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PEPTIDE SYNTHESIS BY A COMBINATION OF SOLID-PHASE AND SOLUTION METHODS IV

MINIMUM-RACEMIZATION COUPLING OF N_α-9-FLUORENYLMETHYLOXYCARBONYL AMINO ACIDS TO ALKOXY BENZYL ALCOHOL TYPE RESINS[1)]

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SUMMARY

The cesium salts of N_α-9-fluorenylmethyloxycarbonyl (Fmoc) amino acids couple smoothly to new chloro derivatives of alkoxy benzyl alcohol resins. While high loading is attained under mild reaction conditions racemization can be suppressed even with Cys and His derivatives.

The attachment of the first amino acid to the resin is one of the crucial steps in solid-phase peptide synthesis. If brought about by esterification to a resin bearing hydroxymethyl groups it can be accompanied by racemization[2)] and/or dipeptide formation[3a)]. As an alternative, carboxylate anions of N_α-protected amino acid derivatives can be coupled to a resin bearing halomethyl groups by alkylation. This method avoids carboxyl activation and concomitant racemization.

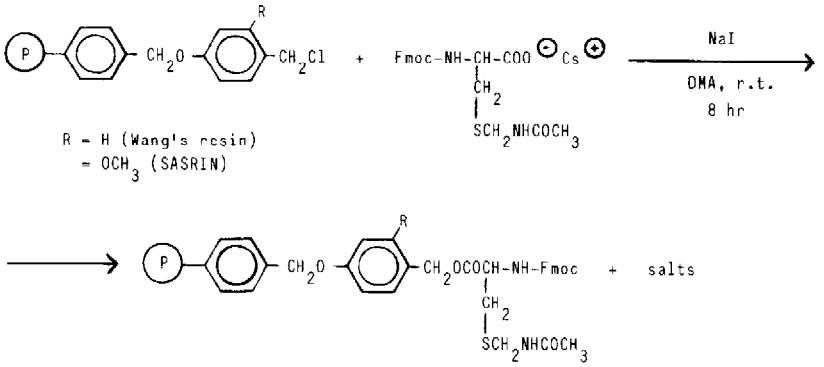
Thus, cesium salts of N_α-protected amino acids react smoothly with chloromethylated polystyrene in very polar solvents, although these solvents do not properly swell the resin [4)]. Excellent coupling results have also been reported with potassium fluoride as base in dimethylformamide (DMF) solution[5)].

These alkylations actually proceed with negligible racemization[6)]. In the preceding paper[1)] we described a method to convert alkoxybenzyl alcohol resins into their halides. In still another paper[7)] we have examined the esterification of N_α-Fmoc amino acids to 2-methoxy-4-alkoxybenzyl alcohol resin (SASRIN). Applying optimized conditions racemization could be kept low in most cases. The present study bears on those derivatives which had resisted our efforts to suppress racemization. Significant improvements could be attained by alkylating cesium salts with the resin halides mentioned above.

The alkylative attachment of N_α-t-butyloxycarbonyl amino acids to solid-phase supports is a well-established procedure[4),5),6a)], however only rarely used with Fmoc amino acids[6b)].

The base-lability of the Fmoc group is a crucial factor in these reactions since catalytic amounts of base are sufficient for complete removal of this protecting group. The alkylation medium therefore has to be kept neutral. Dimethylacetamide (DMA) turned out to be the solvent of choice[3b].

Scheme I exemplifies alkylation employing optimized conditions[8]:



SCHEME I

Complete displacement of chlorine and no detectable Fmoc-cleavage were observed.

The Fmoc-amino acid resins obtained were analyzed for % D-enantiomer content[10].

A comparison of the results obtained by either esterification or alkylation is presented in table I.

