Solution-phase oligosaccharide synthesis in a cycloalkane-based thermomorphic system†

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The cycloalkane-based thermomorphic (CBT) system is a convenient and practical method for oligosaccharide synthesis, and hydrophobically modified oligosaccharides have a remarkable affinity for CBT solutions composed of methylcyclohexane and propionitrile.

Over the past few decades, oligosaccharide synthesis based on solid-phase technology has been a powerful tool in automated synthesis and combinatorial chemistry, allowing effective library construction of targeted carbohydrates.[1] However, solid-phase synthesis has serious shortcomings associated with the nature of the heterogeneous conditions required. Recently, solution-phase methodologies[2] have gained significant attention as an alternative to traditional solid-phase oligosaccharide synthesis. Their many advantages include high reactivity of the soluble species and the possibility of using routine analytical methods to monitor the reaction process and directly determine structures, even when the molecules are attached to phase-tags. In particular, the hydrophobic phase-tagging strategies based upon solid-phase-extraction (SPE) by C18 silica have been well-recognized as useful methods that can permit the effective generation of target carbohydrates.[3] While most procedures for the recovery of target molecules emphasize SPE, liquid–liquid extraction (LLE) may also have broad potential, because suitably phase-tagged compounds in a biphasic system often show a particular affinity for one phase without further supports. Among various methods, oligosaccharide synthesis on fluorous supports with a high fluorine content has opened the door to the implementation of simple LLE techniques commonly used in classical organic synthesis. This methodology is a useful approach toward automated and large-scale synthesis of oligosaccharides.[4] Since the target products can be efficiently separated from other reaction components by the highly fluorinated protecting groups, a key structural feature, much effort has been made to address the issues concerning cost, solubility and reactivity.[5]

Bergbreiter *et al.* have successfully developed a thermomorphic liquid–liquid reaction–separation process in which various soluble polymer-bound catalysts and ligands can be efficiently recovered, based on selective solubility, from *n*-heptane or certain polar miscible biphasic solutions after completion of a reaction under monophasic conditions.[6] As an alternative, we have found that a mixture of typical organic solvents, composed of cyclohexane and aprotic polar organic solvents, may be used as an effective thermomorphic system to enable the practical application of liquid-phase peptide synthesis using a cyclohexane-soluble platform.[7] With the use of an appropriate thermomorphic combination of solvent and cycloalkane-soluble phase tag for oligosaccharide preparation, this methodology could overcome the substrate solubility and interfacial reactivity issues associated with fluorous and other biphasic conditions, which can cause kinetic limitations. We envisioned that hydrophobic modification could render oligosaccharides highly soluble in cycloalkane-based thermomorphic (CBT) solutions, and also result in better reactivity due to the lower molecular weight and size of a sequence of small, hydrophobic CH_2 units. Additionally, a wide variety of lipophilic chains can readily be prepared, allowing efficient construction of cycloalkane-soluble molecules with the desired functions. As the CBT system allows for effective simplification of oligosaccharide synthesis *via* LLE, it may prove useful in certain areas of liquid-phase combinatorial chemistry and in fully automated systems. Herein we report the development of an effective oligosaccharide synthesis method using a CBT system (Fig. 1).

Initially, a hydrophobic monosaccharide **1** was synthesized and its solubility in thermomorphic solvents was tested to evaluate the viability of the proposed approach and its efficiency.[4b] The results are summarized in Fig. 2. Although compound **1** displayed low solubility in *n*-heptane (8.1 mM), its solubility increased to 84 mM in MCH (methylcyclohexane). Surprisingly, it was found that the addition of EtCN to the MCH solvent enhanced the solubility of **1** to 179 mM. As expected, its solubility in EtCN was low. Because nitrile solutions are known to promote glycosyl bond formation,[8] it was thought likely that a CBT solution composed of MCH

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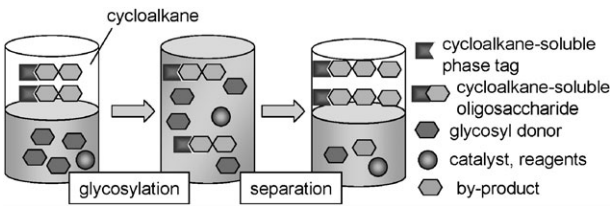
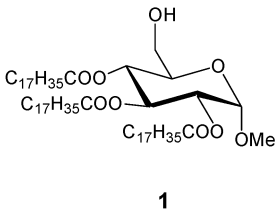


Fig. 1 General view of CBT oligosaccharide synthesis.



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Solvent	Solubility (mM)
n-Heptane	8.1
MCH	84.6
MCH/EtCN(2:1)	179
EtCN	2.6

[a] Determined from a saturated solution of compound **1** at 25 °C

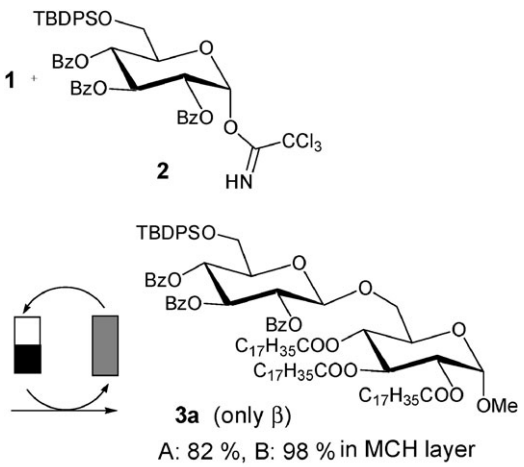
Fig. 2 Solubility of compound **1** in thermomorphic solvents.

and EtCN could be used for targeted glycosylation reactions under miscible conditions, followed by spatial separation of hydrophobic compound **1** in a biphase.

