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Tandem deprotection/coupling for peptide synthesis in water at room temperature†

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A tandem deprotection/coupling sequence is reported for solution-phase peptide synthesis in water under micellar catalysis conditions using the designer surfactant TPGS-750-M. Cbz deprotection followed by peptide coupling in the presence of COMU and 2,6-lutidine afforded polypeptides containing up to 10 amino acid residues. A broad scope characterizes this new technology. No epimerization has been detected. The associated E Factors, as a measure of "greenness" and known to be extremely high for peptide couplings, have been reduced to less than 10 due to the step-economy and minimal amounts of organic solvent needed for product extraction.

Introduction

For decades, amide bond formation especially in medicinal chemistry has been among the most heavily utilized reactions.[1,2] Peptides alone will account for an estimated USD 25.4 billion by 2018 on the global therapeutic market.[3] But with respect to the choice of reaction solvent, peptide synthesis in terms of its environmental footprint is far from benign. Attempts have been made to replace harmful DMF or DCM by less egregious organic solvents such as ethyl acetate, 2-methyl tetrahydrofuran, or *N*-methylpyrrolidine.[4–6] However, massive consumption of organic solvents is still widespread due to the step-by-step nature of these sequences, even though water is the natural peptide biosynthetic medium. Few examples have been described in this "solvent", mainly because of the low solubility of protected amino acids. Various approaches have been designed to mimic Nature; among them, the introduction of water-soluble activating reagents,[7,8] the development of hydrosoluble protecting groups,[9–12] and the pulverization of protected amino-acid to form hydrosoluble nanoparticles.[13–16] And although use of elevated temperatures is known to jeopardize the stereointegrity of sensitive amino acids,[17] microwaves were also proposed as a source of energy to accelerate the coupling step in water, or in the absence of solvent.[18–20] Nonetheless, in most of these reports the scope of the reaction is limited and large amounts of co-solvents are often needed to prevent aggregation.

Another approach to reduce waste creation during peptide synthesis is to rely on step-economy. Katoh *et al.* described a one-pot tripeptide synthesis where the Fmoc protecting group was removed by tetrabutylammonium fluoride (TBAF) hydrate, followed by *in situ* peptide bond elongation using the HOBt analog TBTU. The reaction takes place in either THF or in DMF, along with a thiol to scavenge the resulting dibenzofulvene.[21] Zorn *et al.* applied a 1-pot process to arrive at an Alloc-protected peptide. Deprotection was performed by $Pd(PPh_3)_4$ and DABCO, and was followed by *in situ* coupling in the presence of either a Boc- or Fmoc-protected amino acid and EDC/HOBt in dichloromethane.[22]

Our group has previously introduced an environmentally friendly method for amide/peptide bond formation in an aqueous micellar medium.[23] The reaction takes place within the core of nanomicelles, formed by a 2 wt% aqueous solution of TPGS-750-M (Fig. 1). COMU and 2,6-lutidine were found to be the most efficient combination for the coupling step between two protected amino acids at room temperature. After deprotection, performed in organic solvent, a convergent [2 + 2] synthesis led, for example, to the *Streptocidin C* precursor Cbz-Leu-Phe-Pro-Leu-OEt in good (87%) yield.[23]

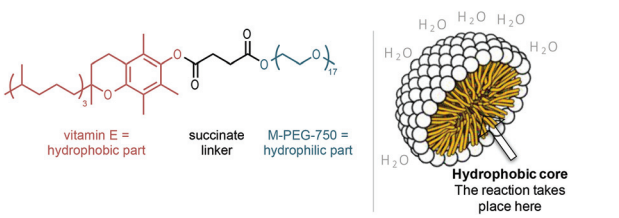

Fig. 1 Structure of TPGS-750-M and micelle formation.

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Herein, we describe an investigation into related peptide syntheses in water, focusing now on both *N*-terminus deprotection and subsequent coupling in a 1-pot fashion at room temperature, together with a greatly enlarged substrate scope of both polar and apolar amino acids leading to elongated peptide lengths.

