62. Lithium-Salt Effects in Peptide Synthesis

Part II

Improvement of Degree of Resin Swelling and of Efficiency of Coupling in Solid-Phase Synthesis

by **Adrian Thaler**[1]) and **Dieter Seebach***

Laboratorium für organische Chemie der Eidgenössischen Technischen Hochschule, ETH-Zentrum, Universitätstr. 16, CH–8092 Zürich

and **Francis Cardinaux**

Präklinische Forschung, *Sandoz Pharma AG*, CH–4002 Basel

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The course of solid-phase peptide-coupling reactions as well as the swelling properties of a peptide-resin are influenced by the addition of inorganic salts (LiCl, LiBr, $LiClO_4$, KSCN). Used as additives, these salts can *i*) improve coupling yields (*e.g.*, for Fmoc-$(Ala)_5$-Phe-resin→ Fmoc-$(Ala)_6$-Phe-resin in DMF/CH_2Cl_2 1:1 from 89.4 to 97.1% (for poly(ethylene oxide) on polystyrene (= PEO-PS) resin) or from 77.5 to 93.8% (for poly-(*N, N'*-dimethylacrylamide) on 'Kieselgur' (= PDMAA-KG) resin) without and with 0.4M LiCl, respectively), *ii*) increase resin swelling (*e.g.* for Fmoc-$(Ala)_5$-Phe-(polystyrene resin) from 2.42- to 5.71-fold in 1-methylpyrrolidin-2-one (= NMP) without and with LiCl, respectively), and *iii*) change coupling rates. Examples of coupling reactions and swelling behaviour (degree *and* rate) in different solvents (DMF, DMF/CH_2Cl_2 1:1, THF, NMP, *N,N*-dimethylpropyleneurea (= DMPU) with and without salts) using different resins (polystyrene (PS); PEO-PS, and PDMAA-KG) and an improved analysis of alanine oligomers up to Ala_{12}-Phe by HPLC and FAB-MS are reported.

Introduction. – The equivalent to the problem of poor solubility of peptide intermediates in solution synthesis is insufficient solvation of peptide chains in solid-phase peptide synthesis[2]). Its symptoms are a sharp decrease in reactivity towards coupling reagents and often a macroscopic change in the swelling properties of the peptide-resin [3–8]. It is frequently observed at a length of between five and about twelve amino acid residues and has been associated with the onset of intra- or interchain aggregation and the concomitant decrease of solvation of the peptide chains on the resin support. Another important factor in solid-phase peptide synthesis is the extent of peptide-resin swelling in the reaction medium; only solvents which are able to swell the peptide-resin sufficiently will allow for rapid and complete coupling reactions [9].

Especially hydrophobic amino acids such as alanine, valine, or isoleucine in oligopeptides show a transition from random coil to β-structure for chain lengths between five and *ca.* twelve in most organic solvents and in H_2O [8] [10]. α-Helix-forming peptides such as $[L\text{-Glu(OBzl)}]_n$, $[L\text{-Lys(Z)}]_n$, and $[L\text{-Met}]_n$ also show a decrease in solubility for chain lengths $n \geq 7$ caused by the transition from random coil to β-structure, whereas for $n > 11$ a transition of β-structure to α-helix is observed [10]. Some of these oligopeptides were also studied attached to poly(ethylene glycol) where similar transitions were observed [11]. Additional aggregation may occur in regions of

[1]) Part of the projected Ph. D. thesis of *A. T.*, ETH Zürich.

[2]) For reviews addressing the problem of solvation in solid-phase peptide synthesis, see [1] [2].

peptides containing apolar side-chain protecting groups; this may lead to 'collapsed structures' [12]. NMR investigations [2] [13] lead to the conclusion that the peptide chains on cross-linked polystyrene (PS) support are as accessible as free in solution. This must not be true for other supports such as poly(*N,N*-dimethylacrylamide) on 'Kieselgur' (PDMAA-KG), poly(ethylene oxide) on polystyrene (PEO-PS; 'graft polymer') or poly(ethylene glycol) alone ('liquid-phase method' [14]). There are examples in which poor solvation has been overcome by using dimethylformamide (DMF) [15], 1-methylpyrrolidin-2-one (NMP) [16], dimethylacetamide (DMA) [17], or CF_3CH_2OH [18]. Recent studies show efficient β-sheet disruption by the use of hexafluoropropan-2-ol, dimethyl sulfoxide (DMSO), hexamethylphosphoramide (HMPA), and trimethyl phosphate [19]. The addition of soluble ureas may also disrupt intermolecular aggregation, as shown by IR absorption [20]. The swelling of a peptide-resin in a given solvent depends upon the peptide/resin ratio; apolar solvents such as CH_2Cl_2 will swell a polystyrene resin better than polar solvents because of hydrophobic interactions, whereas the peptide chain prefers polar solvents [21–23]; both effects determine the extent of swelling. Maximum swelling can, therefore, sometimes only be obtained by the use of mixed solvents [23]. However, the favourable influence of a highly solvated growing resin bound peptide chain on the free energy always enhances swelling of the cross-linked resin and *vice versa* [2]. It is thought that maximum swelling of the interpenetrating polymer network helps minimize the formation of intermolecular peptide chain aggregates [6]. Such aggregates can immobilize the polystyrene matrix [13c] or even lead to a resin shrinkage (by a *de facto* increase of resin cross-linking).

Previously, we have shown that Li-salts very much enhance the solubility of peptides in certain solvents [24], and that they can change the conformation of a peptide by complexation [25]. The question, therefore, arose whether this 'Li-effect' can also be used to enhance solvation of peptides on polymeric supports, and thus improve reactivity in solid-phase synthesis.

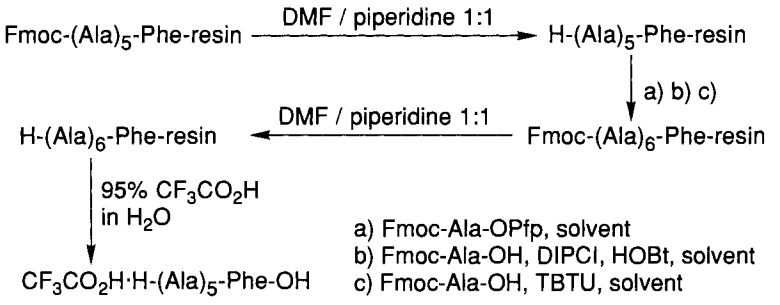
As a model for this investigation, we chose the synthesis of $(Ala)_nPhe$, n ranging from 5 to 12. $(Ala)_n$ is known to adopt a β-structure at $n \geqslant 5$ even in a CF_3CH_2OH solution [10] [26]. Coupling efficiency decreases during the synthesis of $(Ala)_n$-Val on a solid support, and this is attributed to the onset of β-structure formation of the growing peptide chain [7] [8]. Chemical analysis is simplified in this model in that deletions or truncations at any position in the sequence will all lead to the same series of products. Phenylalanine served as an internal standard in amino-acid analysis and as a chromophoric group to facilitate HPLC analysis. Reaction conditions which would effectively prevent ordered conformations or intermolecular aggregation during the synthesis are expected to yield a more homogeneous, monodisperse product. By increasing the size of the target sequence up to the 12-mer, we expected to assess the efficiency of any modification in increasingly difficult coupling steps.

The polymeric support, conceivably, may also take part in the interactions between peptide, solvent, and an additive, *e.g.* Li-salts, and may contribute to the solvation effect. We, therefore, compared chemically different supports in our model syntheses: a polystyrene (PS) support [27], a poly(ethylene oxide) on polystyrene (PEO-PS) support [28], and a poly(*N,N*-dimethylacrylamide) on 'Kieselgur' (PDMAA-KG) support [29] (*Scheme*). PS is still the most widely used support for solid-phase peptide synthesis. The PDMAA support is chemically similar to the preferred polar solvent in solid-phase peptide synthesis, *i.e.* DMF, and to the peptide backbone, while the PEO support may possibly substitute in a way for the ether solvents in which the Li-effects on peptide solubility were first noted [24]. Both of the latter supports are beeing utilized in the more recent 'continuous-flow' variant of solid-phase peptide synthesis ([30] and [31], respectively). For our model syntheses, we selected coupling reactions which were shown to be tolerant to the addition of Li-salt and other salt additives[3]), notably the active-ester,

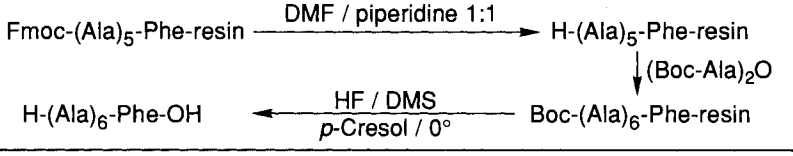
[3]) For Li-salt effects on peptide reactions in solution, see the accompanying paper [32].

