Pergamon

Tetrahedron Letters, Vol. 37, No. 7, pp. 1101-1104, 1996

0040-4039/96 $15.00 + 0.00

0040-4039(95)02315-1

Lanthionines for Solid Phase Synthesis.

John M. Probert, Denise Rennex and Mark Bradley*

Department of Chemistry, University of Southampton, Highfield, Southampton, SO17 1BJ, UK.

Abstract: The synthesis of orthogonally protected lanthionine (Boc, Fmoc, Allyl/Methyl), suitable for combinatorial and solid phase peptide chemistry is described. Three routes were attempted the most suitable proved to be the Michael addition between Fmoc-L-Cys-allyl ester and Boc dehydroalanine-methyl ester.

Lanthionine, the thioether analogue of cystine was first isolated from wool hydrosylates in 1941[1] and synthesised from cysteine and β-chloroalanine in the same year.[2] It has recieved a considerable amount of attention, firstly because it is an interesting, non-reducable mimic of cystine and hence when incorporated into peptides introduces a number of desirable properties,[3] and secondly because lanthionines have been found quite widely in nature - for example in place of meso-DAP in bacterial cell walls[4] and in the family of ribosomally-derived bio-active peptides typified by nisin, subtilin and cinnamycin.[5] This family of so called Lantibiotics contain both 2S, 5R-lanthionines as well as 2S, 3S ,5R methyllanthionines and it is these residues which give these compounds their fascinating cyclic structures and which help to impart a resistance to proteolytic degradation. Since small fragments of nisin are known to be biologically active[6] it suggested that a combinatorial approach[7] to the synthesis of the some of the rings of nisin or subtilin could yield interesting compounds, perhaps with the antibacterial and immunostimmulant properties associated with the parent lantibiotics, in effect a template combinatorial approach.[7] To undertake this synthesis we required an orthogonally protected lanthionine moiety suitable for solid phase chemistry and protected such that the orthogonality of Fmoc, Boc and Allyl chemstries or equivalent could be utilised during the synthesis (see figure 1). Although routes to protected lanthionines are known (eg Cbz, Boc, Methyl ester lanthionine) they did not provide the amino acid in a suitably protected form for Fmoc solid phase peptide synthesis.[8]

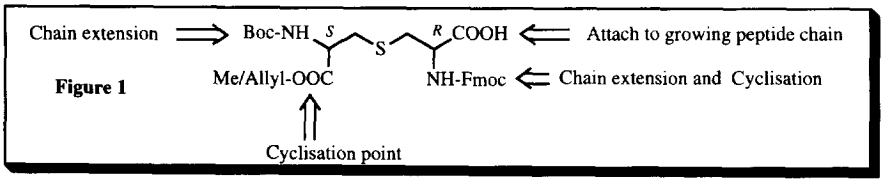

Figure 1

The major problem in the preparation of lanthionines suitable for solid phase synthesis is one of defined regioselective protection.[8] The solid phase desulphurisation of cystine residues using $P(NEt_2)_3$ was initially considered as the method of choice. However trial reactions under the originally reported conditions[9] using $P(NEt_2)_3$ in benzene at reflux gave at most a 32% yield. The choice of solvent was important since all other solvents utilised gave dehydoalanine in up to 45% yield except scrupiously dried DMF which upon optimisation gave the desired lanthionine in a yield of 44%. This low yield, even upon optimisation, precluded this route as a viable synthetic method for the solid phase synthesis of lanthionines.

We therefore decided to generate the required lanthionines by the stereospecific opening of the protected D-serine-β-lactones of Vederas.[10] The β-lactones were cleanly ring opened during trial studies using Boc or Z-L-cysteine methyl ester in (MeCN + 1eq Cs_2CO_3) to give the desired product in up to 48% yield (this method has very recently been reported by others).[8a] However when Fmoc-L-Cys-allyl ester was utilised to generate lanthionine suitable for Fmoc SPPS the ring opening reaction failed, although a wide variety of conditions (pH, base, solvent) were utilised, decomposition of Fmoc-L-Cys allyl ester or of the serine-β-lactone were the only reactions observed.

