The Synergy of ChemMatrix Resin® and Pseudoproline Building Blocks Renders RANTES, A Complex Aggregated Chemokine

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Abstract*: Traditionally, solid-phase synthesis has relied on polystyrene-based resins for the synthesis of all kinds of peptides. However, due to their high hydrophobicity, these resins have certain limitations, particularly in the synthesis of complex peptides. and in such cases, poly(ethylene glycol)-based resins, such as ChemMatrix®, are often found to give superior results. Another powerful strategy to expedite the assembly of complex peptides is to employ pseudoproline dipeptides. These derivatives disrupt the interactions among chains that are usually the cause of poor coupling yields in aggregated sequences. Here we report an efficient stepwise solid-phase synthesis of RANTES (1-68) by combining the advantages of the totally PEG-based ChemMatrix® resin and pseudoproline dipeptides.*

Keywords: ***RANTES, chemokines, PEG-based resins, pseudoproline dipeptides, aggregating peptides***

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INTRODUCTION

In recent years, several peptides, such as T-20, Eptifibatide, Ziconotide, Pramlintide, Exenatide, and Bivalirudin have been approved by the FDA and are on the market for use in the treatment of various diseases. More importantly, at the end of 2004, more than 600 peptides were either in chemical or advanced pre-clinical phases.[1] This explosion of peptides onto the pharmaceutical market has been possible, in part, because chemical synthetic methods, mainly solid-phase approaches, have reached a high level of efficacy. A large arsenal of chemical tools is now available for the synthesis of almost all peptides up to ca 40 residues. However, several small-medium size peptides and many large peptides and/or proteins are still unavailable by classical methods. One example is RANTES, a 68 amino acid chemokine (Figure 1) that has a high propensity to aggregate.[2,3]

This protein has been obtained mainly by intracellular expression in *Escherichia Coli* or secretion from insect cells.[4] An analogue, the aminooxypentane oxime of [glyoxylyl[1]] RANTES (2-68) (AOP-RANTES) has been synthesized by native chemical ligation.[5,6] Here the full peptide was divided in two fragments, AOP-RANTES (2-33)-α-thioester and RANTES (34-68), which were assembled separately by Boc chemistry and then coupled. The stepwise synthesis of the full peptide has been previously described by one of us.[7] That study showed that, at elevated temperature, a PEG-PS resin in combination with HATU as coupling reagent and "magic mixture" as a solvent allowed an effective synthesis of 40-60 residues. Unfortunately, HPLC analysis of RANTES (1-68) indicated that the coupling and deprotection procedures employed did not provide a definitive optimal method.[7]

Here we report that the combined application of the ChemMatrix® resin, a totally PEG-based solid support, and pseudoproline dipeptides allows the stepwise solid-phase synthesis of RANTES (1-68).

H-Ser-Pro-Tyr-Ser-Ser-Asp-Thr-Thr-Pro[9]-Cys-Cys-Phe-Ala-Tyr-Ile-Ala-Arg-Pro-Leu[19]-Pro-Arg-Ala-His-Ile-Lys-Glu-Tyr-Phe-Tyr[29]-Thr-Ser-Gly-Lys-Cys-Ser-Asn-Pro-Ala-Val[39]-Val-Phe-Val-Thr-Arg-Lys-Asn-Arg-Gln-Val[49]-Cys-Ala-Asn-Pro-Glu-Lys-Lys-Trp-Val-Arg[59]-Glu-Tyr-Ile-Asn-Ser-Leu-Glu-Met-Ser[68]-OH

Figure 1. Amino acid sequence of RANTES (1-68).

EXPERIMENTAL SECTION

General

Amino methyl-ChemMatrix® resin was provided by Matrix Innovation, Canada. Pseudoproline dipeptides and other protected amino acids and coupling reagents were obtained from Novabiochem (Laüfelfingen, Switzerland). HPLC was performed on a reversed-phase C_{18} (4.6 × 150 mm, 5 μm) or C_8 column (2.1 × 50 mm, 3.5 μm) as indicated in each case. Linear gradients of 0.045% TFA and 0.036% in CH_3CN were run at flow rates of 1.0 and 0.5 mL/min for the C_{18} and the C_8 column, respectively. HPLC-MS was performed on a reversed-phase C_{18} column (3.9 × 150 mm, 5 μm). Aqueous (0.1%) formic acid and formic acid (0.07%) in CH_3CN were used as eluents. For both

HPLC and HPLC-MS, linear gradients from 10 to 60% were run over 15 min, unless otherwise indicated. MALDI-TOF MS was performed on a MALDI Voyager DE RP time-of-flight (TOF) spectrometer (PE Biosystems, Foster City, CA).

Cleavage

The peptides were cleaved from the resins with simultaneous removal of side-chain protective groups by treatment with reagent K (TFA–phenol–H_2O–thioanisole–1,2-ethanedithiol, 82.5:5:5:5:2.5) for 2 and 3 h at room temperature. A ratio of 100 μL of cleavage cocktail per mg of resin was used. Following the cleavage reaction, peptides were precipitated by adding cold tert-butylmethyl ether. The solution was then decanted, and the solid was triturated with cold tert-butylmethyl ether, which was decanted again. This process was repeated twice, and peptides were finally dissolved in 10% acetic acid and lyophilized.

Attempts to synthesize RANTES

No.	Resin	ΨPro	Fragment obtained
1	PS (manual)	No	44-68
2	PS	No	43-68
3	PS	Yes	24-68
4	PEG	Yes (3 ΨPro´s)	1-68 (21%)
5	PEG	Yes (4 ΨPro´s)	1-68 (31%)

Table 1. Strategies to synthesize RANTES peptide.

Manual Synthesis. Polystyrene resin. Without ΨPro. (1)

Solid-phase peptide elongation was performed in polypropylene syringes, each fitted with a polyethylene porous disk. Solvents and soluble reagents were removed by suction.