Results and discussion

Among the multitude of known amine protecting groups, the Cbz residue was chosen for study as its hydrogenation is the preferred method of deprotection, rather than other alternatives (*e.g.*, Fmoc),[24] typically being both clean and without by-product formation that could affect the subsequent coupling step. We also took advantage of the lipophilicity of this commercial and readily available protecting group, which should enhance its localization inside the hydrophobic micellar core. To achieve this *N*-terminus deprotection, a screening of sources of both palladium as well as hydrogen donors was conducted on model dipeptide Cbz-L-Phe-L-Leu-OEt, **2a** (Table 1).

We have previously described the *in situ* generation of hydrogen gas during Pd-catalyzed silylations and hydrodehalogenations of aryl halides from $PdCl_2$ and tetramethyldisiloxane (TMDS) on water at room temperature.[25] These conditions were first evaluated on dipeptide **2a** (Table 1, entry 1). Total deprotection was observed within 2 h. Unfortunately, several by-products were also detected. Another silane, triethylsilane, as well as sodium borohydride were tested but found to be less efficient (entries 2 and 4; time to completion: 21 h). Hydrogen gas was used directly and the reaction was fast and clean (entry 3). The palladium source was then investigated (entries 5–7). In all cases, deprotection was complete in less than 2 h. From an economics point of view, palladium-on-charcoal and hydrogen gas were selected for this tandem process.[26]

From this model dipeptide (**2a**), Cbz deprotection was performed using 10 wt% Pd/C and hydrogen gas immediately followed by the coupling of Cbz-Ala-OH in the presence of COMU and 2,6-lutidine. To prevent loss of the Cbz group on this third amino acid, the hydrogen gas was removed by argon bubbling through the mixture in between both steps (Fig. 2).

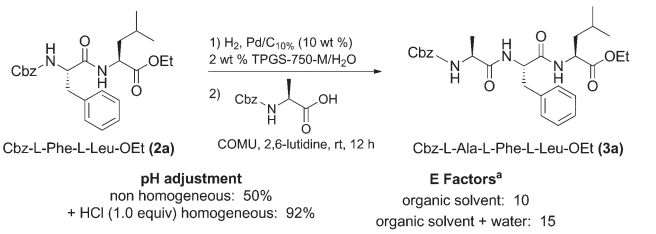


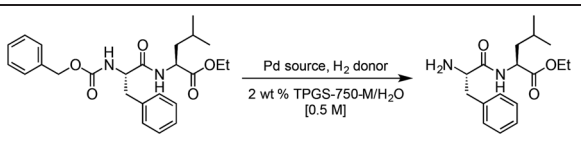
Fig. 2 Importance of pH on the tandem deprotection/coupling sequence for tripeptide synthesis, and E Factor calculations for the 2-step process. [a] Single run on a 3.5 mmol scale; 20 mL of MTBE were used for extraction, isolated yield: 86% (1.53 g).

Initially, the non-homogeneous nature of the reaction during deprotection led ultimately to the tripeptide in yields as low as 50% over the two steps. The free amine was also suspected of strong chelation, occupying the active sites of the catalyst. In this regard, acetic acid is often used as co-solvent to generate the ammonium salt and prevent loss of palladium activity, as well as aggregation. Hence, an aqueous solution of HCl (1.0 equiv. *vs.* peptide) was added resulting in a homogeneous reaction mixture ($pH_{t=0} \approx 1 \rightarrow pH_{t=2\,h} \approx 4$) leading to the desired tripeptide in a global yield of 92%. To verify the importance of the surfactant, the coupling step leading to dipeptide **2a** was conducted solely in deionized water. The yield dropped to 40% compared to a nearly quantitative outcome in aqueous TPGS-750-M. It should also be noted that the same procedure was tested in the presence of EDC and HOBt. Thus, in addition to safety concerns raised by the use of HOBt, slightly lower yields were obtained confirming our previous results and the efficiency of the COMU/2,6-lutidine system.

