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MC-1. A "designer" surfactant engineered for peptide synthesis in water at room temperature†

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Aqueous micellar catalysis has previously been shown to be an enabling technology for green peptide synthesis. Nonetheless, in response to limitations associated with use of selected amino acids in peptide constructions, a new surfactant has been designed, inspired by the commonplace use of the environmentally egregious dipolar aprotic solvent DMSO. A new amphiphile, MC-1, introduces a highly polar sulfone component into the otherwise nonpolar region of a surfactant, and thus, into the inner cores of its derived nanomicelles. This has led not only to a solution to solubility issues, but also to high yields, ease of handling of reaction mixtures, and elimination of co-solvents. Gram scale reactions are also described herein.

Micellar catalysis facilitates organic transformations in an aqueous medium, relying on self-assembling of a surfactant above its critical micellar concentration (CMC). The inner cores of the newly formed nanomicelles create a suitable lipophilic environment for substrates and catalysts, while the associated hydrophobic effect[1] tends to accelerate reactions and enables transformations to occur typically under milder conditions than those common to traditional reactions run in organic solvents. Over the last 10+ years, our designer surfactants (*e.g.*, TPGS-750-M;[2] Fig. 1a) have enabled numerous transformations as well as 1-pot, tandem processes, all featuring water as the reaction medium. The micellar core, composed of racemic vitamin E, easily accommodates organic substrates and catalysts. These nanoreactors exist as part of a unique arrangement of smaller micelles within a larger (45–60 nm) nanoparticle.[3] While initially developed for metal-assisted cross-coupling reactions involving substrates and catalysts of relatively low polarity, more recent examples include oxidations,[4,5] reductions[6] and, in particular, peptide synthesis.[7] This latter application is of special interest from the green chemistry perspective, since peptide synthesis is known for its extensive dependence on, and use of, deleterious organic solvents.[8] Use of 2 wt% TPGS-750-M in water for peptide and amide bond formation has been shown to be both an efficient and green alternative to organic solvents (*e.g.*, DCM, DMF, NMP, *etc.*). After extraction with a minimal amount of EtOAc, the workup usually consists of a simple extraction, followed by acidic/basic aqueous washings, leaving the surfactant and any by-products in the original aqueous layer. Nevertheless, it was observed, on occasion, that polypeptide formation involving polar amino acids led to reaction mixtures becoming pasty, impacting stirring and thereby lowering levels of conversion and ultimately, isolated yields. Use of 10% (v/v) THF[9] led to enhanced solubility, increasing somewhat the extent of conversion, but yields oftentimes still remained modest.[10] To better accommodate polar substrates, such as protected amino acids, adjustment of the nature of the lipophilic portion of the surfactant seemed appropriate. Hence, a library of eight newly engineered surfactants was prepared (Fig. 1b and c), each with an HLB (Hydrophilic Lipophilic Balance) higher than that of TPGS-750-M (Fig. 2). Guidance was provided by the polar organic solvents usually employed for peptide synthesis, such as DMSO, and hence, an even more polar sulfone moiety was selected and introduced into the inner core of each.

Two series of surfactants have been developed, differing by the position of the sulfone along the lipophilic, inner core-forming chain. The sulfone is closer to the hydrophilic PEG portion in the DSP series (Fig. 1b), while it is further away in the PSD series (Fig. 1c). To evaluate each surfactant, comparison reactions were run *versus* the corresponding surfactant containing the pure hydrocarbon (tridecyl)-containing inner core (Fig. 1d). The performance of these new surfactants has been evaluated based on the synthesis of di- tri- and pentapeptides, known for their cosmetic activities.[11–14] Eight dipeptides were prepared according to the general scheme shown in Fig. 3 (top).

