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SOLID PHASE SYNTHESIS OF N-CARBOXY ALKYL-CONTAINING PEPTIDES DERIVED FROM ENANTIOPURE α -KETO- β -AMINOACIDS.

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Abstract: α -Keto- β -aminoacids 5a-c can be reductively aminated with the peptide sequence H₂N-Leu-Val-Phe-Phe on a solid support to afford N-carboxy alkyl peptides 1a-c. The N-carboxy alkyl lysine derivative 7 was subsequently extended from the N-terminus with glutamine and histidine residues. © 1998 Elsevier Science Ltd. All rights reserved.

During attempts to prepare putative inhibitors of ' α -secretase,' the as yet unidentified enzyme thought to be responsible for the cleavage of the amyloid precursor protein (APP) associated with the onset of Alzheimer's disease,¹ we have synthesised N-carboxy alkyl peptides of the type shown in Figure 1 using solid phase techniques. These peptides were designed as putative inhibitors to mimic the amino acid sequence at the Lys-Leu bond in APP, the proposed cleavage site.²

Reductive amination using peptides on solid supports and the preparation of N-carboxy alkyl peptides by reductive amination in solution are both documented.³ However, we believe that the work described here represents the first attempt, using solid phase methods, to (a) apply reductive amination to α -keto- β -amino acid derivatives of N-protected amino acids and (b) extend such N-carboxy alkyl peptides from the N-terminus.

Figure 1:



Initially, we used N-Boc-6-aminohexanoic acid (Scheme 1, R=H) as the substrate but later showed that the method could be extended to the formation of the N-protected (L)-lysines derivatives 1b and 1c.

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0960-894X/98/\$19.00 © 1998 Elsevier Science Ltd. All rights reserved. PII: S0960-894X(98)00054-7 The direct conversion of carboxylic acids into keto ylides was carried out using the conditions described by Wasserman *et al.*⁴ The coupling reaction between carboxylic acids **2 a-c** and the (cyanomethylene)triphenylphosphorane⁵ in the presence of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCl) gave the cyanoketoylides **3 a-c** in excellent yields (67, 86, and 77%, respectively). The oxidation of **3 a-c** was achieved with ozone or dimethyldioxirane (DMD) at -78°C in dichloromethane giving similar yields. The unstable diketonitriles⁶ **4 a-c** thus produced were trapped hydrolytically with water leading to α -ketoacids **5a-c**.⁷

To facilitate the preparation of the pentapeptides 1a-c, a reductive amination of the ketone function in 5 was attempted using a solid phase method. The α -keto- β -aminoacids 5 a-c (3 eq) were each added to a suspension of the Rink Amide MBHA resin (loading level= 0.55 mmol/g), containing the peptide sequence Leu-Val-Phe-Phe 6, in DMF and acetic acid (0.5M overall concentration)⁸ and the intermediate Schiff bases were reduced to the desired *N*-carboxyalkyl derivative 1 a-c by the addition of NaCNBH3.

Scheme 1



The progress of the reaction, which required the cleavage of small amounts of peptides from the support by treatment with TFA (95%), was monitored by HPLC (Bio-Rad RP318 reverse phase HPLC column, 5-70% MeCN/ 0.1% TFA over 20 min at 1mL/min). A significant difference in rate of the reductive amination was observed depending on the R group in 5a-c. Whereas the product 1a was obtained in quantitative yield after 12h, for 1b only around 60% conversion was observed after 12h reaction time. In the case of 5c, the reaction time had to be increased to 24h by which time HPLC shown a single peak corresponding to 1c, with no evidence of 5c remaining. Although no isomer separation was seen in HPLC analysis, we presume that 1b and 1c are a diastereoisomeric mixture, because, in the case of 1a a 1:1 mixture of diastereoisomers was observed

by carbon-13 NMR spectra. The differential shifts were significant for the carboxylic carbon (163.19 and 162.92 ppm) and the carbon in the new chiral center (55.14 and 54.95 ppm).

Following Fmoc deprotection in 20% piperidine/DMF, the AcHis(Trt)-Gln(Trt) sequence was attached to the N-carboxy alkyl peptide 7 on the Rink Amide support (loading level = 0.55 mmol/g) using HBTU (1 eq)/ HOBT (1 eq) and DIPEA (3 eq) to activate the N-terminus. Detritylation of the glutamine and histidine moieties, Boc deprotection of the lysine residue and cleavage from the resin by exposure to TFA (94%), H₂O (5%) and triisopropylsilane (1%) gave the crude peptide 1d which was purified by chromatography on a Waters µBondpak C18 HPLC column (20-40% MeCN/ 0.1% TFA over 30 min at 1.5 mL/min) and characterized by electrospray mass spectroscopy. Further details of these peptide sequences, including the stereochemistry outcome, are currently being investigated.

Scheme 2



In summary, we have shown that α -keto- β -aminoacid derivatives can be reductively aminated and solid phase methods can be applied to the synthesis of N-carboxy alkyl peptides.