Scheme. *Solid-Phase Synthesis of $CF_3CO_2H \cdot H\text{-}(Ala)_6\text{-}Phe\text{-}OH$ Using Different Resins*

Conditions for Resin **A, C,** *and* **D**:

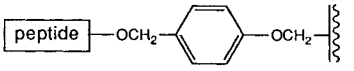


Conditions for Resin **B**:



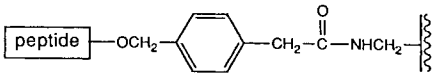
PS Resin **A**[a]):

poly(styrene/1% divinylbenzene) (PS/1%DVB)



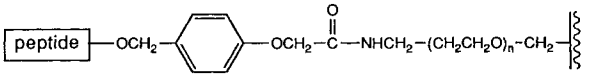
PS Resin **B**[b]):

PS/1%DVB



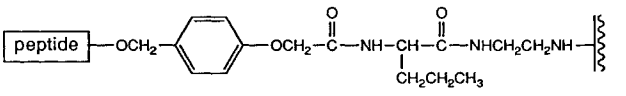
PEO-PS Resin **C**:

poly(ethylene oxide) on (PS/1%DVB)



PDMAA-KG Resin **D**:

poly(*N*,*N*-dimethylacrylamide) on 'Kieselgur'



[a]) [4-(Oxymethyl)phenyloxy]methyl anchor.

[b]) {[4-(Oxymethyl)phenyl]acetamido}methyl anchor.

symmetrical anhydride, diisopropylcarbodiimide (DIPCI)/1-hydroxy-1*H*-benzotriazole (HOBt) [33], and 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium-tetrafluoroborate (TBTU) method [34].

Here, we report on the effects of Li-salts and other salt additives on the solid-phase synthesis of the model peptide, $(Ala)_n$-Phe ($n = 6$–12), on three chemically different polymeric supports.

Results. – In a first series of experiments, we synthesized $CF_3COOH \cdot H\text{-}(Ala)_{12}\text{-}Phe\text{-}OH$ starting from Fmoc-$(Ala)_5$-Phe-(PS resin[4])[5]) and using Fmoc-Ala-OPfp[6]) for coupling[7]). The HPLC showing the separation of oligomers of $CF_3COOH \cdot H\text{-}(Ala)_n\text{-}Phe\text{-}OH$ ($n = 5$–12) obtained from this synthesis is presented in *Fig. 1*. Severe problems must

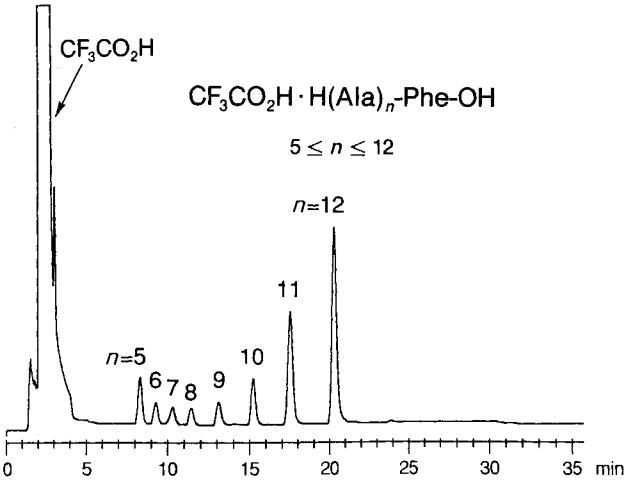


Fig. 1. *HPLC of $CF_3CO_2H \cdot H\text{-}(Ala)_n\text{-}Phe\text{-}OH$* ($5 \leq n \leq 12$). Column: *LiChrosorb 60 RP-8 select B* (4.5 × 250) 10 μm; UV detection at 205 nm; flow 1.5 ml · min^{-1}; gradient: 0–20% *B* in CF_3CO_2H within 25 min; solvent *A*: H_2O/MeCN/H_3PO_4(85%)/Me_4NOH(10%) 900:100:2:20; solvent *B*: H_2O/MeCN/H_3PO_4(85%)/Me_4NOH (10%) 300:700:2:20.

have occurred during elongation of $(Ala)_5$-Phe-resin to $(Ala)_6$-Phe-resin, since $(Ala)_5$-Phe was still present in the product after seven consecutive coupling steps! Addition of Li-salts to the coupling reagent did not significantly improve the product distribution (results not shown). The product mixture was insoluble in all solvents tested, therefore, samples for HPLC analysis and fast-atom-bombardment (FAB) mass spectral analysis (see *Fig. 2*) were dissolved in CF_3COOH.

In further experiments, we focused on the elongation of resin-bound $(Ala)_5$-Phe to $(Ala)_6$-Phe, since a major change in reactivity at this step was evident. The experiments

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[4]) [4-(Oxymethyl)phenyloxy]methyl was used as an anchoring group, see **A** in the *Scheme*.

[5]) Fmoc = [(9*H*-fluoren-9-yl)methoxy]carbonyl.

[6]) Pfp = pentafluorophenyl.

[7]) Fmoc-$(Ala)_5$-Phe-resins as starting materials were prepared on an automated solid-phase peptide synthesizer (PS resin) or a custom-made apparatus for continuous-flow peptide synthesis (PEO-PS resin and PDMAA-KG resin).