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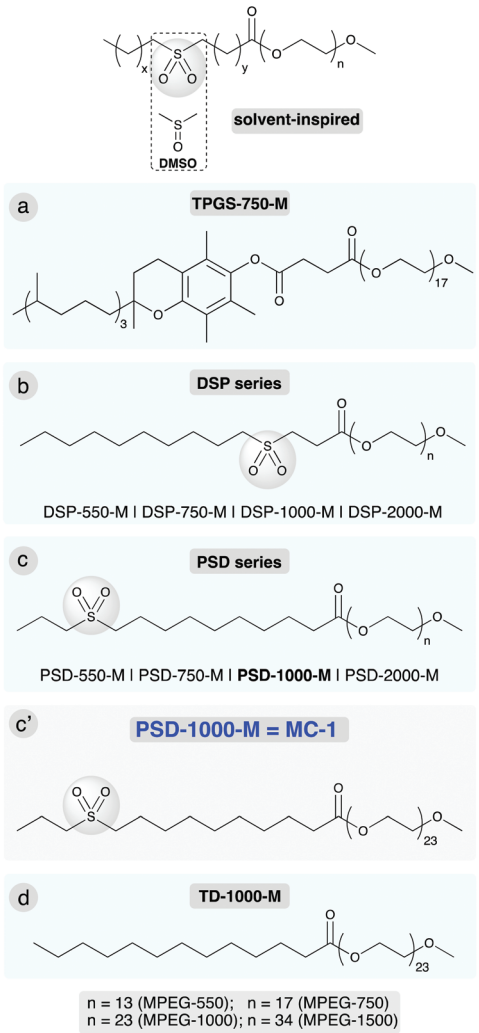


Fig. 1 Two new series of surfactants investigated for peptide synthesis in water (b and c). (a) Structure of TPGS-750-M; (b) structure of the DSP surfactant series (Decyl-, Sulfonyl, Propoyl); (c) structure of the PSD surfactant series (Propyl-, Sulfonyl, Decoyl); (c') highlight on **MC-1** from the PSD series; (d) structure of the sulfone-free TD-1000-M surfactant (TriDecoyl-).

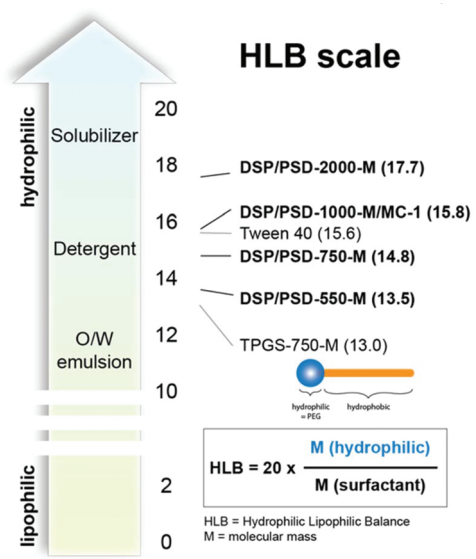


Fig. 2 HLB scale and values for the DSP/PSD series of surfactants, compared to TPGS-750-M and Tween 40. HLB values are indicated in parenthesis.

Comparisons were made between the eight surfactants of the DSP/PSD series, including **MC-1** (Fig. 1c'), TD-1000-M (Fig. 1d), and TPGS-750-M, as well as using water only (Fig. 3). Clearly, these data document that the structure of the surfactant impacted greatly the outcome of each reaction. While all showed improvement relative to water alone, only DSP-1000-M and **MC-1** consistently outperformed TPGS-750-M (with dipeptide **4** being the exception). This suggests that the length of the MPEG chain is an important parameter.

The HLB value of both DSP-1000-M and **MC-1** surfactants is 15.8. Use of Tween 40, a commercially available surfactant with a similar HLB value (15.6), was tested in the synthesis of peptides **4** and **7**. The former was obtained in only 56% yield *vs.* 79% using PSD-1000-M, while **7** was obtained in 74% yield *vs.* 88 and 86% for PSD-1000-M and **MC-1**, respectively. Clearly, the HLB value is not the critical parameter driving the efficiency of surfactants. As determined by cryo-TEM and/or nanoparticle tracking analysis (NanoSight), the micellar diameter for DSP/PSD-550- to -1000-M ranges from 40–80 nm, with an average size of 57 nm (see ESI†). Micelles from DSP/PSD-2000-M are bigger, with an average diameter *ca.* 100 nm, presumably reflecting a greater extent of clustering of smaller particles.[3] It has been shown that *ca.* 50 nm in diameter for typical nonionic nanoreactors (*e.g.*, TPGS-750-M and Nok[15]) leads to a general, very effective arrangement in water, enabling a multitude of reactions to take place. Both sulfonated surfactants bearing MPEG 1000 (*i.e.*, *ca.* 23 ethyleneoxy units) fall within this range.