The peptide was manually synthesized on Fmoc-Ser(^{t}Bu)-PS-Wang resin (0.036 mmol, 0.24 mmol/g). Coupling reactions were carried out with Fmoc-amino acids (3 equiv), HATU (3 equiv), HOAt (3 equiv) and DIEA (6 equiv). Fmoc was removed with piperidine–DMF (1:4) (2 × 5 min, 2 × 10 min). Double couplings were performed at Tyr^{61}, Ala^{51}, Arg^{47}, Asn^{46}, Lys^{45}, and Arg^{44}. Washings between deprotection, coupling and subsequent deprotection steps were performed with DMF (5 × 0.5 min) and CH_2Cl_2 (5 × 0.5min) using 10 mL of solvent/g of resin each time. The coupling and deprotection steps were monitored by Kaiser and De Clercq tests and Fmoc was measured by UV every two or three amino acids. To verify the course of the synthesis, four samples were taken and characterized by HPLC and MALDI-TOF MS. An acceptable HPLC profile was obtained only until Arg^{59}. The sample at Arg^{44} was analyzed by HPLC and a complex chromatogram was obtained.

HPLC-MS showed a minor peak corresponding to the expected mass (t_R 7.14; m/z calcd. for $C_{132}H_{217}N_{42}O_{39}S_2$, 3077.6; found, 3079.0 $[M + H]^+$), and MALDI-TOF MS (m/z calcd. for $C_{132}H_{217}N_{42}O_{39}S_2$, 3077.6; found, 3078.2 $[M + H]^+$) (see Figure 4).

General method for automatic synthesis

Peptide chains were elongated by means of an ABI 433A peptide synthesizer (Applied Biosystems, Foster City, CA) using standard Fmoc chemistry and FastMoc MonPrevPk'. Syntheses were conducted on a 0.1 or 0.04 mmol scale, indicated in each case, with a ten-fold excess of Fmoc-protected L-amino acids and pseudoproline dipeptides (Fmoc-Asn(Trt)^{63}-Ser(ψMe,MePro)^{64}-OH, Fmoc-Val^{42}-Thr(ψMe,MePro)^{43}-OH, Fmoc-Tyr(^{t}Bu)^{30}-Thr(ψMe,MePro)^{29}-OH and Fmoc-Asp(O^{t}Bu)^6-Thr(ψMe,MePro)^7-OH, as indicated in each attempt. HATU 0.28 or 0.16 M (depending on the scale) in DMF was used as coupling reagent. A 35-min coupling was performed on all amino acids except for Val^{38}, for which a double coupling was required. Fmoc was removed by a 15-min treatment with piperidine–DMF (1:4). At one point of the synthesis, indicated in each case, the volume of resin had grown considerably and it was necessary to remove half the resin. From this residue onwards, the concentration of coupling reagent was reduced by half. After the assembly was completed, the peptide-resin was washed with CH_2Cl_2.

1. Polystyrene resin. Without Ψ Pro. **(2)**

Synthesis was performed on Fmoc-Ser(^{t}Bu)-PS-Wang resin (0.036 mmol, 0.24 mmol/g) and using HATU (0.16 M in DMF) as coupling reagent. After Arg^{43}, half of the resin was separated. From this step on, the concentration of coupling reagent was decreased to 0.081 M. During the synthesis, nine samples were taken to verify the course of the reaction. These samples, together with the final one, were characterized by HPLC and MALDI-TOF MS. The sample at Arg^{44} gave a peak (t_R 6.62) that corresponded to the peptide of interest: HPLC-MS (*m/z* calcd. for $C_{132}H_{217}N_{42}O_{39}S_2$, 3077.6; found, 1539.0 $[M + H]^+/2$, 1026.6 $[M + H]^+/3$; 769.7 $[M + H]^+/4$, 615.4 $[M + H]^+/5$) (see Figure 6a). Characterization of the sample at Ser^{30} by HPLC-MS did not give the peptide of interest (*m/z* calcd. for $C_{189}H_{308}N_{57}O_{56}S_3$, 4368.2). The major peak (t_R 7.18 min) corresponded to the peptide until Thr^{43} (*m/z* calcd. for $C_{136}H_{224}N_{43}O_{41}S_2$, 3178.6; found, 1060.5 $[M + H]^+/3$, 635.9 $[M + H]^+/5$).

2. Polystyrene resin. With Ψ Pro. **(3)**

This approach is the same as before, except that ψPro Fmoc-Asn(Trt)^{63}-Ser(ψMe,MePro)^{64}-OH, Fmoc-Val^{42}-Thr(ψMe,MePro)^{43}-OH and Fmoc-Tyr(^{t}Bu)^{30}-Thr(ψMe,MePro)^{29}-OH were used during the synthesis. After Arg^{44}, half of the resin was separated. From this step on, the concentration of coupling reagent was decreased to 0.081 M. During the synthesis, nine samples were taken to verify the course of the reaction. These samples, together with the final one, were characterized by HPLC and MALDI-TOF MS. The sample at Arg^{44} gave a major peak (t_R 6.13 min¸63%) by HPLC and the mass was verified by HPLC-MS (*m/z* calcd. for $C_{132}H_{217}N_{42}O_{39}S_2$, 3077.6; found, 1540.0 $[M + H]^+/2$, 1026.6 $[M + H]^+/3$; 769.9 $[M + H]^+/4$, 616.7 $[M + H]^+/5$, 513.6 $[M + H]^+/6$) (see Figure 6b). For the sample at Ile^{24}, analytical HPLC gave a major peak (t_R 7.43 min, 44%), which was characterized by HPLC-MS (*m/z* calcd. for $C_{237}H_{372}N_{65}O_{68}S_3$, 5311.7; found, 886.3 $[M + H]^+/6$,

759.6 $[M + H]^+$/7, 664.6 $[M + H]^+$/8, 591.1 $[M + H]^+$/9) and MALDI-TOF MS (*m/z* calcd. for $C_{237}H_{372}N_{65}O_{68}S_3$, 5311.7; found, 5312.7 $[M + H]^+$) corresponding to the peptide of interest (see Figure 7).