We therefore resorted to a regio but non-stereospecific synthesis and carried out a Michael reaction of Fmoc-Cysteine allyl ester onto Boc-dehydroalanine methyl ester[11] (figure 2). The reaction proceeded smoothly in MeCN using a catalytic amount of Cs_2CO_3 to give orthogonally protected lanthionine in 72% yield. The two diastereoisomers were then found to be easily separable in a 2:3 ratio by ODS column chromatography.[12]

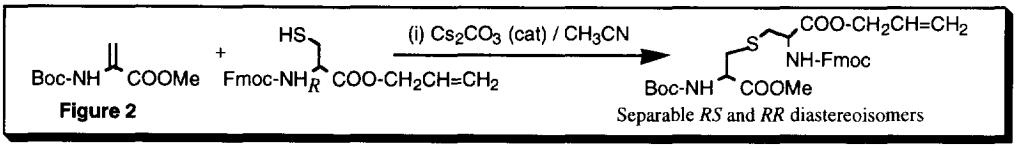

Figure 2

The allyl and methyl esters were subsequently removed under standard conditions[13,14] to give protected lanthionine suitable for solid phase and combinatorial chemistry. This orthogonally protected lanthionine and its ease of synthesis makes it an ideal building block for use as a cystine mimic in SPPS and combinatorial chemistry to produce cyclic peptide libraries. Work is now in progress aimed at the combinatorial synthesis of subtilin and nisin and will be reported in due course.

REFERENCES AND NOTES

1. Horn, M. J.; Jones. D. B.; Ringel. S. J.; *J. Biol. Chem.*, **1941**, *138*, 141

2. Brown, G. B.; du Vigneaud, V.; *J. Biol. Chem.*, **1941**, *140*, 767 and Brown. G. B.; du Vigneaud. V.; *J. Biol. Chem.*, **1941**, *138*, 151.

3. Nutt, R. F.; Veber, D. F.; Saperstein, R.; *J. Am. Chem. Soc.*, **1980**, *102*, 6539, Polinsky, A.; Cooney, M. G.; Toy-Palmer, A.; Osapay, G.; Goodman, M.; *J. Med. Chem.*, **1992**, *35*, 4185.

4. Richaud, C.; Mengin-Lecreulx, D.; Pochet, S.; Johnson, E. J.; *J. Biol. Chem.*, **1993**, *268*, 26827.

5. Alderton, G.; Fevold, H. L.; *J. Am. Chem. Soc.*, **1951**, *73*, 463, Gross, E.; Morell, J. L.; *J. Am. Chem. Soc.*, **1971**, *93*, 4634, Jung, G.; *Angw. Chem.,Int. Ed. Engl*, **1991**, *30*, 1051.

6. Gross, E.; Morell, J. L.; *J. Am. Chem. Soc.*, **1970**, *93*, 2919, Gross, E.; Witkop, B.; *J. Biol. Chem.*, **1962**, *237*, 1856.

7. Gallop, M. A.; Barrett, R. W.; Dower, W. J.; Fodor, S. P. A.; Gordon, E. M.; *J. Med. Chem.* **1994**, *37*, 1233.

8. (a) Shao, H.; Wang, S. H. H.; Lee, C-W.; Osapay, G.; Goodman, M.; *J. Org. Chem.*, **1995**, *60*, 2956, (b) Osapay, G.; Goodman, M.; *J. Chem. Soc. Chem. Comm.*, **1993**, 1599 and (c) Cavelier-Frontin, F.; Daunis, J.; Jacquier, R.; *Tetrahedron Asymmetry*, **1992**, *3*, 85.

9. Harpp, D. N.; Gleason, J. G.; *J. Org. Chem.*, **1971**, *36*, 73, Harpp, D. N.; Gleason, J. G.; *J. Am. Chem. Soc.*, **1968**, *90*, 4181, Wakamiya, T.; Shimbo, K.; Sano, A.; Fukase, K.; Shiba, T.; *Bull. Chem. Soc. Jpn.*, **1983**, *56*, 2044, Moore. C.; Treger, B.; *Tetrahedron*, **1962**, *18*, 205.

