Libraries of non-covalent hydrogen-bonded assemblies; combinatorial synthesis of supramolecular systems

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Libraries of hydrogen-bonded assemblies formed by mixing the individual components under thermodynamically controlled conditions are characterized by ^{1}H NMR spectroscopy and Ag$^+$ assisted MALDI-TOF mass spectrometry.

The combinatorial strategy to create structural diversity has revolutionized the field of chemistry over the past decade.[1] Instead of synthesizing a single molecule, combinatorial chemistry allows the simultaneous synthesis of large libraries of structurally well-defined molecules.[2] For this purpose combinatorial methodologies have been developed for a variety of synthetic transformations (*e.g.* peptide,[3] oligonucleotide,[4] heterocycle[5] and carbohydrate[6] synthesis) either on solid supports[7,8] or in solution.[9,10] Recently, libraries of synthetic receptor molecules, *e.g.* for sequence-selective peptide binding, were reported using a similar combinatorial strategy.[11,12]

Libraries of organic molecules are prepared by statistical combination of reactive molecular fragments *via* the irreversible formation of covalent bonds. In principle, one could also use non-covalent interactions, like hydrogen bonding, to build in a reversible way libraries of assemblies of small complementary molecules. Non-covalent synthesis is a valuable alternative to the classical covalent synthesis of complex supramolecular systems.[13] Recently, Lehn,[14] Sanders[15] and others[16] reported libraries that were obtained *via* reversible formation of covalent (imines, esters) or coordinative (Zn- or Fe-complexes) bonds under conditions of thermodynamic equilibrium.

Here we describe the first example of a non-covalent synthesis of combinatorial libraries of hydrogen bonded assemblies under thermodynamically controlled conditions. *N* different assemblies [**1x**]$_3$ (**x** = **a,b,c**, . . .,*N*; Fig. 1) were mixed in solution under conditions that allow the reversible exchange of the components **1x** to give *M* heteromeric assemblies in addition to the *N* homomeric assemblies (see Fig. 2). The total number of assemblies *P* (*i.e.* $N + M$) present in such a library rapidly increases with increasing *N* [eqn. (1)].

$$P = N + N(N-1) + [N(N-1)(N-2)]/6 \qquad (1)$$

For the smallest possible library ($N = 2$, $P = 4$) we have studied the combination of calix[4]arene bismelamines **1a**,**b** with 5,5-diethyl barbiturate. The individual homomeric assemblies [**1a**]$_3$ and [**1b**]$_3$ form spontaneously in apolar solvents by mixing 3 equiv. of **1a** (or **1b**) with 6 equiv. of 5,5-diethyl barbiturate and are stable down to submillimolar concentrations.[17] The assembly process is driven by the formation of 36 cooperative hydrogen bonds, resulting in significant downfield shifts for the various NH protons [Fig. 3(*a*) and (*b*)].

Mixing of 5 mM solutions of [**1a**]$_3$ and [**1b**]$_3$ at 0 °C (ratio 2 : 1) in [$^2H_8$]toluene gave only a mixture of the two separate homomeric assemblies [Fig. 3(*c*)]. Exchange of the components **1a** and **1b** between the two assemblies is extremely slow at this temperature and, as a consequence, formation of the heteromeric assemblies [**1a**]$_2$[**1b**] and [**1a**][**1b**]$_2$ is not observed.‡ Only at ⩾15 °C do the heteromeric assemblies start to form slowly. This reflects the relatively high kinetic stability of the separate assemblies in this apolar solvent. After 2.5 h at 25 °C the system has reached the thermodynamic equilibrium [Fig. 3(*d*)]. In the more polar solvent $CDCl_3$ the exchange of components **1a** and **1b** is much faster. The [**1a**]$_3$ and [**1b**]$_3$ mixture equilibrates within seconds at 25 °C. Only below −50 °C are the homomeric assemblies [**1a**]$_3$ and [**1b**]$_3$ kinetically inert.

