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Synthetic chemistry in water: applications to peptide synthesis and nitro-group reductions

Margery Cortes-Clerget[1,2], Nicholas R. Lee[1,2] and Bruce H. Lipshutz[1*]

Amide bond formation and aromatic/heteroaromatic nitro-group reductions represent two of the most commonly used transformations in organic synthesis. Unfortunately, such processes can be especially wasteful and hence environmentally harmful, and may present safety hazards as well, given the reaction conditions involved. The two protocols herein describe alternative technologies that offer solutions to these issues. Polypeptides can now be made in water at ambient temperatures using small amounts of the designer surfactant TPGS-750-M, thereby eliminating the use of organic solvents as the reaction medium. Likewise, a safe, inexpensive and efficient procedure is outlined for nitro-group reductions, using industrial iron in the form of carbonyl iron powder (CIP), an inexpensive item of commerce. The peptide synthesis will typically take, overall, 3–4 h for a simple coupling and 8 h for a two-step deprotection/coupling process. The workup usually consists of a simple extraction and acidic/basic aqueous washings. The nitro reduction procedure will typically take 6–8 h to complete, including setup, reaction time and workup.

Introduction

Our group is working toward developing new technologies that reduce the environmental impact of many types of reactions in synthetic organic chemistry. One general approach being taken is to perform reactions that would normally require the dissolution of reactants and catalysts in organic solvents that now take place in aqueous media within the inner lipophilic cores of nanomicelles. We have found, for several important transition metal–mediated processes, that lower loadings of precious metal catalysts not only suffice but can be used under far milder reaction conditions. In this protocol we illustrate this general approach to organic synthesis by describing in detail two chemical reactions: peptide synthesis and nitro-group reduction. The former was chosen because it represents not only an especially important type of bond formation found in both academic and industrial labs but also one that falls under the category of homogeneous catalysis. By contrast, Procedure 2 describes the conversion of a readily available, albeit potentially dangerous, nitro group into its especially valued corresponding primary amine derivative. This process uses carbonyl iron powder as a heterogeneous reagent, also under aqueous micellar conditions. Hence, these procedures document the potential for water to serve as the gross reaction medium, accommodating both homo- and heterogeneous catalysis. And, unlike traditional procedures of today in organic media, they satisfy several of the fundamental 12 Principles of Green Chemistry.

Peptide synthesis (Procedure 1)

On the basis of a paper by Brown and Bostrom from AstraZeneca[1], the most used reaction in medicinal chemistry performed within the pharmaceutical industry over a recent 30-year period (1984–2014) is amide-bond formation. Unfortunately, the most commonly described methods to generate peptides are generally considered neither safe nor green. Despite their known toxicities[2,3], dimethylformamide (DMF), *N*-methyl-2-pyrrolidone (NMP) and dichloromethane (DCM (CH_2Cl_2)) remain to this day the solvents of choice. Over the past few years, many efforts have been made to provide more sustainable amide/peptide synthesis[4]. Some focused on atom economy, with the use of borate esters[5] or silicates[6] as activating reagents, with a few examples trying to avoid the use of protecting groups, but still relying on organic solvents (e.g., toluene) at reflux. The quest for greener organic solvents is also a hot topic, especially for solvent-consuming, step-by-step processes such as solid-phase peptide synthesis, where solvents such as DMF and DCM have been replaced by

[1]Department of Chemistry & Biochemistry, University of California, Santa Barbara, Santa Barbara, CA, USA. [2]These authors contributed equally: Margery Cortes-Clerget, Nicholas R. Lee. *e-mail: Lipshutz@chem.ucsb.edu

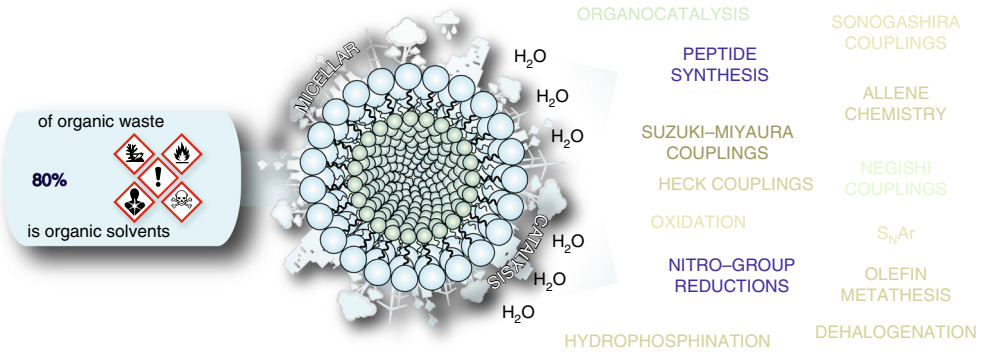

Fig. 1 | Overview of aqueous micellar catalysis applied to several common organic reactions, by-passing organic solvents and the waste created by their use.

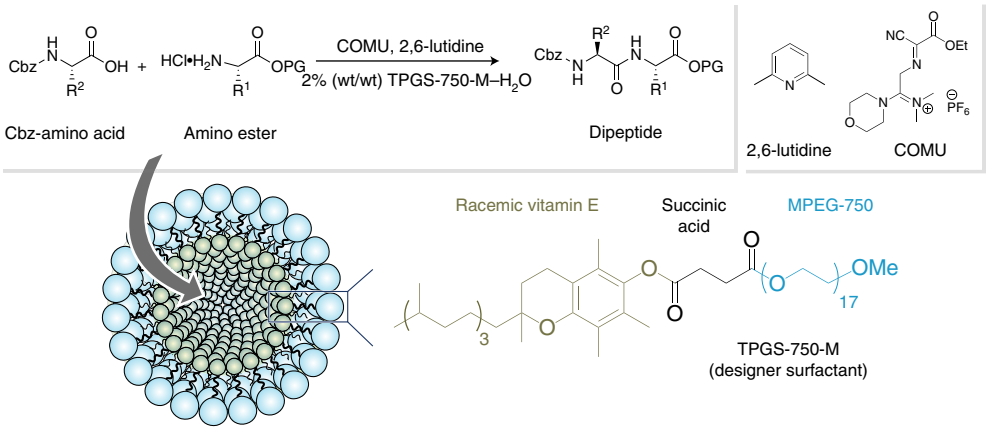

Fig. 2 | Peptide synthesis in micelles composed of TPGS-750-M-H_2O.

methyltetrahydrofuran (Me-THF), ethyl acetate (EtOAc), *N*-butylpyrrolidinone, cyclic carbonates or cyrene[7–10]. This does not solve the problem of organic waste generation, mainly attributable to organic solvents (Fig. 1). Other issues of concern include their flammability and safety in transportation. Mechanochemistry is another appealing method to avoid organic solvents, with encouraging results[11–13].

The use of water, the natural peptide biosynthetic medium, is a challenge for organic chemists, mainly for solubility reasons. The use of water as a reaction solvent in peptide-bond-forming reactions that, formally, extrude water is also counter-intuitive. Attempts involving water-soluble protecting groups[14] (not commercially available) or nano-dispersed amino acids[15] have been described in that regard, but necessitate extra steps.

This is precisely where micellar catalysis in water has been shown to provide a viable solution to what is currently an environmentally egregious situation. That is, nanomicelles engineered to be of the empirically determined ideal shape and size contain a hydrophobic inner core, within which the desired chemistry presumably takes place (Fig. 2). Hence, the lipophilic portion of the self-aggregating surfactant (e.g., TPGS-750-M) functions as the reaction medium, while water is the external, surrounding medium. This technology takes great advantage of the hydrophobic effect and the high concentration of substrates (typically 0.5 M) to offer faster reactions and milder conditions. Our earlier investigations into peptide synthesis focused on the identification of optimal conditions for the coupling between amines and carboxylic acids in TPGS-750-M–H_2O[16]. (1-cyano-2-ethoxy-2-oxoethylidenaminooxy)dimethylamino-morpholino-carbenium hexafluorophosphate (COMU) proved to be more efficient than other, well-established coupling reagents (such as EDC, HATU, PyBOP and MMTM) and, paired with 2,6-lutidine, led to amides in high yields. This choice of base is important, as it allows for the use of mild conditions (pH 6–7), decreases side reactions, and eliminates concerns about epimerization. Numerous nonpolar dipeptides including primary and secondary alkyl amides were prepared in excellent yields. Beyond reducing the extent of waste

Table 1 | Pros and cons of peptide synthesis in micellar media

Pros	Cons
- Reactions run in water (sustainable) - Commercially available reagents - Suitable for convergent strategy, which facilitates access to longer peptides (up to ten residues) - No racemization detected - High yields - Fast reaction (coupling in <1 h) - Low E factors - Applicable to gram-scale syntheses - Step-economical (sustainable) - Avoidance of benzotriazole-based activator	- Poor atom economy (coupling step) - Requires Pd/C for deprotection - Requires H_2 gas for deprotection - Lower yields with glycine as reaction partner

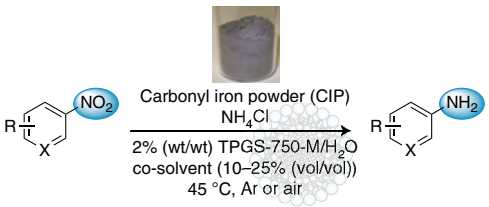

Fig. 3 | Nitro-group reduction by carbonyl iron powder in 2% (wt/wt) TPGS-750-M-H_2O.

generation as reflected by very low environmental (E) factors, making the 'switch'[17] to an aqueous medium likewise addresses the safety issue typically associated with use of benzotriazole-based activators (e.g., HOBt is routinely used to reduce epimerization in amide-bond formation)[18]. The latest developments relying on micellar conditions in water have focused on the introduction of polar amino acids as part of a one-pot deprotection/coupling strategy to access an array of polypeptides[19]. This new technology shows a broad scope (i.e., applicable to a range of amino acids: apolar, polar, basic, acid or hydroxylic), and protecting groups tolerance (Cbz, Boc, Fmoc, tBu and Pbf) has been demonstrated, including the synthesis of an acyclic precursor to decapeptide antibiotic gramicidin S, via a convergent strategy. Unnatural D- or β-amino acids are well tolerated as well. A scale-up to 4 mmol (1.54 g, or 84%) has been applied to the two-step/one-pot synthesis of Cbz-L-Phe-L-Leu-OEt without substantial loss of reactivity. To illustrate this approach to peptide construction in water, here we describe the coupling between two amino acid partners involving a step-economical deprotection/coupling sequence at room temperature (20–25 °C). The method described in this protocol is, in terms of environmental impact and scope of reaction, probably one of the most promising. Advantages and limitations can be found in Table 1.