10. Arnold, L. D.; Kalantar, T. H.; Vederas, J. C.; *J. Am. Chem. Soc.* **1985**, *107*, 7105.

11. Boc-dehydroalanine methyl ester was prepared by the method of Miller, M. J.; *J. Org. Chem.*, **1980**, *45*, 3131.

12. To a stirred solution of Boc-dehydroalanine methyl ester (430mg, 2.14mmol) in MeCN (10ml) was added Fmoc-L-cysteine allyl ester (825mg, 2.15mmol) and a catalytic amount of caesium carbonate (ca.20mg). The reaction was stirred for 2hrs then diluted with water (10ml) and extracted with EtOAc (2x20ml). The EtOAc layers were combined, washed with water (2x20ml), saturated sodium chloride solution (20ml), dried over anhydrous magnesium sulphate and the solvent removed *in vacuo*. The product was then purified by column chromatography (silica gel, 75:25:1 petrol: EtOAc: AcOH) to give a gum. Yield 900mg (72%), R_f = 0.32 (80:20:1) petrol: EtOAc: AcOH, m/z (FAB): 607 ($[M+Na]^+$, 585 $[M+H]^+$, HR MS, expected for $C_{30}H_{36}O_8N_2SNa$ 607.2090, found 607.2099, $[\alpha]_D^{20°}$ = 3.5 (c 20, $CHCl_3$), IR υ_{max}/cm^{-1} (NaCl, nujol mull): 3363(br, m, NH), 1744(m, sh, CO), 1720(m, sh, CO), 1714(m, CO), 1690(w, sh, CO), δ_H (300MHz, $CDCl_3$); 1.45(s, 9H, ^tBu), 2.99(m, 4H, 2xCH_2S), 3.71(s, 3H, $COOCH_3$), 4.23(t, 1H, *J* 7, CH Fmoc), 4.40(d, 2H, *J* 7, CH_2 Fmoc), 4.66(m, 4H, 2xCH_α, $C\underline{H}_2CH=CH_2$), 5.29(m, 2H, $CH=C\underline{H}_2$), 5.47(d, 1H, *J* 7.5, NH), 5.90(m, 1H, $C\underline{H}=CH_2$), 6.03(d, 1H, *J* 8, NH), 7.31(t, 2H, *J* 7.5, 2xCHAr), 7.39(t, 2H, *J* 7.5, 2xCHAr), 7.61(d, 2H, *J* 7, 2xCHAr), 7.75(d, 2H, *J* 7.5, 2xCHAr), δ_C (75MHz, $CDCl_3$, DEPT analysis): 28.5($C(\underline{C}H_3)_3$), 33.3 and 33.5(2x$\underline{C}H_2S$), 47.2($\underline{C}H$ Fmoc),

52.8($COOCH_3$), 53.5 and 54.0(2xCH_α), 66.6(CH_2 Fmoc), 67.4($CH_2CH=CH_2$), 80.4($C(CH_3)_3$), 119.4($CH=CH_2$), 120.2($CH=CH_2$), 125.3, 127.3, 127.9, 131.3(8xCHAr), 141.4, 143.9(4xCAr), 155.4(CONH), 156.0(CONH), 170.3 ($COOCH_2CHCH_2$), 171.4(COOMe). The diastereoisomers were separable by RP-HPLC (ODS) eluting with 60% MeCN/H_2O/0.1%TFA), λ = 270nm. The retention factors (ratio of void time/elution time) for the two isomers were 0.34 and 0.17 (12 and 24 min respectively).

13. Bloomberg, G. B.; Askin, D.; Gargaro, A. R.; Tanner, M. J. A.; *Tetrahedron Letters*, **1993**, *34*, 4709.

14. 1 equivalent of LiOH in cold dioxane will selectively remove a methyl ester in the presence of the Fmoc group, see for example Ye, B.; Burke, T.R. Jr.; *Tetrahedron Letters*, **1993**, *34*, 4733.

Acknowledgements: We would like to thank the Wellcome Trust for an Equipment Grant (039094/Z/93/Z) (MB) the Royal Society for a University Research Fellowship (MB), and the BBSRC for grant GR/H61353.

(*Received in UK* 19 *October* 1995; *accepted* 7 *December* 1995)