Complementary double helix formation through template synthesis†

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The dimerization of carboxylic acid derivatives bearing an amino or a formyl group at one end was significantly enhanced in benzene in the presence of an optically active amidine dimer to afford a complementary double helix stabilized with salt bridges.

An artificial self-replicating system is the ultimate goal for synthetic chemists, who have continued to determine and to identify the principal mechanism of biological systems and to translate it into synthetic molecular systems.[1] Since von Kiedrowski's ground-breaking report of the first artificial self-replicating system,[2] several examples of chemical self-replicating systems have been reported over the last three decades, although it has not yet been completely achieved. They involve oligonucleotide analogues,[2,3] peptides,[4] and small abiotic organic molecules[5] as templates. One of the key factors necessary for a self-replicating system is a complementary interaction, which enables the recognition and transfer of molecular information. Recently, we reported the rational design and synthesis of artificial hetero-stranded double helices that consist of complementary molecular strands intertwined through amidinium-carboxylate salt bridges.[6] Owing to the well-defined geometry and high association constants of the salt bridges, the double helices have a significant possibility for further designs and various types of multiple-stranded helices have already been successfully prepared. In addition, the helicity of the double helices is controlled by the optically active substituents on the amidine groups. As a preliminary step toward truly artificial self-replication systems, we have started a program to investigate the effectiveness of the complementary double helical structure as a replicating field utilizing the salt bridge-based double helices. In this study, we have synthesized an achiral carboxylic acid dimer by linking through the imine-bond formation in the presence of its complementary, optically active dimeric amidine strand (Fig. 1), and have observed an acceleration of the imine-bond formation during the complementary duplex formation. We chose the imine-bond forming reaction for this study, since it does not necessarily require catalysts and is well established in modern organic chemistry to form sophisticated nanostructures.[7,8]

We designed and prepared the two achiral carboxylic acid monomers, **1**† and **2**†, bearing an aldehyde and an amino group at one end, respectively (Scheme 1). The optically active dimeric amidine with an azobenzene linker, **T**,[6d] was chosen as the template strand (Scheme 1), because **T** is compatible in length to **3**. A restricted Hartree–Fock calculation study suggested that **3** and **T** could form the hetero-stranded double helix without any stress or strain (Fig. S5†). The optically active monomeric amidine, **A**,[6a] was also employed for the control experiments to evaluate the template effect of **T** on the complementary duplex formation.

The imine-bond forming reactions between **1** and **2** in the presence of **T** or **A** were conducted in benzene-d_6 at 30 and 50 °C. The reaction progress was first monitored by ^{1}H NMR spectroscopy (Fig. 2, S1†, and S2†). The ^{1}H NMR (500 MHz, benzene-d_6, 30 °C) spectra of the mixtures of **1** and **2** (0.5 mM) in the presence of **T** (0.5 mM) or **A** (1.0 mM) showed the resonances of the NH protons in the low magnetic field of *ca.* 14.5 ppm, indicating the salt bridge formation (Figs. S1† and S2†). The peak intensity of the aldehyde proton (H_a) decreased with time, while the benzyl proton peaks (H_b and H_c) newly appeared and increased with time, both suggesting the formation of the imine-bond, that is, the complex **3**·**T** and **3**·$\mathbf{A_2}$, respectively. The formation of the complex **3**·**T** was also supported by the negative-mode electron-spray ionization mass (ESI-MS) spectra, which exhibited a molecular ionic peak at m/z = 2573.4 corresponding to $[\mathbf{3}\cdot\mathbf{T}-\mathrm{H}]^-$ (Fig. S3†). Unfortunately, the peaks derived from the resulting imine proton of the complex **3**·**T** or **3**·$\mathbf{A_2}$ overlapped with the aromatic protons. The benzyl protons (H_b and H_c in Fig. 2) of the complex **3**·$\mathbf{A_2}$ appeared as a singlet peak (*ca.* 4.5 ppm), indicating that they are virtually chemically and magnetically equivalent because of the free rotation at the imine-bond linker of **3**. In contrast, the benzylic proton signals of the complex **3**·**T** appeared as a pair of doublets (*ca.* 4.3–4.5 ppm). The non-equivalency of these protons of **3**·**T** is attributed to the formation of the double helical structure that produces the chiral environment as well as restricting the rotation at the linkers.