The low-field region of the ^{1}H NMR spectrum is particularly diagnostic as it exclusively displays the hydrogen bonded NH_{barb} protons (δ 13–15) and NH_{arom} protons (δ 8–9). The [$^2H_8$]toluene ^{1}H NMR spectrum of the four component library shows 12 of the 16 NH_{barb} proton signals as separate peaks and 7 of the 8 NH_{arom} proton signals [Fig. 3(*d*)]. The four different assemblies were identified by means of 2D NOESY, ROESY, COSY and TOCSY analysis.§ The relative concentration of the four different assemblies in the mixture was subsequently determined as 1.0 ([**1a**]$_3$) : 3.0 ([**1a**]$_2$[**1b**]) : 3.0 ([**1a**][**1b**]$_2$) : 1.0 ([**1b**]$_3$) (±10%), in agreement with the statistical distribution of

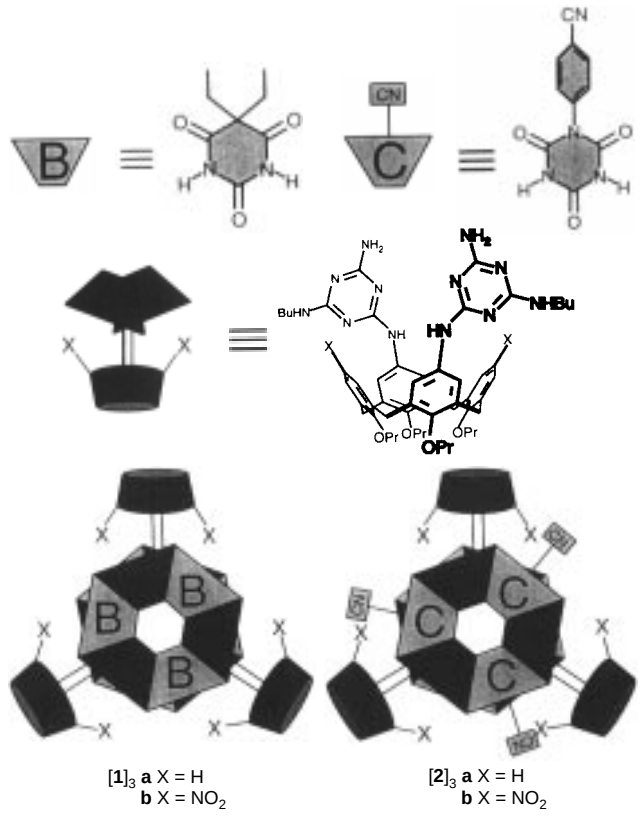

Fig. 1 Schematic representation of hydrogen bonded assemblies [**1**]$_3$ and [**2**]$_3$

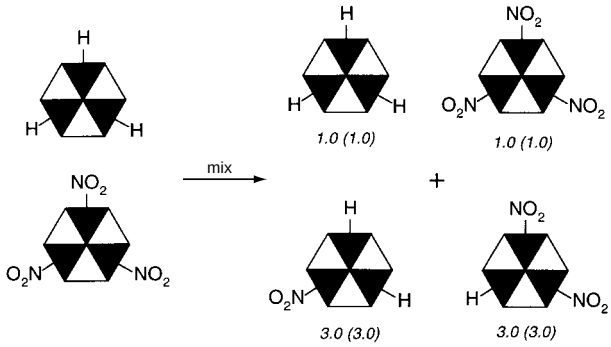

Fig. 2 Non-covalent synthesis of library of assemblies [**1a**]$_3$, [**1a**]$_2$[**1b**], [**1a**][**1b**]$_2$, and [**1b**]$_3$; experimentally determined ratio is given with statistical ratio in parentheses

