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Smart peptide synthesis: Synthesis of Rantes

Rantes (CCL5) is an 8 kD chemokine that has been implicated in the suppression of macrophage-tropic HIV infection by CD8+ T-cells [1]. It is thought to work by blocking or causing downmodulation of the CCR5 co-receptor necessary for viral fusion. N-terminal variants of Rantes have been developed that show promise as topical inhibitors of vaginal viral transmission [2].

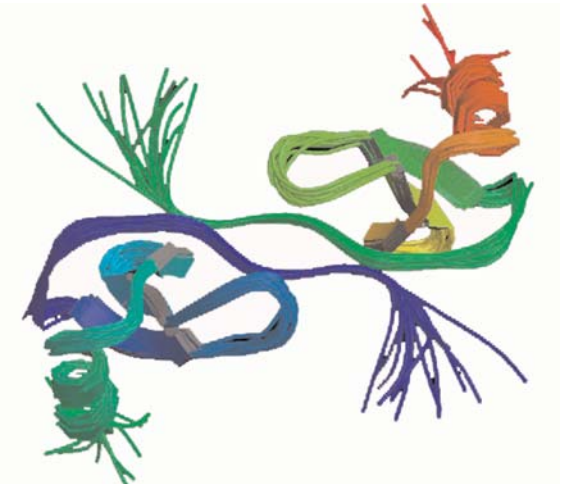

Fig. 1: ^{1}H nmr structure of Rantes dimer [3].

Rantes comprises a single 68 residue polypeptide chain with two disulfide bridges. Analogs of this protein have been obtained by native chemical ligation of two fragments produced by Boc SPPS [2]. An attempted step-wise Fmoc SPPS of this long peptide has been previously described by J. F. McNamara, using a combination of HATU activation, elevated temperatures and the “magic mixture” solvent system [4]. However, despite these forcing conditions no material of the correct mass was obtained by this approach.

In this innovation, the development of a successful Fmoc SPPS is described. This work [5], reproduced here by the kind permission of Wiley and Prof. Albericio, demonstrates how the use of pseudoproline dipeptides when combined with a polar support can act synergistically to provide an efficient synthesis of this difficult peptide.

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"In our hands, pseudoproline derivatives have proven very effective - particularly in the synthesis of peptides with difficult and long sequences. Using pseudoprolines, we saved time and money for repeat synthesis of failed sequences. We now routinely use pseudoproline derivatives for our peptide synthesis. I would highly recommend using them for peptide synthesis in the manufacturing industry as well. I am glad Novabiochem took the lead in manufacturing pseudoprolines in bulk".

Ved Srivastava, Amylin Pharmaceuticals Inc, San Diego, CA

"Pseudoproline dipeptides have greatly increased our success rate for synthesizing both long and difficult peptides. If we are able to integrate pseudoprolines into our syntheses, we can easily machine-synthesize peptides up to 80 amino acids in length. Routine use of pseudoprolines in our peptide syntheses has considerably increased the yield and purity, as well as decreased the number of failed syntheses. They are wonderful products!"

Yingwei He, Protein Chemistry Dept., Abgent, San Diego, CA.

"Biomol started incorporating pseudoproline derivatives into its everyday schedules for routine peptide synthesis some eight years ago. Over the intervening years, the use of these reagents on a routine basis has led to a dramatic reduction in the necessity for repeat synthesis. When coupled with an undoubted improvement in the yield and purity of crude peptides obtained, this has meant considerable financial savings in terms of both synthesis and purification costs. We are firmly of the opinion that the benefits of incorporation of pseudoproline analogs into peptide synthesis protocols is fully justifiable on both scientific and commercial grounds and is to be recommended on a routine basis."

Paul Sheppard, Biomol International Lp, Exeter, UK.

Synthetic Procedures

All syntheses of Rantes (Figure 2) were carried out using an ABi 433A peptide synthesizer. All amino acid additions were performed using single 35 minutes couplings of Fmoc-amino acids activated with HATU/DIPEA, only Val^{39} was coupled twice. Fmoc removal was achieved using a 15 minutes treatment with piperidine/DMF (1:4).

All peptides were cleaved from the resin by treatment with Reagent K* for 2 or 3 h.