Nitro-group reduction (Procedure 2)

The reduction of aromatic nitro compounds is an important transformation commonly used in organic synthesis, and in the pharmaceutical industry in particular. Typical approaches are often notorious for their harshness, as well as their hazardous nature, with nearly all literature approaches taking place in traditional organic solvents. Many variations in nitro reduction methodologies have been developed; common classes include catalytic hydrogenation (H_2/Pd), non-metal reducing agents ($NaBH_4$/catalyst or hydrazine/catalyst) and stoichiometric metal reducing agents ($SnCl_2$, Zn, Fe)[20,21]. Drawbacks to common methods include the need for pressurized equipment (H_2), fire hazard (H_2, hydrazine, $NaBH_4$), high temperature (many methods), toxicity (Sn) and hazardous or troublesome solvents (THF, DMF). As an alternative method that eliminates these troublesome issues, this reduction utilizes CIP (Fig. 3) in aqueous TPGS-750-M[22]. Although CIP has been applied to several industrial needs, its use for nitro-group reductions was unknown. The method is readily scaled, and extensive purification (such as chromatography or recrystallization) is usually not necessary to obtain a high-purity product. Because of these attributes, as well as its functional group compatibility, the use of CIP represents a practical alternative procedure to existing know-how, while simultaneously

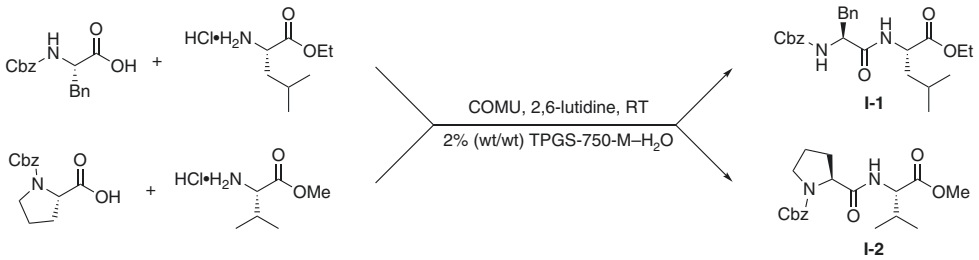

Fig. 4 | Reaction pathway for two representative dipeptides synthesized in 2% (wt/wt) TPGS-750-M–H_2O.

reducing the environmental footprint associated with such reductions. Owing to the crystalline nature of nitro compounds, the inclusion of limited amounts of a co-solvent is necessary to drive most reactions to completion. A guide to select the best co-solvent partner is described in the 'Experimental design' section, as is an adaptable, representative procedure.

Experimental design

Peptide synthesis

In the procedure, we describe the preparation of Cbz-L-Ala-L-Phe-L-Leu-OEt (**II-1**) as a model peptide, and Cbz-L-Lys(Boc)- L-Pro-L-Val-OMe (**II-2**) as a precursor to the C-terminal tripeptide portion of α-MSH-13, known for its anti-inflammatory activity[23]. Two stages are demonstrated: the preparation of the dipeptide precursors Cbz-L-Phe-L-Leu-OEt (**I-1**) and Cbz-L-Pro-L-Val-OMe (**I-2**), and their one-pot deprotection/coupling with the third protected amino acid (respectively, Cbz-L-Ala-OH and Cbz-L-Lys(Boc)-OH).

Because of the high concentrations involved (0.50 M for coupling and 0.25 M for the one-pot deprotection/coupling process), the yields are generally higher if most of the volume in the vial is occupied by the reaction mixture to maximize stirring. Typically, reactions are run in a 1-dram glass vial on a 0.3–1.0 mmol scale. Reactions are run under aerobic conditions, except for the Cbz-deprotection, where an H_2 atmosphere is required.

Preparation of dipeptides

Syntheses of the two precursors Cbz-L-Phe-L-Leu-OEt (**I-1**) and Cbz-L-Pro-L-Val-OMe (**I-2**) are described, demonstrating applications of the COMU/2,6-lutidine coupling step (Fig. 4). Disappearance of the free amine can be monitored by thin-layer chromatography (TLC) using ninhydrin. The coupling reaction is typically complete in <1 h. All by-products are soluble in water, so purification requires only a simple extraction with methyl *t*-butyl ether (MTBE) or EtOAc, followed by acidic and basic aqueous washings. No further purification is required if the dipeptide is directly engaged in a two-step, one-pot deprotection/coupling sequence. For final products, a quick filtration through a pad of silica eliminates all traces of remaining TPGS-750-M, yielding the desired pure product. This method is applicable to a broad range of amino acids and aliphatic amines/carboxylic acids and has proven to be efficient on a gram scale, affording similar yields. Peptides including glycine often lead to lower yields, likely owing to poorer solubility, but their syntheses can be improved with a co-solvent (see the section 'Guidelines for use of co-solvents in peptide synthesis'). For scale-up and industrial purposes, the use of 10% (vol/vol) of a co-solvent (e.g., THF) is highly recommended for a better handling of the reaction.

Preparation of tripeptides

In this method, the prepared Cbz-dipeptides **I-1** and **I-2** are deprotected with Pd/C and H_2 gas (Fig. 5). The initial deprotection reaction typically takes ~2 h to reach completion and can be monitored by TLC. One equivalent of HCl is required to form the ammonium salt in situ and prevent catalyst poisoning. After argon sparging to release hydrogen, the next *N*-protected amino acid/peptide can then be coupled in the same vial following the optimized coupling method, with the addition of another portion of 2% (wt/wt) TPGS-750-M–H_2O solution that reduces the overall concentration to 0.25 M. The carboxybenzyl (Cbz) residue was selected for its lipophilicity, which increases the solubility of the amino acid within the core of the micelles. Moreover, the use of this protecting group led to its clean deprotection, as only toluene and CO_2 by-products are generated, leaving the

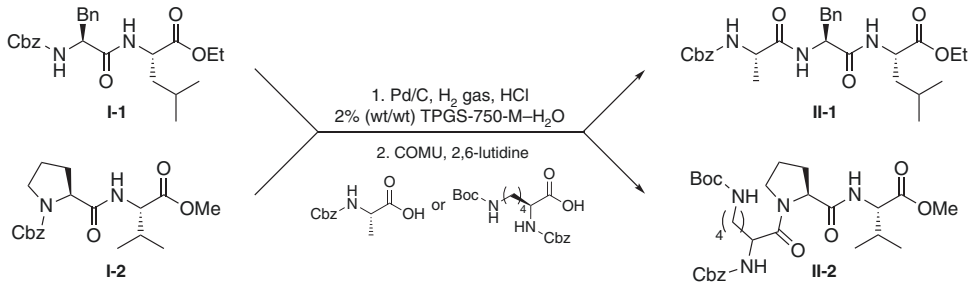

Fig. 5 | Reaction pathway for two representative tripeptides synthesized in a two-step, one-pot fashion in 2% (wt/wt) TPGS-750-M-H_2O.

Table 2 | Influence of the amount of co-solvent on the yield of Cbz-L-Lys(Boc)- L-Pro-L-Val-OMe

Amount of co-solvent (vol/vol %)	Isolated yield (%)
0	47
10	**84**
20	81

subsequent step unaffected. In cases where solubility issues may exist, especially when the resulting product is a solid, the addition of 10% (vol/vol) THF can help to improve both yields and handling of reaction mixtures. The final product is extracted with EtOAc, and the extracts are filtrated through Celite to eliminate Pd/C. After acidic and basic aqueous washings, we carried out a small purification on silica gel, which afforded the desired tripeptides in good yields. This methodology is extremely efficient for accessing short peptides with high yields and can be applied to up to a decapeptide through an [8+2] convergent approach.

Guidelines for use of co-solvents in peptide synthesis

Depending on the nature of the coupling partners, solubility issues can arise owing to the formation of solid dipeptides. The resulting viscous medium affects stirring and may lead to lower yields. This is especially the case when a protected glycine is involved. We screened co-solvents and identified THF as the best candidate. The addition of 10% (vol/vol) THF at Step 3 (Procedure 1), along with the solution of 2% (wt/wt) TPGS-750-M–H_2O, can dramatically improve the outcome of the reaction.

A co-solvent can also be required at Step 18 (Procedure 1), when the dipeptide is poorly soluble in the 2% (wt/wt) TPGS-750-M–H_2O solution. Whereas Cbz-L-Phe-L-Leu-OEt dissolved readily, Cbz-L-Pro-L-Val-OMe needed a small amount of co-solvent to facilitate dispersion and, ultimately, to increase the efficiency of the reaction. It is not necessary to add more than 10% THF, as shown in Table 2. Increasing the amount to 20% (vol/vol) THF did not improve the yield or handling of the reaction.

Experimental design—nitro-group reduction

The procedure details how to set up and run the reduction of aromatic nitro compounds, as well as how to carry out the workup and purification. Included are directions on how to address 'difficult-to-reduce' compounds and the selection of co-solvents. The co-solvent selection guide should be followed when you are screening conditions for an unknown substrate at 0.5-mmol scale. These have been organized to decrease the amount of time and number of trials needed to find optimal conditions. To illustrate this method, we describe the synthesis of 4-bromoaniline (**III-1**) and 2-isopropoxy-5-methyl-4-(pyridin-4-yl)aniline (**III-2**) on a 0.5-mmol scale (Fig. 6). A scale-up to a 13-mmol scale is also illustrated by the synthesis of *N*1-(1-benzylpiperidin-4-yl)benzene-1,4-diamine (**III-3**). The scale-up (>1 mmol) required the use of a mechanical stirrer. All reactions were

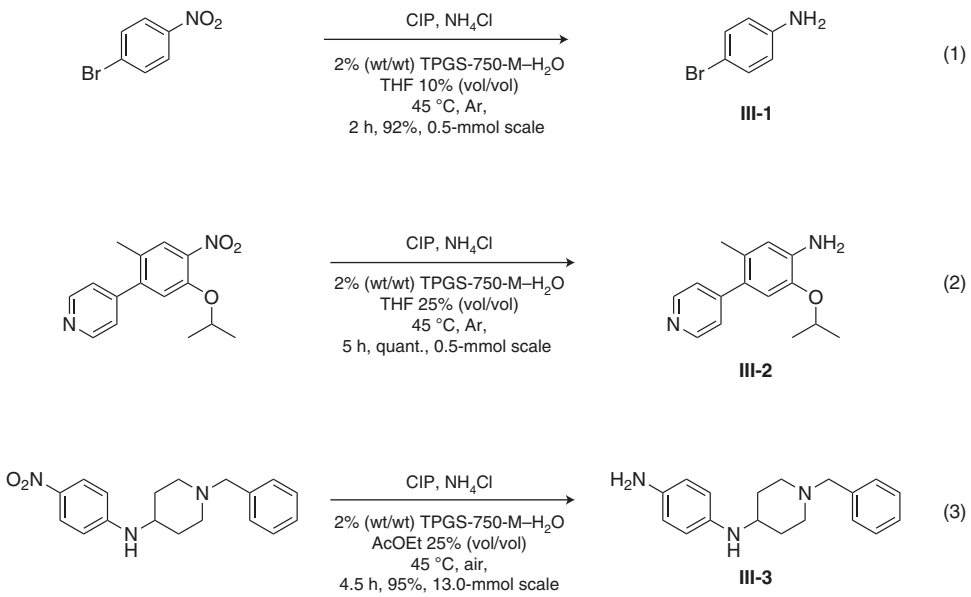

Fig. 6 | Reaction pathway for three representative nitro-group reductions in 2% (wt/wt) TPGS-750-M-H_2O.

monitored by TLC. Different strategies are proposed for the reaction and the workup to make the method suitable for a broad range of substrates.