Several dipeptide building blocks were prepared, including both apolar and polar amino acids (Table 2). Thus, peptides containing, *e.g.*, ornithine (in peptide **2c**), aspartic acid (in peptide **2m**) or tyrosine (in **2r** and **2t**) could all be fashioned in water with good-to-excellent yields. Both tyrosine and serine[27] (in peptide **2o**) were used without a protecting group on the side chain.

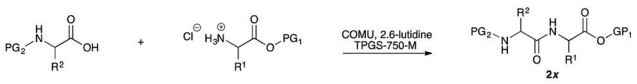
In some cases, especially when the final product was not readily soluble in the core of the micelle, the media turned into a paste, affecting stirring and the efficiency of the reaction. We also observed that glycine-containing peptides showed lower yields. As previously demonstrated,[28] the addition of a co-solvent could improve both yield and handling of a given reaction mixture, and potentially facilitate an industrial process. Addition of 10% THF was noted to increase the yield significantly from 41% to 75% in the case of Z-Arg(Pbf)-Ala-OEt (to compound **2s**), while the reaction failed completely under classic conditions (in DCM with EDC and HOBt). The co-solvent effect played an even greater role in the case of Cbz-Pro-Gly-OEt (compound **2d**) and Cbz-Ser-Ile-OMe (compound **2o**), leading to an increase of approximately 40% yield. Thus, the precursor of the C-terminal tripeptide portion of α-MSH-13, Lys-Pro-Val (Fig. 3; Table 3; peptide **3c**), known for

Table 1 Cbz deprotection conditions: screening of catalysts and sources of H_2



Entry	Catalyst	Hydrogen donor	Time (h)
1	$PdCl_2$ (5 mol%)	TMDS (1.5 equiv.)	2
2	$PdCl_2$ (5 mol%)	Et_3SiH (1.5 equiv.)	21
3	$PdCl_2$ (5 mol%)	H_2 gas	2
4	$PdCl_2$ (5 mol%)	$NaBH_4$ (1.2 equiv.)	21
5	**Pd/$C_{10\%}$ (10 wt%)**	**H_2 gas**	**2**
6	$Pd(OH)_2$ (20 mol%)	H_2 gas	2
7	$Pd(OAc)_2$ (10 mol%)	H_2 gas	2

Table 2 Dipeptides synthesized in 2 wt% aqueous TPGS-750-M solution



	Peptide	Yield (%)		Peptide	Yield (%)
2a	Cbz-Phe-Leu-OEt	99	**2k**	Cbz-Pro-Leu-OEt	94
2b	Cbz-Val-Gly-OEt	26 (95)[a]	**2l**	Cbz-D-Phe-Pro-OMe	83
2c	Cbz-Orn(Boc)-Leu-OMe	91	**2m**	Cbz-Asp(*t*Bu)-Ala-OMe	74 (83)[a]
2d	Cbz-Pro-Gly-OEt	42 (85)[a]	**2n**	Cbz-Tyr[b]-Tyr[b]-OMe	84
2e	Cbz-Val-Ala-OEt	72	**2o**	Cbz-Ser[b]-Ile-OMe	44 (82)[a]
2f	Boc-Pro-Leu-OEt	84	**2p**	Cbz-Phe-Ala-OEt	85
2g	Cbz-D-Phe-Pro-OMe	30 (63)[a]	**2q**	Cbz-Pro-Ala-OEt	65
2h	Cbz-Ile-Val-OMe	70	**2r**	Cbz-Pro-Tyr[b]-OMe	71
2i	Cbz-Pro-Val-OMe	88	**2s**	Cbz-Arg(Pbf)-Ala-OEt	41 (75)[a]
2j	Cbz-Ala-Phe-OEt	82			

[a] Performed in presence of 10% THF as co-solvent. [b] Unprotected side chain. Nature of the residue: Aliphatic/Acidic/Basic/Aromatic/Hydroxylic.

its anti-inflammatory activity,[29] was obtained in better yield in the presence of THF (65% *vs.* 51%). In addition, the more lipophilic Cbz protecting group leads to better yields compared to Boc; *e.g.*, in the case of PG-Pro-Leu-OEt (compounds **2k** and **2f**; 94% *vs.* 84%, isolated yields).

