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Solid Phase Synthesis of Peptide *C*-Terminal Semicarbazones and Aldehydes

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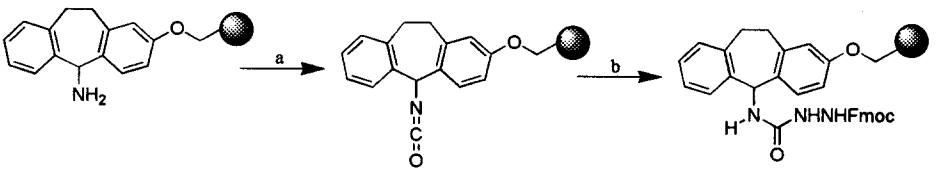
Abstract: A new linker based on the dibenzosuberyl system was developed in order to synthesise peptide *C*-terminal semicarbazones which can be readily converted into peptide *C*-terminal aldehydes. The method uses Fmoc-methodology and proceeds with no loss of stereochemical integrity.

Keywords: solid phase synthesis, peptide analogues/mimetics, enzyme inhibitors

Several examples of peptide aldehydes have been found to be potent inhibitors of enzymes including serine,[1-2] cysteine[3-4] and aspartyl[5-6] proteases and prohormone convertases.[7] Thus it is highly desirable to develop reliable routes to peptide *C*-terminal aldehydes which can also be used further in a wide range of chemistry including chemical ligation[8] and generation of reduced bond peptide isosteres.[9]

Although there are several methods for the synthesis of peptide aldehydes using SPPS,[10-18] we sought to extend the versatility of the dibenzocyclohept-1,4-diene (dibenzosuberyl) linker previously reported,[19] by introducing a semicarbazide moiety which would allow the synthesis and isolation of peptide *C*-terminal semicarbazones. Such derivatives are inherently more stable and easier to purify than the corresponding peptide aldehydes and, indeed, could have interesting biological properties. These peptide semicarbazones may be stored at 4°C until conversion into the peptide aldehyde is required.

The requisite semicarbazide linker can be synthesised from the corresponding amide linker in two simple steps (Figure 1) the course of which can be followed using IR and, in this way, routinely functionalities of 0.2-0.25 mmol/g (by UV determination of Fmoc) can be obtained.



a) DIEA (1eq), triphosgene (3eq), DCM, sonicate 1hour;
b) $FmocNHNH_2$† (2.5eq), DCM, sonicate 2 hours

Figure 1

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† Fmoc-hydrazine is prepared according to the method outlined in Zhang, Z. E.; Cao, Y. L.; Hearn, M. W. *Anal. Biochem.* **1991**, *195*, 160-170.

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The *C*-terminal residues, Fmoc-protected amino aldehydes, were derived from Fmoc-amino acids as previously reported.[20-21] These were loaded onto the linker, in the presence of DIEA, in good yields based on start and end functionalities (Table 1).

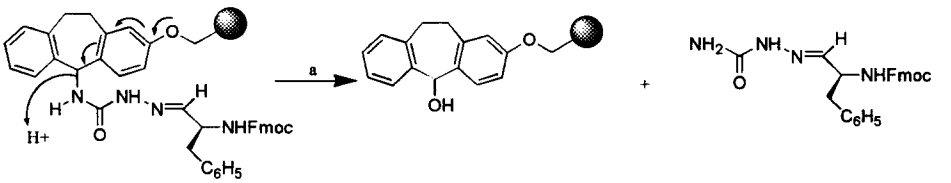
Compound	Loading Level (%)	Loading Time (Hours)
Fmoc-(L)Ala-H	100	5
Fmoc-(D)Ala-H	70	5
Fmoc-Phe-H	90	5
Fmoc-Trp-H	100	4
Fmoc-Asp(O^{t}Bu)-H	90	5

Table 1

To determine the extent, if any, of racemisation taking place under cleavage conditions (Figure 2), the stability of Fmoc-phenylalaninal semicarbazone to TFA treatment was checked (Table 2). It was proposed to use pyruvic acid exchange to convert the semicarbazones into aldehydes. This step was also examined for racemisation (Table 2). For each set of compounds identical tlc, MS, IR, ^{1}H and ^{13}C NMR were also obtained.

