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Advances on the green synthesis of Etelcalcetide

[1]Koula Achilleos, [2]Andreas Sergides, [1]Styliana Mirallai, [2]Charalambos Ioannou, [3]Vicky Nicolaidou, [1]Christos Petrou, [2]Christakis Sergides, [1]Yiannis Sarigiannis

[1] Department of Health Sciences, School of Life & Health Sciences, University of Nicosia, Nicosia, Cyprus

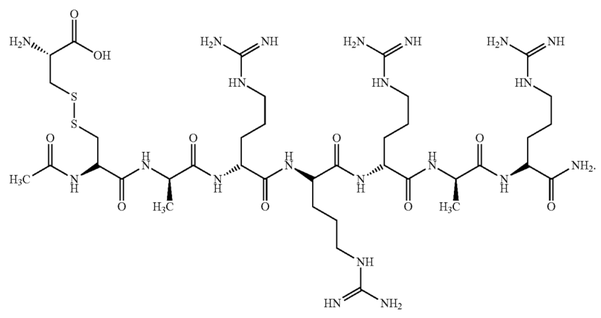
[2] Biogena API LTD, Limassol, Cyprus

[3] Department of Life Sciences, School of Life & Health Sciences, University of Nicosia, Nicosia, Cyprus

Introduction

Background

Treatment of Secondary Hyperparathyroidism (SHPT) includes Etelcalcetide (ETC), a calcium-sensing receptor (CaSR) agonist, which reduces the levels of Parathyroid Hormone (PTH) levels in serum leading to increased bone mass. However, the effects of combined treatment with osteoporosis drugs such as teriparatide (TPTD) remain unclear. Meanwhile ETC is under investigation for the treatment of other rare diseases caused from abnormal calcium sensing and signaling like autosomal dominant hypocalcemia (ADH) or Bartter-syndrome type V.



Objective

Etelcalcetide is a linear octapeptide with a disulfide bridge consisting of seven D-amino acids, four of them are D-Arginine, while at the N terminal site there is a D-Cysteine which forms a disulfide bridge with an L-Cysteine. It is really a synthetic challenge because of the three arginine in a row. Arginine is a sensitive amino acid which produces epimerization or other side reactions. This demands special treatment during synthesis and particular purifications steps as reported in most of the patents. The new EU regulations restrict the use of DMF (Dec 2023), a commonly used solvent in SPPS for manufacturing of peptides. This green wind of change presents challenges and opportunities for peptide manufactures. Solvents which is among the crucial parameters are especially considered as they may affect the total process. Here, we present some of our efforts for the synthesis of etelcalcetide in a greener way.

Materials - Methods

Our plan was to use ChemMatrix ® resin which is an established PEG type resin and exhibits great performance especially in high temperatures and stability under Microwave irradiation. However, this resin became recently unavailable for industrial scale. So, it was decided to work with other resins currently available in the market in bulk quantities making the future transfer from lab to industrial scale easier. In short and ultra short peptides like Etelcalcetide in industrial scale resins should have high loading capacity or else the peptide should be synthesized in liquid phase. We tried several combinations of resins (Polystyrene or PEG type or Hybrids) and green solvents and Fmoc-deprotection solutions. For the protection of Cysteine side group Trt or Mmt protecting group was selected. Depending on the side group selection of Cysteine, different way of disulfide bridge formation was selected. Here we present some of our findings elaborating with the following solid supports: Rink Amide Protide ®, PL-Rink Amide (Fmoc-Rink Amide AMS), Rink Amide MBHA, and PL Rink Amide. All couplings for Arg and Cys had been performed at temp 40-45 min while Fmoc deprotections occurred in similar conditions. The peptides were cleaved from the resin with TFA:TIS:H_2O (92:4:4, v/v).

