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Divalent metal ions-directed release of *C*-terminally modified peptides at 60 °C under mild conditions

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Introduction

C-terminally modified peptides (*C*-TMPs) are of great interest in the field of therapeutic peptides as modification of such macromolecules can favorably impact its potency, chemical stability, hydrophobicity and biological barriers permeability.[1] The synthesis of *C*-TPMs can be complex and time-consuming. In fact, some steps of the synthetic routes employ acidic or basic conditions, leading to *C*-terminal amino acid enantiomerization[2a,b] and other side-reactions.

In the 1990s, Machini-Miranda et al. first observed transesterification of the *N*-acylated *C*-esterified tripeptide Moz-Asn-Leu-Gly-OEt in MeOH/H_2O containing calcium salts. These authors also proved the applicability such Ca^{2+}-promoted transesterification by using this new procedure to produce various di- and tripeptide esters.[3a] Soon after, they first demonstrated that the peptide-resin (PR) ester bond present in growing PRs made by Boc chemistry was susceptible to Ca^{2+}-promoted methanolysis, releasing the corresponding the *N*- and side-chain protected peptide methyl ester.[3b] In the following years, the group investigated Ca^{2+}- or Zn^{2+}-promoted peptide detachment from the Kaiser oxime resin (KOR) to give the corresponding *C*-TMPs. Examples included: i) transesterification of X-KOR bond from Ac-Ala-Gly-X-KOR (X: Gly, Ala, Phe or Lys (2-Cl-Z)) and from Ac-Ile-Ser(Bzl)-Asp(OZ)-KOR (Z: Bzl or cHex) to yield 85-96 % of the corresponding Ac-peptide-OMe;[4] ii) aminolysis of the Asp(X)-KOR bond from Ac-Ile-Ser(Bzl)-Asp(X)-KOR (X: OBzl or OcHex) to yield > 50 % of the corresponding Ac-peptide-amide;[4] iii) hydrolysis of X-KOR bond from Ac-Ile-Ser(Bzl)-X-KOR (X: Gly, Asp, Phe, Lys or Leu (2-Cl-Z)) to yield 19–90 % of the corresponding Ac-peptides-OH;[5] iv) thiolysis of the Asp(OcHx)-KOR bond from Ac-Ile-Ser(Bzl)-Asp(OcHx)-KOR to yield > 90 % of the corresponding Ac-peptide-SR.[6]

Objectives

With the intention of expanding this alternative single-step synthetic approach to SPPS-Fmoc chemistry, we have been studying peptide detachment from HMBA resins because the ester bond P-HMBA from these PRs is also susceptible to nucleophilic attack[7] and could be promoted by Ca^{2+}, Zn^{2+} or Cu^{2+} ions.

Experimental

The synthesis of aminoacyl (Fmoc-Asp(O^{t}Bu)-HMBA; Fmoc-Phe-HMBA, and Fmoc-Gly-HMBA) and PRs (Fmoc-Ile-Ser(But)-Asp(O^{t}Bu)-HMBA, Ac-Met-Ser-Leu-Phe-HMBA, and Fmoc-Gly-Gly-His(Trt)-Gly-HMBA) were performed using traditional or microwave-assisted synthesis at 60 °C and our customized protocols for Fmoc chemistry.[8] Their substitution levels (SL) were determined by total acidic hydrolysis of PRs at 130 °C for 24 h followed by amino acid analysis of the hydrolysates. The alcoholysis reactions were performed using Ca^{2+}, Zn^{2+} or Cu^{2+} ions as additive and RP-HPLC/LC-MS for monitoring. Identities of the *C*-esterified side-chain protected peptides formed were confirmed by electrospray ionization mass spectrometry (ESI-MS). Reaction yields were calculated from the difference between initial and final SLs.

Results and Discussion

Initially, we investigated alcoholysis of the ester bond of Fmoc-aminoacyl or Fmoc-peptidyl-resin varying the HMBA resins, the divalent metal ion (Ca^{2+} and Zn^{2+}), and the solvent system (20–50 % ROH/DMF; R: Et, ^{i}Pr and $(CH_2)_2CH_3$). The results showed that HMBA-AM and HMBA-PEGA resins were appropriate, $ZnCl_2$ was better metal salt than

$Ca(OAc)_2$ in 50% ROH/DMF at 60 °C for 48 h at molar ratio of 1:2 (peptide: metal ion). The Zn^{2+}-promoted alchoholyis of amino acid-resin ester bond from Fmoc-Gly-HMBA-AM produced the desired Fmoc-Gly-OR with yields > 97 % and methanolysis of the PR ester bond from Fmoc-Ile-Ser(^{t}Bu)-Asp(O^{t}Bu)-HMBA-AM furnished the desired product Fmoc-Ile-Ser(^{t}Bu)-Asp(O^{t}Bu)-OMe with yield of 87 %. All reactions were monitored by RP-HPLC; it was observed slow formation of the DBF (dibenzofulvene) resulting from removal of Fmoc group that led to little amounts of contamination. Aiming to overcome this undesirable side-reaction, the Fmoc group was replaced by acetyl group, so Zn^{2+}-promoted alcoholyses of the PR ester bond from Ac-Ile-Ser(^{t}Bu)-Asp(O^{t}Bu)-HMBA-AM using 50 % MeOH, EtOH, ^{i}PrOH, butan-1-ol or benzyl alcohol/DMF at 60 °C for 48 h formed the corresponding Ac-peptides-OR with yields of 73–95%, except for ^{i}PrOH that yielded only 20 % of the product due to sterical hindrance.

Afterwards, we studied the Zn^{2+}-promoted transesterification of Ac-Ile-Ser-Asp-OMe in Ac-Ile-Ser-Asp-OR (R:Et, But, Benzyl and ^{i}Pr)] under the same conditions. Such reactions produced the desired *C*-esterified peptides with yields of 73 – 92 %, except for that using ^{i}PrOH that formed the product with yield of 30 %. At last, to verify the tolerance of other functional groups, such as sulfur, in peptide sequences by our alternative method, we synthesized Ac-Met-Ser-Leu-Phe-HMBA, sequence analogous to that of the anti-inflammatory tripeptide For-Met-Ser-Leu-Phe.[9] Zn^{2+}-promoted methanolysis in 50 % MeOH/DMF, at 60 °C for 48 hfurnished the Ac-Met-Ser-Leu-Phe-OMe with yield of 93.5 % and high purity (83 %).

Currently, we are studying Shepherin I (Shep I)-derived PRs because this unique Gly- and His–rich peptide has 7 repetitions of the ATCUN motif GGH in its structure. which chelates Cu^{+2} and Ni^{2+} ions.[10] Once again, Cu^{2+}, Ca^{2+} and Zn^{2+}-promoted methanolyses of the PR ester bond from Fmoc-Gly-Gly-His(Trt)-Gly-HMBA-PEGA were effectively carried-out in 20% MeOH/DMF at 60 °C for 24 h to give the desired products with reaction yields >50 %.

In short, our alternative single-step method to produce *C*-TMPs based on divalent metal ion-promoted detachment of the peptide from resins showed to be compatible to Fmoc chemistry and HMBA resins, is efficient, quite selective, employs very mild conditions and neutral pH, being greener than the traditional.

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References

1. Tanaka, S.; Kanno, M; Tashiro, Y. *Explor Drug Sci.* **2023**, *1*, 322.

2. a) Halpern, B.; Chew, L.; Close, V. et al. *Tetrahedron Lett.* **1968**, 5163. b) Weber, J. R.; Lokey, R. S. *Org. Letters.* **2010**, *12*, 1852.

3. a) Miranda, M. T. M.; Morita, H.; Tominaga, M. *Int. J. Peptide Prot. Res.* **1991**, *37*, 299. b) Miranda, M. T. M.; Theobaldo, F.C.; Tominaga, M. *Int. J. Peptide Prot. Res.* **1991**, *37*, 451.

4. Moraes, C.M., Bemquerer, M.P. & Miranda, M.T.M. *J. Peptide Res.* **2000**, *55*, 279.

5. Proti, P. B.; Remuzgo, C. Miranda, M. T. M. *J. Pept. Sci*. **2007**, *13*, 386.

6. Proti, P. B. & Miranda, M. T. M. *Tetrahedron Lett.* **2008**, *49*, 3853.

7. Alsina, J. & Albericio, F. *Biopolymers (Peptide Science)*, **2003**, *71*, 454-477.

8. Loffredo, C. et al. *J. Pept. Sci.* **2009**, *15*, 808.

9. Freire, T. S., Master Dissertation, Institute of Chemistry, University of São Paulo, **2013**.

10. a) Park. C. J. et al. *Plant Mol. Biol*. **2000**, *44*, 187. b) Remuzgo. C. et al. *Amino Acids*. **2014**, *46*, 2573. c) Nunes, L.G.P. et al., *Int. J. Pept. Res. Ther.* **2021**, *27*, 2945.