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Smart peptide synthesis: Synthesis of PADRE-Muc chimeric peptide

Strategies advocated for disrupting peptide aggregation during solid phase synthesis either use structure-breaking derivatives such as pseudoproline [1] or Dmb dipeptides [2] to prevent peptide self-association or attempt to generally improve the overall solvation of the peptide-resin complex. The use of dipolar aprotic solvents such as DMF, NMP or DMSO, chaotropic salts [3] or special solvent cocktails like "Magic Mixture" [4] has been found to be efficacious in the latter instance. However, two of the most important factors that can benefit peptide-resin solvation are the nature of the resin itself and the extent of resin functionalization. PEG-based resins have been found to be particularly effective in this regard, owing to the amphipathic nature of the polymer backbone [5, 6], whereas the use of low-loaded resins has been shown to minimize the effects of aggregation [7].

The two approaches described above for overcoming aggregation are complementary as they work by different mechanisms. The effects of applying them together in a single "Smart" synthesis can be synergistic, resulting in remarkable improvements in peptide quality and yield that could not be achieved using either alone. In this innovation, the development of the Fmoc SPPS of chimeric peptide consisting of the Muc1 peptide and the PADRE epitope is described [8]. This work, reproduced here by the kind permission of Wiley, demonstrates how resin loading, resin polarity and the use of pseudoproline dipeptides can influence synthetic efficiency and shows how these factors can be combined to provide an optimized approach to the synthesis of a "difficult peptide".

PADRE epitope

H-D-Ala-Lys-Cha-Val-Ala-Ala-Trp^{7}-Thr^{8}-Leu-Lys-Ala-D-Ala-NH$_2$ **1**

Synthesis of the PADRE epitope **1** was carried out on an ABi 433 synthesizer using 10-fold excesses of Fmoc-amino acids and standard FastMoc activation protocols. After each coupling step, unreacted amino groups were capped with Ac_2O. Fmoc removal was effected by three piperidine treatments. The release of dibenzofulvene-piperidine adduct

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was followed by UV spectroscopy. If the optical density of the solution from the third treatment was more than 3% of the first, two further piperidine treatments were carried out automatically.

As capping was carried out after each coupling step, the total Fmoc content of the resin after addition of the final residue provides an estimate of the total elongation yield for each synthesis step.

Influence of loading

PADRE was assembled on Rink Amide resin of three different substitutions: 0.76, 0.4, 0.26 mmole/g. Figure 1 shows the UV traces for the Fmoc deprotection reactions obtained from these syntheses. The syntheses on 0.76 and 0.4 mmole/g resins showed a marked slowing down after 9 cycles, resulting in products containing numerous truncation peptides. In contrast, the low substitution resin gave a good elongation yield and afforded the product in high purity (Table 1).

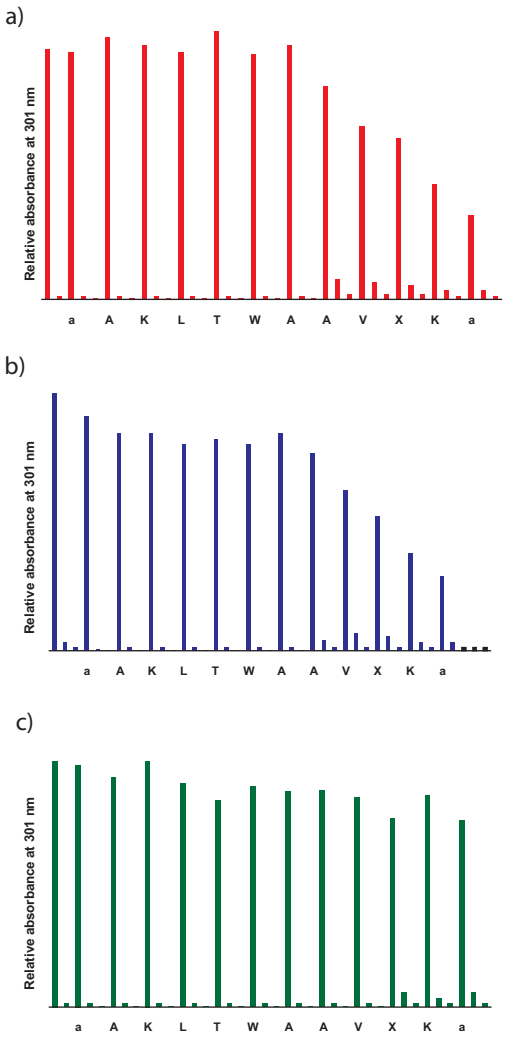
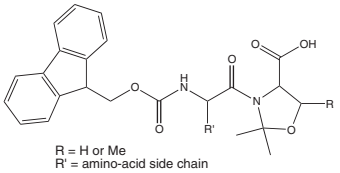