Results

Swelling test

Swelling of the resin is crucial for the success of a synthesis in Solid Phase Peptide Synthesis as it permits the molecules to come closer and easily react. In the following figures we present an example of the swelling tests. **A remarkable notice is that the green solvents swell better PEG Type resins than PS type resins like the Amphispheres 20 RAM (PS PEG600 Fmoc-Rink Amide).**

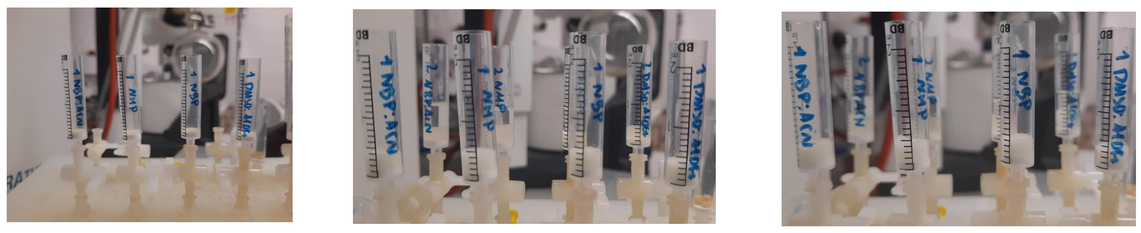


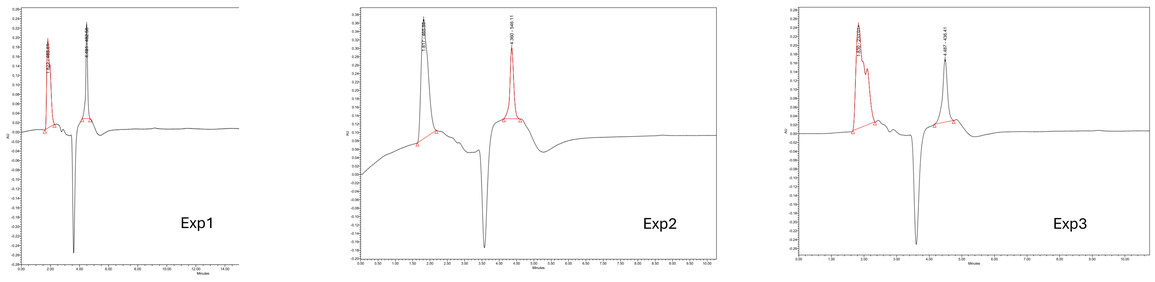
Fig 1. Swelling test for 50mg resin Amphispheres 20 RAM (loading 0.6mmoL/g) and 1 mL of solvent for 15min and 30min

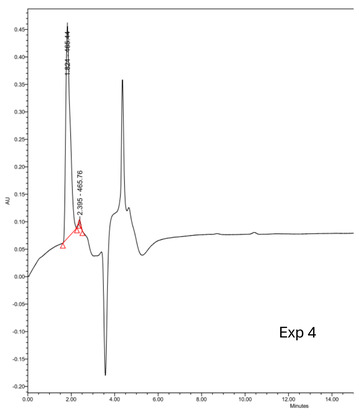
Table 1 (left) presents the combinations of Rink Amide MBHA resin and solvents systems for the synthesis of Etelcalcetide as well as occurred problems during synthesis while **Table 2** (right) presents combinations of PEG Type resins as well as problems occurred during synthesis.

Resins	Coupling conditions	Solvent Systems	Deprotection Conditions	Results
Rink Amide MBHA resin (200-400 mesh)	Fmoc-D-Arg (Pbf)-OH: Coupling: 1 min 40°C, 4 min 45°C	DMSO: EtOAc (1:9)	5% piperazine + 2% DBU in NMP	Arg and Cys needed recouplings
Rink Amide MBHA resin (200-400 mesh)	Fmoc-D-Arg (Pbf)-OH: Coupling: 1 min 40°C, 4 min 45°C	DMSO: EtOAc (1:9)	5% piperazine + 2% DBU in NMP	*Increase of coupling time resolve the previous problem*
Rink Amide MBHA resin (200-400 mesh)	Fmoc-D-Arg (Pbf)-OH: Coupling: 1 min 40°C, 4 min 45°C	NBP	5% piperazine + 2% DBU in NMP	The 3rd Arg need recoupling. *Elongation of the coupling time resolve the problem*
Rink Amide MBHA resin (200-400 mesh)	Fmoc-D-Arg (Pbf)-OH Coupling: 3 min 40°C, 7 min 45 °C	NBP: ACN (5:5)	5% piperazine + 2% DBU in NMP	After 3rd arginine each AA needed recoupling
Rink Amide MBHA resin (200-400 mesh)	Fmoc-D-Arg (Pbf)-OH Coupling: 3 min 40°C, 7 min 45 °C	NMP	5% piperazine + 2% DBU in NMP	Each Arginine demands recoupling