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- 9. All compounds had spectral data consistent with the proposed structures. Compound 3a: ¹H NMR (CDCl₃, 300 MHz) 7.68-7.52 (m, 15H), 4.22 (br s, 1H), 3.10 (m, 2H), 2.91 (t, 2H, J= 7.2 Hz), 1.67 (m, 2H, J= 7.2, 7.4 Hz), 1.45 (s, 9H), 1.55-1.32 (m, 4H) ppm; IR (neat) 3300 (NH), 2170 (CN), 1712 (CO) cm⁻¹. Compound 3b: ¹H NMR (CDCl₃, 500MHz) 7.68-7.53 (m, 15H), 7.34 (s, 5H), 5.63 (d, 1H, J= 7.9 Hz), 5.09 (m, 2H), 4.93 (m, 1H), 4.68 (m, 1H). 3.06 (m, 2H), 1.67 (m, 1H), 1.56-1.30 (m, 14H) ppm; IR (neat) 3340 (NH), 2178 (CN), 1714 (CO) cm⁻¹; MS, m/e 663(M⁺, 1) 555 (3), 482 (5), 328 (20), 262 (68), 183 (33), 108 (100), 91 (48), 79 (99), 59 (54). Compound 3c: ¹H NMR (CDCl₃, 500MHz) 7.85-7.6 (m, 23H), 5.67 (br d, 1H), 4.96 (m, 1H), 4.66 (m, 1H), 4.35 (d, 2H, J= 7.1 Hz), 4.20 (dd, H, J= 7.1 Hz), 3.0 (m, 2H), 1.74 (m. 1H), 1.55 (m, 1H), 1.41 (s, 9H), 1.4 (m, 4H) ppm; ¹³C NMR (CDCl3, 125 MHz) 22.36, 28.44, 33.46, 40.48, 47.25, 48.07, 56.07, 66.70, 78.86, 119.88, 122.27, 122.99, 125.25, 127.0, 127.57, 128.44, 128.54, 129.22, 129.32, 131.91, 132.04, 132.12, 132.97, 133.38, 133.51, 133.59, 141.27, 143.93, 144.17, 155.91, 156.02, 194.11 ppm; IR (neat) 3344 (NH), 2177 (CN), 1710 (CO) cm⁻¹; MS, m/e 768 (M+17, 1), 713 (4), 529 (63), 472 (25), 456 (20), 429 (23), 328 (100), 300 (22), 277 (40), 262 (57), 183 (44), 52 (10), 128 (13), 108 (24), 91 (63). Compound 5a: ¹H NMR (CDCl₃, 300 MHz) 3.10 (m, 2H), 2.91 (t, 2H, J= 7.1Hz), 1.67 (m, 2H, J= 7.1, 7.3 Hz), 1.44 (s, 9H), 1.55 -1.28 (m, 4H) ppm; ¹³C NMR (CDCl₃, 75 MHz) 22.44, 25.86, 28.26, 29.80, 38.37, 40.22, 80.00, 156.36, 156.38, 162.24, 195.92 ppm; MS, m/e 277 (M+18, 25), 259 (M⁺, 9), 241 (14), 200 (5), 186 (5), 158 (22), 140 (37), 131 (15), 114 (24), 96 (10), 86 (24), 74 (17), 69 (24), 57 (100), 44 (10), 41 (33). Compound 5b: ¹H NMR (CDCl₃, 300 MHz) 7.33 (s, 5H), 5.62 (m, 1H), 5.25 (m, 1H), 3.10 (m, 2H), 1.98 (m, 1H), 1.58 (m, 1H), 1.55-1.30 (m, 13H) ppm; ¹³C NMR (CDCl₃, 75 MHz) 22.28. 28.29, 33.46, 40.00, 40.50, 56.90, 67.05, 78.90, 118.50, 128.05, 128.10, 128.15, 128.40, 135.92, 156.21, 157.75, 162.08, 194.26 ppm; MS, m/e 407 (M⁺, 29), 346 (18), 324 (14), 307 (2), 277 (18), 200 (20), 183 (20), 156 (12), 139 (30), 128 (10), 108 (25), 99 (14), 91 (97), 79 (19), 71 (7), 65 (9), 57 (100), 41 (36). Compound 5c: ¹H NMR (CDCl₃, 300 MHz) 7.72 (m, 2H), 7.56 (m. 2H), 7.41-7.27 (m, 4H), 5.68 (br m, 1H), 5.04 (m, 1H), 4.39 (m, 2H), 4.21 (m, 1H), 3.18 (m, 2H), 1.58-1.29 (m, 15H) ppm. Compound 1a: ¹H NMR (D₂O, 500 MHz) 7.40 (m, 4H), 7.35 (m, 2H), 7.28 (m, 4H), 4.16 (m, 1H), 3.99 (m, 1H), 3.63 (m, 1H), 3.13 (m, 1H), 3.05-2.95 (m, 5H), 1.95 (m, 3H), 1.73 (m, 4H), 1.47 (m, 5H), 0.95 (m, 9H), 0.83 (m, 3H) ppm; ¹³C NMR (D₂O, 125 MHz) 18.53, 19.02, 21.47, 22.98, 24.54, 25.76, 26.84, 28.94, 30.67, 35.88, 36.20, 38.00, 54.95, 55.14, 58.41, 59.82, 60.87, 127.64, 127.76, 129.22, 129.29, 129.62, 136.54, 136.89, 162.92, 163.19, 168.81, 172.01, 172.38, 175.26 ppm; ES M⁺ 667.5. Compound 1b: ES M⁺ 816.6. Compound 1c: ES M⁺ 904.7. Compound 1d: ES M⁺ 989.6.