Co-solvent selection guide

In most cases, the use of a 2% (wt/wt) TPGS-750-M–H_2O solution as the reaction medium is not sufficient for the solubilization of nitro compounds. Their highly crystalline nature and typically poor solubility necessitated exploration of co-solvents to address this issue. Although THF was found to be the best candidate, EtOAc was sometimes as effective, and may be preferred in cases where it is also the solvent for extraction.

The co-solvent percentage needs to be tuned for each substrate to get the best possible results. Too little co-solvent will not be enough to dissolve the nitro compound, and will ultimately lead to slow or no reaction, and too much co-solvent has been found to slow reactions dramatically as well. Optimal conditions for one substrate are not necessarily optimal for another; therefore, we recommend an initial screening of the following conditions when reducing a new substrate.

Suggested co-solvent screening protocol (use with general procedure—0.5-mmol scale)

When optimizing conditions for an unknown substrate, set up these seven trials with varying ratios of co-solvent, then use the ratio that gives the best results for further studies such as scale-up experiments:

Trial 1: no co-solvent (1.0 mL of 2% (wt/wt) TPGS-750-M/–H_2O)

Trial 2: 10% (vol/vol) THF (0.9 mL of 2% (wt/wt) TPGS-750-M–H_2O + 0.1 mL of THF)

Trial 3: 20% (vol/vol) THF (0.8 mL of 2% (wt/wt) TPGS-750-M–H_2O + 0.2 mL of THF)

Trial 4: 25% (vol/vol) THF (0.75 mL of 2% (wt/wt) TPGS-750-M–H_2O + 0.25 mL of THF)

Trial 5: 10% (vol/vol) EtOAc (0.9 mL of 2% (wt/wt) TPGS-750-M–H_2O + 0.1 mL of EtOAc)

Trial 6: 20% (vol/vol) EtOAc (0.8 mL of 2% (wt/wt) TPGS-750-M–H_2O + 0.2 mL of EtOAc)

Trial 7: 25% (vol/vol) EtOAc (0.75 mL of 2% (wt/wt) TPGS-750-M–H_2O + 0.25 mL of EtOAc)

The two procedures as outlined above, while very useful, are merely representative of the potential for organic chemistry to follow nature's lead[13]: that is, to correct the unfortunate choice made at the beginnings of modern organic synthesis (roughly 150–200 years ago) to pursue organic solvents as the media in which to run organic reactions. How unfortunate! Organic chemistry could have, and should have, been developed in water using nature as the ideal model, having had hundreds of millions of years to perfect its trade. Today, we can only speculate on the current status of organic chemistry had our predecessors chosen more wisely: no dependence on, and depletion of, our petroleum reserves, little to no waste that is mostly solvent-derived, elimination of overconsumption of endangered metals essential for use in today's catalytic processes, and the inherent safety that comes with the use of a nonflammable reaction medium. So, while we continue to pay the price for

such unfortunate historical decisions that have put organic chemistry on a clearly unsustainable path, the beginnings of long overdue technological solutions are, in fact, on display, complete with details. Based on these two typical reactions herein, what are the broader implications for doing organic chemistry, in general, in water? Is it really as simple as replacing each of the many commonly used organic solvents with water containing small numbers of nanomicelles serving as 'nanoreactors' in which more familiar homogeneous catalysis is taking place? The answer may actually be 'yes', at least at this most fundamental of levels. For the newcomer to the field, a succesful initial trial met with success is all that is necessary to show that this is highly effective approach. Moreover, it is now possible to run many types of valued reactions under the same set of conditions: in water under mild conditions (i.e., between 21 and 45 °C). Fine-tuning, as with any methodology, may require screening of variables such as surfactant, ligands and global reaction concentration, but such parameters are easily determined in relatively short order. Reactions that are driven by transition metal catalysis, such as Pd-catalyzed name reactions (e.g., Suzuki-Miyaura, Sonogashira, Heck, Stile), Au-catalyzed processes (e.g., hydration, cyclizations), Rh catalysis (e.g., asymmetric conjugate additions), olefin metathesis (catalyzed by Ru complexes), and Cu and Ni catalysis can all be done using the same aqueous micellar catalysis conditions. The list, which continues to grow, now includes two other key, non-metal-mediated processes that are also amenable: amide/peptide bond formation and SNAr reactions. All are easily adapted to this very simple and yet environmentally responsible approach to doing synthesis. Overall, the payoff to making this switch is huge: organic chemistry is diverted from its current status as an unsustainable discipline as currently practiced in organic solvents into one that is modeled on nature, doing chemistry in water, thereby guaranteeing an unlimited future.

Materials

Reagents

Solvents

! CAUTION Use all organic solvents in a well-ventilated area away from dust/fume/gas/vapor/spray. Wear protective clothes, protective gloves, eye protection and face protection. Store all solvents in tightly closed bottles in a cool place. **▲ CRITICAL** Solvents were used as purchased, without degassing or drying. All reagents are stored as prescribed and used without further purification.

- Methyl *t*-butyl ether (MTBE; Fisher Chemical, cat. no. MX0826-1) **! CAUTION** MTBE is flammable and irritates the skin.
- Ethyl acetate (EtOAc; Fisher Chemical, cat. no. E145-20) **! CAUTION** EtOAc is highly flammable.
- Hexane (Fisher Chemical, cat. no. H292-20) **! CAUTION** Hexane is highly flammable. It enters the airways, and may be fatal if swallowed. It causes skin and eye irritation and may cause respiratory irritation, drowsiness or dizziness. It is suspected of damaging fertility, and causes damage to the organs through prolonged and repeated exposure.
- Hexane, HPLC grade (Fisher Chemical, cat. no. H302)
- 2-propanol, HPLC grade (Fisher Chemical, cat. no. A451) **! CAUTION** 2-propanol is highly flammable. It enters the airways, and may be fatal if swallowed. It causes skin irritation and serious eye irritation, and may cause drowsiness or dizziness. It is suspected of damaging fertility, and causes damage to the organs through prolonged or repeated exposure.
- *n*-butanol (Fisher Chemical, cat. no. A399) **! CAUTION** *n*-butanol is flammable in liquid and vapor form. It is harmful if swallowed. It causes skin irritation and serious eye damage.
- Tetrahydrofuran (THF; Fisher Scientific, cat. no. T397) **! CAUTION** THF is highly flammable in liquid and vapor form. It is harmful if swallowed. It causes serious eye irritation, and may cause respiratory irritation, drowsiness or dizziness. It is suspected of causing cancer.
- Methanol (Fisher Scientific, cat. no. A412) **! CAUTION** Methanol is highly flammable in liquid and vapor form. It is toxic if inhaled or swallowed and if it comes in contact with the skin. It causes damage to the organs through prolonged or repeated exposure.
- Dichloromethane (Fisher Scientific, cat. no. D37) **! CAUTION** Dichloromethane causes skin irritation and serious eye irritation. It may cause drowsiness or dizziness and cancer. It may cause damage to the organs through prolonged or repeated exposure.

Common for both protocols

- Water—HPLC grade, submicrometer-filtered (H_2O; Fisher Chemical, cat. no. W5)
- TPGS-750-M (wax; Sigma-Aldrich, cat. no. 763896), stored between 0 and 8 °C; or 2% (wt/wt) in H_2O (Sigma-Aldrich, cat. no. 733857), stored under argon

Peptide synthesis

▲ **CRITICAL** Amino acids, COMU and 2,6-lutidine are stored between 0 and 8 °C.

- L-leucine ethyl ester hydrochloride (HCl·H-L-Leu-OEt, ≥99% (AT); Sigma-Aldrich, cat. no. 61850). Store between 0 and 8 °C.
- *N*-carbobenzyloxy-L-phenylalanine (Cbz-L-Phe-OH, 99+%; Acros Organics, cat. no. 275960250). Store between 0 and 8 °C.
- L-valine methyl ester hydrochloride (HCl·H-L-Val-OMe, 99%; Sigma-Aldrich, cat. no. 860271). Store between 0 and 8 °C.
- *N*-carbobenzyloxy-L-proline (Cbz-L-Pro-OH; Chem-Impex International, cat. no. 02237) Store between 0 and 8 °C.
- *N*α-carbobenzyloxy-*N*ε-*t*-butoxycarbonyl-L-lysine (Cbz-L-Lys(Boc)-OH; Chem-Impex International, cat. no. 03510). Store between 0 and 8 °C.
- 2,6-lutidine (98%; Alfa Aesar, cat. no. A10478) **! CAUTION** 2,6-lutidine is flammable, is harmful if swallowed, and causes skin and eye irritation. Wear protective gloves, protective clothing and eye protection.
- (1-cyano-2-ethoxy-2-oxoethylidenaminooxy)dimethylamino-morpholino-carbenium hexafluorophosphate (COMU; >96.5%; Sigma-Aldrich, cat. no. 712191). Store between 2 and 8 °C.
- Palladium on activated charcoal (Pd/C 10% Pd basis; Sigma-Aldrich, cat. no. 75990) **! CAUTION** Palladium causes skin irritation and serious eye irritation. Wear protective gloves, protective clothing and eye protection. Store under an inert atmosphere.
- Compressed hydrogen gas (H_2; ≥99.9999% 6.0; Praxair, cat. no. HYK 6.0RS) **! CAUTION** Compressed H_2 is an extremely flammable gas under pressure. It may explode if heated, and displace oxygen and cause rapid suffocation. It may form an explosive mixture with air and burns with an invisible flame. Wear leather safety gloves and safety shoes when handling the cylinders. Keep away from heat, open flames, sparks and hot surfaces. Use and store only outdoors or in a well-ventilated place.
- Hydrochloric acid (HCl; 12 M; EMD, cat. no. HX0603P) **! CAUTION** HCl causes severe skin burns and eye damage. It may cause respiratory irritation. Wear protective gloves, protective clothing, eye protection and face protection.

Nitro-group reduction

- Carbonyl iron powder (average particle size, 5–9 μm; CIP; 99.5%; Sigma-Aldrich, cat. no. 44890) **! CAUTION** CIP is a flammable solid.
- Ammonium chloride (NH_4Cl; Fisher Scientific, cat. no. A661-500) **! CAUTION** NH_4Cl is harmful if swallowed. It causes serious eye irritation. It may cause respiratory irritation and damage to the organs through prolonged or repeated exposure.