3. ChemMatrix® resin. With ΨPro.

This synthesis was performed on the Wang-ChemMatrix® PEG-resin (0.09 mmol, 0.60 mmol/g). The first amino acid was coupled manually as follows: Fmoc-Ser(^{t}Bu)-OH (4 equiv), MSNT (4 equiv), NMI (8 equiv) and DIEA (12 equiv) in CH_2Cl_2 at room temperature for 5 h Next, an acetylation step of the *N*-terminal group was carried out using Ac_2O–DIEA (50 equiv each) in DMF for 15 min. On the basis of Fmoc quantification, a loading of 0.42 mmol/g was determined. The remaining peptide chain elongation was done on an automatic synthesizer using HATU (0.28 M) as coupling reagent. Four samples were taken and analyzed. Until the sample at Ser^{31}, two ΨPro dipeptides were introduced, Fmoc-Asn(Trt)^{63}-Ser(ψMe,MePro)^{64}-OH and Fmoc-Val^{42}-Thr(ψMe,MePro)^{43}-OH. Up to this point, half the resin was split and the synthesis continued separately.

A. Using three ΨPro dipeptides. (4). The synthesis was continued on half of the resin without using Fmoc-Tyr(^{t}Bu)^{30}-Thr(ψMe,MePro)^{29}-OH, which was replaced by the corresponding Fmoc-L-amino acids. Another sample was taken to verify the course of the synthesis. The final peptide was characterized by HPLC (t_R 7.97, 21 %) and MALDI-TOF MS (*m/z* calcd. for $C_{350}H_{539}N_{96}O_{100}S_5$, 7846.8; found, 7847.3 $[M + H]^+$) (see Figure 8).

B. Using four ΨPro dipeptides. (5). The synthesis continued with the remaining half of the resin separated at Ser^{31}, in this case incorporating Fmoc-Tyr(^{t}Bu)^{29}-Thr(ψMe,MePro)^{30}-OH. A final sample was taken and checked. The final peptide was characterized by HPLC (t_R 7.99, 31%) and MALDI-TOF MS (*m/z* calcd. for $C_{350}H_{539}N_{96}O_{100}S_5$, 7846.8; found, 7851.0 $[M + H]^+$) (see Figure 9).

Purification. An aliquot of the crude RANTES was solved in 7.3 M guanidinium HCl, 0.5% EDTA, 0.1 M Tris·HCl, pH 8.2 and purified on an analytical reverse-phase C_8 column, using a linear gradient of 0.045% aqueous TFA-0.036% TFA in CH_3CN, from 10 to 60% over 15 min, with a flow rate of 0.5 mL/min. The purified peptide was characterized under reducing conditions by HPLC (t_R 7.14, 90%) and MALDI-TOF MS (*m/z* calcd. for $C_{350}H_{539}N_{96}O_{100}S_5$, 7846.8; found, 7848.5 $[M + H]^+$) (see Figure 10).

RESULTS AND DISCUSSION

Although the synthesis of peptides containing hin- dered amino acids can be accomplished by the con-course of highly reactive coupling methods[10,11] such as HATU[12] or N,N,N^0,N^0-tetramethylformamidinium hexafluorophosphate (TFFH),[13] that of large and well-structured peptides requires additional synthetic tools. In such cases, intra- and interchain interactions can be minimized by special solvents, such as the so-called magic mixtures[14] or those containing chaotropic salts,[15] by structure disrupters such as Pro residues[16] or protected amide bonds,[17] and by resins that facilitate the solvation of peptide chains.[18,19]

The present work explores the application of pseudoproline (ΨPro) dipeptides in combination with a total PEG–resin to overcome aggregation and thus achieve the desired peptide. Pro dipeptides consist of a dipeptide in which the Ser, Thr, or Cys residue has been reversibly protected as proline-like TFA-labile oxazolidine (Figure 2).[20,21] Just like Pro, the insertion of a pseudoproline residue into a sequence disrupts the aggregation thought responsible for the problems encountered during peptide assembly.[22] Once peptide chain elongation has been accomplished, the final acidic treatment that detaches the peptide from the resin and removes the side-chain protecting groups of the amino acids also opens the oxazolidine ring, thereby releasing the natural amino acids. ΨPro residues are incorporated using preformed dipeptides because the poor nucleophilicity of the secondary oxazolidine amino function would prevent the solid-phase incorporation of the next amino acid.

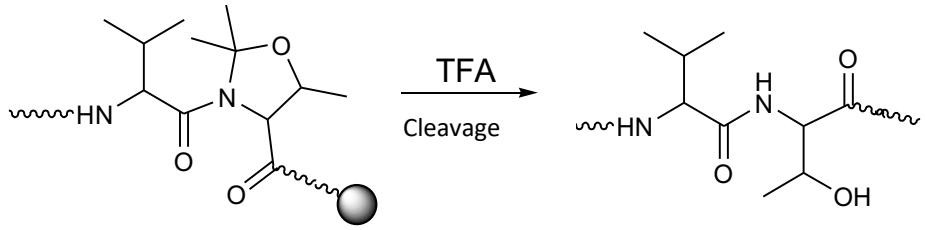

Figure 2. Val[42]-Thr(ψMe,MePro)[43] on RANTES synthesis. Upon cleavage the pseudoproline renders Val[42]-Thr[43].

Furthermore, our groups have recently succeeded in obtaining difficult short- and medium-sized peptides using ChemMatrix resin (Figure 3), a total PEG resin.[8] PEG-based resins were independently developed by Meldal and coworkers[23,24] and Cote.[25] The optimal properties of the PEG are due to the vicinal arrangements of carbon–oxygen bonds throughout the chain, which make the PEG assume helical structures with gauche interactions between polarized bonds.[26] PEG can exhibit three helical arrangements: the first, largely hydrophobic; the second, of interme- diate hydrophobicity; and the third, hydrophilic. The amphiphilic nature of PEG makes the resin well sol- vated in both polar and nonpolar solvents and disrupts backbone interactions,[26] and gives usually superior results when compared with PS resins.[8,23,24,27]

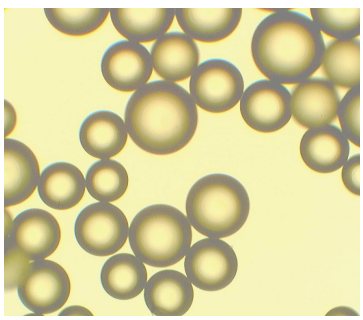

Figure 3. ChemMatrix® bead resins by Electronic Microscopy.