Fig. 1: Trace from UV monitoring of Fmoc removal during synthesis of PADRE on Rink Amide resin: a) at 0.76 mmole/g substitution; b) 0.4 mmole/g substitution; c) 0.26 mmole/g substitution.

Influence of pseudoproline dipeptide

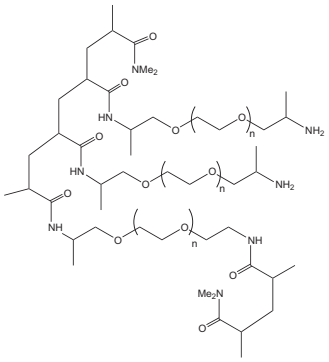
Fmoc-Aaa-Ser/Thr($\Psi^{Me,Me}$pro)-OH



PADRE was prepared as above on 0.4 mmole/g Rink Amide resin, except that residues Trp-Thr were introduced using Fmoc-Trp(Boc)-Thr($\Psi^{Me,Me}$pro)-OH. The UV trace obtained from this synthesis indicated a significant improvement in synthetic efficiency compared with the same synthesis carried out using standard Fmoc amino acid building blocks (Table 1). Nevertheless, sluggish Fmoc removal from Lys was still observed (Fig 2).

Influence of polymer matrix

PEGA resin



PADRE was also prepared on Rink Amide PEGA resin. This highly polar PEG-polyacrylamide-based resin had a loading of 0.36 mmole/g, enabling direct comparisons to be made with the 0.4 mmole/g polystyrene-based Rink Amide resin. The elongation yield for this synthesis was 87% against 45% for the comparable synthesis on polysyrene resin, indicating a positive influence of the polar PEGA resin.

Table 1: Elongation yields from synthesis of PADRE **1** *and PADRE-Muc* **2**.

Peptide	Resin	Loading (mmole/g)	Pseudoproline dipeptide	Elongation yield (%)
1	Polystyrene	0.76	-	37
1	Polystyrene	0.4	-	45
1	Polystyrene	0.26	-	82
1	Polystyrene	0.4	WT	89
1	PEGA	0.36	-	87
2	PEGA	0.36	-	82
2	PEGA	0.36	WT	97