Stain materials

- Ninhydrin (Combi-Blocks, cat. no. QA-7417) **! CAUTION** Ninhydrin is harmful if swallowed. It causes skin irritation and serious eye irritation. It may cause respiratory irritation. Wear protective clothes, protective gloves, eye protection and face protection.
- Cerium IV sulfate ($Ce(SO_4)_2$; Millipore Sigma, cat. no. 359009) **! CAUTION** $Ce(SO_4)_2$ causes skin irritation and serious eye irritation. Wear protective gloves, eye protection and face protection. Store in a dry place.
- Ammonium molybdate tetrahydrate ($(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$; ≥99.0%; ACS reagent; HoneyWell Fluka, cat. no. 09880) **! CAUTION** Repeated or prolonged exposure to $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$ may irritate the eyes, skin and respiratory system. Wear protective gloves, protective clothing, eye protection and face protection.
- Acetic acid, glacial (AcOH; EM Science, cat. no. AX0073) **! CAUTION** AcOH is flammable as a liquid or vapor. It may be corrosive to metals. It causes severe skin burns and eye damage. Wear protective gloves, protective clothing, eye protection and face protection. Store at >16.7 °C (62 °F), but away from direct heat.
- Sulfuric acid (H_2SO_4; EMD Millipore, cat. no. SX1244PC-5) **! CAUTION** H_2SO_4 may be corrosive to metals. It causes severe skin burns and eye damage. It may cause respiratory irritation and drowsiness or dizziness. Wear protective gloves, protective clothing, eye protection and face protection.

Inorganic salts

- Magnesium sulfate, anhydrous ($MgSO_4$; EMD, cat. no. MX0075-1)
- Sodium carbonate, anhydrous (Na_2CO_3; Fisher Scientific, cat. no. S263-500) **! CAUTION** Na_2CO_3 causes serious eye irritation. Wear eye and face protection. Store in a dry and well-ventilated place.

Miscellaneous

- SiliaFlash F60 (40–63 μm (230–400 mesh); Silicycle, cat. no. R10030B). Store in a dry place
- Celite (545 FilterAid; Fisher Chemical, cat. no. C212). **! CAUTION** Celite may cause cancer. It causes damage to the organs through prolonged or repeated exposure. Use personal protective equipment. Store locked up in a well-ventilated place.
- Cotton balls
- Sand, sea washed (SiO_2; Fisher Chemical, cat. no. S25-10)

Equipment

- Analytical balance (Mettler Toledo, cat. no. AB 204-S)
- Hot plate magnetic stirrer (IKA, cat. no. RCT basic S1)
- Reaction block, 1-dram vials, 48 positions (ChemGlass, cat. no. CG-1991-04)
- DuoSeal vacuum pump (Welch Vacuum, cat. no. 1400)
- Rotavapor (Büchi, cat. no. R-114)
- Waterbath (Büchi, cat. no. B-480)
- Diaphragm vacuum pump (VacuumBrand, cat. no. MD1C)
- Isotemp recirculating heating water bath chiller (Fisher Scientific, cat. no. 1016D)
- Vacuum/argon gas system
- Analytical HPLC system (Shimadzu LC-20AT Prominence coupled with Shimadzu SPD-M20A Prominence diode array detector)
- HPLC column (Lux 5-μm Cellulose-2, 250 × 4.6 mm; Lux, cat. no. 00G-4457-E0)
- NMR spectrometers (Varian Unity Inova 400 MHz, 500 MHz, 600 MHz)
- ESI-MS spectrometer (Waters Micromass LCT TOF ES+ Premier mass)
- Dual-temperature heat gun (Black & Decker, cat. no. HG1300)
- Ultrasonic cleaning bath (Fisher Scientific, cat. no. FS20H)
- Glass vial (short-form style with Phenolic cap on, 1 Dr; VWR; cat. no. 66011-041)
- Pyrex narrow-mouth graduated Erlenmeyer flasks (25 and 50 mL; Sigma-Aldrich, cat. nos. CLS498025 and CLS498050)
- Graduated cylinders (10 and 25 mL; Fisher Scientific, cat. nos. S63455 and S63456)
- Round-bottom flasks (single neck, 14/20 50 mL and 24/40 100 mL; VWR, cat. nos. 89426-460 and 89426-454)
- Separatory funnel (60 mL; Fisher Scientific, cat. no. 10-437-10A)
- Glass column for chromatography (1.5-cm diameter)
- Sleeve stoppers (14 × 20 and 24 × 40; VWR, cat. nos. 89097-554 and 89097-558)
- Egg-shaped PTFE magnetic stir bars (5 mm × 9.5 mm; VWR; cat. no. 58949-012)
- Weighting paper (Fisher Brand, cat. no. 09-898-12B)
- Spatula
- Microsyringe Microliter (25 μL–1 mL; Hamilton)
- Norm-Ject 4010-200V0 syringe (1 mL; Henke-Sass, Wolf)
- Precision glide needle (20-gauge × 1.5; 0.9 mm × 40 mm; BD, cat. no. 305176)
- 5 3/4-inch Pasteur pipettes (Fisher Scientific, cat. no. 13-678-6A)
- 9-inch Pasteur pipettes (Fisher Scientific, cat. no. 13-678-6B)
- Parafilm M paraffin film (Sigma-Aldrich, cat. no. P7794)
- Rubber bulbs (2-mL amber latex; Fisher Scientific, cat. no. 150-005-06)
- TLC silica gel 60-gauge F_{254} glass plates (Merck, cat. no. 100390)
- TLC cutter (7 inch/175 mm; BosiTools, cat. no. BS302192)
- UV lamp (Fisher Scientific, cat. no. PI95034)
- Glass capillary tubes (0.3 mm × 100 mm; Amazon, cat. no. B01M5LJF2Z)
- Mechanical stirring motor (Surgent, cat. no. S-S764456) with impeller

Reagent setup

TPGS-750-M (2% (wt/wt); wax)

Dissolve the wax in degassed HPLC-grade water under an argon atmosphere. Stir the solution under argon overnight until complete dissolution (an oil bath set at 40 °C can help accelerate the dissolution of the surfactant). This solution can be stored at room temperature for months if stored under argon.

Procedure 1

Synthesis of dipeptides (0.3-mmol scale) ● Timing 1 h

▲ **CRITICAL** This procedure is illustrated by the synthesis of Cbz-L-Phe-L-Leu-OEt (**I-1**; Fig. 7) and Cbz-L-Pro-L-Val-OMe (**I-2**; Fig. 8) (Supplementary Information) as shown in Fig. 4, but can be applied to a broad range of amino acids. We provide a step-by-step tutorial in the video 'Peptide synthesis in water—episode 1: coupling', previously published online (https://youtu.be/WvRVfaR7dwM).

1 Add the first protected amino acid partner (0.3 mmol, 1.0 equiv.) to a 1-dram (~4-mL) vial.
▲ **CRITICAL STEP** The order of addition is not crucial. We suggest weighing any viscous compound first to facilitate its handling. Make sure that the compound is well dissolved in the 2% (wt/wt) TPGS-750-M–H_2O solution and does not stick to the wall of the vial.
▲ **CRITICAL STEP** As the reaction mixture is highly concentrated, an appropriate vial size (the total reaction should occupy at least one-third of the vial volume) is beneficial for stirring, and leads to higher yields.
? TROUBLESHOOTING

2 Add the second protected amino acid partner (0.3 mmol, 1.0 equiv.) to the vial.

3 Add 0.6 mL of a 2% (wt/wt) solution of TPGS-750-M–H_2O. The reaction concentration is 0.50 M of the amino acids.
▲ **CRITICAL STEP** 10% (vol/vol) THF can be added at this stage in the case of a low isolated yield. This is highly recommended for glycine-containing peptides. See the section 'Guidelines for use of co-solvents in peptide synthesis'.
▲ **CRITICAL STEP** To increase the storage life of the 2% (wt/wt) TPGS-750-M–H_2O solution, keep it under argon and purge the syringe twice before measuring the desired amount.
? TROUBLESHOOTING

4 Add 105 µL of 2,6-lutidine (0.92 mmol, 3.05 equiv.).
▲ **CRITICAL STEP** Though COMU releases 1.0 equiv. of morpholine during amide-bond formation, use of only 2.05 equiv. of 2,6-lutidine leads to lower yields and a heterogeneous mixture. The solution can be stirred for 1 min to ensure good dispersion of the protected amino acids, leading to a transparent-to-milky solution (depending on the nature of the protected amino acids).
? TROUBLESHOOTING

5 Add 0.135 g of COMU (0.32 mmol, 1.05 equiv.). The solution should turn bright yellow instantly.

6 Stir vigorously at room temperature for 1 h.
▲ **CRITICAL STEP** Cooler temperatures (15–20 °C) do not affect the reaction outcome.

7 Monitoring the reaction after 1 h: In two separate tubes, dissolve a sample of the reaction and the starting amino ester in 0.5 mL of a saturated aqueous solution of Na_2CO_3 and 0.5 mL of EtOAc to

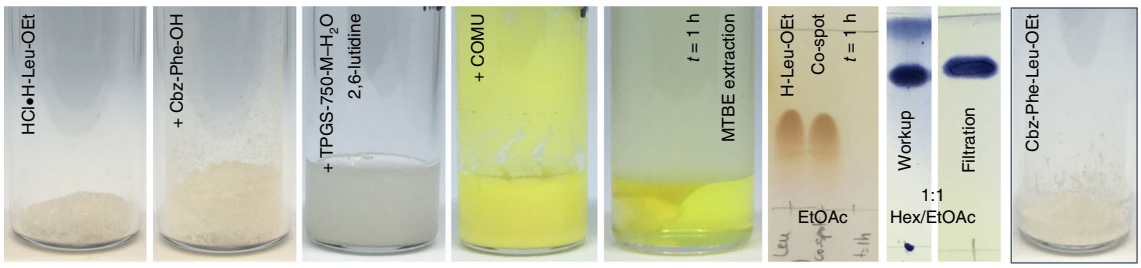

Fig. 7 | Step-by-step synthesis of Cbz-L-Phe-L-Leu-OEt.

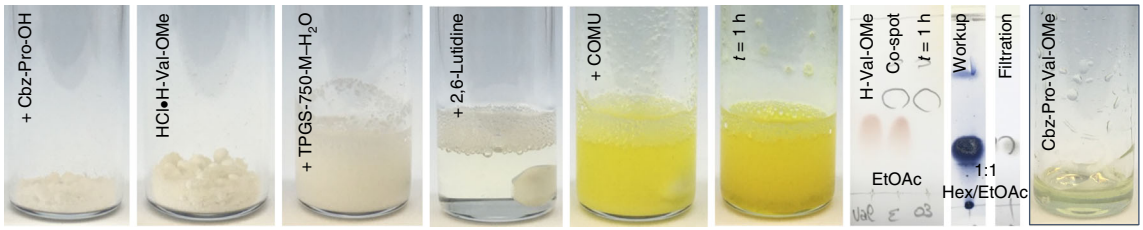

Fig. 8 | Step-by-step synthesis of Cbz-L-Pro-L-Val-OMe.

ensure a consistent ionization state. Spot the organic layer on the TLC plate (amino ester/co-spot/reaction). Elute the TLC plate with EtOAc as the mobile phase, using $UV_{254\ nm}$ and ninhydrin as a stain. When the red spot corresponding to the amino ester does not appear in the reaction spot, this indicates that the reaction has reached completion.

8 Add 2.5 mL of MTBE to the vial, stir gently, collect the upper layer with a pipette and transfer to a separatory funnel. Repeat this operation one more time (5 mL total).
▲ **CRITICAL STEP** The full contents of the vial can be directly transferred to the separatory funnel, but the method described above helps to prevent the formation of an emulsion during the subsequent washing step, and thus helps to reduce the needed amounts of solvent/aqueous solutions. EtOAc is a suitable solvent for the extraction but tends to dissolve the oxime by-product and TPGS-750-M better than MTBE, thereby increasing the needed number of aqueous washings.