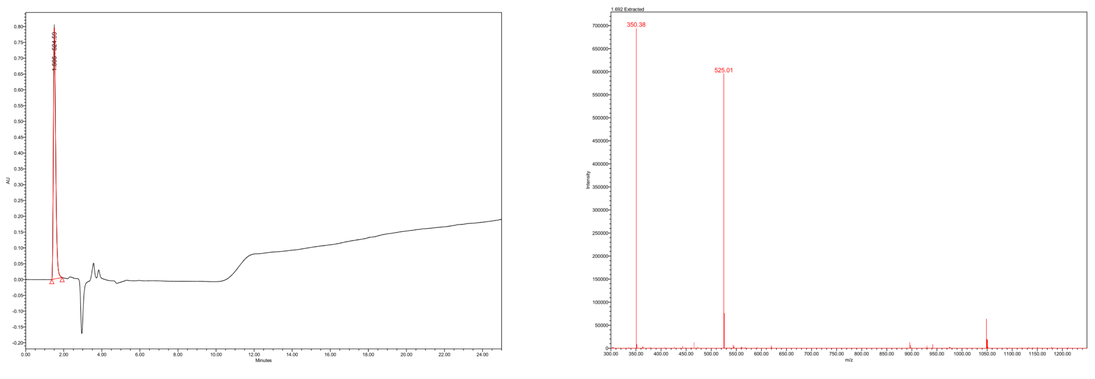
Resins	Coupling conditions	Solvent Systems	Deprotection Conditions	Results
PL-Rink: Fmoc Rink amide AMS resin	Fmoc-D-Arg (Pbf)-OH: Coupling: 3 min 40°C, 7 min 45°C	NBP: ACN (5:5)	5% piperazine + 2% DBU in NMP	**No need for any recoupling**
PL-Rink: Fmoc Rink amide AMS resin	Fmoc-D-Arg (Pbf)-OH: Coupling: 3 min 40°C, 7 min 45°C	NMP	5% piperazine + 2% DBU in NMP	*Repeat couplings*
Rink Amide Protide Resin	Fmoc-D-Arg (Pbf)-OH: Coupling: 3 min 40°C, 7 min 45°C	NBP: ACN (5:5)	5% piperazine + 2% DBU in NMP	**No need for any recoupling**
PS PEG600 Fmoc Rink amide	Fmoc-D-Arg (Pbf)-OH Coupling: 3 min 40°C, 7 min 45 °C	NBP: ACN (5:5)	5% piperazine + 2% DBU in NMP	**No need for recoupling**
Rink Amide Protide Resin	Fmoc-D-Arg (Pbf)-OH Coupling: 1 min 40°C, 5 min 45 °C	NBP: ACN (5:5)	5% piperazine + 2% DBU in NMP	**No need for recoupling**

Under these conditions we didn't manage to obtain products of the heptapeptide without epimerization. Another issue is the high polarity of the acetyl heptapeptide which is responsible for the early elution of the peptide (~ 2 min). This demands other analytical approach which is under investigation.





It is obvious how the resin selection and the rest conditions affect the synthesis of the linear heptapeptide. **Figure 3** presents the synthesis of heptapeptide with Rink Amide MBHA (200-400 mesh) and using NMP as solvent. **On the opposite PL Rink Amide resin either with NMP or NBP-ACN reduces the epimerization of the heptapeptide. Moreover, Rink Amide Protide also displays high epimerization level under these conditions. We have some preliminary results of other conditions at higher temperatures limiting the epimerization. Soon we are going to present them.**



LC-MS of isolated Etelcalcetide

LC-PDA-MS analysis was performed using a Luna 5um C18 100 A (150 x 3 mm, 5um) column and eluents A: H_2O 0.1FA% v/v, B: MeCN 0.1FA % v/v (5% B to 65% B in 25min);
Detection at 220 nm. $[M+H]^{+}$: 1047.53, $[M+2H]^{2+}$: 525.01, $[M+3H]^{3+}$: 350.38

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