Compound	$[\alpha]_D$ (c g/100ml DMF)	Mpt (^{0}C)
Fmoc-Phenylalaninal Semicarbazone (Initially)	-24.7^0 (0.288)	144-145
Fmoc-Phenylalaninal Semicarbazone (TFA Treated)	-24.0^0 (0.325)	143-145
Fmoc-Phenylalaninal (Reduction of Weinreb Amide)	-43.3^0 (1.146)	100-102
Fmoc-Phenylalaninal (Pyruvic Acid Exchange)	-41.8^0 (0.467)	102-103

Table 2



a) TFA/H_2O (9:1), 1.5 hours

Figure 2

As a final check, the test peptide sequences Fmoc-Phe-Val-(L)Ala-H and Fmoc-Phe-Val-(D)Ala-H were synthesised. It has previously been reported[22] that racemisation in a peptide aldehyde, containing three residues, or more, will be indicated by more than one signal for the aldehydic protons in the NMR spectrum. For each of these peptides only one signal (at 9.50 ppm for the L-isomer and at 9.34 ppm for the D-isomer) was observed.

A series of test peptide semicarbazones have been prepared to assess the success of this method (Table 3) and these were subsequently converted to the corresponding peptide aldehydes (Table 4). Among the sequences prepared was an inhibitor of caspase 3[23-24] (final entry, Table 4).

Sequence[‡]	Yield[§]	Mass[¶] (Found)	Mass (Calc)	AAA (24 Hrs)
Fmoc-FV(L)A-sc	25	599.21 (MH^+)	599.71	$Phe_1$1.00, $Val_1$1.00
Fmoc-FV(D)A-sc	24	599.37 (MH^+)	599.71	$Phe_1$0.91, $Val_1$1.09
Fmoc-GAKGF-sc	40	763.46 $(M-H)Na^+$	763.83	$Gly_2$1.86, $Ala_1$1.09, $Lys_1$0.98
Fmoc-HLDIIW-sc	27	1082.04 (MNa^+)	1082.23	$Asp_1$1.03, $Ile_2$0.96, $Leu_1$1.09, $His_1$0.88
Ac-AAVALLPAVL LALLAPDEVD-sc	29	2079.52 (MNa^+)	2079.41	$Asp_1$1.14, $Glu_1$1.02, $Pro_2$1.92, $Ala_6$5.68, $Val_3$2.80, $Leu_6$5.99

Table 3

Sequence	Yield[^]	Mass[¶] (Found)	Mass (Calc)	AAA (24Hrs)	NMR[†]
Fmoc-FV(L)A-H	50	542.61 (MH^+)	542.65	$Phe_1$1.16, $Val_1$0.86	9.50 ppm
Fmoc-FV(D)A-H	56	654.29 ($M^+CF_3CO_2^-$)	654.66	$Phe_1$1.13, $Val_1$0.87	9.34 ppm
Fmoc-GAKGF-H	62	761.74 $(M-H)K_2^+$	761.98	$Gly_2$1.86, $Ala_1$1.10, $Lys_1$0.97	9.34 ppm
Fmoc-HLDIIW-H	38	779.66 (MH^+-Fmoc)	779.94	$Asp_1$1.04, $Ile_2$0.96, $Leu_1$1.09, $His_1$0.88	9.29 ppm
Ac-AAVALLPAVL LALLAPDEVD-H	50	1998.16 $(M-H)^-$	1998.35	$Asp_1$1.07, $Glu_1$1.05, $Pro_2$1.90, $Ala_6$5.61, $Val_3$3.10, $Leu_6$5.80	9.36 ppm

Table 4

In conclusion, this methodology is indeed very effective in producing peptide *C*-terminal semicarbazones and aldehydes with no epimerisation occurring at the *C*-terminal chiral centre.

[‡] The suffix -sc has been adopted to indicate that the sequence is the semicarbazone of the *C*-terminal aldehyde.
[§] Yield quoted is based on theoretical maximum based on Fmoc-loading on completion of the sequence and is calculated for isolated product after purification by preparative HPLC.
[¶] All masses were determined using a Perseptive Biosystems Voyager™ MALDI-TOF mass spectrometer.
[^] Yield quoted is for isolated product after purification by preparative HPLC.
[†] The signal quoted is that for the aldehydic proton and was the only signal observed in that region.

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