9 Add 2.5 mL of a 1 M HCl solution. Shake the separatory funnel and allow the two layers to separate. Collect the lower layer in a beaker. Repeat this step once (5 mL total).

10 Add 3 mL of a saturated aqueous Na_2CO_3 solution. Shake the separatory funnel and allow the layers to separate. Collect the lower layer in a second beaker. Repeat twice (9 mL total).
▲ **CRITICAL STEP** Do not collect the acidic and basic layers in the same beaker, to prevent a potentially violent acid–base reaction. The first basic aqueous layer is bright yellow. Basic aqueous washings must be repeated until the aqueous layer is colorless (typically two times).

11 Collect the organic layer in a beaker. Add $MgSO_4$ until some free powder can be observed, and filter the solution through a funnel filled with a piece of cotton into a round-bottom flask.

12 If the product is an intermediate in the one-pot deprotection/coupling process, remove the volatiles via rotatory evaporation to dryness in a bath set up at 40 °C for 30 min and then proceed to Step 17 (Procedure 1). The product can be used without further purification, as the only impurities are traces of TPGS-750-M. (Optional) Filter the product formed if it should be isolated (Steps 13–16 (Procedure 1)).

13 Add 0.5 g of silica to the round-bottom flask containing the peptide solution, and then dry-load the product using the rotatory evaporator with a bath set up at 40 °C until the silica is dry and free-flowing.
▲ **CRITICAL STEP** Celite is also suitable for dry loading, especially if acid-sensitive moieties are present.
▲ **CRITICAL STEP** If you used EtOAc for the extraction, we recommend that you evaporate the solvent first, and then dissolve the crude in a solvent with a lower boiling point to facilitate dry loading.
▲ **CRITICAL STEP** As some powder can stick to the wall of the round-bottom flask after the dry-loaded material has been poured, we recommend sonication to collect the entire amount.

14 Add 3 g of silica in 10 mL of EtOAc to a 1.5-cm-diameter chromatographic column. Elute the EtOAc. Rinse the silica with 10 mL of hexane. Keep 1–2 cm of the hexane above the silica front.

15 Pour the dry-loaded product on top of the column. Add 1 cm of sand to the top. Elute 10 mL of a 90:10 mixture of hexane and EtOAc, followed by 20 mL of a 1:1 mixture of hexane and EtOAc. Collect all the fractions in disposal tubes (13 × 100 mm). In a previously tared round-bottom flask, collect the tubes containing the product (checked by TLC—(1:1) hexanes:EtOAc–CAM stain) and evaporate the solvent to dryness using the rotatory evaporator with a bath set up at 40 °C.
▲ **CRITICAL STEP** This exact elution works for almost all dipeptides. For highly polar dipeptides, the second elution can be replaced by a 25:75 hexane-to-EtOAc ratio.

16 Keep the flask under high vacuum overnight. Confirm the product by $^1H/^{13}C$ NMR (in $CDCl_3$).
■ **PAUSE POINT** Store the product under argon between 0 and 8 °C. In these conditions, the products can be stored for several months without degradation.

Synthesis of tripeptides (0.3-mmol scale) ● Timing 4 h

▲ **CRITICAL** Any Cbz-dipeptide ester can be used as a starting point. We chose to illustrate this synthesis with the dipeptide obtained in Step 13 (Procedure 1). Any length (up to eight residues) can be involved in the second in situ coupling step. For the Cbz-deprotection of peptides of more than two residues, up to 50% (wt/wt) Pd/C is required.

▲ **CRITICAL** This step is illustrated by the synthesis of Cbz-L-Ala-L-Phe-L-Leu-OEt (**II-1**; Fig. 9) and Cbz-L-Lys(Boc)- L-Pro-L-Val-OMe (**II-2**; Fig. 10) (Supplementary Information) as shown in Fig. 5, but can be applied to a broad range of amino acids and peptides. We provide a step-by-step tutorial in the

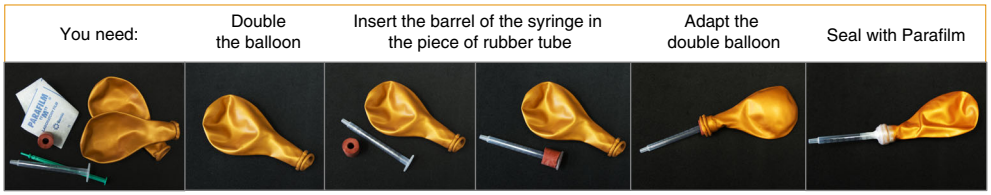

Fig. 9 | Preparation of a double-walled balloon for hydrogen gas.

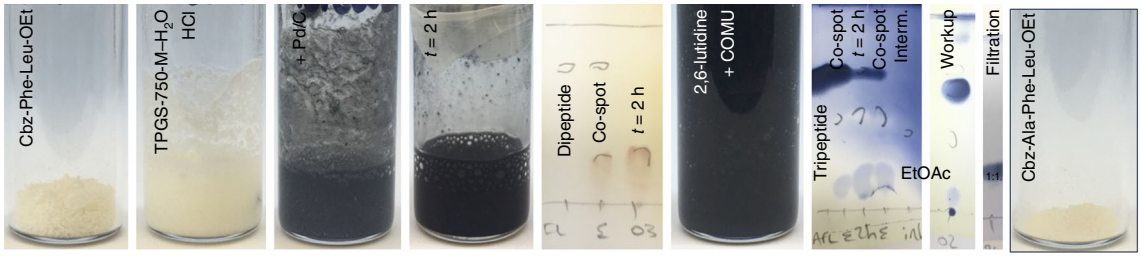

Fig. 10 | Step-by-step Cbz-L-Ala-L-Phe-L-Leu-OEt synthesis.

video 'Peptide synthesis in water—episode 2: 1-pot deprotection/coupling', previously published online (https://youtu.be/eNqan3L4LrQ).

17 Add the Cbz-dipeptide ester (0.3 mmol, 1.0 equiv.) to a 1-dram (~4-mL) vial.
▲ **CRITICAL STEP** As the reaction mixture is highly concentrated, an appropriate vial size (the total reaction should occupy at least one-third of the vial volume) is beneficial for stirring of the reaction, and leads to higher yields.
? TROUBLESHOOTING

18 Add 0.6 mL of a 2% (wt/wt) solution of TPGS-750-M–H_2O (0.50 M in dipeptide).
▲ **CRITICAL STEP** If the dipeptide is not soluble in the 2% (wt/wt) solution of TPGS-750-M–H_2O, add 10% THF (TPGS-750-M–H_2O:THF = 0.48:0.12 instead of 0.6 mL) to help dissolution and improve the efficiency of the reaction. See the section 'Guidelines for use of co-solvents in peptide synthesis'.
? TROUBLESHOOTING

19 Add 25 µL of concentrated HCl (0.3 mmol, 1.0 equiv.).
▲ **CRITICAL STEP** The addition of HCl (1.0 equiv.) is essential to prevent catalyst poisoning.
? TROUBLESHOOTING

20 Add $Pd/C_{10\ wt\%}$ (10% (wt/wt) with respect to the dipeptide, e.g., 0.0132 g out of 0.1322 g of Cbz-L-Phe-L-Leu-OEt, or 0.0109 g out of 0.1089 g of Cbz-L-Pro-L-Val-OMe). Close the vial with a septum and seal it with paraffin film.

21 Purge a balloon mounted on a syringe barrel (see preparation in Fig. 11) with H_2 gas. Fill the balloon with H_2 gas.

22 Attach a needle to the tip of the barrel. Insert into the vial through the septum. Use a second needle to flush the vial with H_2 gas. When the balloon is almost empty, remove the vent needle first, then the balloon. Repeat this step twice.
▲ **CRITICAL STEP** Remove the vent needle first to prevent any loss of H_2 gas or air entry in the vial. Use Teflon tape at the barrel–needle junction to prevent any gas leak.

23 Stir the reaction vigorously at room temperature for 2 h.
▲ **CRITICAL STEP** When the reaction is completed, the medium is completely fluid and black (versus dark gray at $t = 0$). This is a good indication of completion.

24 Monitoring of the reaction after 2 h: dissolve in methanol, into two separate tubes, a sample of the reaction, drawn with a syringe through the septum, and the starting dipeptide. Filter the reaction sample through a pad of Celite to remove Pd/C. Spot the solutions on the TLC plate (dipeptide/co-spot/reaction). Elute the TLC plate with a 1:1 mixture of hexane and EtOAc as the mobile phase, using UV_{254nm} and ninhydrin as a stain. The UV-active spot corresponding to the dipeptide will not appear in the reaction spot, indicating completion. A pink spot with a lower R_f is revealed by ninhydrin.
▲ **CRITICAL STEP** Keep the reaction sample for monitoring the next in situ coupling step.

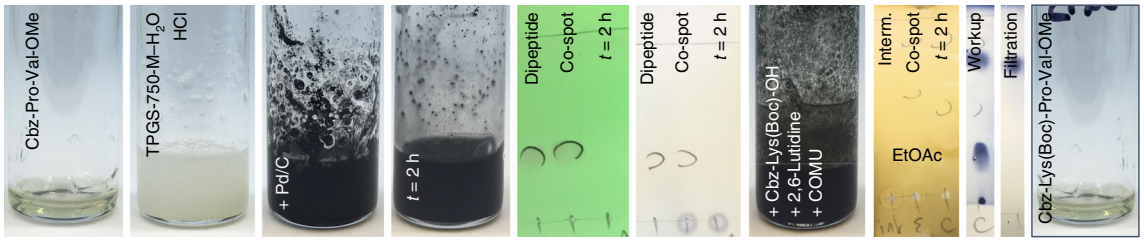

Fig. 11 | Step-by-step Cbz-L-Lys(Boc)-L-Pro-L-Val-OMe synthesis.

25 Purge the vial with argon for 0.5 h to remove the H_2 gas.
▲ **CRITICAL STEP** As Pd/C remains in the flask, the elimination of H_2 gas is required to prevent any partial Cbz-deprotection for the next amino acid/peptide coupling partner.
? TROUBLESHOOTING

26 Add the following Cbz amino acid or Cbz peptide (0.315 mmol, 1.05 equiv.).

27 Add 0.141 g of COMU (0.33 mmol, 1.1 equiv.).

28 While stirring, add 108 µL of 2,6-lutidine (3.1 equiv.).

29 Add 0.6 mL of a 2% (wt/wt) solution of TPGS-750-M–H_2O (concentration is now 0.25 M in starting dipeptide).

30 Stir vigorously at room temperature for 2 h.
▲ **CRITICAL STEP** The medium can be viscous at first, so vigorous stirring is required.

31 Monitoring of the reaction after 2 h: dissolve in methanol a sample of the reaction and filter it through a pad of Celite to remove Pd/C. Spot this solution, as well as the previous HCl·H dipeptide ester intermediate, on the TLC plate (HCl·H-peptide ester/co-spot/reaction). Elute the TLC plate with a 1:1 mixture of hexane and EtOAc as the mobile phase, using UV_{254nm} and ninhydrin as a stain. The red spot corresponding to the HCl·H-peptide ester will not appear in the reaction spot, indicating completion.