The position of the sulfone along the lipophilic chain backbone is also of major consequence, and while no clear trend has arisen from these data, **MC-1** is the most efficient surfactant in seven out of the eight examples studied (see Fig. 3; the highest isolated yield is highlighted in green). Just how the difference in location of the sulfone along the lipophilic chain alters the properties of the derived nanomicelles, and the observed effectiveness towards peptide construction, remains clouded at this time. While the absence of sulfone functionality (as in TD-1000-M) is not detrimental to peptide coupling, this "standard" type of surfactant did not afford the best results.

Challenging sequences involving tyrosine, histidine, and arginine, as well as numerous protecting groups (Pbf, *t*-Bu, Boc, Trt) are well-tolerated by **MC-1**. No co-solvent is needed, notably for the synthesis of peptide **3** (Cbz-Arg(Pbf)-Tyr(*t*Bu) OMe), whereas all other surfactants needed 10% THF to avoid formation of a precipitate.

To further investigate the potential of these surfactants, tri- and penta-peptides were synthesized *via* the 1-pot, 2-step deprotection/coupling method developed previously from our

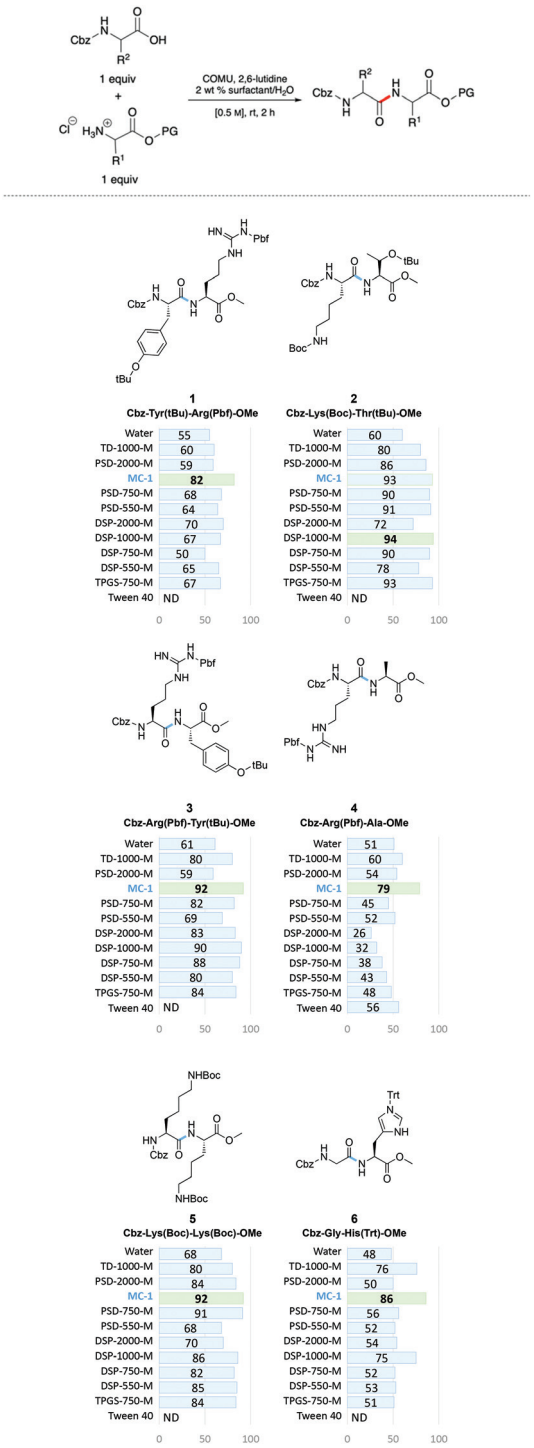


Fig. 3 Comparison of surfactant performance on dipeptide synthesis in water. The general scheme of the coupling reaction is depicted (top). Isolated yields are reported in each aqueous surfactant solution (bottom). ND = not determined.

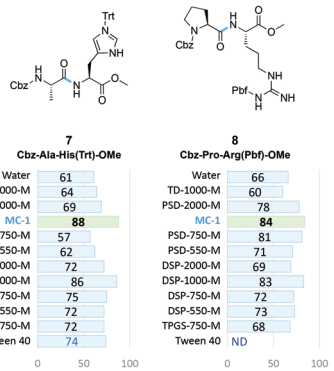


Fig. 3 *(Contd).*

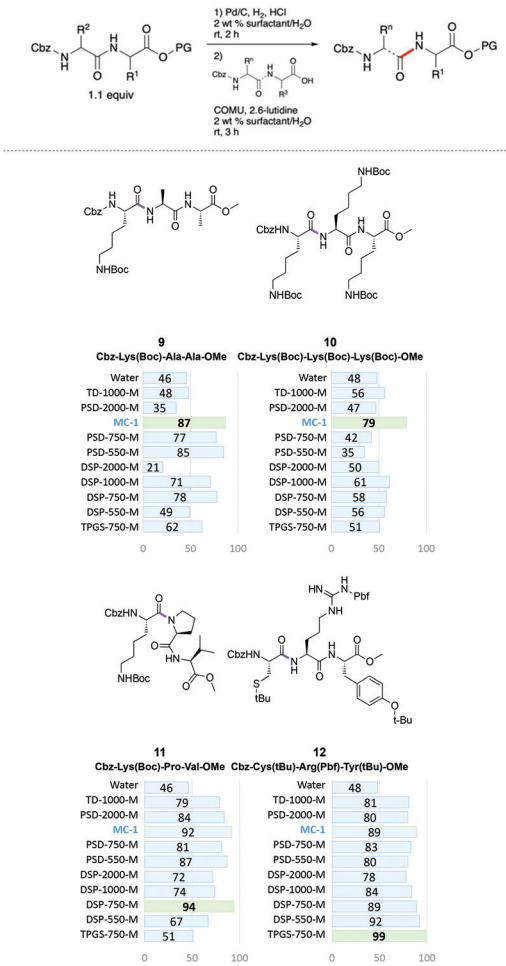


Fig. 4 Comparison of surfactants in a 1-pot, 2-step deprotection/coupling. The general scheme of the 1-pot, 2-step reaction is depicted (top). Isolated yields are reported in each aqueous surfactant solution (bottom).

group.[10] The deprotection of the Cbz-protected N-terminal amine was performed in the presence of Pd/C and hydrogen gas. Cryo-TEM images of Pd/C in a 2 wt% **MC-1**/H_2O (see ESI†) revealed that the supported catalyst was too large to fit into these nanomicelles, although the micelles tend to agglomerate around the solid. After argon flushing, the next coupling partner as well as COMU and 2,6-lutidine were added and the reaction stirred for 3 h at rt. In the synthesis of tripeptides **9** and **10**, **MC-1** was the most effective, with 16–18% higher yields compared to DSP-1000-M (Fig. 4). Insofar as peptides **11** and **12** are concerned, DSP-750-M and TPGS-750-M led to the

highest yields (94 and 99%, respectively), although **MC-1** was still quite enabling (92% and 89%).

Beyond the overall efficiency of each reaction, handling of the mixture is also an important consideration for scale-up and industrial purposes, in general. As illustrated in Fig. 5, plot I, dispersion of the protected amino acids was faster in the sulfonated surfactants compared to that in TPGS-750-M. In Fig. 5, plot II, differences attributed to the surfactant are readily observable. In water only (vial I), a precipitate formed, while in vial C the 1-pot, 2-step reaction appears to be homogeneous after complete conversion to Cbz-Lys(Boc)-Ala-Ala-OMe (**9**). In Fig. 5, plot III, surfactants are compared following deprotection of Cbz-Arg(Pbf)-Tyr(*t*-Bu)-OMe (**3**) *en route* to tripeptide **12**. While the PSD series led to high reaction mixture homogeneity (especially in vials F to H), all other surfactants gave heterogeneous mixtures. This state of affairs did not necessarily affect the outcome of the coupling, although handling of the reaction mixture was much easier.

A [3 + 2] convergent synthesis to access pentapeptide Cbz-Gly-Pro-Arg(Pbf)-Pro-Ala-OMe (**13**) has also been performed in 2 wt% **MC-1**/H_2O, following the 1-pot, 2-step deprotection/coupling protocol (Fig. 6). This important anti-wrinkle "botox-like" peptide, obtained in 76% isolated yield in its protected form, inhibits acetylcholine receptor release.[16]

Assessing the potential for use of **MC-1** in larger scale reactions, peptides **2**, **4**, **5**, **6** and **7** were prepared in gram quantities (Table 1). The isolated yield in each case was similar to that obtained in smaller scale reactions.

Synthesis of **MC-1**, which utilizes inexpensive and readily available starting materials, is very straightforward (Scheme 1). The first step is an S_N2 reaction between propanethiol and 10-bromodecanoic acid. After extraction and precipitation in pentane, the pure sulfide **14** was obtained in 68% yield. Oxidation with Oxone® in a 2 : 1 H_2O/EtOH mixture leads to sulfone **15** in 98% yield. Lastly, esterification with MPEG-1000 (EDC/DMAP) led to the targeted surfactant (88%). None of the three steps necessitated column chromatography, suggesting that this route to **MC-1** may be suitable for industrial purposes.[17,18]

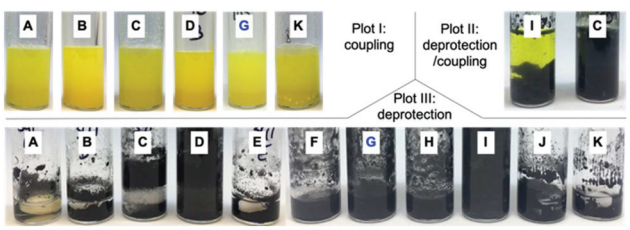


Fig. 5 Handling of the reaction. **Plot I**: Appearance of the reaction at *t* = 20 min for the synthesis of Cbz-Lys(Boc)-Lys(Boc)-OMe (5); **Plot II**: Water *vs.* DSP-1000-M as reaction medium for synthesis of Cbz-Lys(Boc)-Ala-Ala-OMe. Appearance after completion of the 2-step deprotection/coupling process; **Plot III**: En route to Cbz-Cys(*t*-Bu)-Arg(Pbf)-Tyr(*t*-Bu)-OMe (12). Appearance of the reaction after Cbz-Arg(Pbf)-Tyr(*t*-Bu)-OMe (3) deprotection. (A) DSP-550-M; (B) DSP-750-M; (C) DSP-1000-M; (D) DSP-2000-M; (E) PSD-550-M; (F) PSD-750-M; (G) **MC-1**; (H) PSD-2000-M; (I) H_2O; (J) TD-1000-M; (K): TPGS-750-M.

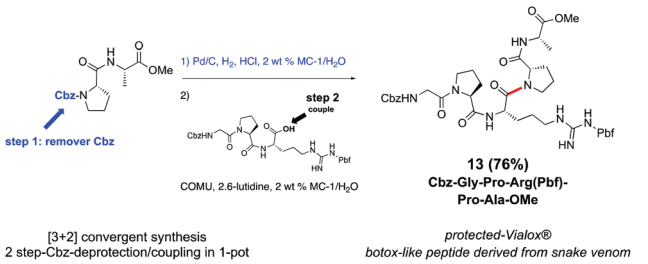
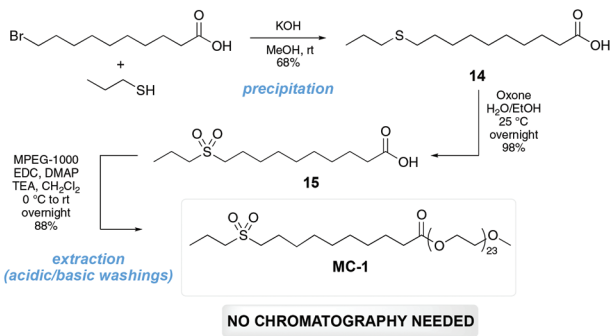


Fig. 6 En route to the synthesis of Vialox, pentapeptide with botox-like activity.

Table 1 Application of **MC-1** to dipeptide synthesis in gram quantities

Peptide	Scale (mmol)	Mass (g)	Yield (%)
2	6.5	3.30	92
4	6.0	3.06	79
5	3.5	1.96	90
6	2.5	1.30	86
7	2.5	1.36	88



Scheme 1 Synthetic pathway to **MC-1**.

Conclusions

A library of surfactants has been prepared, into each of which has been embedded a sulfone residue. These were inspired by the common use of DMSO as solvent for peptide synthesis, and the goal of providing an environmentally responsible alternative means of peptide synthesis, in water. The new amphiphile **MC-1** has emerged as an attractive, unprecedented surfactant containing a highly polar residue within the non-polar portion of the surfactant. This material spontaneously forms nano-particles that accommodate amino acid/peptide partners, together with reagents that facilitate peptide couplings within a polar yet still lipophilic micellar interior. Yields of the resulting, isolated materials tended to be higher than those using other surfactants. Additional features, such as ease of handling of reac-

tion mixtures and avoidance of co-solvents, as well as the consistency of performance by **MC-1** under gram scale conditions, all highlight its potential for industrial applications.

Conflicts of interest

There are no conflicts to declare.

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