Our initial attempts to prepare RANTES by a classi- cal stepwise Fmoc solid-phase strategy on polystyrene resin failed to deliver the expected peptide, despite trying a range of coupling reagents and manual and auto- matic synthesis conditions. For instance, a manual synthesis starting with low-loading Wang–PS–resin [Fmoc–Ser(tBu)–O–Wang–PS–resin, 0.24 mmol/g] and HATU/HOAt, considered one of the most efficient coupling methods,[12] gave an extremely poor yield of RANTES Arg^{44}–Ser^{68} (25 amino acids) (Figure 4).

To overcome these difficulties, the use of Pro dipeptides was then evaluated. When using this strat- egy, the choice of the key residues Thr or Ser to be replaced by Pro residues is crucial because the proline motif provides a deliberate change of native struc- ture, usually in the region of 6–10 residues after its incorporation, thereby improving the efficacy of synthesis. The RANTES sequence contains the following replaceable residues: Ser^{68}, Ser^{64}, Thr^{43}, Ser^{35}, Thr^{30}, Thr^{8}, Thr^{7}, Ser^{5}, and Ser^{4}. Ser^{64} is located close to the C-terminal, and therefore the Pro [Fmoc–Asn (Trt)^{63}–Ser ($\Psi^{Me,Me}$Pro)^{64}–OH] was introduced into his C-terminal part to initially alter chain conformation. According to the structural analysis by Chung et al.,[28] and corroborated by our own preliminary synthesis, the critical part of the sequence falls between residues 53 and 22. In particular, hydrogen bonds formed by Thr^{30} and Thr^{43} are energetically important and crucial during folding, as they are the first to be formed. Incorporation of Pro at the turn positions destabilizes these hydrogen bonds, thereby preventing beta-sheet formation. By taking advantage of these Thr's, two Pro dipeptides were chosen in this region, Val – Thr($\Psi^{Me,Me}$Pro)^{43} and Tyr(tBu)^{29}–Thr($\Psi^{Me,Me}$Pro)^{30}, which are located on distinct sheets in the backbone of the native peptide (Figure 5).

Previous studies on the synthesis of this large and complex peptide concluded that the N-terminal region is also a difficult part.[7] Thus the Pro dipeptide Asp(OtBu)^{6}–Thr($\Psi^{Me,Me}$Pro)^{7} was selected in this region (Alternatively, the Fmoc–Thr(tBu)^{7}–Thr($\Psi^{Me,Me}$Pro)^{8}–OH could have been inserted, but both these beta-branched building blocks are of more difficult preparation and its incorporation as dipeptide on Pro^{9} would also be more difficult).

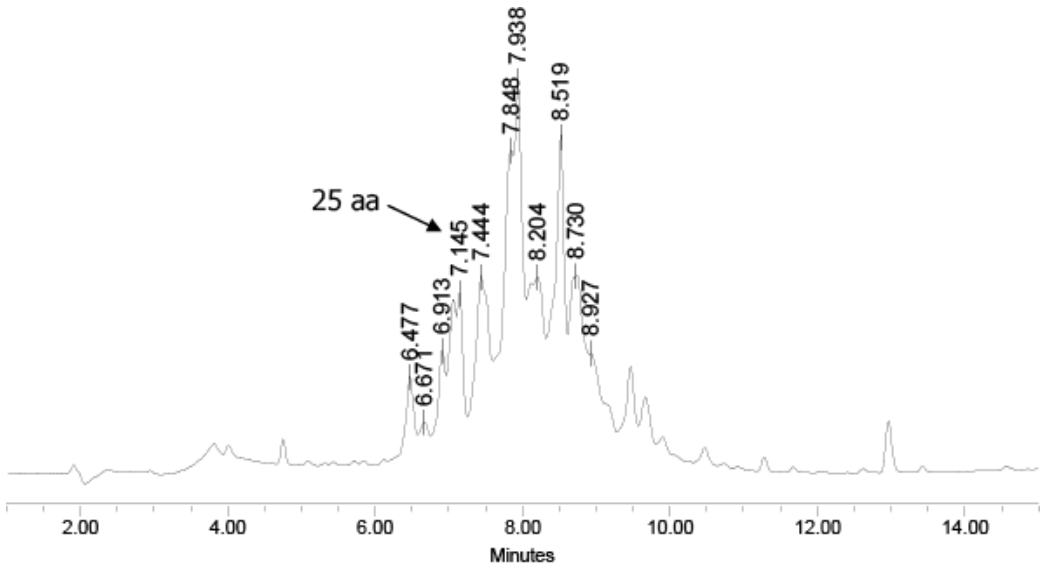

Figure 4. HPLC chromatogram of RANTES (Arg^{44}-Ser^{68}) manually synthesized on PS resin and using HATU/HOAt as coupling reagent. HPLC conditions: C-18 column, linear gradient 0.1% aqueous TFA-0.1% TFA in CH_3CN, from 10 to 60% over 15 min, flow rate of 1.0 mL/min.