Time-dependent circular dichroism (CD) and absorption measurements were then performed to monitor the reaction

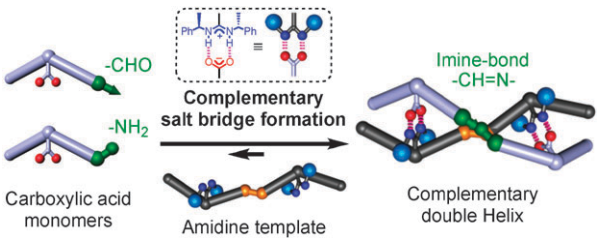

Fig. 1 Schematic illustration of an artificial replication system through complementary double helix formation.

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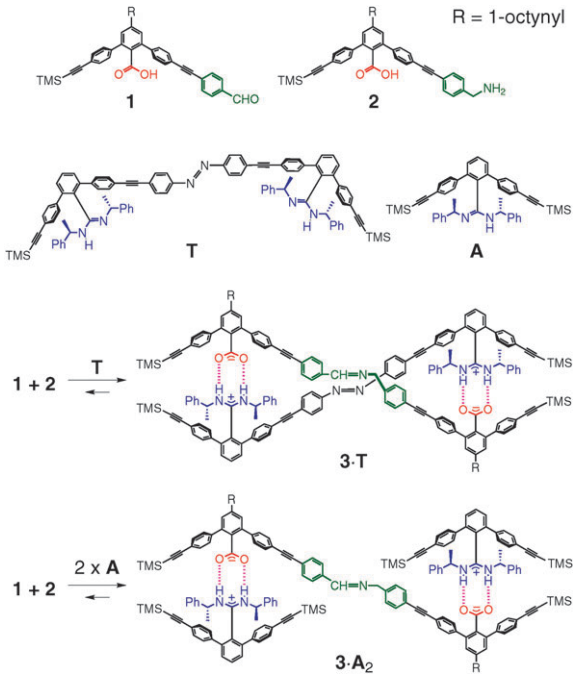

Scheme 1 The imine-bond forming reaction between **1** and **2** in the presence of the template strand **T** and monomeric amidine **A**.

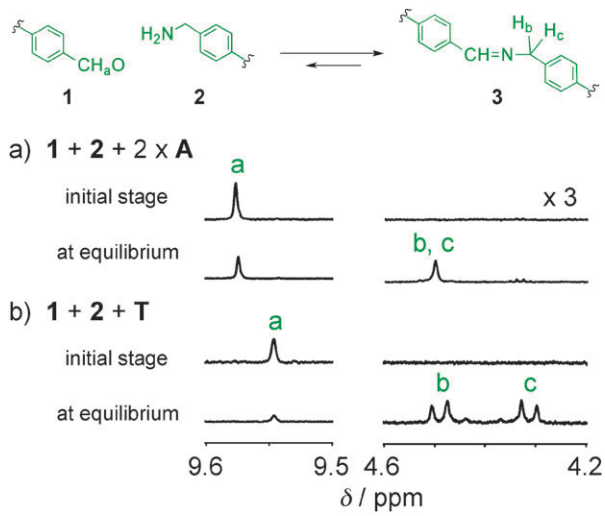

Fig. 2 Partial ^{1}H NMR (500 MHz, benzene-d_6, 30 °C) spectra of the mixtures of **1** and **2** (0.5 mM) in the presence of (a) **A** (1.0 mM) and (b) **T** (0.5 mM) during the initial stage of the reaction (top) and after reaching equilibrium (bottom).