32 With stirring, dilute the reaction with 2 mL of EtOAc and filter through a pad of Celite. Rinse the vial and the pad three times with 2 mL of EtOAc (8 mL total).

33 Add 2.5 mL of a 1 M HCl solution. Shake the separatory funnel and allow the two layers to separate. Collect the lower layer in a beaker. Repeat this step once (5 mL total).

34 Add 3 mL of a saturated aqueous Na_2CO_3 solution. Shake the separatory funnel and allow the two layers to separate. Collect the lower layer in a second beaker. Repeat twice (9 mL total).
▲ **CRITICAL STEP** Do not collect the acidic and basic layers in the same beaker, to prevent a potentially violent acid–base reaction. The first basic aqueous layer is bright yellow. This step must be repeated until the aqueous layer is colorless (typically two times).

35 Collect the organic layer in a beaker. Add anhydrous $MgSO_4$ until some free powder can be observed, and filter the solution through a funnel filled with a piece of cotton into a round-bottom flask.
▲ **CRITICAL STEP** Perform TLC (1:1 hexane:EtOAc) to verify whether purification is necessary.
? TROUBLESHOOTING

36 Evaporate the solvent to dryness via rotatory evaporation with a bath set up at 40 °C. If purification is necessary, dissolve the crude material in a minimal amount (~5 mL) of MTBE (or any low-boiling-point solvent, such as CH_2Cl_2) and proceed to Step 37 (Procedure 1). If no purification is required, proceed to Step 43 (Procedure 1).

37 Add 0.5 g of silica gel to the round-bottom flask containing the peptide solution, and dry-load the product onto a rotatory evaporator until the silica is dry and free-flowing.
▲ **CRITICAL STEP** Celite is also suitable for dry loading, especially if acid-sensitive moieties are present.

38 Add 6.5 g of dry silica (10 cm height) in 15 mL of EtOAc to a 1.5-cm-diameter chromatographic column. Elute the EtOAc. Rinse the silica with 20 mL of hexane. Keep 1–2 cm of the hexane above the silica front.

39 Load the dry-loaded product on the column. Add 1 cm of sand.
▲ **CRITICAL STEP** As some powder can stick to the wall of the round-bottom flask, we recommend sonication to collect the entire solid.

40 Apply a hexane–EtOAc gradient to separate the peptide from impurities. Collect all fractions in disposal tubes (13 × 100 mm). In a previously tared round-bottom flask, collect the tubes

containing the product and evaporate the solvent to dryness via rotatory evaporation with a bath set at 40 °C.

▲ **CRITICAL STEP** Depending on the length and polarity of the peptide, different hexane–EtOAc ratios have to be used. For a crude TLC at 1:1 hexane:EtOAc, follow Steps 41–43 (Procedure 1).

41 If R_f > 0.5: 90/10 (50 mL)–80/20 (50 mL)–70:30 (50 mL)–60:40 (100 mL).

42 If R_f < 0.5: 50/50 (100 mL)–25/75 (100 mL)–0/100 (50 mL).

43 Keep the flask under high vacuum overnight. Confirm the product by $^{1}H/^{13}C$ NMR (in $CDCl_3$).

■ **PAUSE POINT** Store the product under argon at 0–8 °C. In these conditions, the products can be stored for several months without degradation.

Procedure 2

Nitro reduction procedure: 0.5-mmol-scale reaction ● Timing 2–12 h

▲ **CRITICAL** This step is illustrated by the synthesis of 4-bromoaniline (Fig. 12) and 2-isopropoxy-5-methyl-4-(pyridin-4-yl)aniline (Figs. 13 and 14), but can be applied to a broad range of nitro-containing compounds.

1 Add the nitro compound (0.5 mmol, 1.0 equiv.), 139.6 mg of CIP (2.5 mmol, 5.0 equiv.), and 80.2 mg of NH_4Cl (1.5 mmol, 3.0 equiv.) to a 1-dram (~4-mL) vial equipped with a stir bar.

▲ **CRITICAL STEP** As the reaction mixture is highly concentrated, an appropriate vial size (the total reaction should occupy at least one-fourth of the vial volume) is beneficial for the stirring of the reaction.

2 Briefly flush the vial with argon. Add the co-solvent if necessary (see 'Co-solvent screening protocol') via syringe and cap the vial.

▲ **CRITICAL STEP** Although the reduction is not typically air sensitive, some substrates reduce faster under argon. We recommend trying both with new substrates. Some products may, however, be air sensitive and thus need an argon atmosphere.

3 Stir the vial at ~300 r.p.m. at 45 °C for ~2 min to allow the co-solvent to partially dissolve the nitro compound.

▲ **CRITICAL STEP** Avoid splattering by keeping the stirring smooth and slow.

▲ **CRITICAL STEP** 10–20% (vol/vol) co-solvent probably will not be enough to fully dissolve the compound. If it is only enough to wet the surface, that is sufficient to proceed.

4 Open the lid of the vial slightly and add a 2% (wt/wt) TPGS-750-M–H_2O solution via a syringe (total volume of 2% (wt/wt) TPGS-750-M–H_2O and co-solvent: 1.0 mL) and cap the vial.

5 Stir the vial at 45 °C at ~300 r.p.m. until the reaction reaches completion.

▲ **CRITICAL STEP** When a block reactor is used, the internal temperature in the vial is frequently lower than the set temperature for the reactor, thus necessitating a higher temperature setting—that is, the block reactor must be set to 50 °C to maintain an internal temperature of the vial of 45 °C. The sensor can also be placed in a vial containing water for a more accurate measurement.

? TROUBLESHOOTING

6 Monitor the reaction by TLC: briefly open the vial, dip a pipette in the reaction mixture, recap the vial and place the pipette in a small test tube. Add a few drops of EtOAc to the test tube and swirl to mix. Test the mixture by TLC using the upper organic layer, and co-spot versus the starting nitro compound. Elute the TLC plate with EtOAc:hexane (40:60) as the mobile phase for 4-bromoaniline and MeOH:DCM (10:90) for 2-isopropoxy-5-methyl-4-(pyridin-4-yl)aniline, using $UV_{254\ nm}$ and

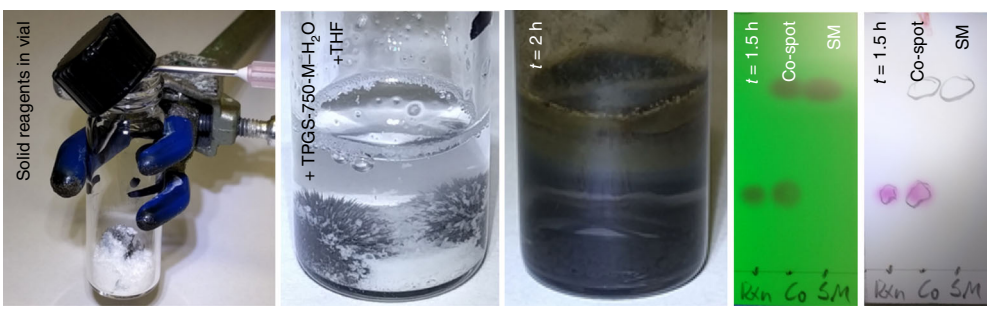

Fig. 12 | Synthesis of 4-bromoaniline, **III-1**.

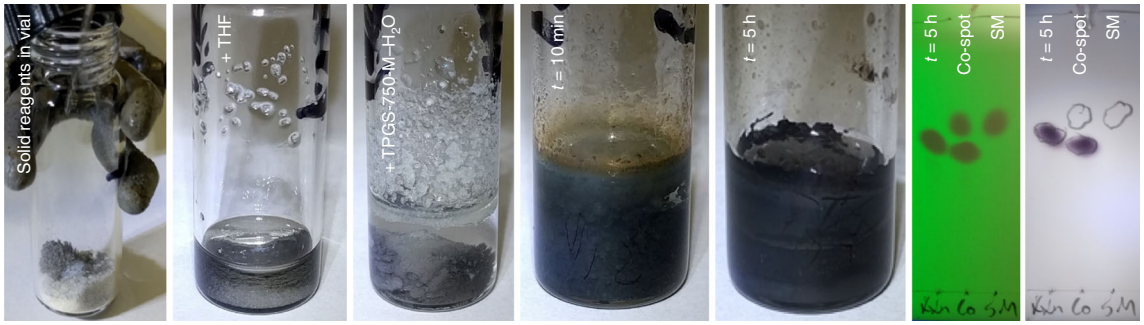

Fig. 13 | Synthesis of 2-isopropoxy-5-methyl-4-(pyridin-4-yl)aniline, **III-2**.

ninhydrin as a stain. The red/gray spot corresponding to the amine product is revealed by ninhydrin while the disappearance of the starting material is observed by UV, indicating completion. The reaction will take between 1.5 and 14 h depending on the substrate.

▲ **CRITICAL STEP** If some of the nitro compound is trapped above the water line, it can lead to a false positive for reaction completion.

■ **PAUSE POINT** The reaction does not need to be worked up immediately. We have left reactions overnight, with no decomposition noted.

? TROUBLESHOOTING

7 Once the reaction reaches completion, isolate the product according to the workup in the section 'Extraction, workup and purification' (Step 11 (Procedure 2)). If the starting material is still present after 14 h, follow the steps under 'Difficult-to -reduce compounds' starting at Step 8 (Procedure 2).

(Optional) Difficult-to-reduce compounds (0.5-mmol scale) ● Timing 2–12 h

▲ **CRITICAL** If after following Steps 1–7 (Procedure 2) and consulting the co-solvent selection guide you still observe poor conversion for a substrate, you can identify that substrate as a 'difficult compound' that might benefit from the addition of HCl.

8 Add 42 µL of concentrated (12 M) HCl (0.5 mmol, 1 equiv.) dropwise via a syringe to the uncompleted reaction and cap the vial.

9 Return the vial to the block reactor or oil bath and stir at 45 °C and ~300 r.p.m. until completion.

▲ **CRITICAL STEP** Monitor the reaction by TLC (see Step 6 (Procedure 2)).

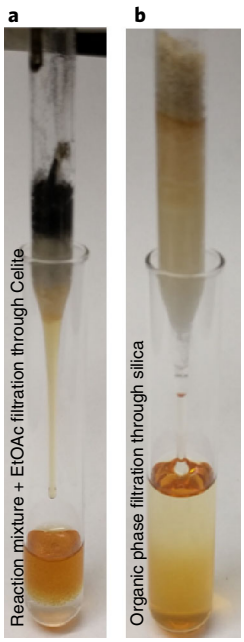

Fig. 14 | Reaction workup and purification.

10 Work up the reaction according to the workup section.
▲ CRITICAL STEP The use of HCl initially lowers the reaction pH, but it typically rebounds within a few minutes, reaching ~5 and staying there until the end of the reaction. When we used HCl, before extraction, we found it beneficial to add 100 µL of saturated $NaHCO_3$ solution to the vial and then stir vigorously to increase the pH, thereby making extraction more efficient.
■ PAUSE POINT The reaction does not need to be worked up immediately; reactions that we left overnight showed no decomposition.