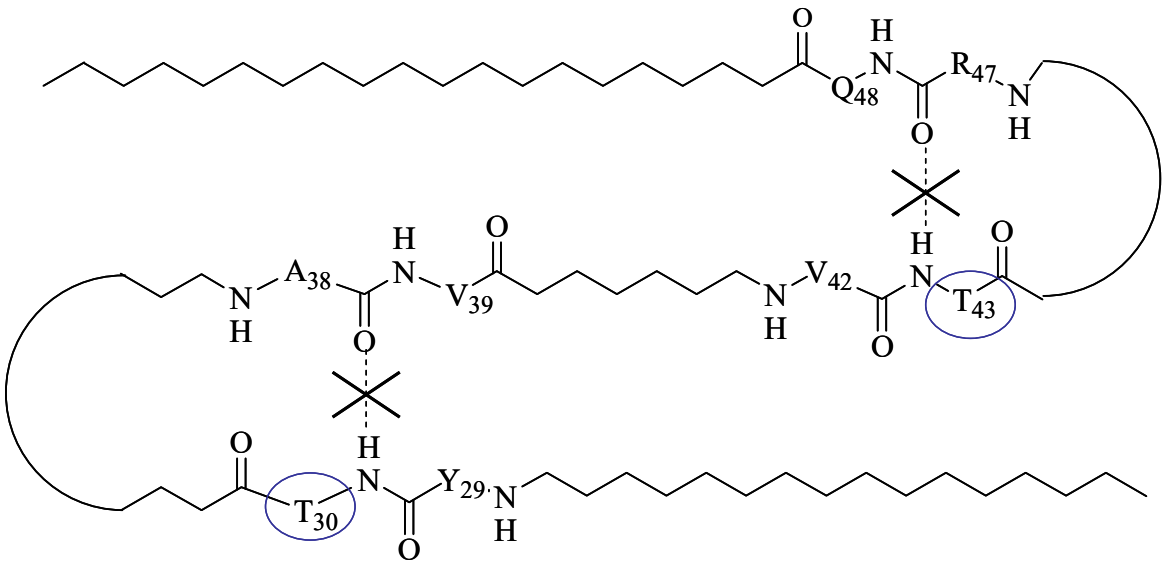

Figure 5. Scheme of part of the ß-sheet of the RANTES structure. Circled amino acids were incorporated as ΨPro dipeptides.

Two automatic syntheses with and without Pro dipeptides of the RANTES sequence were performed on PS using HATU as coupling reagent. Syntheses were analyzed at several points during the process. Thus, in the synthesis carried out with regular amino acids only, the peptide obtained from the sample taken at

Arg^{44} (25 amino acids) showed a peak (t_R 6.36 min) corresponding to the peptide of interest, and should hypothetically allow the isolation of this fragment (Figure 6a) (An additional conclusion of the two syntheses discussed is that automatic synthesis very often performed better than the manual ones). The peptide from the next sample taken at Ser^{31} (38 amino acids), however, did not show a mass that could be identified by HPLC- MS with the target peptide.

In contrast, the synthesis performed using similar experimental conditions but with the use of ΨPro dipeptides progressed more smoothly. Thus, the pep- tide obtained from the sample taken at Arg^{44} (26 amino acids), where the Pro dipeptide $Asn(Trt)^{63}$– $Ser(\Psi^{Me,Me}Pro)^{64}$ was incorporated, showed a major peak (t_R 6.31 min), corresponding to the peptide of interest, which should be easily isolated (Figure 6b). Furthermore, the product from the sample at Ser^{31} (38 amino acids), where the second Pro,Val^{42}– $Thr(\Psi^{Me,Me}Pro)^{43}$ was also incorporated, showed a large peak by HPLC-MS, which corresponded to the target peptide (data not shown). Unfortunately, the product from the sample at Ile^{24} (45 amino acids) was the last one in which a peak corresponding to the target peptide could be identified (Figure 7) (At this stage, a short study of several cleavage methods was performed. Reagent K gave clearly better results than the other methods tested).

These results indicate that although the use of Pro dipeptides clearly benefits the synthesis of RANTES, the effects of their incorporation are insufficient to allow the assembly of the full-length peptide in good yield. Consequently, the synthesis of RANTES was then repeated using Pro dipeptides in combination with the ChemMatrix resin; as in our previous experiences, the use of this amphiphilic resin could be anticipated to significantly improve the synthetic efficiency of this hydrophobic peptide.[23] A new synthesis was therefore undertaken using Fmoc– Ser(tBu)–O–Wang–ChemMatrix resin. The higher substitution of this resin (0.41 mmol/g) when com- pared with the PS resin (0.24 mmol/g) was not anticipated to negatively effect the synthesis, owing to the unique architecture of this polymer. Residues 63 and 64 and 42 and 43 were incorporated using Pro dipeptides $Asn(Trt)^{63}$–$Ser(\Psi^{Me,Me}Pro)^{64}$ and Val^{42}– $Thr(\Psi^{Me,Me}Pro)^{43}$. The resin was divided in two portions at residue 31 to evaluate the beneficial effect of introducing a Pro residue at Thr^{30}. With one portion of the resin, the ‘‘classical’’ amino acid Fmoc– Thr(tBu)–OH was introduced at residue 30, whereas in the second, $Tyr(tBu)^{29}$–$Thr(\Psi^{Me,Me}Pro)^{30}$ was used to introduce Tyr^{29}–Thr^{30}. In both syntheses, the ΨPro dipeptide $Asp(OtBu)^6$–$Thr(\Psi^{Me,Me}Pro)^7$ was introduced in place of Asp^6–Thr^7. In both cases, a major peak corresponding to the desired final RANTES peptide was obtained (Figure 8 and 9). Moreover, the HPLC profile was notably improved when all four pseudoPro dipeptides were used (Figure 9). In this case, the MALDI-TOF MS confirmed the success of the approach that combines the full PEG resin, ChemMatrix, and Pro dipeptides to disrupt backbone interactions.

Finally, the peptide was purified under reducing conditions and the desired sequence was obtained with a 90% purity and was then verified by MALDI- TOF MS using the linear mode (Figure 10).