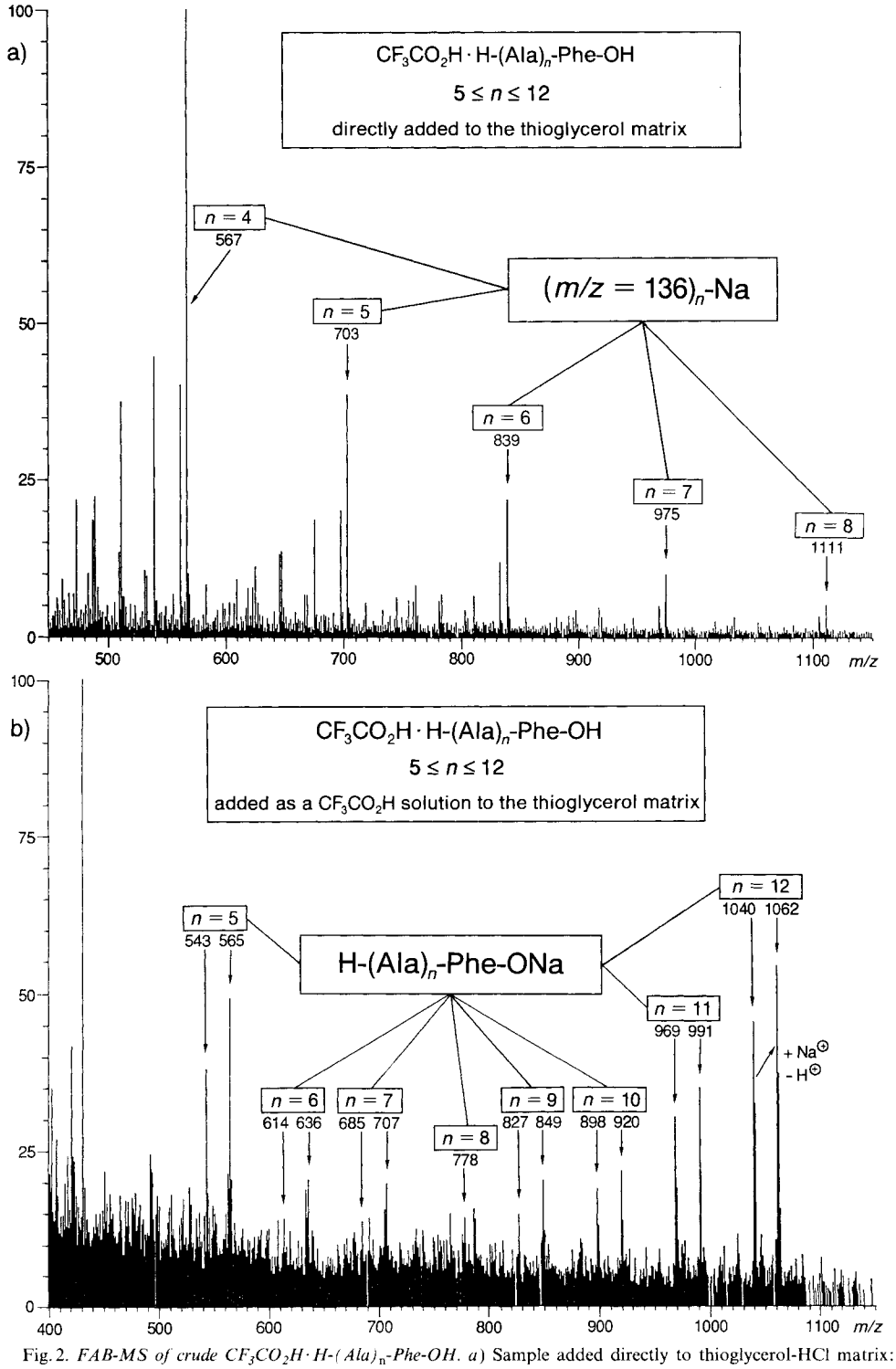


Fig. 2. *FAB-MS of crude $CF_3CO_2H \cdot H\text{-}(Ala)_n\text{-}Phe\text{-}OH$. a)* Sample added directly to thioglycerol-HCl matrix: appearance of these signals depends on the batch of resin (*Bachem*) used, possibly due to an unknown impurity in the anchoring group. *b)* Sample dissolved in neat CF_3CO_2H prior to addition to thioglycerol-HCl matrix: signals correspond to peptide oligomers ($m/z + 1$ (H^+) and $m/z + 23$ (Na^+) series are observed).

were performed using a *RaMPS*[8]) apparatus for multiple syntheses, in which up to 25 different reaction conditions could be tested in parallel syntheses in the same run.

Coupling the next Ala residue to H-$(Ala)_5$-Phe-(PS resin)[4]) (*Scheme 1*) was incomplete for all combinations of activation method, solvent, and additive tested (*Table 1*). This was quite different from the essentially complete coupling observed under standard conditions in the previous steps up to $(Ala)_5$-Phe resin (results not shown). LiCl doubled the coupling yield in 1-methylpyrrolidin-2-one (NMP) to 44% conversion at most. In te-

Table 1. *Coupling of Fmoc-Ala to H-$(Ala)_5$-Phe-(PS- resin*[a])*) by Different Coupling Methods*

Solvent	Method	Added salt[b])	Conversion [%][c])
DMF	Pfp ester	none	38.6
		LiCl	35.2
		$LiClO_4$	42.4
	TBTU reagent	none	47.1
		LiCl	43.0
	DIPCI/HOBt	none	43.1
		LiBr	31.2
DMF/CH_2Cl_2 1:1	Pfp ester	none	36.3
		LiCl	39.9
		LiBr	36.0
		KSCN	42.6
		LiClO4	37.2
THF		none	44.3
		LiCl	27.5
NMP		none	25.8
		LiCl	43.5
		$LiClO_4$	35.5
		KSCN	30.4

[a]) [(4-Oxymethyl)phenyloxy]methyl on poly(styrene/1% divinylbenzene) (*Wang* resin), see **A** in *Scheme*.
[b]) All salt solutions used for pre-equilibration of the peptide resin and for coupling were 0.4M.
[c]) Measured by HPLC (area at 205 nm) after deprotection and cleavage from the resin.

trahydrofuran (THF), the addition of Li-salts decreased coupling yields. Fmoc-$(Ala)_5$-Phe-(PS resin)[4]) swelled much less in DMF and NMP than Fmoc-$(Ala)_4$-Phe-(PS resin)[4]) (*Table 2*). Addition of a further Ala residue to give Fmoc-$(Ala)_6$-Phe-(PS resin)[4]) had a much smaller effect on the swelling behaviour. No effect of peptide length on swelling was observed in THF. Addition of 0.4M LiCl increased the swelling of all peptide resins in NMP: 1.2-fold for Fmoc-$(Ala)_4$-Phe-resin; 2.4-fold for Fmoc-$(Ala)_5$-Phe-resin, and 2.6-fold for Fmoc-$(Ala)_6$-Phe-resin to a record swelling volume of 5.71 relative to the dry state for Fmoc-$(Ala)_5$-Phe-resin. In DMF of THF, there was hardly any salt effect on the swelling behaviour.

In the course of these experiments, we also noticed different kinetics of peptide-resin swelling (see *Fig. 3*). For Fmoc-$(Ala)_4$-Phe-(PS resin), roughly the same time was required to maximally swell the peptide resin (*ca.* 60 min) in the solvents tested. For Fmoc-$(Ala)_5$-Phe-(PS resin), the initial swelling *rate* decreased in the following order: THF > DMF ≈ 0.4M LiCl/NMP > NMP. The rate in pure NMP was much lower for the Fmoc-$(Ala)_5$-

[8]) Trademark of *Du Pont de Nemours*.

Table 2. *Swelling of Fmoc-(Ala)$_n$-Phe-(PS resin[4]))*, n = 4–6[a])

n	Solvent	Added salt[b])	Swelling [*x*-fold][c])	Standard deviation
4	DMF	none	3.65	0.11
		LiCl	3.97	0.06
	THF	none	2.11	0.07
		LiCl	2.33	0.04
	NMP	none	4.46	0.05
		LiCl	5.23	0.07
5	DMF	none	1.92	0.07
		LiCl	1.98	0.04
	THF	none	2.15	0.02
		LiCl	2.20	0.02
	NMP	none	2.42	0.06
		LiCl	5.71	0.12
6	DMF	none	1.81	0.06
		LiCl	1.72	0.04
	THF	none	2.02	0.01
		LiCl	2.13	0.09
	NMP	none	2.09	0.03
		LiCl	5.46	0.05

[a]) The material tested as $n = 6$ was a mixture of 31% of Fmoc-(Ala)$_5$-Phe-(PS resin) and 69% Fmoc-(Ala)$_6$-Phe-(PS resin).

[b]) 0.4M of LiCl, salt dissolved prior to swelling experiment.

[c]) Dry peptide-resin, volume = 1; measurements in triplicate.

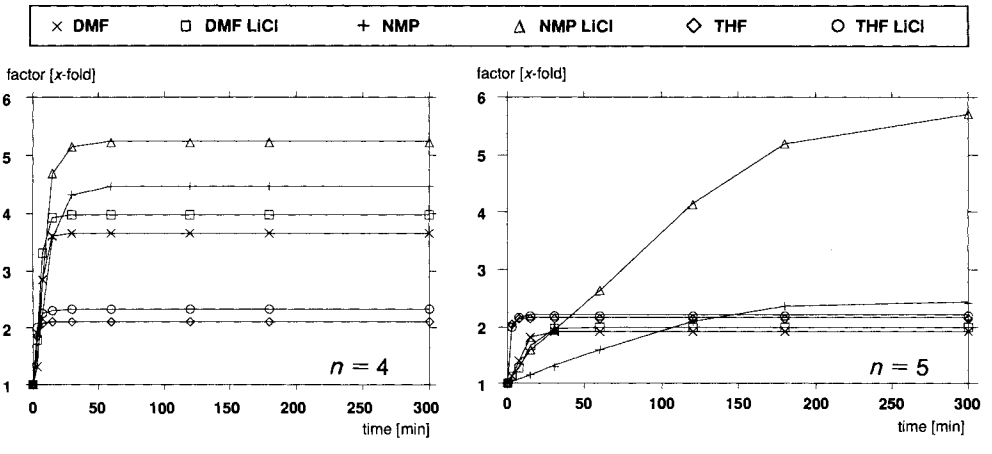


Fig. 3. *Rates of swelling of Fmoc-(Ala)$_5$-Phe-(PS resin[4]))* for n = 4 and n = 5 *in THF, DMF, and NMP with and without 0.4M LiCl*

Phe-resin than for the Fmoc-(Ala)$_4$-Phe-resin. However, 0.4M LiCl in NMP was the only solvent in which Fmoc-(Ala)$_5$-Phe-resin ultimately swelled to the same volume as Fmoc-(Ala)$_4$-Phe-resin. Three to four hours were needed for the Fmoc-(Ala)$_5$-Phe-resin to reach maximal swelling in this solvent. The Fmoc-(Ala)$_6$-Phe-resin behaved similarly to the Fmoc-(Ala)$_5$-Phe-resin in swelling experiments, showing an additional but comparingly small decrease in swelling volume (*Table 2*) and rate (results not shown).

