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

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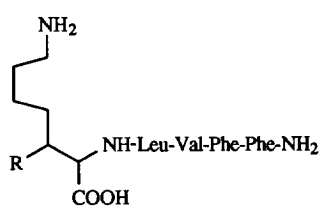
Abstract: α-Keto-β-aminoacids **5a-c** can be reductively aminated with the peptide sequence H_2N-Leu-Val-Phe-Phe on a solid support to afford *N*-carboxy alkyl peptides **1a-c**. The *N*-carboxy alkyl lysine derivative **7** was subsequently extended from the *N*-terminus with glutamine and histidine residues.

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:



1 a R= H
1 b R= NHCbz
1 c R= NHFmoc
1 d R= Ac-His-Gln

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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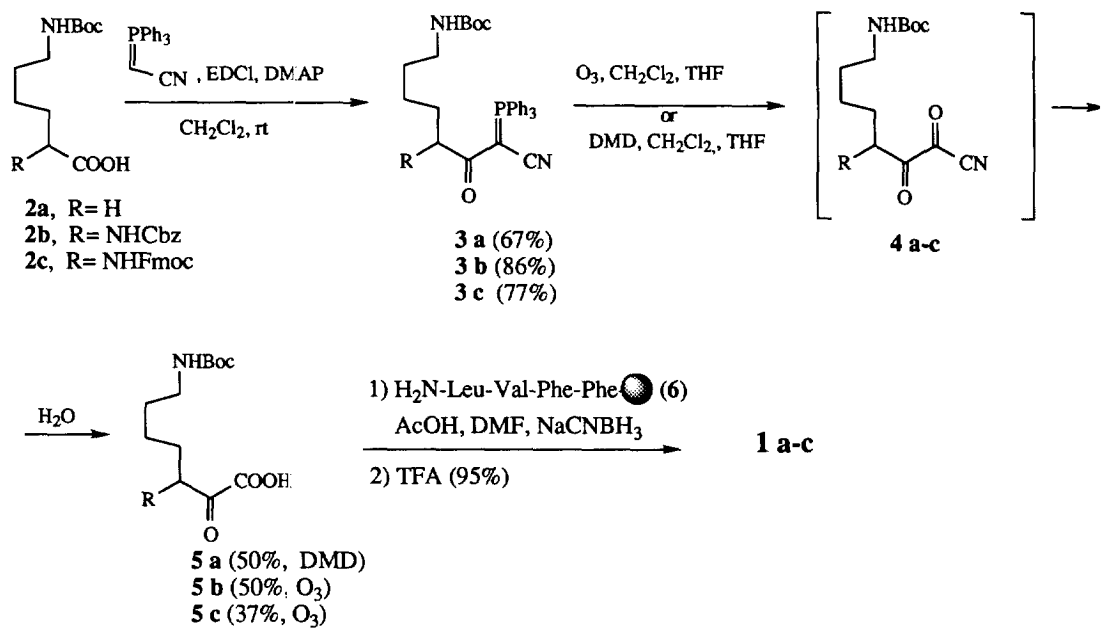
The direct conversion of carboxylic acids into keto ylides was carried out using the conditions described by Wasserman *et al.*.[4] The coupling reaction between carboxylic acids **2 a-c** and the (cyanomethylene)-triphenylphosphorane[5] in the presence of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI) 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 diketonitriles[6] **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 $NaCNBH_3$.

Scheme 1

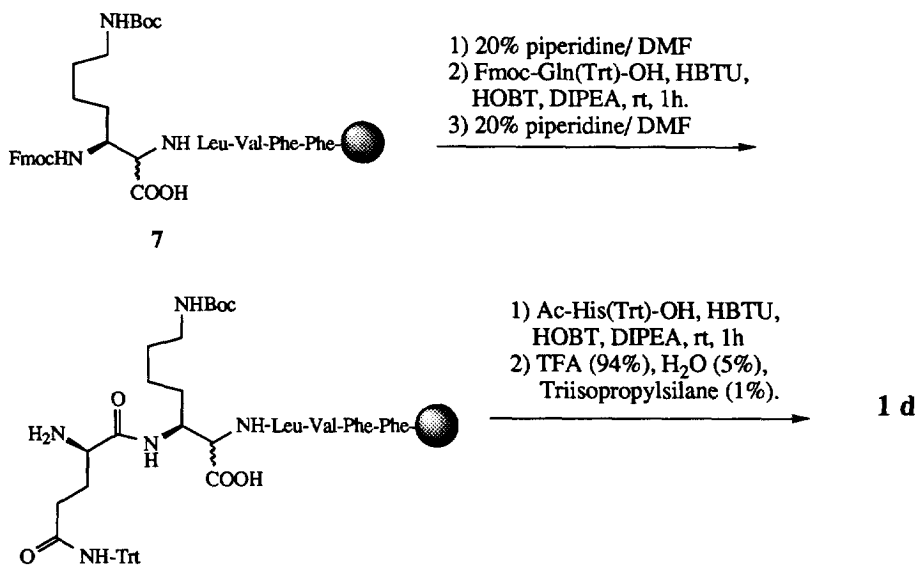


The progress of the reaction, which required the cleavage of small amounts of peptides from the support by treatment with TFA (95%), was monitored by HPLC (Bio-Rad RP318 reverse phase HPLC column, 5-70% MeCN/ 0.1% TFA over 20 min at 1mL/min). A significant difference in rate of the reductive amination 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%), H_2O (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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References and Notes:

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7. The (S)-(-)-methylbenzylamine salt of the acetal protected α-ketoacid **5c** was prepared and the proton NMR spectrum showed only one major diastereoisomer, indicating that no substantial epimerization occured during the reaction sequences.
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9. All compounds had spectral data consistent with the proposed structures.
Compound **3a**: ^{1}H NMR ($CDCl_3$, 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**: ^{1}H NMR ($CDCl_3$, 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**: ^{1}H NMR ($CDCl_3$, 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; ^{13}C NMR ($CDCl_3$, 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**: ^{1}H NMR ($CDCl_3$, 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; ^{13}C NMR ($CDCl_3$, 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**: ^{1}H NMR ($CDCl_3$, 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; ^{13}C NMR ($CDCl_3$, 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**: ^{1}H NMR ($CDCl_3$, 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**: ^{1}H NMR (D_2O, 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; ^{13}C NMR (D_2O, 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.