Extraction, workup and purification (0.5-mmol scale) ● Timing 1–2 h

11 The reaction can be either filtered through Celite and then extracted (filtering before extraction eliminates emulsions and is the preferred workup; option A) or extracted directly in the vial with EtOAc (option B).
(A) **Filtration before extraction**
(i) After completion of a reaction, add ~0.5–1.0 mL of EtOAc to the vial and stir briefly.
(ii) Via syringe, transfer the contents of the vial to a cotton-plugged pipette with ~1 cm of Celite. Use a pipette bulb or compressed air to filter the reaction contents through the Celite plug (Fig. 14a).
▲ CRITICAL STEP Filtration without prior addition of EtOAc to the vial can be very difficult, especially with poorly soluble anilines, as the Celite is easily clogged.
? TROUBLESHOOTING
(iii) Add ~0.5–1.0 mL of EtOAc to the reaction vial, and stir briefly.
(iv) Repeat Steps 11A(ii) and 11A(iii) (Procedure 2) two additional times.
(v) Via pipette, transfer the organic layer to a new test tube and extract the remaining aqueous phase with EtOAc, if needed.
(B) **In-vial extraction**
(i) Add ~0.5–1.0 mL of EtOAc to the vial, stir briefly and allow contents to settle.
▲ CRITICAL STEP Either centrifuging or placing a magnet against the bottom of the vial helps pull solids to the bottom, leading to separation of layers (for difficult emulsions, the vial can be heated to ~50 °C and then allowed to settle).
(ii) Remove the organic layer with a pipette or syringe and repeat the extraction as needed (typically three extractions of 0.5–1.0 mL are conducted).

12 A small amount of TPGS-750-M will be present in the organic phase. Depending on the future use of the aniline product, this may or may not be problematic. If the impurity must be removed (e.g., if the aniline will be used in a reaction incompatible with the presence of PEG), then add ~1 mL of deionized water to the test tube containing the organic phase and mix thoroughly with a pipette. After allowing the layers to settle, remove the aqueous layer via a pipette and discard this layer. If a small amount of TPGS-750-M contamination is acceptable, then proceed directly to Step 13 (Procedure 2).

13 Add drying agent (anhydrous Na_2SO_4 or $MgSO_4$) to the combined organic phases and mix via a pipette.

14 Using a pipette, transfer the organic phase to a cotton-plugged pipette with ~5 cm of silica gel (Fig. 14b). Using a pipette bulb or compressed air, push the organic phase through the silica plug into a round-bottom flask, rinsing the silica with a solvent mix such as the one suitable for TLC of this compound.
▲ CRITICAL STEP If the silica is not thoroughly rinsed with a sufficiently polar solvent mixture, some compound may be left on the silica, decreasing the yield.

15 Remove the solvent by rotary evaporator and dry the product under vacuum to constant mass.

Scale-up procedure (3.5-g scale) ● Timing 2–12 h

▲ CRITICAL This reduction is illustrated by the synthesis of *N*1-(1-benzylpiperidin-4-yl)benzene-1,4-diamine (**III-3**) (Fig. 15) but can be applied to a broad range of nitro-group-containing compounds.
▲ CRITICAL When scaling, first perform the reaction according to Procedure 2 at 0.5 mmol to determine the optimal co-solvent ratio, reaction time and expected yield. Do not proceed to a scale-up trial without first obtaining satisfactory results on a 0.5-mmol-scale trial.

16 Add 2.09 g of NH_4Cl (39 mmol, 3 equiv.), 3.63 g of CIP (65 mmol, 5 equiv.) and 4.05 g of finely ground 1-benzyl-*N*-(4-nitrophenyl)piperidin-4-amine (13.0 mmol, 1 equiv.) to a 100-mL round-bottom flask.

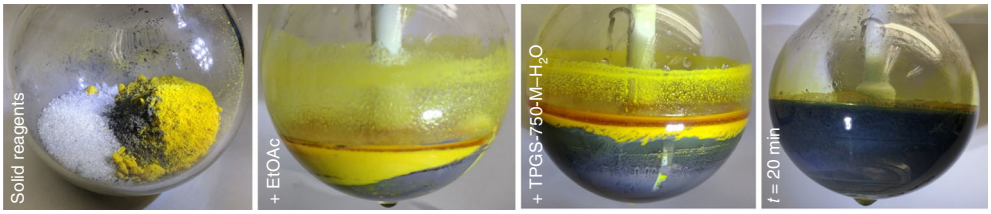

Fig. 15 | Reaction at a larger scale.

▲ **CRITICAL STEP** Because of the typically poor solubility of nitro compounds in aqueous media, the material must be as finely ground as possible. The presence of large lumps/crystals can result in unreacted starting material in the final reaction mixture.

17 Fit the flask with a mechanical stirrer and add 6.5 mL of EtOAc via a syringe. Stir at 45 °C for 2 min to partially dissolve the nitro compound.

18 Add 19.5 mL of 2% (wt/wt) TPGS-750-M–H_2O solution via a syringe and stir the reaction vigorously at 45 °C.

19 Monitor the reaction by TLC (see Pause point of Step 6) to confirm completion.
? TROUBLESHOOTING

20 Upon completion, filter the reaction mixture in a Buchner funnel and rinse the flask and residue with EtOAc. Pour the filtrate into a separatory funnel and separate the organic layer. Extract the aqueous layer with EtOAc.
? TROUBLESHOOTING

21 Dry the combined organic over anhydrous Na_2SO_4 and filter through a short plug of silica (fritted funnel with ~2 inches of silica) with MeOH–DCM (10:90) (for this particular substrate).

22 Remove the solvent via rotary evaporation and dry the product under vacuum to constant mass (3.48 g, 95%).

Troubleshooting

Troubleshooting advice for peptide synthesis (Procedure 1) can be found in Table 3.

Table 3 | Troubleshooting for peptide synthesis

Step	Problem	Possible reason	Solution
1, 17	Low isolated yield	The volume of the vial is inadequate	Use a vial or a round-bottom flask allowing the reaction to occupy at least one-third of its volume
		A viscous starting material is stuck on the wall of the vial	Ensure proper distribution/mixing by dropping any viscous reagent directly to the bottom of the vial
3, 4	Low isolated yield	All the solids were added before the liquids, forming a gum in the bottom of the vial	Before adding COMU, make sure that the amino acids are well dispersed in the basic micellar solution
3, 17	Incomplete deprotection	The starting materials are not readily soluble in the surfactant solution	If the reagent-surfactant solution is pasty, the addition of 10% THF can help the dissolution and facilitate deprotection
3, 18	Low isolated yield	The starting materials are not readily soluble in the surfactant solution	If the reagent-surfactant solution is pasty, the addition of 10% THF can help the dissolution and facilitate stirring
19	Low isolated yield	The deprotection was not performed in the presence of HCl	If the solution is increasingly pasty along the deprotection, verify that HCl (1.0 equiv.) was added. Otherwise, an aggregate with the Pd forms, affecting the outcome of the reaction
20	Incomplete deprotection	The starting peptide is longer than two residues	Use 50% (wt/wt) Pd/$C_{10\%}$ for the deprotection of longer peptides
35	Purity	More polar by-products are present after the workup of the one-pot deprotection/coupling	Purge the vial with argon after deprotection to prevent Cbz deprotection of the incoming coupling partner (Step 25)

Troubleshooting advice for nitro-group reduction (Procedure 2) can be found in Table 4.

Table 4 | Troubleshooting: potential problems and solutions for nitro-group reduction

Step	Problem	Possible reason	Solution
5	Reaction will not proceed or stalls	Low solubility or co-solvent incompatibility	See co-solvent screening protocol; try the seven recommended ratios of co-solvent to TPGS-750-M-H_2O
		This is a 'difficult' compound	See 'Difficult-to-reduce compounds' in Procedure 2. The addition of HCl has been found to improve conversion with difficult substrates. HCl should be used only if necessary, as it often results in decreased yields and difficulty in filtration
		Reaction preference for air or argon	Some substrates were found to give better conversion under argon than air, and in some cases vice versa. Try the TPGS-750-M/co-solvent ratios under argon as well as under air
5, 6	TLC of the reaction shows complete conversion, but the starting material remains in the product after workup	Lumps or large crystals of nitro compound were used and remained undissolved	Especially important for scale-up reactions: finely grind/crush nitro compound before using
	Reaction will not proceed or stalls	Scale and stirring concerns	Small-scale (0.5 mmol) trials are best conducted with magnetic stirring. Upon scaling (>1.0 mmol), CIP clumps on the stir bar, impeding conversion, and the reaction must be conducted with overhead mechanical stirring
5, 19	Reaction material splattering up walls of vial	Stirring too fast	Stir the reaction more slowly; 300 r.p.m. (on 0.5-mmol scale) has been found to be sufficient to mix the reaction without spreading material far up the inside of the vial
		Stirring uneven	Place the vial near the center of the stir plate; placing the vial far from the center of the plate can increase splattering
		'Ring' of material forming above water line	Periodically use a magnet to 'wipe' the sides of the glass (through the glass) using the CIP clump. Or hold a magnet to the bottom of the flask to keep the CIP on the bottom and gently shake the vial to rinse the walls of the glass
		Vial/reaction vessel not full enough	On a small scale (e.g., 0.5 mmol), the reaction is very messy, but the appearance improves substantially when the reaction is scaled up. Keeping a reaction vessel as full as possible decreases the amount of surface area that the material can stick to
	TLC of the reaction shows complete conversion, but the starting material remains in product after workup	Nitro compound was trapped above the water line	Be careful to prevent nitro compound from becoming trapped above the water line, stir more slowly or rinse compound into the reaction
	Reaction material splattering up walls of vial	Co-solvent amount/choice	EtOAc tends to help rinse the walls of the vial; this is especially helpful when the reaction is run on a larger scale. Try EtOAc as co-solvent or other water-immicible organic solvents
11A	Filtering of the completed reaction is difficult/slow	Use of HCl produced 'goo'	Use of HCl tends to make 'gooey' solids, which are difficult to filter. Use a filtration apparatus with a larger surface area, or pre-filter the reaction through cotton before trying to filter through Celite
11A, 20	Filtering of the completed reaction is difficult/slow	Aniline is poorly soluble	Add EtOAc first to the reaction and stir briefly before trying to filter. If the aniline product is poorly soluble, it tends to clog the Celite and make filtration difficult
		Cotton is packed too tightly in the pipette filter	Make a new pipette filter, but with a larger, looser cotton plug
11A, 20	Filtering of the completed reaction is difficult/slow	Filtration apparatus is too small	For scaled reactions, increase the size of the Buchner funnel/filtration apparatus as necessary

Timing

Preparation of the 2% (wt/wt) TPGS-750-M–H_2O solution: overnight

Procedure 1: peptide synthesis

Steps 1–5, preparation of reagents: 0.5 h
Step 6, reaction time: 1 h
Steps 7–11, reaction workup: 1 h
Steps 12–15, purification: 1 h
Step 16, drying: overnight
Steps 17–22, preparation of reagents: 0.5 h
Step 23, reaction time: 2 h
Step 25, argon flushing: 0.5 h
Steps 26–29, reagent preparation: 0.5 h; can be done during Step 25
Step 30, reaction time: 2 h
Steps 31–36, reaction workup: 1 h
Steps 37–42, purification: 1.5 h
Step 43, drying: overnight

Procedure 2: nitro-group reduction

Steps 1–4, reaction setup: 0.5 h
Steps 5 and 6, reaction time: 2–12 h
Step 8, adding HCl: 5 min
Step 9, reaction time: 1–12 h
Steps 10–14, extraction, workup and purification: 1 h
Step 15, drying: 1–2 h
Step 16, reagent preparation: 0.5 h
Steps 17–19, reaction time: 2–12 h
Steps 20 and 21, workup and purification: 1–2 h
Step 22, drying: overnight

Anticipated results

Cbz-L-Phe-L-Leu-OEt·H_2O (I-A)

Cbz-L-Phe-L-Leu-OEt·H_2O is obtained as an off-white powder (119.6 mg, 87%).