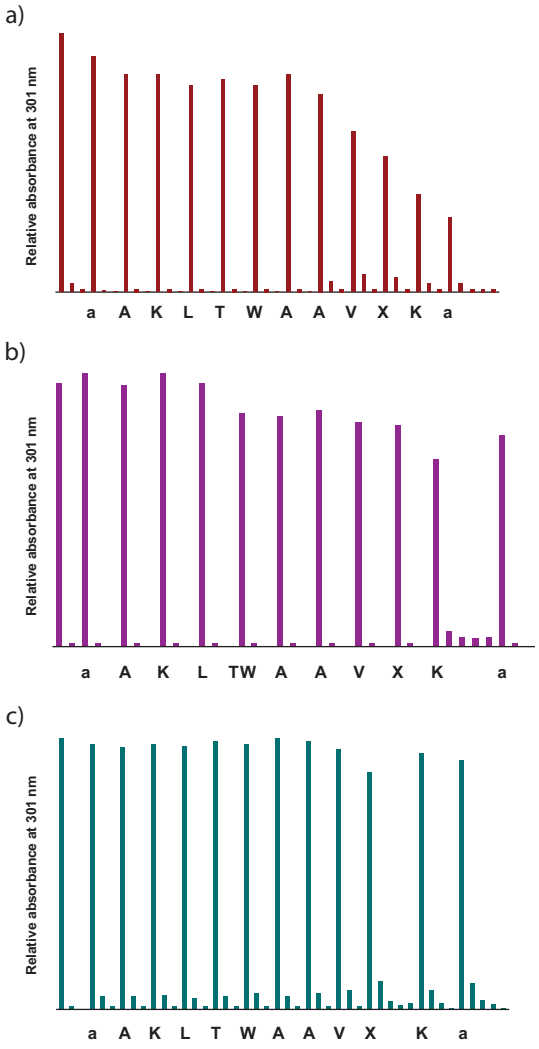


Fig. 2: Trace from UV monitoring of Fmoc removal during synthesis of PADRE on a) Rink Amide resin 0.4 mmole/g substitution; b) Rink Amide resin 0.4 mmole/g substitution. Substitution of WT with pseudoproline dipeptide; c) Rink Amide PEGA resin 0.36 mmole/g substitution.

PADRE-Muc

H-D-Ala-Lys-Cha-Val-Ala-Ala-Trp^{7}-Thr^{8}-Leu-Lys-Ala-D-Ala-Pro-Pro-Ala-His-Gly-Val-Thr-Ser-Ala-Pro-Asp-Thr-Arg-Pro-Ala-Pro-Gly-Ser-Thr-Lys-Ala-NH_2 **2**

In view of the excellent previous results obtained in the synthesis of PADRE, PEGA resin was selected as the solid support for the preparation of the PADRE-Muc chimera. As the desired peptide was required as a C-terminal hydrazide for subsequent hydrazone ligation, synthesis was carried out on a PEGA resin loaded with a hydrazine cleavable Boc-Ala-PAM linker. The elongation yield obtained was 86% but the UV monitoring indicated a slowing of Fmoc release from Thr of the PADRE sequence. However, re-synthesis using a combination of PEGA resin with the pseudoproline Fmoc-Trp(Boc)-Thr($\Psi^{Me,Me}$pro)-OH led to an excellent elongation yield and very even rates of Fmoc removal rates (Fig. 3). HPLC analysis of the crude PADRE gave a major peak (87%) corresponding to the target peptide and a minor peak (10%) which appears to be an acetylated by-product (Fig. 4).

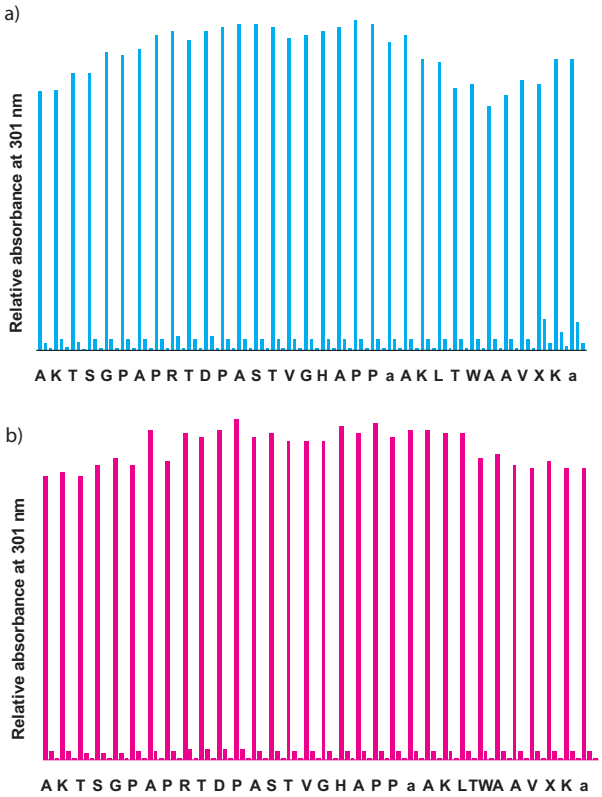


Fig. 3: Trace from UV monitoring of Fmoc removal during synthesis of PADRE-Muc on a) Rink Amide PEGA resin 0.36 mmole/g substitution; b) Rink Amide PEGA resin 0.36 mmole/g substitution. Substitution of WT with pseudoproline dipeptide.

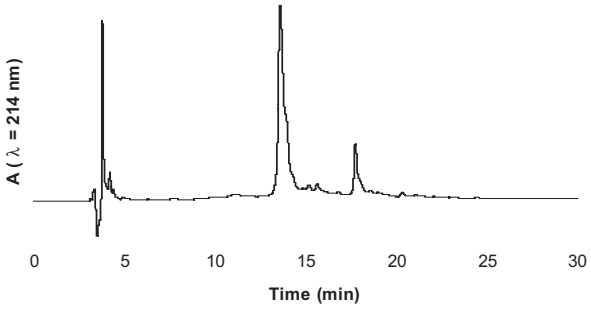


Fig. 4: HPLC profile of crude PADRE-Muc [8].