* Reagent K = TFA/phenol/H_2O/thioanisol/1,2-ethanedithiol, 82.5:5:5:5:2.5

Synthesis on polystyrene-based resins

The synthesis of Rantes was initially attempted using Fmoc-Ser(tBu)-Wang resin with standard amino-acid building blocks. The product obtained after 25 cycles was analyzed by HPLC (Figure 3) and found to be of poor quality. The synthesis was abandoned after 38 cycles, as the desired peptide could not be identified by LC-MS in the crude product obtained after TFA treatment of a sample peptidyl resin after addition of Ser^{31}.

The difficulties encountered in the synthesis may be ascribed to Rantes adopting antiparallel β-strands between residues 22 and 53, as such structures are likely to have a negative impact on the assembly of this region of the peptide. As incorporation of pseudoproline dipeptides could be anticipated to dramatically improve the efficiency of such a peptide, the synthesis was repeated using pseudoproline dipeptides inserted at the positions indicated in Figure 2. Figure 4 shows the HPLC profile of the peptide isolated after 45 cycles; the product corresponding to the major peak was identifed by MS to be the correct material. Unfortunately, despite these improvements in synthetic efficiency provided by the use of pseudoproline dipeptides, this sample was the last one in which the desired product could be identified.

Fig. 3: HPLC profile of crude Rantes (44-68) prepared using standard Fmoc amino acids on Wang resin.

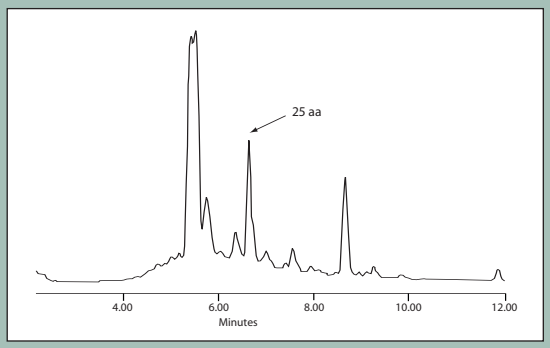


Fig. 4: HPLC profile of crude Rantes (24-68) prepared using 4 pseudoproline dipeptides.

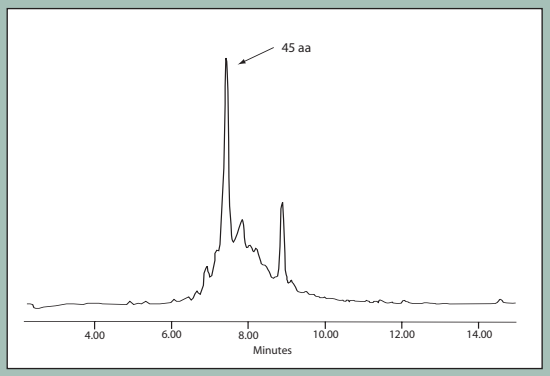
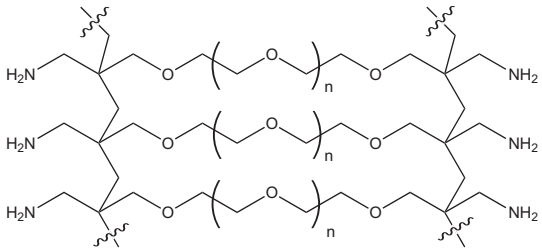


Fig. 2: Primary sequence of reduced Rantes (1-68). Sites of pseudoproline substitution are highlighted in red.

H-Ser[1]-Pro-Tyr-Ser-Ser-Asp-Thr-Thr-Pro-Cys[10]-Cys-Phe-Ala-Tyr-Ile-Ala-Arg-Pro-Leu-Pro[20]-Arg-Ala-His-Ile-Lys-Glu-Tyr-Phe-Tyr-Thr[30]-Ser-Gly-Lys-Cys-Ser-Asn-Pro-Ala-Val-Val[40]-Phe-Val-Thr-Arg-Lys-Asn-Arg-Gln-Val-Cys[50]-Ala-Asn-Pro-Glu-Lys-Lys-Trp-Val-Arg-Glu[60]-Tyr-Ile-Asn-Ser-Leu-Glu-Met-Ser[68]-OH

Synthesis on NovaPEG resin

NovaPEG



The use of NovaPEG resin has been shown to lead to dramatic improvements in peptide quality, particularly of hydrophobic, aggregated sequences. In view of the difficulties encountered on polystyrene-based supports, the synthesis of Rantes was then repeated on NovaPEG Wang resin derivatized with Fmoc-Ser(tBu). The assembly was performed as previously described. The resin was divided at cycle 36 to evaluate the influence of insertion of a pseudoproline residue at residue-30 on synthetic efficiency. Residue 30 was introduced to one half of the resin using Fmoc-Thr(tBu)-OH, whereas with the remaining half, residues 29 and 30 were incorporated in a single step using Fmoc-Tyr(tBu)-Thr($\Psi^{Me,Me}$pro)-OH. Assembly was then continued on both portions of resin in an identical manner.

LC-MS analysis (Figure 5) indicated that in both cases the major product obtained after cleavage and side-chain deprotection was Rantes. However, the product produced using 4 pseudoproline residues was of significantly higher quality and could be easily purified (Figure 6).

These results demonstrate that there is a remarkable synergy between pseudoprolines and NovaPEG resins which should greatly assist in the synthesis of large and complex peptides. Indeed, similar enhancements in synthetic efficiency have also been observed in the synthesis of CCL4-L1 by B. G. de la Torre, *et al.* [6]

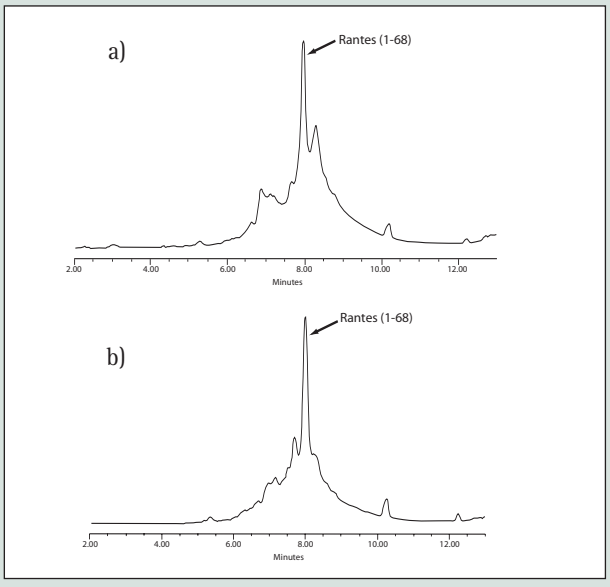


Fig. 5: HPLC profiles of crude reduced Rantes (1-68). a) Product prepared with 3 pseudoproline dipeptides; b) Product prepared with 4 pseudoproline dipeptides. a) and b) both prepared on NovaPEG Wang resins.

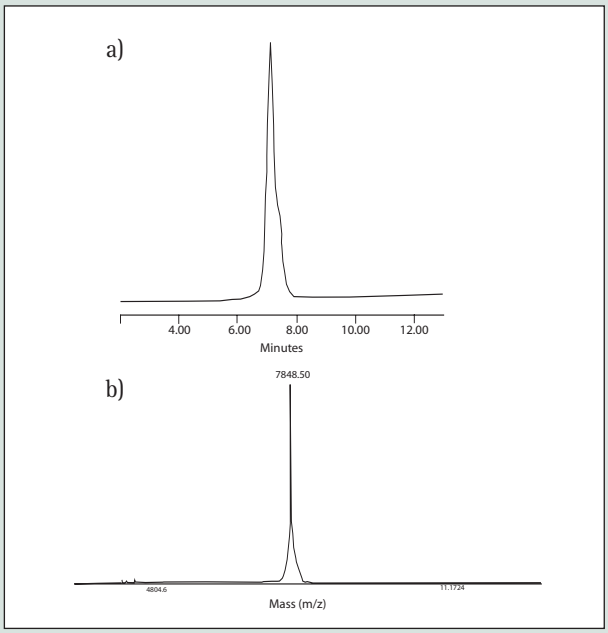


Fig. 6: a) HPLC profile of purified reduced Rantes (1-68); b) MALDI-TOF MS of reduced Rantes (1-68).

Ordering information

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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