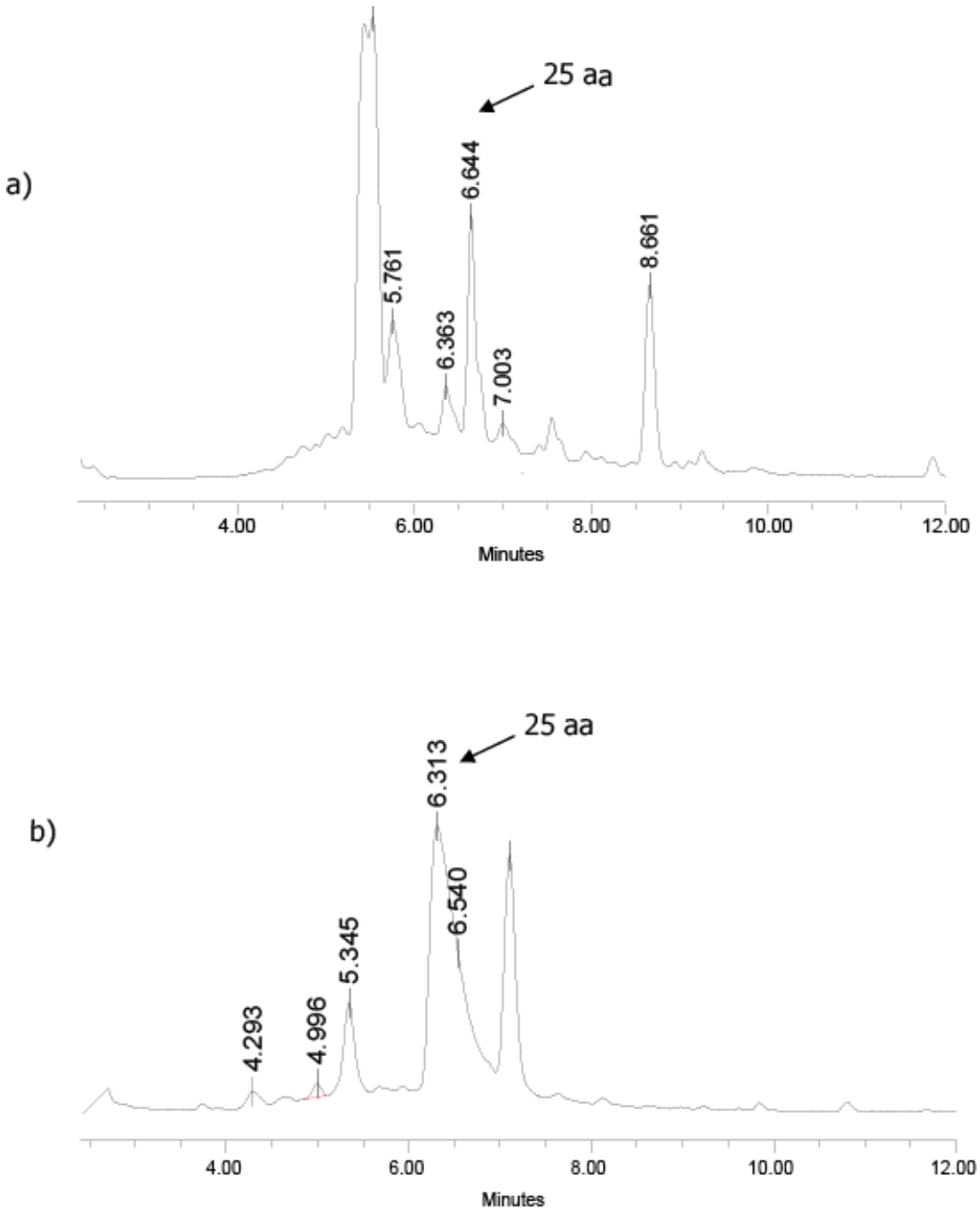

Figure 6. Synthesis of RANTES. **Sample Arg[44]. a)** PS resin, without using ΨPro.

b) PS resin, using ΨPro. HPLC conditions: C-18 column, linear gradient 0.1% aqueous TFA-0.1% TFA in CH_3CN, from 10 to 60% over 15 min, flow rate of 1.0 mL/min.

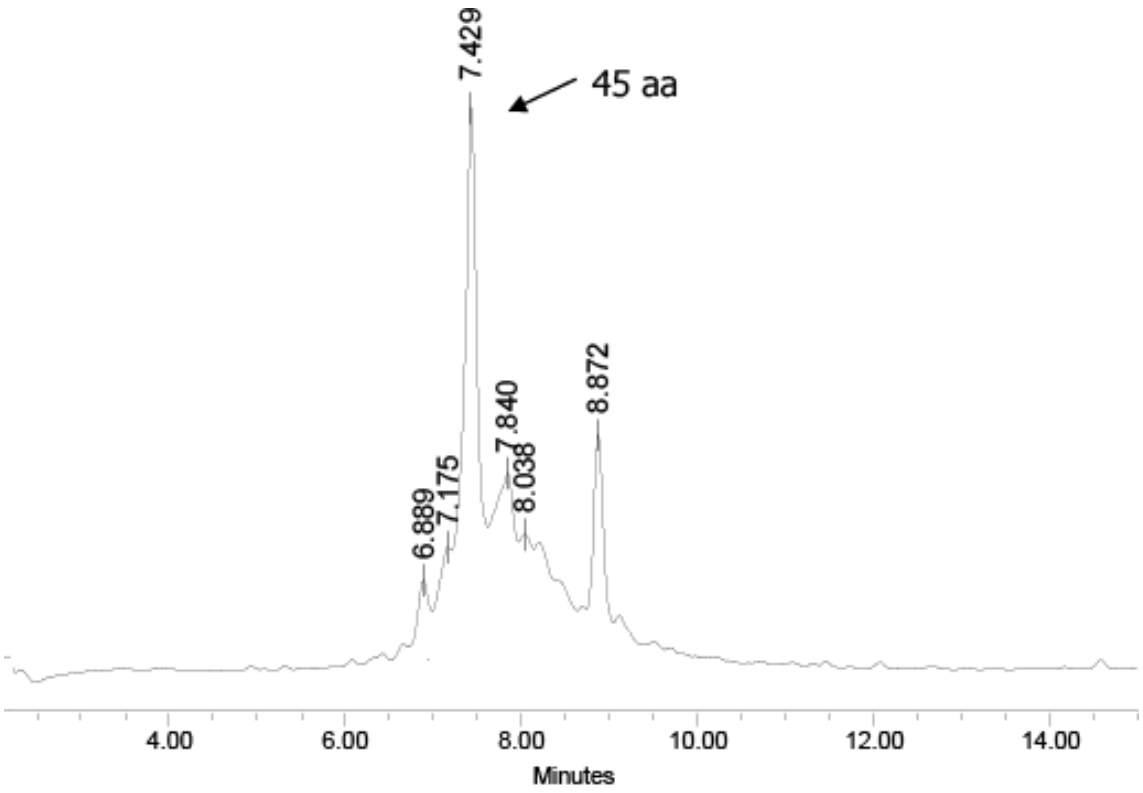

Figure 7. Synthesis of RANTES using ΨPro dipeptides on PS resin. **Sample Ile[23]**. HPLC conditions: C-18 column, linear gradient 0.1% aqueous TFA-0.1% TFA in CH_3CN, from 10 to 60% over 15 min, flow rate of 1.0 mL/min.