A few experiments were done with (*tert*-butoxy)carbonyl (Boc) instead of Fmoc protection using a different anchoring group[9]) on a PS support (see **B**) and symmetrical anhydrides for coupling (*Scheme*). Coupling yields were generally higher in these experiments possibly due to the use of a different coupling method, and were increased by salt additives but remained unsatisfactory (*Table 3*). LiCl increased swelling of the starting peptide-resin 1.20-fold in the solvent mixture DMF/CH_2Cl_2 1:1.

Table 3. *Coupling of (Boc-Ala)$_2$O to H-(Ala)$_5$-Phe-(PS resin[a])) in DMF/CH_2Cl_2 1:1 and Swelling of the Starting Fmoc-peptide-resin*

Added salt[b])	Conversion [%][b])	Swelling [x-fold][c])	Standard deviation (swelling)
none	66.0	2.09	0.06
KSCN	78.0	–	–
LiBr	75.5	2.29	0.09
LiCl	–	2.50	0.07

[a]) {[4-(Oxymethyl)phenyl]acetamido}methyl on poly(styrene/1% divinylbenzene) (PAM 'resin').
[b]) Same as in *Table 1*.
[c]) Dry peptide-resin, volume = 1; measurements in triplicate.

Table 4. *Coupling of Fmoc-Ala-OPfp to H-(Ala)$_5$-Phe-(PEO-PS resin[a])) and Swelling of the Starting Fmoc-peptide-resin*

Solvent	Added salt[b])	Conversion [%][b])	Swelling [x-fold][c])	Standard deviation (swelling)
DMF/CH_2Cl_2 1:1	none	89.4	2.91	0.03
	LiCl	97.1	3.22	0.04
	$LiClO_4$	88.8	–	–
	KSCN	98.0	–	–
THF	none	78.5	2.49	0.11
	LiCl	53.0	2.31	0.20
NMP	none	92.0	3.34	0.11
	LiCl	97.8	3.32	0.09
	$LiClO_4$	96.3	–	–
	KSCN	96.1	–	–
DMPU	none	94.2	2.65	0.10
	LiCl	98.1	3.43	0.07

[a]) {{[4-(Oxymethyl)phenyloxy]acetamido}propyl}poly(ethylene oxide) on poly(styrene/1% divinylbenzene) graft copolymer, see **C** in *Scheme*.
[b]) Same as in *Table 1*.
[c]) Same as in *Table 3*.

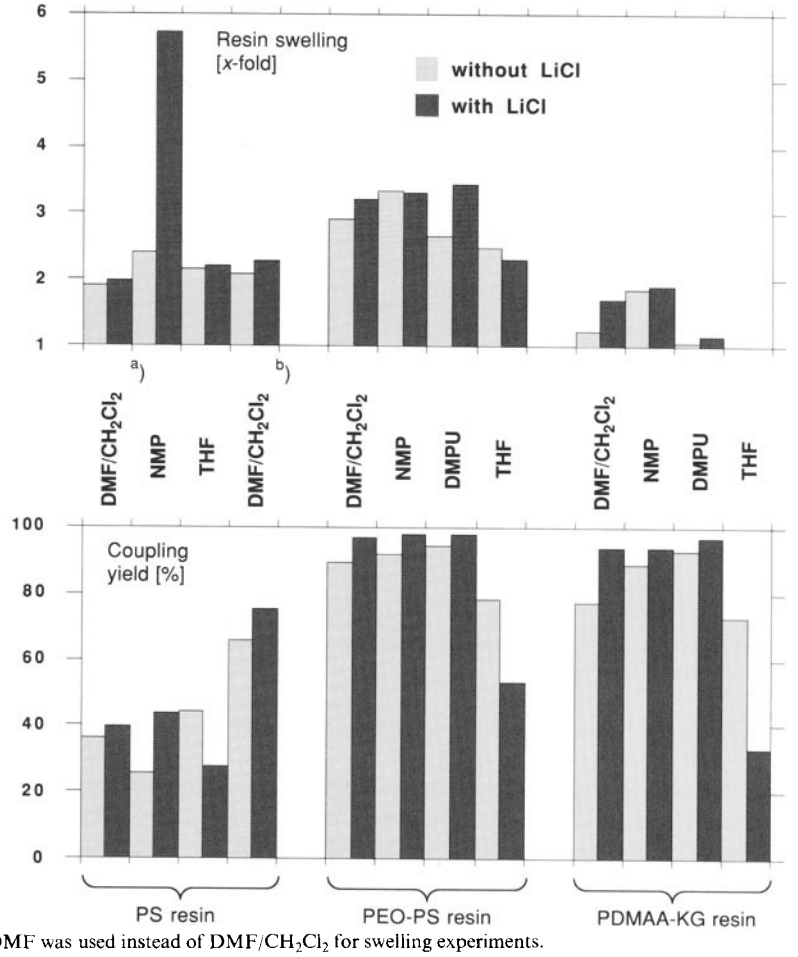
The most promising resin was the newly developed poly(ethylene oxide)-poly(styrene/1% divinylbenzene) support [28] (see **C**, *Scheme*). Yields for coupling of Fmoc-Ala-OPfp to H-(Ala)$_5$-Phe-(PEO-PS resin) were much higher (89–98% in polar solvents) for this support than for standard PS (*Table 4*)[10]). In *N,N'*-dimethylpropyleneurea[11]) (DMPU)

[9]) {[4-(Oxymethyl)phenyl]acetamidomethyl anchor [35], see **B** in the *Scheme*.
[10]) It should be noted that loading was 0.097 mmol/g for the PEO-PS resin compared to 0.541 mmol/g for the PS resin.
[11]) DMPU (= 3,4,5,6-tetrahydro-1,3-dimethylpyrimidin-2(1*H*)-one) is a non-mutagenic substitute for HMPA [36].

Table 5. *Coupling of Fmoc-Ala-OPfp to H-(Ala)$_5$-Phe-(PDMAA-KG resin*[a])*) and Swelling of the Starting Fmoc-peptide-resin*

Solvent	Added salt[b])	Conversion [%][b])	Swelling [x-fold][c])	Standard deviation (swelling)
DMF/CH_2Cl_2 1:1	none	77.5	1.22	0.03
	LiCl	93.8	1.70	0.02
THF	none	72.9	1.00	0.00
	LiCl	33.0	1.00	0.00
NMP	none	89.1	1.85	0.05
	LiCl	93.9	1.90	0.06
DMPU	none	92.8	1.06	0.03
	LiCl	97.1	1.15	0.01

[a]) {{2-{[4-(Oxymethyl)phenyloxy]acetamido}pentanamido}ethylamino}poly(*N*,*N*-dimethylacrylamide) on a 'Kieselgur' support, see **D** in *Scheme*. [b]) Same as in *Table 1*. [c]) Same as in *Table 3*.



[a]) DMF was used instead of DMF/CH_2Cl_2 for swelling experiments.
[b]) LiBr was used instead of LiCl and 'PAM'- instead of 'WANG'-anchor.