To highlight the robustness of this 2-step, 1-pot process, longer peptides were also investigated (Table 3). To access a peptide of more than four residues, we first adopted a [*x* + 2] convergent strategy where only 10 wt% Pd/$C_{10\%}$ was needed for deprotection of the first dipeptide. As the length and the polarity of the peptide increased, the global concentration had to be reduced to 0.25 M to achieve better stirring and solubility. Cbz-Val-Gly-Val-Ala-OEt (Fig. 3; peptide **4c**), a tetrapeptide precursor of the anti-microbial Dermaseptin[30] was thus obtained by a [2 + 2] tandem sequence in 60% yield over two steps. This process also tolerated both polar and nonpolar amino acids and allowed the preparation of decapeptide Cbz-D-Phe-Pro-Val-Orn(Boc)-Leu-D-Phe-Pro-Val-Orn(Boc)-Leu-OMe, the linear precursor of the antibiotic gramicidin S *via* a convergent [8 + 2] approach (Fig. 4; peptide **10a**, 82% isolated yield). For polypeptides longer than two units, 50 wt% of Pd/$C_{10\%}$ was required. The same decapeptide **10a** could thus be obtained *via* a [5 + 5] convergent strategy in 72% yield over two steps. Likewise, hexapeptide **6a** was prepared using a [4 + 2] and a [3 + 3] approach in 89% and 76% yields, respectively.

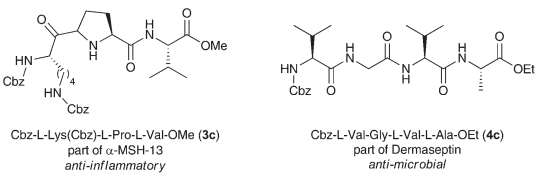
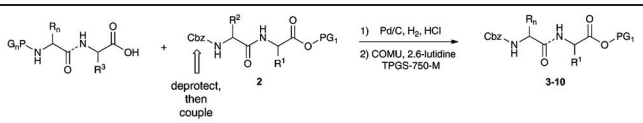


Fig. 3 Precursors of biologically active peptides *via* a 2-step, 1-pot process.

Table 3 Tri- to deca-peptides synthesized by a deprotection/coupling tandem process in 2 wt% aqueous TPGS-750-M



	Peptide	Yield (%)		Peptide	Yield (%)
3a	Cbz-Ala-Phe-Leu-OEt	92	**4a**	Cbz-Phe-Leu-Ile-Val-OMe	70
3b	Cbz-Gly-Phe-Leu-OEt	82	**4b**	Cbz-Pro-Leu-Phe-Leu-OEt	89
3c	Cbz-Lys(Cbz)-Pro-Val-OMe	51 (65)[a]	**4c**	Cbz-Val-Gly-Val-Ala-OEt	60
3d	Cbz-Val-Orn(Boc)-Leu-OMe	51 (79)[a]	**4d**	Cbz-Pro-Val-Pro-Tyr[b]-OMe	86
3e	Cbz-D-Phe-Pro-Val-OMe	66	**5a**	Cbz-D-Phe-Pro-Val-Orn(Boc)-Leu-OMe	88

	Peptide	Yield (%)
5b	Cbz-Ala-Phe-Leu-Asp(tBu)-Ala-OMe	89
6a	Cbz-Pro-Leu-Phe-Leu-Phe-Ala-OEt	89 (76)[c]
6b	Cbz-D-Phe-Pro-Val-Orn(Boc)-Leu-D-Phe-OMe	75
8a	Cbz-D-Phe-Pro-Val-Orn(Boc)-Leu-D-Phe-Pro-Val-OMe	86
10a	Cbz-D-Phe-Pro-Val-Orn(Boc)-Leu-D-Phe-Pro-Val-Orn(Boc)-Leu-OMe	82 (72)[d]