Ordering information

01-64-0101	Rink Amide PEGA resin	1 g 5 g
01-64-0470	Rink Amide AM resin LL (100 - 200 mesh)	1 g 5 g 25g
01-64-0467	Rink Amide MBHA resin LL (100 - 200 mesh)	1 g 5 g 25g

05-20-1000	Fmoc-Ala-Ser($\Psi^{Me,Me}$pro)-OH	1 g 5 g
05-20-1005	Fmoc-Ala-Thr($\Psi^{Me,Me}$pro)-OH	1 g 5 g
05-20-1010	Fmoc-Asn(Trt)-Ser($\Psi^{Me,Me}$pro)-OH	1 g 5 g
05-20-1008	Fmoc-Asn(Trt)-Thr($\Psi^{Me,Me}$pro)-OH	1 g 5 g
05-20-1011	Fmoc-Asp(OtBu)-Ser($\Psi^{Me,Me}$pro)-OH	1 g 5 g
05-20-1126	Fmoc-Asp(OtBu)-Thr($\Psi^{Me,Me}$pro)-OH	1 g 5 g
05-20-1115	Fmoc-Gln(Trt)-Ser($\Psi^{Me,Me}$pro)-OH	1 g 5 g
05-20-1125	Fmoc-Gln(Trt)-Thr($\Psi^{Me,Me}$pro)-OH	1 g 5 g
05-20-1002	Fmoc-Glu(OtBu)-Ser($\Psi^{Me,Me}$pro)-OH	1 g 5 g
05-20-1122	Fmoc-Glu(OtBu)-Thr($\Psi^{Me,Me}$pro)-OH	1 g 5 g
05-20-1127	Fmoc-Gly-Ser($\Psi^{Me,Me}$pro)-OH	1 g 5 g
05-20-1124	Fmoc-Gly-Thr($\Psi^{Me,Me}$pro)-OH	1 g 5 g
05-20-1119	Fmoc-Ile-Ser($\Psi^{Me,Me}$pro)-OH	1 g 5 g
05-20-1118	Fmoc-Ile-Thr($\Psi^{Me,Me}$pro)-OH	1 g 5 g
05-20-1004	Fmoc-Leu-Ser($\Psi^{Me,Me}$pro)-OH	1 g 5 g
05-20-1009	Fmoc-Leu-Thr($\Psi^{Me,Me}$pro)-OH	1 g 5 g
05-20-1003	Fmoc-Lys(Boc)-Ser($\Psi^{Me,Me}$pro)-OH	1 g 5 g
05-20-1116	Fmoc-Lys(Boc)-Thr($\Psi^{Me,Me}$pro)-OH	1 g 5 g
05-20-1121	Fmoc-Phe-Ser($\Psi^{Me,Me}$pro)-OH	1 g 5 g
05-20-1128	Fmoc-Phe-Thr($\Psi^{Me,Me}$pro)-OH	1 g 5 g
05-20-1012	Fmoc-Ser(tBu)-Ser($\Psi^{Me,Me}$pro)-OH	1 g 5 g
05-20-1117	Fmoc-Ser(tBu)-Thr($\Psi^{Me,Me}$pro)-OH	1 g 5 g
05-20-1130	Fmoc-Trp(Boc)-Ser($\Psi^{Me,Me}$pro)-OH	1 g 5 g
05-20-1013	Fmoc-Trp(Boc)-Thr($\Psi^{Me,Me}$pro)-OH	1 g 5 g
05-20-1014	Fmoc-Tyr(tBu)-Ser($\Psi^{Me,Me}$pro)-OH	1 g 5 g
05-20-1007	Fmoc-Tyr(tBu)-Thr($\Psi^{Me,Me}$pro)-OH	1 g 5 g
05-20-1001	Fmoc-Val-Ser($\Psi^{Me,Me}$pro)-OH	1 g 5 g
05-20-1006	Fmoc-Val-Thr($\Psi^{Me,Me}$pro)-OH	1 g 5 g

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