Fig. 4. *Illustrative examples of salt effects on coupling yield and resin swelling.* The full collection of data obtained with PS, PEO-PS, and PDMAA-KG resin is given in *Tables 1–3, 4,* and *5,* resp. The methods by which these data (and the standard deviations in the case of resin swelling) were determined are described in the *Exper. Part.*

without any additives the coupling reached a respectable 94% level which was further increased to 98% by the addition of 0.4M LiCl. A beneficial effect of salt additives was also observed in DMF/CH_2Cl_2 mixtures and in NMP as solvent. In THF, the addition of LiCl decreased coupling efficiency which in THF alone was already lower than in the other solvents. Changes in swelling upon addition of salts were modest except for DMPU, where a 1.29-fold increase was measured. The swelling *rate* was very high when compared to PS resins. Swelling was complete within 15 min, whereas PS resins needed 1–5 h for complete swelling. Similar results as for the PEO-PS resin were obtained for the PDMAA-KG support (see **D** in the *Scheme*; results in *Table 5*). A large salt-effect was observed for DMF/CH_2Cl_2, where the coupling yield increased from 77.5 to 93.8% in the presence of LiCl. The highest coupling yield (97%) was achieved in DMPU with LiCl. As for PEO-PS resin, a negative effect of LiCl on coupling was observed in THF.

Some illustrative coupling experiments showing the effect of salt additives are shown in *Fig. 4*.

Kinetics were studied for the reaction of a Fmoc-Ala-OPfp with H-$(Ala)_5$-Phe-(PEO-PS resin). The ratio of the UV (205 nm) absorption of starting material and product was measured in samples of the reaction mixture by HPLC after deprotection and cleavage from the resin. An interesting effect is seen in *Fig. 5*. The initial reaction was slower in the

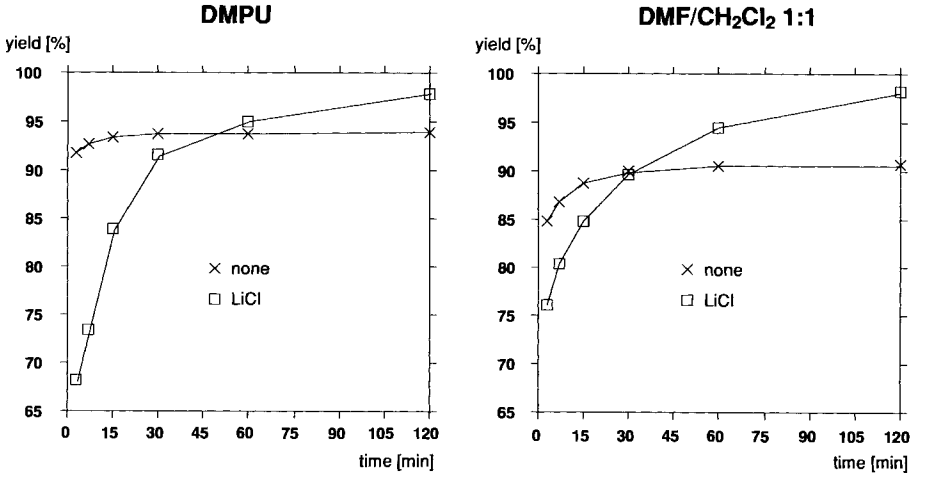


Fig. 5. *Kinetics of the coupling of Fmoc-Ala-OPfp with H-$(Ala)_5$-Phe-(PEO-PS resin) in DMF/CH_2Cl_2 1:1 and DMPU with and without LiCl* (0.4M) *added*

presence of LiCl, but the 'salt curves' crossed the 'non salt-curves' after 30 min (DMF/CH_2Cl_2) or 50 min (DMPU) towards 100% conversion, whereas, without salts, no further increase of product could be detected after 60 min (DMF/CH_2Cl_2) or 30 min (DMPU).

Discussion. – During the synthesis of oligo(L-alanine) by solid-phase peptide synthesis, we observed a drastic reduction in coupling efficiency to below 50% for the $(Ala)_5$-Phe-(PS resin) compared to the $(Ala)_4$-Phe-(PS resin). The same effect was reported for the synthesis of $(Ala)_n$-Val on a PS [7] and a PDMAA [8] support, respectively. It shows

the dramatic consequence conformational and solvation effects [3–8] [37] can have on the course of any solid-phase peptide synthesis. These effects are highly sequence-dependent and, for $(Ala)_n$ oligomers, can be overcome by the insertion of either D-Ala [7] or Pro or Gly residues [10] in the sequence. Our results show that on a PS support neither the use of polar solvents nor of salt additives was able to overcome the barrier to $(Ala)_6$-Phe synthesis[12][13]), although salt additives brought some improvement.

Structure and physicochemical properties of the resin support had a decisive influence on the reactions studied. Using the more polar (PEO-PS or PDMAA-KG) resins, generally much better coupling yields were observed, which were further improved by salt additives (*Fig. 4*). In part, this may be a consequence of the lower loading of these resins compared to PS supports, since loading is known to influence coupling efficiency [22] [41]. On the polar resins, coupling reactions could be brought near completion by the addition of LiCl to DMF/CH_2Cl_2, NMP, or DMPU for PEO-PS resin and by LiCl to DMPU for PDMAA-KG resin. Without salt additives, DMPU was superior to all other solvents tested. LiCl/DMPU was the best solvent especially for PDMAA-KG resin. In THF – the solvent it all began with [24] – coupling reactions were very incomplete on all resins, and salt-additives had a negative effect. This was also true for PEO-PS resins where one might have expected a favourable interaction between the ether solvent, the polyether moiety of the support, and Li-salts [24].

The drastic decrease in coupling yields of the reaction leading from $(Ala)_5$-Phe to $(Ala)_6$-Phe on PS resin was paralleled by a remarkable decrease in resin-swelling (*Fig. 3* and *Table 2*). Interestingly, not only the final *swelling volume* was affected, but also the *swelling rate*. Especially in NMP, a large decrease in swelling rate was observed for Fmoc-$(Ala)_5$-Phe-resin compared to Fmoc-$(Ala)_4$-Phe-resin. Addition of Li-salts increased peptide-resin swelling volume and rate[14]). Measuring swelling could thus be a simple method to test peptide-resins for reactivity problems, whereas solvent and salt additives should be optimised with coupling experiments. The slow rate of swelling particularly observed with PS resins can easily be the time-critical event in a synthesis-cycle in solid-phase peptide synthesis. For PEO-PS resin, the swelling rate would be high enough to allow for short cycle-times.

On the other hand, the rate of the coupling reaction must also be considered. Interestingly, the addition of LiCl to an active-ester coupling in DMF/CH_2Cl_2 or DMPU led to decreased coupling rates as measured for PEO-PS resin, but resulted in a higher final coupling yield (*Fig. 5*). Coupling times longer than 2 h would probably lead to even higher conversions than the ones observed.

The analytical measurement of the conversion of a particular coupling step on a solid support is subject to the same limitations as the coupling itself: the ninhydrin test [42]

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[12]) While this work was in progress, it was reported, that chaotropic salts can accelerate coupling reactions in solid-phase peptide synthesis, as shown in a synthesis of Ac-Ala-Glu-Thr-Ala-Ala-Ala-Lys-Phe-Leu-Arg-Ala-His-Ala-resin, an α-helical sequence [38].

[13]) Peptide cleavage from the *Kaiser*'s oxime resin (nucleophilic cleavage with *N*-hydroxypiperidine [39]) is facilitated in 2M LiBr/THF, whereas in CH_2Cl_2 or DMF, only moderate cleavage yields are obtained [40]. We thank Prof. *P. Lansbury* for providing us with hitherto unpublished results and for sending a preprint prior to publication.

[14]) Improved swelling of peptide-resins in 2M LiBr in THF is correlated with a transformation of antiparallel β-sheet to unordered structure as observed by FT-IR [40].

seemed not to be satisfactory with our oligoalanine model. In the preparation of Fmoc-$(Ala)_6$-Phe-(PS resin), the ninhydrin test showed no free amine (apparent coupling yield > 99%), whereas our HPLC analysis showed 69% of $CF_3COOH \cdot$ H-$(Ala)_6$-Phe-OH and 31% of $CF_3COOH \cdot$ H-$(Ala)_5$-Phe-OH. The accessibility of the peptide chain to ninhydrin molecules seems to be as hindered as the accessibility to activated amino acids during coupling reaction [37].