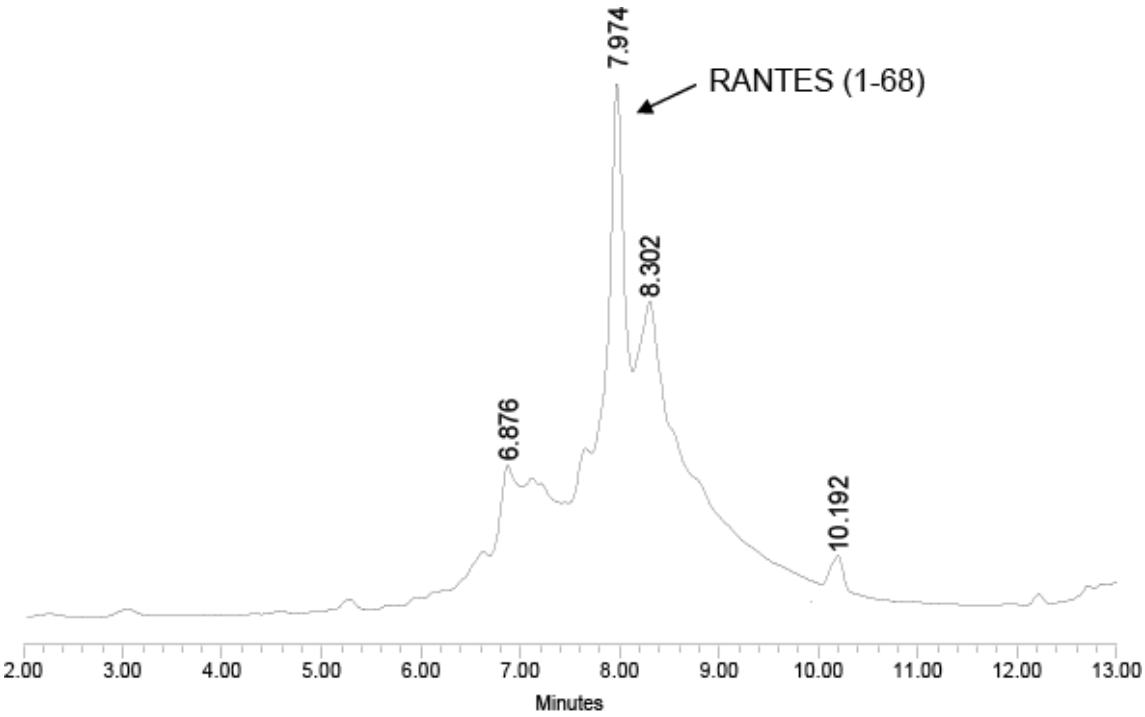

Figure 8. Synthesis of RANTES using 3 ΨPro dipeptides, on ChemMatrix® resin. HPLC conditions: C_8 column, linear gradient 0.1% aqueous TFA-0.1% TFA in CH_3CN, from 10 to 60% over 10 min, flow rate of 1.0 mL/min.

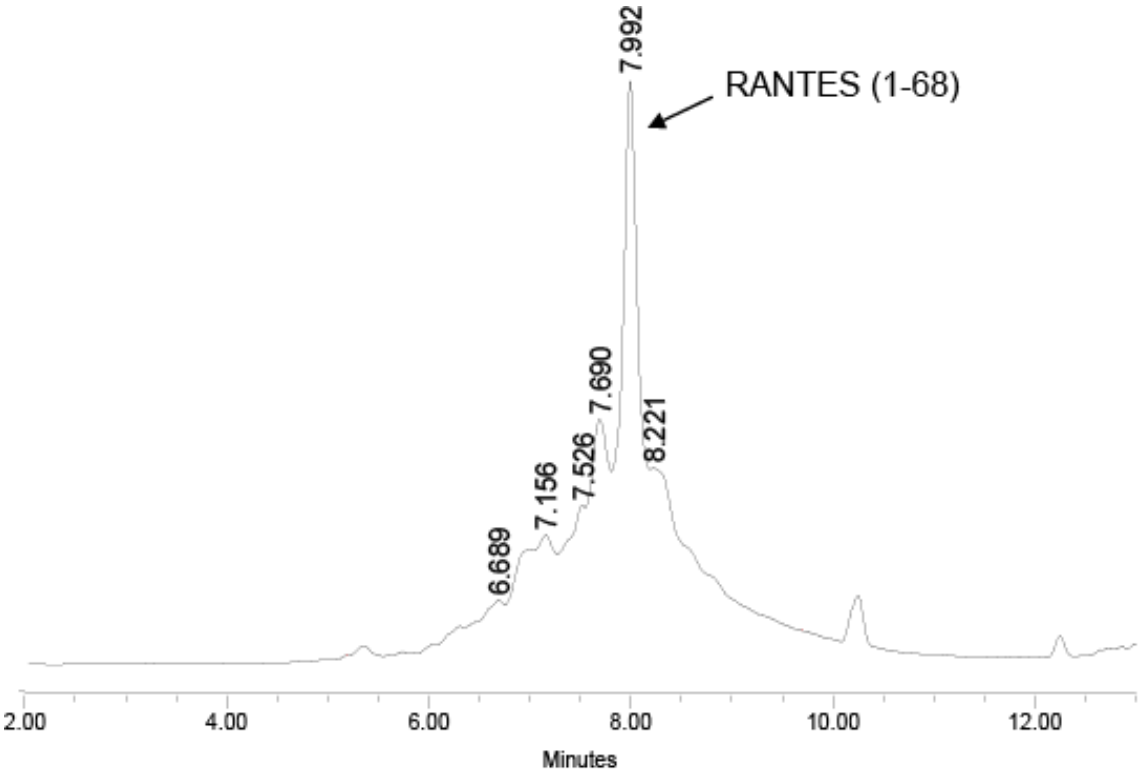

Figure 9. Synthesis of RANTES using PEG-CM and 4 ΨPro dipeptides. HPLC conditions: C-8 column, linear gradient 0.1% aqueous TFA-0.1% TFA in CH_3CN, from 10 to 60% over 10 min, flow rate of 1.0 mL/min.

Finally, the peptide was purified under reducing conditions and the desired sequence was obtained with a 90% purity and was then verified by MALDI-TOF MS using the linear mode (Figure 10).

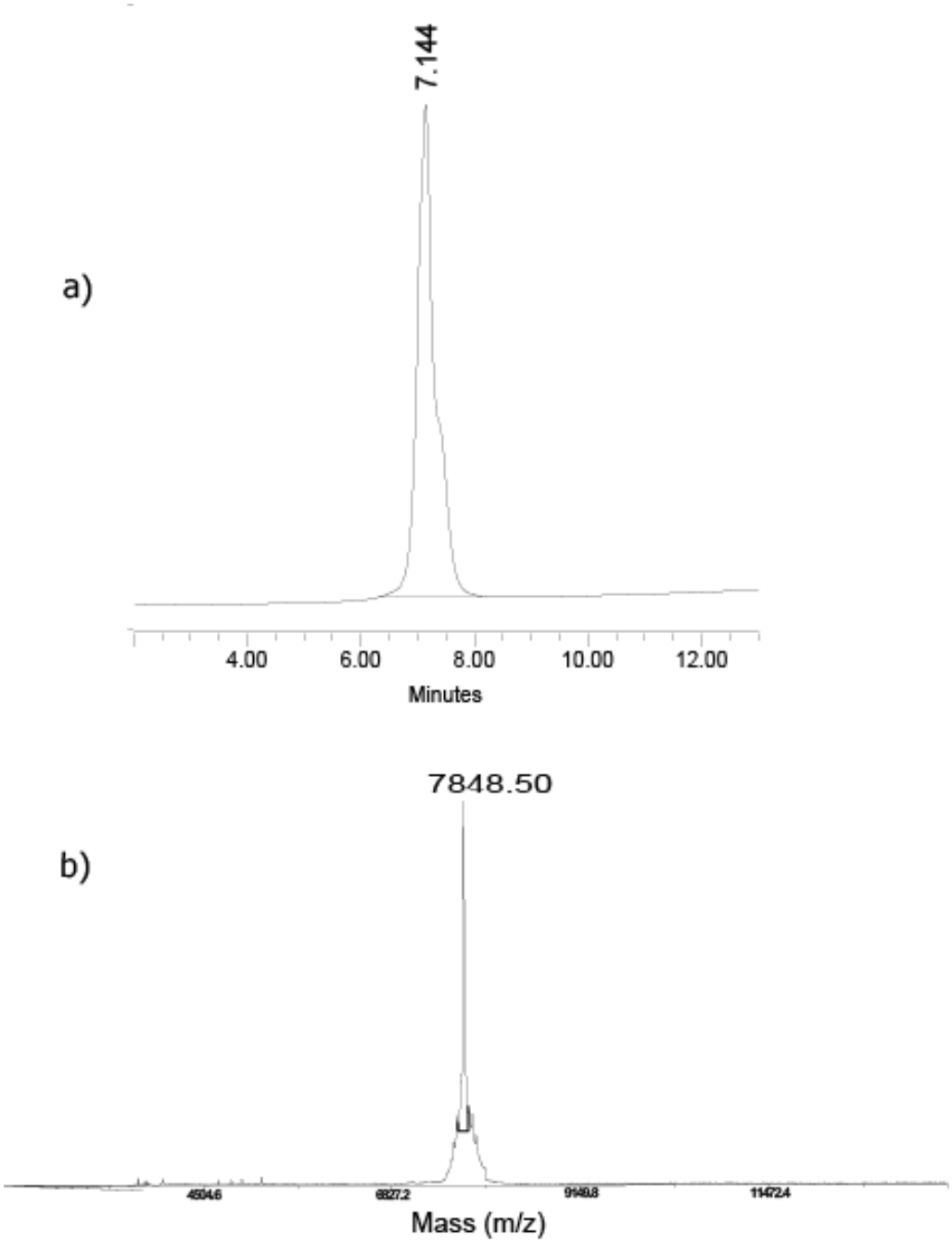

Figure 10. Purified RANTES. HPLC (t_R 7.14, 90%) and MALDI-TOF MS (7848.5, $[M + H]^+$). HPLC conditions: peptide dissolved in 6M guanidine, using a C-8 column, linear gradient 0.1% aqueous TFA-0.1% TFA in CH_3CN, from 10 to 60% over 10 min, flow rate of 1.0 mL/min.

CONCLUSIONS

Although ChemMatrix® resin and ΨPro dipeptides have been separately demonstrated to be excellent strategies for the synthesis of complex and/or large peptides, here we show that the concomitant use of both allows the stepwise synthesis of an even greater range of large peptides and proteins. The synergy between the two methods should boost research in several therapeutic areas.

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ABBREVIATIONS

Abbreviations used for amino acids and the designations of peptides follow the IUPAC-IUB Commission of Biochemical Nomenclature in *J. Biol. Chem*. **1982**, *247*, 977−983. Amino acid symbols denote L-configuration.

Ac2O, acetic anhydride; Boc, *tert*-butyloxycarbonyl; CH3CN, acetonitrile; CM, ChemMatrix®; DIEA, *N*,*N*-diisopropylethylamine; DIPCDI, *N*,*N*′-diisopropylcarbodiimide; MSNT, 1-(2-mesitylenesulfonyl)-3-nitro-1H-1,2,4-triazole; DMF, *N*,*N*-dimethylformamide; EDT, 1,2-ethanedithiol; ESMS, electrospray mass spectrometry; FDA, Food and Drug Administration; Fmoc, 9-fluorenylmethoxycarbonyl; HATU, 1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo-[4,5-*b*]pyridinium hexafluorophosphate 3-oxide; HOAc, acetic acid; HOAt, 1-hydroxy-7-azabenzotriazole (3-hydroxy-3*H*-1,2,3-triazolo-[4,5-*b*]pyridine; HPLC, high performance liquid chromatography; MALDI, matrix-assisted laser desorption ionization; MBHA, *p*-methylbenzydrylamine; MeOH, methanol; MS, mass spectrometry; NMI, *N*-methylimidazole; PEG, polyethylene glycol; PS, polystyrene solid support; SPPS, solid-phase peptide synthesis; ^{t}Bu, *tert*-butyl; TFA, trifluoroacetic acid; TFFH, *N,N,N′,N′*-tetramethylformamidinium hexafluorophosphate; TIS, triisopropylsilane; Trt, trityl; TOF, time-of-flight. UV, ultraviolet. □Pro, pseudo-proline.

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