A glycosylation reaction was carried out using MCH–EtCN (2 : 1, v/v) (Scheme 1). Compound **1** was allowed to react with 2 equivalents of trichloroacetimidate donor **2** in the presence of TMSOTf (trimethylsilyl trifluoromethane sulfonate), affording the corresponding disaccharide **3a** (82% yield), which was separated by extraction of the upper MCH layer after cooling of the homogenous reaction mixture.

With the addition of MeCN, the product was recovered almost quantitatively in MCH and was also readily isolated by precipitation (*via* the addition of methanol to the MCH solution, followed by filtration). In the absence of MCH, the coupling reaction proceeded in unacceptable yield, even with longer reaction times.

These results prompted us to investigate the relationship between the MCN : MeCN : EtCN ratio and the miscible temperature. As the content of EtCN increased, the miscible temperature decreased in a linear fashion (Fig. 3(A)). As shown in Fig. 3(B), the maximum miscible temperature was observed at a MCH : nitrile ratio of 80 : 20 (v/v), and the miscible temperature decreased as the MCH ratio decreased. Clear phase separation was observed for MCH–MeCN–EtCN mixtures between *ca.* 70 and 10 °C. In addition, although MCH : EtCN (1 : 1, v/v) formed a homogenous phase at 25 °C, addition of MeCN resulted in the formation of a biphase without cooling. This allows for an effective CBT process at a



TMSOTf, 2 equiv. of **2**. (A) MCH/EtCN; (B) MCH/EtCN with addition of MeCN

Scheme 1 Preparation of disaccharide **3a** in a CBT system.

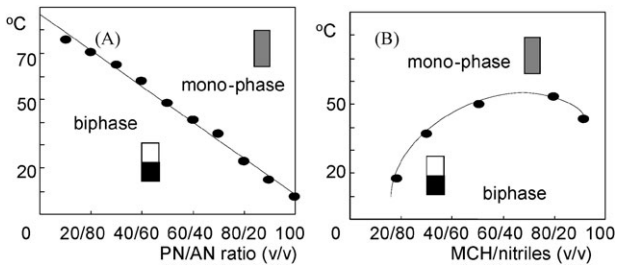


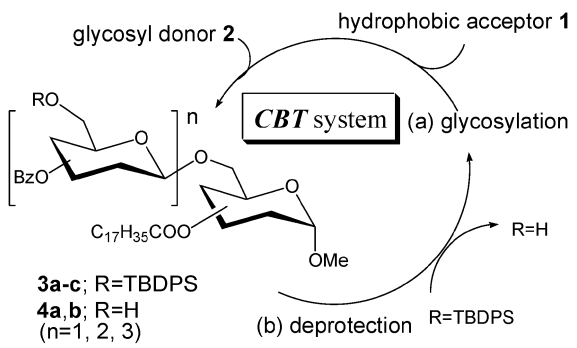
Fig. 3 Effect of solvent composition on miscible temperature. The graphs represent solvent mixtures composed of (A) a 1 : 1 (v/v) mixture of MCH and nitriles with varying AN : PN ratios (v/v), and (B) varying ratios of MCH–nitrile (v/v) at an MeCN : EtCN ratio of 1:1 (v/v).

temperature and solvent ratio suitable for miscible–immiscible phase separation according to target reaction conditions.

Using an MCH : EtCN ratio of 2 : 1 (v/v), we conducted a further investigation of tetrasaccharide synthesis (Scheme 2). The targeted di-tetrasaccharides **3a–c** were successfully prepared under the conditions described. Deprotection of TBDPS (*tert*-butyldiphenyl silyl) groups by treatment with mild TBAF–AcOH also proceeded under the CBT system to afford the acceptors **4a** and **4b** in the MCH phase.

In this system, side-reactions such as the migration of benzoyl groups *via* intramolecular transesterification were not observed. Furthermore, saccharide derivatives attached to phase-tags showed a significant improvement in terms of partition into the separable phase, especially when compared with fluorous modification (Table 1).[9] Although the addition of water was found to result in improved separation in the MCH phase, this procedure was not employed because it is an ineffective strategy in sequential glycosylation. It is notable that simple LLE manipulations *via* the CBT process can effectively facilitate sequential glycosylation and deprotection steps, which should ensure its usefulness in solution-phase oligosaccharide synthesis and in the realization of fully automated processes.

In conclusion, we have demonstrated that the CBT system described here is a convenient and practical method for oligosaccharide synthesis, and that hydrophobically modified oligosaccharides have a remarkable affinity for cycloalkanes.



(a) TMSOTf, 2.0 equiv. of **2**, 25 °C, MCH /EtCN (2:1);
(b) TBAF, AcOH, MCH/EtCN

Scheme 2 Cycloalkane-based thermomorphic oligosaccharide synthesis.

Table 1 Partition ratio of hydrophobic oligosaccharide to MCH : MeCN : EtCN 50 : 25 : 25 (v/v/v) in a biphasic solution

Compound	Phase-tag content[a] (%)	Partition ratio[b] (%)
1	80.8	96
3a	47.0	98
3b	36.8	88
3c	30.2	71

[a] Tag content = phase-tag molecular weight/total molecular weight $\times 100$ (phase tag = $3 \times C_{17}H_{35}CO$). [b] Determined by HPLC analysis.

With the aim of preparing various carbohydrate derivatives, the incorporation of cycloalkane-soluble chains into acceptor and/or donor molecules while maintaining high levels of partition in the cycloalkane phase is now in progress.

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9 Tetrasaccharide synthesis on fluorous supports (Bfp, TfBz = 1006.33, 1599.61) required about 70% phase-tag contents at least; see ref. 3.