[a] Performed in presence of 10% THF as co-solvent. [b] Unprotected side chain. [c] Performed with a [3 + 3] convergent approach. [d] Performed with a [5 + 5] convergent approach. Nature of the residue: Aliphatic/Acidic/Basic/Aromatic/Hydroxylic.

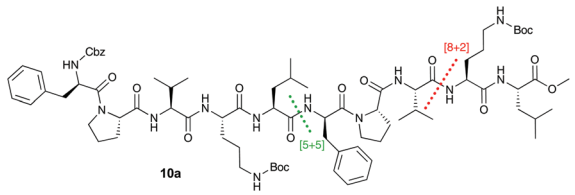
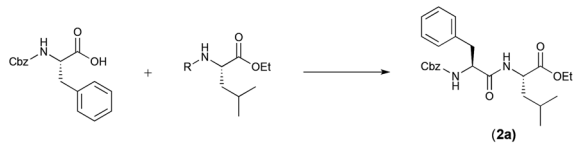


Fig. 4 Synthesis of gramicidin S precursor **10a** *via* a convergent [5 + 5] or [8 + 2], 2-steps, 1-pot approach.

Table 4 Determination of extent of epimerization under optimized conditions



Entry	-R	Conditions	ee[a]
1	H·HCl	COMU, 2,6-lutidine, TPGS-750-M/H_2O	>99%
2	CBz	(1) Pd/C, H_2, HCl, TPGS-750-M/H_2O (2) COMU, 2,6-lutidine	>99%

[a] Determined by chiral HPLC analysis.

Attempts to obtain the tripeptide **3a** in a coupling/deprotection/coupling 1-pot sequence using COMU/2,6-lutidine gave very poor results (<20% yield). Addition of up to 80% Pd/C failed to fully deprotect the newly formed polypeptide. This may be attributable to the oxime by-product interfering with the catalyst. The resulting pasty mixture was not optimal for proper stirring. EDC-HOBt/TEA gave encouraging results but no more than 50% yield overall was achieved.

The stereointegrity of the process was evaluated based on a representative coupling step. The mild conditions employed favored maintenance of optical purity, as epimerization was not observed in the formation of dipeptide Z-L-Phe-L-Leu-OEt (**2a**) (Table 4, entry 1).[31] Likewise, the tandem deprotection/coupling sequence applied to the preparation of the same dipeptide (**2a**), starting from Cbz-L-Leu-OEt, led to the same conclusion: that the chiral integrity was not compromised (Table 4, entry 2).

While E Factors for pharmaceuticals are typically between 25 and 100, due to the stepwise nature of chemical peptide synthesis, values are estimated to be 100 times larger.[32] Here, the reduced amounts of organic solvent used for extraction, and the step-economy involved, led to E Factors of 15 and 10 based on organic solvent used both with, and without, water in the calculations, respectively (Fig. 2).

Conclusion

An efficient technology has been developed that dramatically reduces the environmental impact of traditional solution-based polypeptide synthesis that relies on a tandem deprotection/peptide coupling under mild aqueous micellar conditions. This approach is broadly applicable to several types of amino acids that contain differing polarities, as well as an array of varying substitution patterns on their side chains, including phenyl or alkyl moieties, protected amine or carboxylic acid, and unprotected alcohol and phenolic groups. The polypeptide length has been extended to ten amino acid residues without significant loss of efficiency. Elimination of two environmentally egregious organic solvents (DCM or DMF) has been demonstrated, while the associated E Factors as a measure of waste generated have dropped considerably for this 2-step sequence. Access to cyclic peptides by this methodology is under active investigation.

Acknowledgements

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