 (d, *J* 5.7, 3H) and 0.82 (d, *J* 5.7, 3H).

‡ δ_H (250 MHz, $(CD_3)_2SO$) 8.82 (d, *J* 7.7, 1H), 8.21 (d, *J* 8.5, 1H), 8.02 (d, *J* 7.3, 1H), 7.43 (br, 1H), 6.61 (dt, *J* 15.4, 6.8, 1H), 6.03 (d, *J* 15.4, 1H), 4.76–4.69 (m, 1H), 4.21–4.18 (m, 1H), 4.12–4.06 (m, 1H), 3.10–3.03 (m, 2H), 2.17–2.10 (m, 2H), 1.82–1.48 (m, 8H), 1.32–1.23 (m, 2H), 1.15 (d, *J* 6.7, 3H), 0.91–0.89 (m, 12H).

References

1 For part 95 of the series Amino Acids and Peptides, see U. Schmidt and F. Stäbler, *Synthesis* in press; for part 27 of the series Cyclopeptides, see U. Schmidt, A. Kleefeldt and R. Mangold, *J. Chem. Soc., Chem. Commun.*, 1992, 1687.
2 N. Fusetani, S. Matsunaga, H. Matsumoto and Y. Tukebayashi, *J. Am. Chem. Soc.*, 1990, **112**, 7053.
3 M. Nagi, K. Ogawa, Y. Muraoka, H. Naganawa, T. Aoyagi and T. Takeuchi, *J. Antibiotics*, 1991, **44**, 956.
4 S. Toda, C. Kotake, T. Tsuno, Y. Narita, T. Yomasaki and M. Konishi, *J. Antibiotics*, 1992, **45**, 1580.
5 M. Hagihara and S. L. Schreiber, *J. Am. Chem. Soc.*, 1992, **114**, 6570.
6 H. Eckert and B. Forster, *Angew. Chem.*, 1987, **99**, 922.
7 U. Schmidt, A. Lieberknecht, H. Griesser and J. Talbiersky, *J. Org. Chem.*, 1982, **47**, 3261.
8 U. Schmidt, A. Lieberknecht, H. Bökens and H. Griesser, *Angew. Chem.*, 1981, **93**, 1121; *Angew. Chem., Int. Ed. Engl.*, 1981, **20**, 1026; U. Schmidt, A. Lieberknecht, H. Bökens and H. Griesser, *J. Org. Chem.*, 1983, **48**, 2680.
9 U. Schmidt and U. Schanbacher, *Angew. Chem.*, 1983, **95**, 150; *Angew. Chem., Int. Ed. Engl.*, 1983, **22**, 152; U. Schmidt and U. Schanbacher, *Liebigs Ann. Chem.*, 1984, 1205.
10 U. Schmidt, H. Bökens, A. Lieberknecht and H. Griesser, *Tetrahedron Lett.*, 1982, **22**, 4949; U. Schmidt, H. Bökens, A. Lieberknecht and H. Griesser, *Liebigs Ann. Chem.*, 1983, 2153.
11 U. Schmidt, A. Lieberknecht, H. Griesser and J. Häusler, *Angew. Chem.*, 1981, **93**, 272; *Angew. Chem., Int. Ed. Engl.*, 1981, **20**, 281; U. Schmidt, A. Lieberknecht, H. Griesser and J. Häusler, *Liebigs Ann. Chem.*, 1982, 2153.
12 U. Schmidt, M. Zäh and A. Lieberknecht, *J. Chem. Soc., Chem. Commun.*, 1991, 1002.
13 U. Schmidt, A. Lieberknecht, H. Griesser and F. Bartkowiak, *Angew. Chem.*, 1984, **96**, 310; *Angew. Chem., Int. Ed. Engl.*, 1984, **23**, 318; U. Schmidt, A. Lieberknecht, H. Griesser, R. Utz, T. Beutler and F. Bartkowiak, *Synthesis*, 1986, 361.
14 U. Schmidt, U. Beutler and A. Lieberknecht, *Angew. Chem.*, 1989, **101**, 344; *Angew. Chem., Int. Ed. Engl.*, 1989, **28**, 333.
15 U. Schmidt, A. Kleefeldt and R. Mangold, *J. Chem. Soc., Chem. Commun.*, 1992, 1687.
16 U. Schmidt and P. Gleich, *Angew. Chem.*, 1985, **97**, 606; *Angew. Chem., Int. Ed. Engl.*, 1985, **24**, 569.
17 U. Schmidt and D. Weller, *Tetrahedron Lett.*, 1986, **27**, 3495.
18 U. Schmidt, R. Utz and P. Gleich, *Tetrahedron Lett.*, 1985, **26**, 4367; U. Schmidt and H. Griesser, *Tetrahedron Lett.*, 1986, **27**, 163.
19 U. Schmidt and R. Utz, *Angew. Chem.*, 1984, **96**, 723; *Angew. Chem., Int. Ed. Engl.*, 1984, **23**, 725; U. Schmidt, R. Utz, A. Lieberknecht, H. Griesser, B. Potzolli, J. Bahr-Thielemann, K. Wagner and P. Fischer, *Synthesis*, 1987, **233**, 236.
20 U. Schmidt, M. Kroner and H. Griesser, *Tetrahedron Lett.*, 1988, **29**, 3057; U. Schmidt, M. Kroner and H. Griesser, *Synthesis*, 1991, 294.
21 U. Schmidt, V. Leitenberger, H. Griesser, J. Schmidt and R. Meyer, *Synthesis*, 1992, 1248.
22 U. Schmidt, R. Meyer, V. Leitenberger, H. Griesser and A. Lieberknecht, *Synthesis*, 1992, 1025.
23 U. Schmidt, D. Weller, A. Holder and A. Lieberknecht, *Tetrahedron Lett.*, 1989, **29**, 3227.
24 U. Schmidt and F. Stäbler, *J. Chem. Soc., Chem. Commun.*, 1992, 1353.