Incomplete deprotection of Fmoc groups should be considered an additional source of product heterogeneity in hindered peptide-resins. We have not investigated the effect of our reaction conditions and supports on the deprotection reaction as yet.

Conclusions. – Li-Salts exhibit a strong influence on solid-phase peptide-coupling reactions. Salt additives can increase resin swelling and improve coupling yields (see *Fig. 4*). Best results were obtained with the more polar PEO-PS and PDMAA-KG supports using DMPU/LiCl or NMP/LiCl as solvents (pure DMPU was also superior to other solvents, when no additives were used).

For best results, the choice of resin, solvents, and salt additives should be adjusted to the problem. Swelling, which can be easily determined with the intermediate of a difficult coupling reaction, may help to find the optimum salt/solvent combination for a particular coupling step. A simple method is given for measuring swelling parameters and swelling rates on small resin samples (see *Exper. Part*).

We thank Mr. *Ch. Beerli* and Mr. *A. Mosimann* (Preclinical Research, *Sandoz Pharma AG*, Basel) for the preparation of Fmoc-$(Ala)_5$-Phe-resins.

Experimental Part

General. See [32]. Solid-phase peptide syntheses; *RaMPS* apparatus (multiple peptide-synthesis system; *Du Pont de Nemours*, Biotechnology Systems Division) for manual multiple synthesis.

Procedure 1. The following steps were carried out: 2 × washing with DMF (1 ml) each; 2 × deprotecting with DMF/piperidin 1:1 (1 ml) each, 3 and 7 min, resp.; 8 × washing with DMF (1 ml) each; 3 × washing with H_2O (1 ml) each; 4 × washing with DMF (1 ml) each; 6 × washing with CH_2Cl_2 (1 ml) each. Then the resins were transfered into flasks and stirred with 95% CF_3CO_2H (2 ml) for 1 h. After precipitation of the free peptide in Et_2O (50 ml) each, $CF_3CO_2H \cdot$ H-$(Ala)_n$-Phe-OH as a mixture (n = 5 and 6) was filtered off and analysed in CF_3CO_2H by HPLC (0–10% *B* in *A*, within 10 min [32]).

1. *Peptide-Resin Starting Materials.* 1.1. *Fmoc-(Ala)*$_n$*-Phe-{{[4-(oxymethyl)phenyloxy]methyl}-PS resins*} (n = 4, 5, 6) were prepared from a {[4-(hydroxymethyl)phenyloxy]methyl}-PS resin[15]) using an Fmoc-synthesis protocol on an automated synthesizer[16]) [27].

1.2. *Fmoc-(Ala)*$_5$*-Phe-{{{[4-(oxymethyl)phenyl]acetamido}methyl}-PS resin*} was prepared from a Boc-Phe-{{{[4-(oxymethyl)phenyl]acetamido}methyl}-PS resin}[17]) using the same Fmoc-synthesis protocol after removal of the Boc group [43].

1.3. *Fmoc-(Ala)*$_5$*-Phe-(PEO-PS resin)*[18]) was prepared from an amino-resin[19]) by attaching, first, [4-(hydroxymethyl)phenyloxy]acetic acid using DIPCI/HOBt, then, Fmoc-Phe by a dicyclohexylcarbodiimide/

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[15]) 4-Alkoxybenzyl-alcohol resin obtained from *Bachem Feinchemikalien AG*, Bubendorf, Switzerland.

[16]) Model *A430, Applied Biosystems*, Foster City, California, USA.

[17]) PAM-phenylalanine obtained from *Applied Biosystems*, Foster City, California, USA.

[18]) '(PEO-PS resin)' stands for '{{{[4-(oxymethyl)phenyloxy]acetamido}propyl}poly(ethylene oxide) on poly(styrene/1%divinylbenzene)}', see **C** in the *Scheme*.

[19]) *TentaGel*-resin amine obtained from *Rapp Polymere*, Tübingen, FRG.

HOBt/4-(dimethylamino)pyridine-mediated esterification and assembling the peptide on a custom-made apparatus for continuous-flow synthesis using Fmoc-Ala and coupling by DIPCI/HOBt [44].

1.4. *Fmoc-(Ala)$_5$-Phe-(PDMAA-KG resin)*[20]) was prepared from a resin already loaded with Fmoc-Phe (0.088 mequiv./g)[21]) and using the same apparatus and Fmoc-synthesis protocol [29].

2. *Coupling Experiments.* 2.1. *CF_3CO_2H· H-(Ala)$_6$-Phe-OH from Fmoc-(Ala)$_5$-Phe-{{[4-(oxymethyl)-phenyloxy]methyl}-PS resin}*. Batches of Fmoc-(Ala)$_5$-Phe-(PS resin[4]) (50 mg, 27 μmol peptide, loading: 0.541 mmol peptide/g resin) were weighed into *RaMPS* cartridges. For the experiments *a*)–*s*), the solvents *A*–*S*, resp., were prepared (salt solns. were 0.4M each): *A* (DMF), *B* (DMF/LiCl), *C* (DMF/$LiClO_4$), *D* (DMF), *E* (DMF/LiCl), *F* (DMF), *G* (DMF/LiBr), *H* (DMF/CH_2Cl_2(1:1)), *I* (DMF/CH_2Cl_2(1:1)/LiCl), *K* (DMF/CH_2Cl_2(1:1)/LiBr), *L* (DMF/CH_2Cl_2(1:1)/KSCN), *M* (DMF/CH_2Cl_2(1:1)/$LiClO_4$), *N* (THF), *O* (THF/LiCl), *P* (NMP), *Q* (NMP/LiCl), *R* (NMP/$LiClO_4$), and *S* (NMP/KSCN). The following steps were carried out: 2 × washing with DMF (1 ml) each; 2 × deprotecting with DMF/piperidin 1:1 (1 ml) each, 3 and 7 min, resp.; 8 × washing with DMF (1 ml) each; 2 × washing with *A*–*S*, resp.; 1 × pre-equilibration, 30 min, with *A*–*S* (1 ml), resp.; 1 × coupling, 2 h, with *a*)–*c*) Fmoc-Ala-OPfp (77 mg, 0.162 mmol) each in *A*–*C* (2 ml), resp. with *d*) and *e*) Fmoc-Ala-OH · 3/4H_2O (53 mg, 0.162 mmol), TBTU (52 mg, 0.162 mmol), and 4-methylmorpholine (17.9 μl, 0.162 mmol) each in *D* and *E* (2 ml), resp., with *f*) and *g*) Fmoc-Ala-OH (53 mg, 0.162 mmol), DIPCI (25.1 μl, 0.162 mmol), and HOBt (22 mg, 0.162 mmol) each in *F* and *G* (2 ml), resp., and with *h*)–*s*) Fmoc-Ala-OPfp (77 mg, 0.162 mmol) each in *H*–*S* (2 ml), resp.; 2 × washing with *A*–*S* (1 ml), resp.; 7 × washing with DMF (1 ml) each; 10 × washing with i-PrOH (1 ml) each. The analysis of *a*)–*s*) was carried out according to *Procedure 1*. This led to the conversions shown in *Table 1*.