Melting point: 106–107 °C.

R_f = 0.85 (1:1 hexane/EtOAc)—cerium ammonium molybdate stain.

^{1}H-NMR (500 MHz, $CDCl_3$): δ 7.40–7.28 (m, 6H), 7.26–7.16 (m, 4H), 6.11 (bd, J = 8.2 Hz, 1H), 5.28 (bd, J = 6.1 Hz, 1H), 5.10 (d, J = 2.7 Hz, 2H), 4.54 (td, J = 8.5, 5.3 Hz, 1H), 4.44 (bd, J = 8.2 Hz, 1H), 4.16 (qd, J = 7.1, 3.5 Hz, 2H), 3.14 (dd, J = 13.9, 6.4 Hz, 1H), 3.06 (dd, J = 13.9, 6.9 Hz, 1H), 1.54–1.41 (m, 3H), 1.27 (t, J = 7.1 Hz, 3H), 0.90 (dd, J = 8.5, 6.3 Hz, 6H).

^{13}C-NMR (126 MHz, $CDCl_3$): δ 172.3, 170.5, 155.9, 136.3, 136.1, 129.4, 128.6, 128.5, 128.2, 128.0, 127.0, 67.0, 61.3, 56.0, 50.9, 41.5, 38.4, 24.7, 22.7, 22.0, 14.1.

Cbz-L-Pro-L-Val-OMe (I-B)

Cbz-L-Pro-L-Val-OMe is obtained as a pale yellow oil (90.2 mg, 83%).

R_f = 0.39 (1:1 hexane/EtOAc)—cerium ammonium molybdate stain.

^{1}H-NMR (600 MHz, $CDCl_3$): δ 7.50–7.26 (m, 4H), 7.26–7.18 (m, 1H), 6.40 (s, 1H), 5.26–5.05 (m, 2H), 4.56–4.30 (m, 2H), 3.84–3.39 (m, 5H), 2.49–1.82 (m, 6H), 0.87 (dd, J = 13.9, 6.8 Hz, 6H).

^{13}C-NMR (126 MHz, $CDCl_3$): δ 171.9, 171.4, 155.7, 154.7, 136.3, 67.0, 60.4, 60.0, 57.1, 56.7, 51.7, 47.2, 46.7, 30.8, 27.9, 24.4, 23.4, 18.7, 17.4.

Cbz-L-Ala-L-Phe-L-Leu-OEt (II-A)

Cbz-L-Ala-L-Phe-L-Leu-OEt is obtained as an off-white powder (92%, 141.1 mg).

Melting point: 129–130 °C.

R_f = 0.39 (1:1 hexane/EtOAc)—cerium ammonium molybdate stain.

^{1}H-NMR (600 MHz, $CDCl_3$): δ 7.40–7.29 (m, 5H), 7.27–7.15 (m, 5H), 6.81 (bd, J = 7.7 Hz, 1H), 6.56 (bd, J = 7.7 Hz, 1H), 5.41 (d, J = 7.2 Hz, 1H), 5.16–4.99 (dd, J = 50.0, 12.2 Hz, 2H), 4.73 (q, J = 7.2 Hz, 1H), 4.52 (td, J = 8.5, 5.3 Hz, 1H), 4.24 (d, J = 7.1 Hz, 1H), 4.15 (qd, J = 7.1, 1.6 Hz, 2H), 3.08 (d, J = 6.7 Hz, 2H), 1.54 (dddd, J = 42.2, 21.4, 9.9, 6.5 Hz, 3H), 1.31 (d, J = 7.1 Hz, 3H), 1.26 (t, J = 7.1 Hz, 3H), 0.88 (d, J = 6.4 Hz, 6H).

^{13}C-NMR (151 MHz, $CDCl_3$): δ 172.4, 172.4, 170.5, 156.0, 136.4, 136.3, 129.4, 128.6, 128.5, 128.3, 128.1, 127.0, 67.1, 61.3, 54.2, 51.0, 50.7, 41.4, 38.2, 24.8, 22.8, 22.1, 18.7, 14.2.

Cbz-L-Lys(Cbz)- L-Pro-L-Val-OMe (II-B)

Cbz-L-Lys(Cbz)- L-Pro-L-Val-OMe is obtained as a pale yellow oil (149.6 mg, 84%).

R_f = 0.71 (100% EtOAc)—cerium ammonium molybdate stain.

^{1}H-NMR (500 MHz, $CDCl_3$): δ 7.39–7.29 (m, 5H), 7.08–7.01 (m, 1H), 5.63 (s, 1H), 5.10 (d, J = 2.0 Hz, 2H), 4.85 (s, 1H), 4.59 (dd, J = 8.1, 3.1 Hz, 1H), 4.51 (ddd, J = 19.3, 8.5, 4.8 Hz, 2H), 3.74 (s, 3H), 3.73–3.65 (m, 1H), 3.58 (ddd, J = 16.7, 9.9, 5.5 Hz, 1H), 3.09 (dd, J = 13.2, 6.8 Hz, 2H), 2.31 (d, J = 9.6 Hz, 1H), 2.15 (ddt, J = 12.3, 9.7, 6.2 Hz, 2H), 2.09–1.90 (m, 2H), 1.88–1.58 (m, 3H), 1.53 (dt, J = 12.9, 7.0 Hz, 3H), 1.47 (s, 9H), 0.90 (dd, J = 12.4, 6.9 Hz, 6H).

^{13}C-NMR (126 MHz, $CDCl_3$): δ 172.3, 172.0, 171.1, 156.2, 156.2, 136.4, 128.6, 128.2, 128.1, 79.0, 67.0, 60.5, 60.1, 57.4, 52.2, 52.2, 52.2, 47.5, 40.1, 32.6, 31.2, 29.5, 28.5, 27.6, 25.2, 22.3, 21.1, 19.1, 17.7, 14.3.

4-Bromoaniline (III-1)

4-Bromoaniline is obtained as a tan solid (80.2 mg, 92%).

Melting point = 60–61 °C.

R_f = 0.60 (Et_2O)—UV and ninhydrin stain.

^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.23 (d, J = 8.6 Hz, 2H), 6.56 (d, J = 8.6 Hz, 2H), 3.57 (s, 2H).

^{13}C-NMR (101 MHz, $CDCl_3$): δ 145.5, 132.2, 116.9, 110.4.

2-Isopropoxy-5-methyl-4-(pyridin-4-yl)aniline (III-2)

2-Isopropoxy-5-methyl-4-(pyridin-4-yl)aniline is obtained as a pale yellow solid (122 mg, quant).

Melting point = 90–92 °C.

R_f : 0.50 (90:10 DCM/MeOH)—UV and ninhydrin stain.

^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.59 (dd, J = 4.5, 1.6 Hz, 2H), 7.25 (dd, J = 4.5, 1.6 Hz, 2H), 6.68 (s, 1H), 6.63 (s, 1H), 4.50 (dt, J = 12.1, 6.1 Hz, 1H), 3.97 (s, 2H), 2.18 (s, 3H), 1.34 (d, J = 6.1 Hz, 6H).

^{13}C-NMR (101 MHz, $CDCl_3$): δ 150.3, 149.6, 143.7, 137.8, 128.8, 128.0, 124.6, 117.4, 115.3, 71.3, 22.5, 19.7.

*N*1-(1-benzylpiperidin-4-yl)benzene-1,4-diamine (III-3)

*N*1-(1-benzylpiperidin-4-yl)benzene-1,4-diamine is obtained as a red solid (3.48 g, 95%).

Melting point: 30–40 °C.

R_f: 0.40 (90:10 DCM/MeOH)—UV and ninhydrin stain.

^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.33 (d, J = 4.3 Hz, 11H), 7.27 (dd, J = 7.7, 3.3 Hz, 4H), 6.60 (d, J = 8.6 Hz, 6H), 6.51 (d, J = 8.6 Hz, 6H), 3.54 (s, 6H), 3.23 (d, J = 9.0 Hz, 9H), 3.18 (dd, J = 9.3, 5.2 Hz, 3H), 2.86 (d, J = 11.7 Hz, 6H), 2.13 (t, J = 11.6 Hz, 6H), 2.02 (d, J = 12.3 Hz, 6H), 1.45 (td, J = 13.7, 3.5 Hz, 6H).

^{13}C-NMR (101 MHz, $CDCl_3$): δ 140.1, 138.3, 137.9, 129.2, 128.3, 127.1, 117.0, 115.7, 63.20, 52.5, 51.3, 32.8.

Reporting Summary

Further information on research design is available in the Nature Research Reporting Summary.

Data availability

The authors declare that all data supporting the findings of this study are available within the article and its Supplementary Information files.

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Author contributions

M.C.-C., N.R.L. and B.H.L. wrote, reviewed and edited the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

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Key references using this protocol

Gabriel, C. M., Keener, M., Gallou, F. & Lipshutz, B. H. *Org. Lett.* **17**, 3968–3971 (2015): https://pubs.acs.org/doi/abs/10.1021/acs.orglett.5b01812

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Field-specific reporting

Please select the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☐ Life sciences ☐ Behavioural & social sciences ☒ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/authors/policies/ReportingSummary-flat.pdf

Ecological, evolutionary & environmental sciences study design

All studies must disclose on these points even when the disclosure is negative.

Study description	Detailed procedures for two common reactions, performed under environmentally responsible conditions in water using nanomicelles as the reaction vessels.
Research sample	N/A
Sampling strategy	N/A
Data collection	All spectral data were obtained by the co-authors.
Timing and spatial scale	N/A
Data exclusions	N/A
Reproducibility	The procedures provided are all fully reproducible, as assessed by co-workers and by several reactions of each type involving different substrates.
Randomization	N/A
Blinding	N/A

Did the study involve field work? ☐ Yes ☒ No

Reporting for specific materials, systems and methods

Materials & experimental systems

n/a	Involved in the study
☒	☐ Unique biological materials
☒	☐ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology
☒	☐ Animals and other organisms
☒	☐ Human research participants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging