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A liquid-phase peptide synthesis in cyclohexane-based biphasic thermomorphic systems†

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Combinations of typical organic solvents composed of cyclohexane and qualified aprotic polar organic solvents were found to realize an effecitive, biphasic thermomorphic system in arbitrary ratios of upper and lower phases that enable a practical application of a liquid-phase peptide synthesis.

Much current research utilizes the solid-phase as a platform for organic reactions.[1] Solid-phase resins offer many advantages with regard to compound isolation and ease of handling. The insoluble nature of the resins has, however, complicated the characterization of compounds attached to them and led to reagent accessibility problems. One successful merging of solid- and liquid-phase chemistry is the use of soluble polymer platforms that selectively remove excess reaction reagents and byproducts.[2] On the other hand, the use of a liquid–liquid biphasic thermomorphic process, in which a reagent or a catalyst is designed as a residue in one of the liquid phases and as a product in the other liquid phase, can be an enabling approach for a commercial application of chemical reactions with high selectivity, efficiency, and ease of handling for the separation of solutes.[3] Based on the immiscibility of perfluorinated hydrocarbons with both organic and inorganic solvents, a novel 'fluorous biphasic system' for catalysis has been proposed.[4] Fluorous biphasic systems consist of a fluorous phase containing a preferentially fluorous soluble reagent and a catalyst with limited solubility in the fluorous phase.[5] The thermomorphic liquid–liquid separation system composed of organic solvents of different polarity might allow for extremely efficient reactions, for example, by the association of apolar products and polar substrates or catalysts in a one-phase solution, which could be spatially separated after the completion of reactions. It should further open the door for the construction of liquid-phase combinatorial chemistry, industrial and green chemistry by the ease of the separation of products, catalyses, reagents, electrolytes, and/or soluble platforms for organic synthesis. This gave us the incentive to explore a novel liquid-phase peptide synthesis system in a thermomorphic system using typical organis solvents. Herein is a report of our findings of the combination of cyclohexane and some typical organic solvents to realize the aimed miscible biphasic system in arbitrary ratios of upper and lower phases. By using the cyclohexane-based biphasic thermomorphic system, an application for liquid-phase peptide synthesis was efficiently accomplished.

We first explored extensively solvent-constructions for thermomorphic solutions that could be reversibly immixed at 'desired' temperatures in 'arbitrary ratios' of upper and lower layer volumes. Among numerous compositions of organic solvents, an appropriate mixture of cyclohexane (CH) and nitroalkane (NA) was initially found to show the desired function, that is, a 75:25 (v/v) mixture of CH and NA [a 20:80 v/v mixture of nitromethane (NM) and nitroethane (NE)]. At 25 °C, the solvent mixture was separated into an upper CH(main) phase and a lower NA(main) phase (Fig. 1, colored by methylene blue, MB). After heating to 45 °C, it was soon immixed to form a stable, homogeneous phase. Furthermore, by cooling back to 25 °C, the solution was immediately separated into the two initial phases, completely recovering the homogeneously dissolved MB into the lower NA layer. This clear partition of a solute suggested the ability of the thermo-controlled solubilization and the partition of designed solutes in this solvent system. It was therefore prompted us to seek the promising property of the biphasic organic liquid system by expanding the solvent combinations and ratios. Fig. 2(a) shows the correlations between the miscible temperature and the ratio of NE and NM. With increasing of the content of NE, the miscible temperature was linearly lowered. For example, a biphasic mixture of CH–NE–NM (CH:NA 17:83, NE:NM 70:30) formed a homogeneous phase at 14 °C and higher

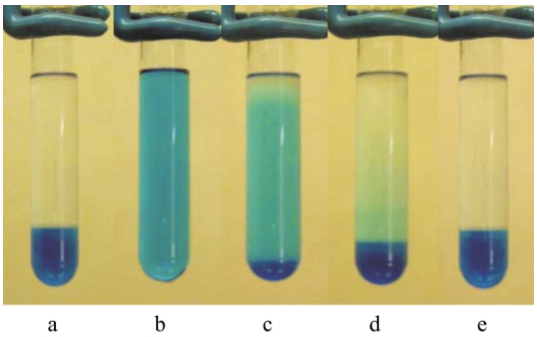

Fig. 1 (a) An organic solvent mixture composed of CH–NE–NM (75:20:5 v/v/v) formed the biphasic system at 25 °C (a lower NA layer was colored by methylene blue). (b) The solvent mixture was immixed at 45 °C. (c,d) After cooling back to 25 °C, it immediately began to exclude the CH phase. (e) Finally, it formed the initial biphasic solution to recover methylene blue in the lower layer.

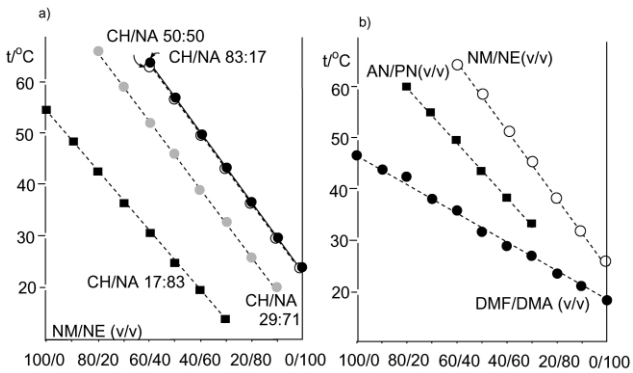

Fig. 2 (a) Effect of the solvent compositions (CH:NA ratios and NM:NE ratios) on the miscible temperatures. Each biphasic solvent mixture (10 mL in an 18 × 200 mm test tube, 10 °C) was gradually heated (*ca.* 3 °C min^{-1}) with stirring to form a homogeneous solution at the temperature plotted above. (b) Effect of solvent composition on the miscible temperature. The solvent mixtures were composed of a 50:50 (v/v) mixture of CH–nitriles or CH–amides with varying ratios of AN:PN or DMF:DMA, respectively.

† Electronic supplementary information (ESI) available: general procedure and spectral data for compounds **2**–**7**. See http://www.rsc.org/suppdata/cc/b2/b205156g/

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temperatures. On the other hand, the miscible temperature increased with an increase in the NM ratio. The miscible temperature was also affected by the ratio of CH, requiring higher temperature with an increase in the CH ratio to complete the one-phase formation. The miscible temperature, however, was not affected when the ratio of CH : NA (v/v) was more than 50 : 50. This shows that the miscible temperature and the ratio of CH : NA can be arbitrarily chosen by controlling the ratio of NE : NM. Furthermore, a similar property was also found in the mixture of CH–dimethylacetamide (DMA)–dimethylformamide (DMF) and of CH–acetonitrile (AN)–propionitrile (PN) (Fig. 2(b)). In the mixture of CH–DMF–DMA, the miscible temperature varied between 18 and 47 °C by changing the DMF : DMA ratio. A clear phase separation was also observed for the mixture of CH–AN–PN between 33 and 61 °C.

In the thermomorphic system, less-polar chemicals or designed less-polar platforms were selectively partitioned in the CH-layer, and a platform, (3,4,5-trioctadecyloxyphenyl)-methan-1-ol **1**[6] effectively worked in the sequential peptide synthesis (Fig. 3). The platform was selectively[7] dissolved in the CH-layer of the biphasic solution composed of CH–DMF–DMA, which allowed the sequential peptide synthesis in the liquid-phase. In the homogenized reaction mixture at 35 °C, peptide-chain elongation was accomplished by treating with only 3 mol equiv. of activated Fmoc-amino acids. After deprotection of the Fmoc moiety, the deprotected products (*e.g.* **3**, **5** and **7**) were immediately 'fished out' from the unreacted reagents just by cooling to form the biphasic solution. The deprotected amino group was then repeatedly treated with another activated Fmoc-amino acid. In these sequential reactions, highly excessive amounts of activated Fmoc-amino acid was not required, and the peptide intermediates (*e.g.* **3**–**7**) were easily isolated in excellent yield and characterized by NMR and/or TOF-MS.

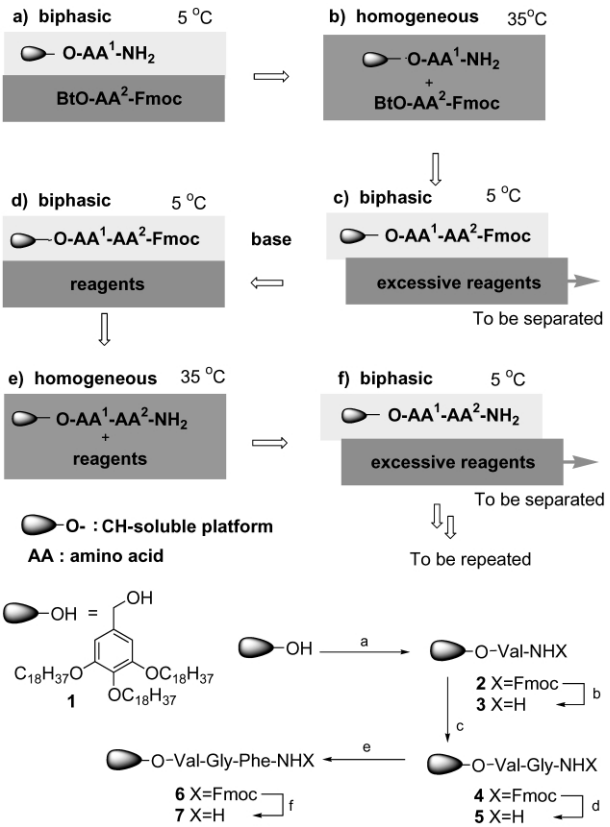

Fig. 3 An example of the liquid-phase peptide synthesis using the biphasic organic solvent mixture. a, (Fmoc-Val-)$_2$O, DMAP in CH–DMF–DMA 50 : 25 : 25 (35 °C), 95%; b, 10% Et_2NH in DMF–DMA 50 : 50 (35 °C), 99%; c, Fmoc-Gly-OBt, DIPCD in DMF–DMA 50 : 50 (5 to 35 °C), 99%; d, 10% Et_2NH in DMF–DMA 50 : 50 (35 °C), 99%; e, Fmoc-Phe-OBt, DIPCD (5 to 35 °C), 97%; f, 10% Et_2NH in DMF–DMA 50 : 50 (35 °C), 99%.

In conclusion, biphasic, thermomorphic liquid–liquid separation systems have been constructed for liquid-phase peptide synthesis by using a CH-soluble platform in CH and typical organic solvents. It is noteworthy that the regulation of the separation and the immixing of solutes can be achieved by a moderate thermo-control in the practical range of 15–65 °C. Furthermore, products can be easily isolated from the CH-layer to complement existing solid-phase peptide synthesis, offering advantages with regard to compound isolation, characterization, and reagent accessibility in reaction mixtures. Since those polar media, NM, DMF and AN, have been widely used for organic reactions with varied catalysts and reagents,[8] the extensive chemical applications can be also assured with the flexibly controllable temperatures and upper/lower layer ratios.

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