2.2. *HF· H-(Ala)$_6$-Phe-OH from Fmoc-(Ala)$_5$-Phe-{{{[4-(oxymethyl)phenyl]acetamido}methyl}-PS resin}*. Batches of Fmoc-(Ala)$_5$-Phe-(PS resin[9])) (100 mg, 58 μmol peptide; loading: 0.579 mmol peptide/g resin) were weighed into *RaMPS* cartridges. For the experiments *a*)–*c*), the solvents *A*–*C*, resp., were prepared (salt solns. were 0.4M each): *A* (DMF/CH_2Cl_2 1:1), *B* (DMF/CH_2Cl_2(1:1)/KSCN), and *C* (DMF/CH_2Cl_2(1:1)/LiBr). The following steps were carried out: 5 × washing with DMF (1 ml) each; 2 × deprotecting with DMF/piperidin 1:1 (1 ml) each, 3 and 7 min, resp.; 10 × washing with DMF (1 ml) each; 5 × washing with *A*–*C*, resp.; 1 × pre-equilibration, 1 h, with *A*–*C* (1 ml), resp.; 1 × coupling, 2 h, with 2 ml of a (Boc-Ala)$_2$O soln. in *A*–*C*, resp. (prepared by addition of DCCI (215 mg, 1.04 mmol) to a soln. of Boc-Ala-OH (394 mg, 2.08 mmol) in CH_2Cl_2 (30 ml), stirring for 1 h, filtration, dividing into 3 equivalent parts, solvent evaporation, and redissolving in *A*–*C* (2 ml), resp.; 5 × washing with *A*–*C* (1 ml), resp.; 5 × washing with DMF/CH_2Cl_2 1:1 (1 ml) each; 10 × washing with i-PrOH (1 ml) each. For analysis of *a*)–*c*), the peptide-resins were treated with Me_2S (0.5 ml) and *p*-Cresol (0.5 g) in HF (5 ml) each for 1 h at 0°, filtrated, and precipitated using Et_2O to yield HF-(Ala)$_n$-Phe-OH as a mixture (n = 5 and 6). Analysis in CF_3CO_2H by HPLC (0–10% *B* within 10 min [32]) indicated the conversions shown in *Table 3*.

2.3. *CF_3CO_2H·H-(Ala)$_6$-Phe-OH from Fmoc-(Ala)$_5$-Phe-(PEO-PS resin)*[19]). Batches of Fmoc-(Ala)$_5$-Phe-(PEO-PS resin)[19]) (50 mg, 5 μmol peptide; loading: 97 μmol peptide/g resin) were weighed into *RaMPS* cartridges. For the experiments *a*)–*m*), the solvents *A*–*M*, resp., were prepared (salt solns. were 0.4M each): *A* (DMF/CH_2Cl_2 1:1), *B* (DMF/CH_2Cl_2(1:1)/LiCl), *C* (DMF/CH_2Cl_2(1:1)/$LiClO_4$), *D* (DMF/CH_2Cl_2(1:1)/KSCN), *E* (THF), *F* (THF/LiCl), *G* (NMP), *H* (NMP/LiCl), *I* (NMP/$LiClO_4$), *K* (NMP/KSCN), *L* (DMPU), and *M* (DMPU/LiCl). The following steps were carried out: 2 × washing with DMF (1 ml) each; 2 × deprotecting with DMF/piperidin 1:1 (1 ml) each, 3 and 7 min, resp.; 8 × washing with DMF (1 ml) each; 2 × washing with *A*–*M*, resp.; 1 × pre-equilibration, 60 min, with *A*–*M* (1 ml), resp.; 1 × coupling, 2 h, with Fmoc-Ala-OPfp (70 mg, 0.147 mmol) each in *A*–*M* (2 ml), resp.; 2 × washing with *A*–*M* (1 ml); 5 × washing with DMF (1 ml) each; 10 × washing with i-PrOH (1 ml) each. The analysis of *a*)–*m*) was carried out according to *Procedure 1*. This led to the conversions shown in *Table 4*.

2.4. *CF_3CO_2H·H-(Ala)$_6$-Phe-OH from Fmoc-(Ala)$_5$-Phe-(PDMAA-KG resin)*[21]). Batches of Fmoc-(Ala)$_5$-Phe-(PDMAA-KG resin)[21]) (100 mg, 8.5 μmol peptide; loading: 85 μmol peptide/g resin) were weighed into *RaMPS* cartridges. For the experiments *a*)–*h*), the solvents *A*–*H*, resp., were prepared (salt solns. were 0.4M each): *A* (DMF/CH_2Cl_2 1:1), *B* (DMF/CH_2Cl_2(1:1)/LiCl), *C* (THF), *D* (THF/LiCl), *E* (NMP), *F* (NMP/LiCl), *G* (DMPU), and *H* (DMPU/LiCl). The following steps were carried out: 2 × washing with DMF (1 ml) each; 2 × deprotecting with DMF/piperidin 1:1 (1 ml) each, 3 and 7 min, resp.; 8 × washing with DMF (1 ml) each; 2 × washing with *A*–*H*, resp.; 1 × pre-equilibration, 60 min, with *A*–*H* (1 ml), resp.; 1 × coupling, 2 h, with

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[20]) '(PDMAA-KG resin)' stands for '{{{2-{[4-(oxymethyl)phenyloxy]acetamido}pentanamido}ethylamino}-poly(*N*,*N*-dimethylacrylamide) on 'Kieselgur'}', see **D** in the *Scheme*.

[21]) Fmoc-Phe-PepSyn KA(100), *Cambridge Research Biochemicals Ltd.*, Cambridge, UK.

Fmoc-Ala-OPfp (73 mg, 0.153 mmol) each in *A*–*H* (2 ml), resp.; 2 × washing with *A*–*H* (1 ml), resp.; 5 × washing with DMF (1 ml) each; 10 × washing with i-PrOH (1 ml) each. The analysis of *a*)–*h*) was carried out according to *Procedure 1* without precipitation with Et_2O (CF_3CO_2H solns. were directly injected into HPLC). This led to the conversions shown in *Table 5*.

3. *Swelling Experiments.* – 3.1. *Fmoc-(Ala)$_4$-Phe-{{[4-(oxymethyl)phenyloxy]methyl}-PS resin}*. Boiling-point tubes (∅ 2 mm) were filled with Fmoc-(Ala)$_4$-Phe-(PS resin[4])) to *ca.* 5-mm height. Solvents *A* (DMF), *B* (0.4M LiCl in DMF), *C* (THF), *D* (0.4M LiCl in THF), *E* (NMP), and *F* (0.4M LiCl in NMP) were added each, air bubbles were removed by mixing with a turned-around melting-point tube, and the heights of the resin were measured after standing for the given time (no further swelling after standing for 1 h was observed) using a slide caliper. Each experiment was carried out three times and the following heights [mm] were obtained after 0 min: *A* (5.3, 5.2, 4.9), *B* (5.0, 5.6, 4.9), *C* (6.3, 6.2, 5.0), *D* (5.3, 6.6, 5.0), *E* (4.6, 5.2, 4.7), *F* (5.0, 5.5, 5.4); 3 min: *A* (6.3, 7.1, 7.0), *B* (7.8, 9.9, 9.8), *C* (11.6, 11.5, 9.6), *D* (11.3, 13.2, 9.4); 7 min: *A* (11.8, 16.2, 15.5), *B* (15.8, 19.3, 16.1), *C* (13.8, 12.4, 10.4), *D* (11.8, 15.1, 11.1); 15 min: *A* (18.8, 19.3, 17.3), *B* (19.2, 22.5, 19.1), *C* (13.8, 12.7, 10.4), *D* (12,4, 15.2, 11.3), *E* (15.7, 18.6, 17.2), *F* (22.9, 26.1, 25.2); 30 min: *A* (19.7, 19.3, 17.3), *B* (20.0, 22.5, 19.1), *C* (13.8, 12.7, 10.4), *D* (12.6, 15.2, 11.5), *E* (19.5, 22.6, 20.6), *F* (25.8, 28.2, 27.9); 60 min: *A* (19.7, 19.3, 17.3), *B* (20.0, 22.5, 19.1), *C* (13.8, 12.7, 10.4), *D* (12.6, 15.2, 11.5), *E* (20.8, 23.0, 20.9), *F* (26.5, 28.4, 28.3).