progress at 30 and 50 °C over a period of 72 and 12 h (Fig. 3 and S4†), respectively. In the presence of **A**, the absorption spectra slightly changed with time, accompanied by negligible changes in their CD spectra. On the other hand, in the presence of **T**, the absorption spectra gradually changed with time, accompanied by the enhancement of the negative Cotton effect, especially in the absorption region of 280–320 nm, indicating that **3·T** most likely adopted a double helical structure with a helix-sense bias induced by the chiral amidine groups of **T**. The restricted Hartree–Fock calculation results suggested that **3·T** could preferably adopt a right-handed double helical structure (Fig. S5†). The conversions to **3·T** and **3·A$_2$** at 30 °C were plotted *versus* the reaction time, as shown in Fig. 4a. The conversions were estimated by the changes in the intensities of the CD and absorption spectra, which were normalized by the CD, absorption, and ^{1}H NMR spectra of the reference materials.[9] As apparent from Fig. 4a, the imine-bond formation in the presence of the template **T** reached equilibrium much faster than that in the presence of **A**. Moreover, the template **T** shifted the equilibrium states to the imine-bond formation arising from the entropic factors of the template. Second-order kinetics was assumed as the most probable mechanism for the present reactions during the initial stages,[10] because the concentration of **1** is equal to that of **2**. The experimental data were fitted to a second-order kinetic model using eqn (1),

$$1/C - 1/C_0 = kt \quad (1)$$

where C is the concentration of **1** or **2**, t is the reaction time, and k is the reaction rate constant. The rate constant for the reaction in the presence of **T** at 30 °C was estimated to be $(1.56 \pm 0.04) \times 10^{-1}$ M^{-1} s^{-1}, while that in the presence of **A** was $(2.58 \pm 0.04) \times 10^{-2}$ M^{-1} s^{-1} by the least-squares curve fitting method. Thus, the reaction was accelerated 6-fold (k_0(**T**)/k_0(**A**) = 6.0) in the presence of **T**. To verify the assumption that the present reactions follow second-order kinetics, the initial rate constants (k_0) were also calculated from the slope (a) of the linear initial part of the reaction profiles in Fig. 4a according to eqn (2),

$$k_0 = a/C_0^2 \quad (2)$$

which is valid when [**1**] = [**2**]. The k_0 values of the reactions with **T** and **A** at a 10% conversion were calculated at 1.31×10^{-1} and 2.28×10^{-2} M^{-1} s^{-1}, respectively, which are in good agreement with those obtained by the curve fitting method, thus supporting that the reactions obey second-order kinetics during the initial stage. Similarly, the reaction progress of the imine-bond formation in the presence of **T** or **A** at 50 °C was plotted *versus* the reaction time (Fig. S4c†). Both reaction mixtures reached equilibrium faster than those conducted at 30 °C, while the equilibrium states did not change very much. The reaction rates during the initial stages were analyzed based on the assumption that they obey a pseudo-second-order kinetics, and were estimated to be 1.27 ± 0.03 and $(2.97 \pm 0.11) \times 10^{-1}$ M^{-1} s^{-1} for those in the presence of **T** and **A**, respectively (Fig. S4d†), which correspond to a 4-times acceleration at 50 °C (k_0(**T**)/k_0(**A**) = 4.3). In addition, the Eyring analysis of the k_0 values obtained at different temperatures (30–50 °C) was used to determine the thermodynamic parameters for the reaction in the presence of **T**: $\Delta H^{\ddagger}$ = 82.9 ± 2.7 kJ mol^{-1}, $\Delta S^{\ddagger}$ = 12.7 ± 9.1 J mol^{-1} K^{-1}, and $\Delta G^{\ddagger}_{298}$ = 79.1 ± 5.4 kJ mol^{-1} (Fig. S8†). It is noted that the enthalpic contribution ($\Delta H^{\ddagger}$) is much higher than the entropic component ($\Delta S^{\ddagger}$). The particularly low entropy of activation indicates organization of the monomers on the template, that is, the formation of the ternary complex **T·1·2** contributes to the acceleration of the reaction.

