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ALDEHYDE COMPONENTS FOR USE IN FOUR-COMPONENT CONDENSATION ("4CC") UGI REACTION PEPTIDE SYNTHESIS

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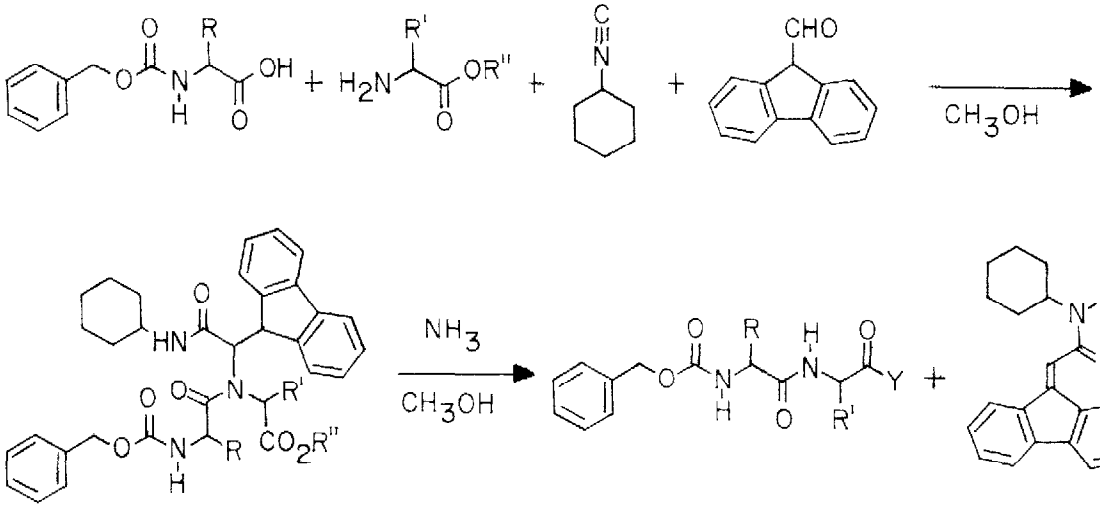
Abstract: The use of 9-formylfluorene as the aldehyde component in four-component condensation (4CC) fragment strategy peptide synthesis has been investigated in the synthesis of model dipeptides.

Four-component condensation peptide synthesis, developed by Ugi et al.,[1-7] has been used for the one-step construction of tripeptide derivatives (4CC synthesis) and for the coupling of two peptide fragments (4CC fragment condensation). Recently, Waki and Meienhofer have reported the synthesis of several di- and tetrapeptides utilizing 4CC fragment condensation strategy.[8] Their investigation explored the use of several aldehyde components in the condensation, and the reaction conditions required for subsequent cleavage of the product amide N-auxiliary substituent. We report our results utilizing 9-formylfluorene as the aldehyde component in 4CC fragment condensation peptide synthesis.

We had anticipated that the base lability of 9-heteroatom methyl substituted derivatives of fluorene[9] would afford a new and useful method for the necessary substituent cleavage step in 4CC fragment condensation peptide synthesis. Our expectations have been borne out. The reaction of an amino-protected amino acid, a carboxyl-protected amino acid, cyclohexylisonitrile and 9-formylfluorene[10] in methanol provided condensation product 1 in moderate to good yield (Table I.).

In a representative condensation reaction, a solution of 2.0 mmol of the amino-protected amino acid and 2.0 mmol of the carboxyl-protected amino acid in 2.0 mL of methanol was treated with equimolar quantities of cyclohexylisonitrile and 9-formylfluorene. The reaction mixture was stirred at room temperature for a period of 16-20 hr. The reaction solvent was evaporated at reduced pressure and the evaporation residue was dissolved in 25 mL of chloro-



1

2

3

1a	R=H	R'=H	R''=CH_3	2a	R=H	R'=H	Y=NH_2
1b	R=H	R'=H	R''=C_2H_5	2b	R=H	R'=H	Y=NH_2
1c	R=H	R'=H	R''=$C(CH_3)_3$	2c	R=H	R'=H	Y=$OC(CH_3)_3$
1d	R=$CH_2\phi$	R'=H	R''=$C(CH_3)_3$	2d	R=$CH_2\phi$	R'=H	Y=$OC(CH_3)_3$

form. The solution was washed with an equal volume each of 0.5M aqueous sodium bicarbonate, 0.5M aqueous citric acid, and distilled water. The organic layer was dried over anhydrous magnesium sulfate and filtered. The filtrate was concentrated under reduced pressure to obtain 1. Final compound purification was accomplished by recrystallization from an appropriate solvent, or by silica gel chromatography. Isolated condensation products were homogeneous as judged by silica gel thin layer chromatography and were characterized by spectroscopic methods.[11]

Removal of the N-(α-cyclohexylcarbamoyl-9-fluorenylmethyl) substituent from 1 was accomplished in excellent yield with ammonia. Reaction of 1 in refluxing ammonia afforded only partial cleavage even after a period of 12 hr. However, when 1 was treated at room temperature with methanol saturated with anhydrous ammonia, the cleavage reaction was complete in 1.5 hr as determined by thin layer chromatographic analysis. Treatment of 1a or 1b with ammonia in methanol at room temperature or with refluxing liquid ammonia, resulted in ammonolysis of the unhindered alkyl ester to provide the corresponding peptide amide, 2a or 2b. Compounds 1c and 1d, peptide tert-butyl esters, did not undergo ammonolysis on reaction with ammonia in methanol. Removal of the auxiliary substituent from 1c and 1d proceeded in excellent yield to provide,

Table I.

Compound No.	% Yield[a]	M.P.[b]	R_f[c]
1a	63	118-120	0.33 (S_1)
1b	46	112-114	0.38 (S_1)
1c	52	—	0.44 (S_1)
1d	47	—	0.56 (S_1)
2a (2b)	82	84-86	0.05 (S_2)
2c	88	—	0.36 (S_2)
2d	87	101-104	0.55 (S_2)
3	80-91	198-200	0.66 (S_1)

[a]Isolated yields. [b]Uncorrected. [c]Thin layer chromatography on E. Merck silica gel $60F_{254}$ using dichloromethane-methanol mixtures of 99:1 (S_1) or 98:2 (S_2).

respectively, 2c and 2d. The N-(α-cyclohexylcarbamoyl-9-fluorenylmethyl) substituent of 1 was unaffected by treatment with trifluoroacetic acid over a period of 90 min.

Ammonia reaction products, 2 and 3, were isolated by preparative thin layer chromatography on silica gel and were indistinguishable from authentic samples prepared by routine alternative procedures. Isolated product yields are shown in Table I.

The choice of an aldehyde component for use in 4CC fragment condensation peptide synthesis determines the efficiency of the condensation step and the method required for cleavage of the product amide N-auxiliary substituent. From previous work,[1,8,12] the most promising aldehyde component appeared to be 1-tert-butyloxycarbonyl-3-formylindole affording condensation yields of 50-60% and auxiliary substituent cleavage yields of 70-75%. Removal of the N-auxiliary substituent was accomplished by two successive treatments with trifluoroacetic acid. The procedure described herein utilizing 9-formylfluorene as the aldehyde component, offers comparable condensation step efficiency and a means of auxiliary substituent cleavage which is orthogonal to methods required for removal of common peptide blocking groups, i.e. benzyloxycarbonyl-, tert-butyl

oxycarbonyl- and tert-butyl ester, and 2-(*p*-biphenyl)-*isopropyloxycarbonyl*-. The investigation of 9-formylfluorene as an aldehyde component in 4CC fragment condensation strategy peptide synthesis will continue.

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