3.2. *Fmoc-(Ala)$_5$-Phe-{{[4-(oxymethyl)phenyloxy]methyl}-PS resin}*. The experiments were carried out as described in *3.1*; heights [mm] after 0 min: *A* (5.4, 5.0, 6.3), *B* (5.4, 5.2, 6.8), *C* (5.0, 5.2, 5.4), *D* (5.8, 4.4, 5.8), *E* (5.5, 5.3, 5.0), *F* (5.3, 5.7, 5.5); 3 min: *A* (6.1, 5.7, 7.1), *B* (6.0, 6.0, 7.5), *C* (10.7, 10.0, 11.0), *D* (11.8, 8.2, 11.9); 7 min: *A* (7.5, 6.9, 9.1), *B* (7.1, 6.6, 8,6), *C* (10.8, 11.1, 11.3), *D* (12.9, 9.2, 12.6); 15 min: *A* (9.6, 9.1, 11.5), *B* (9.1, 8.4, 11.3), *C* (10.7, 11.1, 11.7), *D* (12.9, 9.6, 12.8), *E* (6.5, 5.9, 6.0), *F* (9.5, 8.2, 8.4); 30 min: *A* (10.0, 9.9, 12.1), *B* (10.5, 10.4, 13.2), *C* (10.7, 11.1, 11.7), *D* (12.9, 9.6, 12.8), *E* (7.5, 6.6, 6.6), *F* (11.6, 10.1, 10.3); 60 min: *A* (10.0, 9.9, 12.1), *B* (10.8, 10.4, 13.2), *C* (10.7, 11.1, 11.7), *D* (12.9, 9.6, 12.8), *E* (9.1, 7.9, 8.1), *F* (14.8, 14.1, 14.4); 120 min: *A* (10.0, 9.9, 12.1), *B* (10.8, 10.4, 13.2), *C* (10.7, 11.1, 11.7), *D* (12.9, 9.6, 12.8), *E* (12.1, 10.1, 10.7), *F* (24.1, 21.9, 22.1); 180 min: *A* (10.0, 9.9, 12.1), *B* (10.8, 10.4, 13.2), *C* (10.7, 11.1, 11.7), *D* (12.9, 9.6, 12.8), *E* (13.1, 12.2, 12.0), *F* (28.4, 29.2, 28.2); 300 min: *A* (10.0, 9.9, 12.1), *B* (10.8, 10.4, 13.2), *C* (10.7, 11.1, 11.7), *D* (12.9, 9.6, 12.8), *E* (13.7, 12.6, 12.0), *F* (29.8, 33.3, 31.2).

3.3. *Fmoc-(Ala)$_6$-Phe-{{[4-(oxymethyl)phenyloxy]methyl}-PS resin}*. The experiments were carried out as described in *3.1*; heights [mm] after standing for 5 h (for *A*–*D* no further swelling was observed even after 1 h): *A*: dry (6.3, 6.7, 7.5), swollen (11.6, 11.7, 13.9); *B*: dry (5.6, 6.4, 6.3), swollen (9.6, 10.8, 11.1); *C*: dry (7.2, 6.0, 5.7), swollen (14.6, 12.1, 11.5); *D*: dry (6.1, 6.0, 5.1), swollen (13.5, 12.2, 11.0); *E*: dry (6.0, 6.5, 6.1), swollen (12.7, 13.4, 12.8); *F*: dry (5.5, 5.9, 5.4), swollen (30.0, 32.0, 29.8).

3.4. *Fmoc-(Ala)$_5$-Phe-{{{[4-(oxymethyl)phenyl]acetamido}methyl}-PS resin}*. The experiments were carried out as described in *3.1* with *A* (DMF/CH_2Cl_2 1:1), *B* (0.4M LiCl in DMF/CH_2Cl_2 1:1), and *C* (0.4M LiBr in DMF/CH_2Cl_2 1:1); height [mm] after standing for 1 h (no further swelling was observed): *A*: dry (5.3, 5.9, 5.6), swollen (10.8, 12.7, 11.7); *B*: dry (5.8, 6.4, 5.9), swollen (14.9, 15.6, 14.7); *C*: dry (6.9, 6.1, 7.0), swollen (15.4, 14.6, 15.8).

3.5. *Fmoc-(Ala)$_5$-Phe-(PEO-PS resin)*[19]). The experiments were carried out as described in *3.1* with *A* (DMF/CH_2Cl_2 1:1), *B* (0.4M LiCl in DMF/CH_2Cl_2 1:1), *C* (THF), *D* (0.4M LiCl in THF), *E* (NMP), *F* (0.4M LiCl in NMP), *G* (DMPU), and *H* (0.4M LiCl in DMPU); heights [mm] after standing for 15 min (maximal swelling): *A*: dry (4.2, 4.6, 4.8), swollen (12.1, 13.5, 14.0); *B*: dry (4.5, 4.5, 4.4), swollen (14.6, 14.3, 14.3); *C*: dry (4.1, 5.6, 4.5), swollen (10.7, 13.6, 10.9); *D*: dry (5.3, 4.6, 4.1), swollen (13.3, 9.7, 9.5); *E*: dry (4.5, 5.6, 5.1), swollen (15.4, 18.0, 17.3); *F*: dry (4.8, 4.7, 5.5), swollen (16.3, 15.8, 17.7); *G*: dry (4.4, 4.8, 4.6), swollen (11.8, 12.2, 12.6); *H*: dry (4.3, 4.7, 4.7), swollen (14.6, 16.5, 15.9).

3.6. *Fmoc-(Ala)$_5$-Phe-(PDMAA-KG resin)*[21]). The experiments were carried out as described in *3.1* with *A* (DMF/CH_2Cl_2 1:1), *B* (0.4M LiCl in DMF/CH_2Cl_2 1:1, *C* (THF), *D* (0.4M LiCl in THF), *E* (NMP), *F* (0.4M LiCl in NMP), *G* (DMPU), and *H* (0.4M LiCl in DMPU); heights [mm] after standing for 30 min (maximal swelling): *A*: dry (7.0, 6.7, 7.2), swollen (8.7, 8.2, 8.6); *B*: dry (7.5, 7.5, 7.9), swollen (12.8, 12.6, 13.5); *C*: dry (7.8, 6.8, 6.8), swollen (7.8, 6.8, 6.8); *D*: dry (7.4, 8.1, 8.2), swollen (7.4, 8.1, 8.2); *E*: dry (8.9, 7.4, 6.7), swollen (16.0, 14.0, 12.4); *F*: dry (6.4, 7.5, 7.4), swollen (12.6, 13.9, 13.9); *G*: dry (7.4, 8.2, 8.7), swollen (7.6, 8.8, 9.4); *H*: dry (7.6, 8.6, 7.5), swollen (8.7, 9.9, 8.6).

4. *Kinetics of Solid-Phase Peptide Coupling Reactions on the PEO-PS Resin.* After filling 4 cartridges with Fmoc-(Ala)$_5$-Phe-(PEO-PS resin)[19]) (50 mg) each, the peptide was deprotected as described for the preparation of CF_3CO_2H · H-(Ala)$_6$-Phe-OH (see *2.2*). The resins were transferred into 4 flasks and Fmoc-Ala-OPfp (105 mg, 0.22 mmol) in 3 ml of solvent (*A*: DMF/CH_2Cl_2 1:1; *B*: 0.4M LiCl in DMF/CH_2Cl_2 1:1; *C*: DMPU; *D*: 0.4M LiCl in

DMPU) was added to each. Samples of 0.5 ml were taken after 3, 7, 15, 30, 60, and 120 min, resp., and immediately washed 10 × with DMF (1 ml) each. After deprotection with DMF/piperidine 1:1 (2 ml) each for 10 min, the residues were washed 8 × with DMF (1 ml), 3 × with H_2O (1 ml), 4 × with DMF (1 ml) and 6 × with CH_2Cl_2 (1 ml) each and transferred into flasks, where the peptides were cleaved from the resin with CF_3CO_2H (1 ml) each and directly analysed as CF_3CO_2H solns. using HPLC. This led to the following conversions [%]: *A*: 3 (84.8), 7 (86.8), 15 (88.7), 30 (89.9), 60 (90.5), and 120 min (90.6); *B*: 3 (76.0), 7 (80.3), 15 (84.8), 30 (89.6), 60 (94.4), and 120 min (98.1); *C*: 3 (91.8), 7 (92.6), 15 (93.4), 30 (93.7), 60 (93.8), and 120 min (93.9); *D*: 3 (68.1), 7 (73.4), 15 (83.8), 30 (91.5), 60 (95.0), and 120 min (97.9).

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