Finally, the thermodynamic aspect of the template effects was further investigated by employing a methyl ester derivative

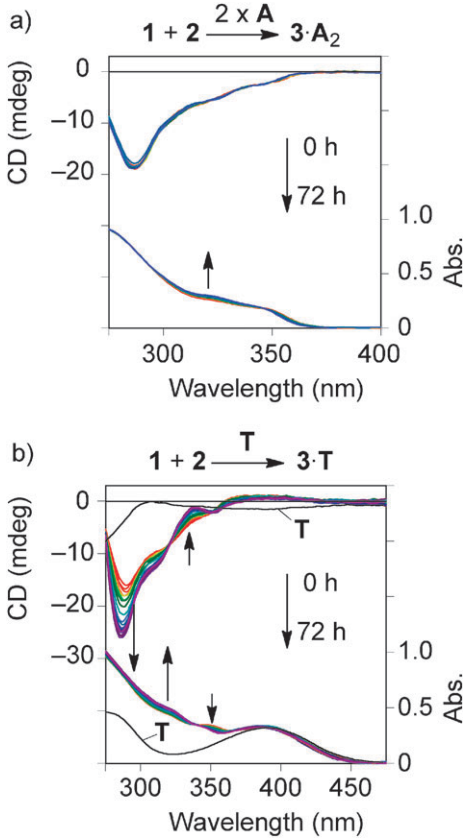

Fig. 3 Time-dependent CD and absorption spectra of the mixtures of **1** and **2** (0.5 mM) in the presence of (a) **A** (1.0 mM) and (b) **T** (0.5 mM) in benzene-d_6 at 30 °C. The CD and absorption spectra of **T** (0.5 mM, benzene-d_6, 30 °C) are also shown in (b). Cell length = 0.1 mm.

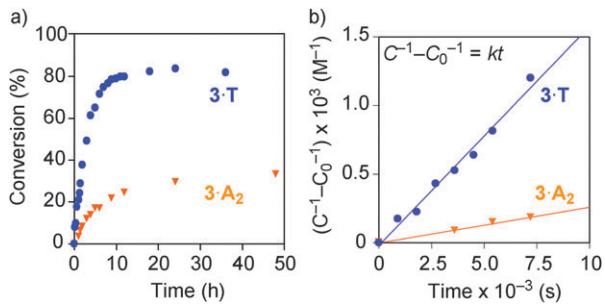

Fig. 4 Time–conversion relationships (a) and kinetic plots (b) of the imine-bond forming reaction between **1** and **2** in the presence of **T** and **A** at 30 °C.

of **1** (**4**) as an antagonistic competitor to **1** (Fig. S9†). A solution of equimolar amounts of **1**, **2**, **T**, and **4** in benzene-d_6 was heated at 30 °C and the reaction reached equilibrium within 1 day. The product distribution was determined by the ^{1}H NMR spectrum, which showed that the carboxylic acid dimer **3** was formed with a high selectivity of *ca.* 95% over the ester-containing strand (**5**).

In summary, we have constructed the complementary double helix through template synthesis, of which the formation was significantly accelerated and stabilized by the template strand. We believe that double helix formation by the combination of salt bridges and an imine-bond used in this study can be applied to the construction of more sophisticated replicating systems, which is now underway in our laboratory.

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9 Because of the reversible nature of the imine-bond formation, we could not obtain **3·T** and **3·A$_2$** in pure forms. Hence, we synthesized **3·T** and **3·A$_2$** with relatively high conversions as reference materials using molecular sieves (MS4A). The conversions of the reference materials were estimated based on the integral ratios between the aldehyde protons of **1** and the benzyl peaks of **3·A$_2$** or **3·T** in the ^{1}H NMR spectra (Fig. S6†). The CD and absorption spectra measurements of the reference materials were also performed (Fig. S7†).

10 Although the imine-bond formation is a reversible reaction, it is reasonable to assume that it obeys a pseudo-second-order kinetics during the initial stage, in which the inverse reaction is negligibly small.