Table I Attachment of Fmoc amino acids to SASRIN[14]: alkylation vs. acylation

Fmoc-AA	% D-Enantiomer formation		Fmoc-AA	% D-Enantiomer formation	
	Alkylation[a]	Acylation[b]		Alkylation[a]	Acylation[b]
Asn(Dod)	0.3	1.3	Cys(Acm)[13]	0.5	4.0[9]
Asn(Tmob)	0.2	1.4	Cys(Acm)**	0.6	
Arg(Pmc)[15]	< 0.1	0.3	Cys(tBu)	0.3	4.7
Gln(Tmob)	0.7	0.8	Cys(Trt)	2.5	18.3
Ile	0.1*	1.1*	His(Trt)	0.4	26.0[11]
Ser(tBu)	< 0.1	0.3			

Conditions a) 1.5 to 3 eq. Cs-salt in DMA, NaI (1 eq.), r.t., 24 hr

b) 1.2 - 1.5 eq. Fmoc-AA-OH, CH_2Cl_2/DMF (3:1), 1.1 - 1.2 eq. dicyclohexyl carbodiimide 0.01 - 0.1 eq. 4-dimethylaminopyridine, -20 to 0° C, 20 hr, capping with benzoyl chloride/pyridine

abbreviations used:

Dod: 4,4'-dimethoxybenzhydryl, Tmob: 2,4,6-trimethoxybenzyl, Pmc: 2,2,5,7,8-pentamethylchromane-6-sulfonyl, Acm: acetamidomethyl, tBu: tert. butyl, Trt: triphenylmethyl (trityl)

*: formation of D-allo isomer; **: to SASRIN bromide

Sodium iodide exerts a strong accelerating effect. The rate of the system Cs-salt/NaI/SASRIN chloride compares well with the rate of Cs-salt/SASRIN bromide[12]. As to be expected Wang's resin halides react more slowly than SASRIN halides[14]. Furthermore the rate is somewhat diminished by bulky protecting groups, e.g. trityl. No differences in purity regardless of the coupling method were observed in Fmoc amino acids cleaved from the resin (TLC, HPLC)[9]. In addition, we also examined the purity of cleaved Fmoc amino acids as a function of the resin type and corresponding cleavage conditions. The purities (HPLC) of amino acids thus obtained are listed in table II:

Table II Purities of Fmoc amino acids cleaved from alkoxybenzylalcohol type resins, determined by HPLC[a)]

	purity (area %)		
Fmoc-AA	educt	SASRIN[b)]	Wang[c)]
Cys(Acm)	99.3	96.5	67.0
Cys(StBu)	99.5	97.9	80.5
Cys(tBu)	99.6	99.6	94.2
Cys(Trt)	99.0	94.1	78.0
His(Trt)	99.5	96.8	→ Fmoc-His-OH
Arg(Pmc)	97.5	97.2	n.d.
Ser(tBu)	99.7	99.5	→ Fmoc-Ser-OH

a) column: Lichrosorb RP-18, (4.6 x 250 mm, 10 µm); eluent: buffer A: H_2O + 0.1 % TFA, buffer B: CH_3CN + 0.1 % TFA; flow: 1 ml/min; gradient: 5 % B - 30' - 100 % (10') or 50 % B - 20' - 100 % (10'); detection: at 260 nm

b) cleavage: 1 % CF_3COOH/CH_2Cl_2

c) cleavage: 50 % CF_3COOH/CH_2Cl_2, treatment of free Fmoc-amino acid derivatives under analogous conditions results in isolation of substances of considerably higher purity.

It appears from the above that Cys derivatives cleaved from SASRIN generally are purer than those cleaved from Wang's resin. Fully protected fragments with protected SH-functions are useful derivatives for oxidative cyclizations. The high purity of the Fmoc-Cys-derivatives cleaved from SASRIN is an excellent prerequisite towards successful synthesis of Cys-containing peptides.

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Literature references and remarks

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12) Comparable results have been obtained with SASRIN mesylate/NaI

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14) Alkylation of Wang's resin: Fmoc-Cys(Acm) 1.0 % D, Fmoc-Cys(tBu) 1.0 % D
Fmoc-His(Trt) 0.6 % D, Fmoc-Cys(Trt) ~20 % D

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