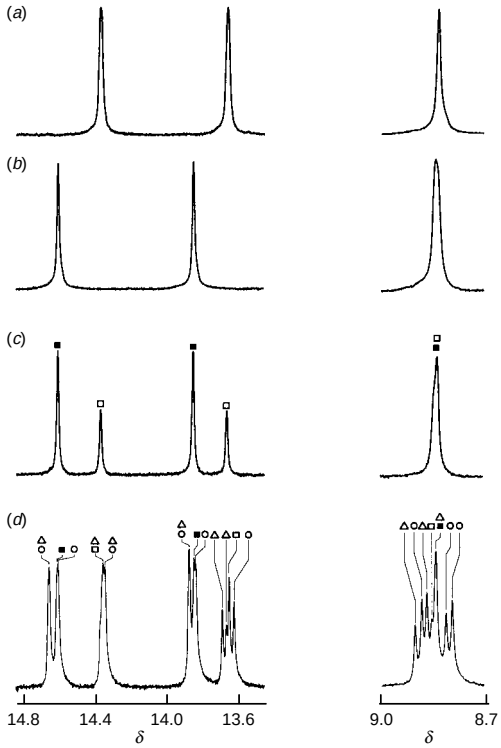

Fig. 3 Low field regions of the ^{1}H NMR spectra of (*a*) homomeric assembly [**1a**]$_3$; (*b*) homomeric assembly [**1b**]$_3$; (*c*) 2 : 1 mixture of homomeric assemblies [**1a**]$_3$ (■) and [**1b**]$_3$ (□) at 0 °C; (*d*) library of assemblies [**1a**]$_3$ (■), [**1a**]$_2$[**1b**] (○), [**1a**][**1b**]$_2$ (△), [**1b**]$_3$ (□) 2.5 h after mixing homomeric assemblies [**1a**]$_3$ and [**1b**]$_3$ at 25 °C (ratio 6 : 4). All spectra were recorded at 400 MHz in [^{2}H$_8$]toluene.

components **1a** and **1b** over the various assemblies. Unfortunately, the $CDCl_3$ spectrum displayed insufficient resolution to determine the relative composition of the mixture.

Characterization of the library was also performed by mass spectrometry using Ag^+ assisted MALDI-TOF MS technique.[18] For this purpose we studied the assemblies [**2a**]$_3$ and [**2b**]$_3$, which contain cyano substituents to coordinate to the Ag^+ labels (Fig. 1). Individual solutions of the assemblies, pretreated with 1.5–2.0 equiv. of CF_3CO_2Ag in $CHCl_3$ for at least 24 h, give intense signals in the MALDI-TOF spectra at *m/z* 4348.1 (calc. for $C_{222}H_{252}N_{60}O_{30}{\cdot}^{107}Ag^+$: 4347.9) and 4620.4 (calc. for $C_{222}H_{246}N_{66}O_{42}{\cdot}^{107}Ag^+$: 4618.8), respectively, for the monovalent Ag^+ complexes. As expected the MALDI-TOF spectrum of a mixture of the CF_3CO_2Ag pretreated $CHCl_3$ solutions (5 mM each) of assemblies [**2a**]$_3$ and [**2b**]$_3$ clearly shows two additional signals for both the monovalent Ag^+ complexes of the heteromeric assemblies [**2a**]$_2$[**2b**] and [**2a**][**2b**]$_2$ at *m/z* 4438.3 (calc. for $C_{222}H_{250}N_{62}O_{34}{\cdot}^{107}Ag^+$: 4437.9) and 4527.7 (calc. for $C_{222}H_{248}N_{64}O_{38}{\cdot}^{107}Ag^+$: 4528.9), respectively.¶ Especially for the characterization of libraries with more components, mass spectrometry will be an important tool.

The reversible library concept presented here can in principle be extended to libraries of any desirable size. For example, by mixing 10 different assemblies [**1x**]$_3$ a library of 220 different hydrogen-bonded assemblies is created. Subsequently, the information in the different assemblies can be stored by a covalent post-modification step, *e.g. via* a metathesis reaction.[19] Covalent post-modification of hydrogen-bonded assemblies is currently under active investigation in our group.

Notes and References

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‡ Two different types of kinetic processes characterize the hydrogen bonded assemblies, *i.e.* (*i*) exchange of 5,5-diethyl barbiturate and (*ii*) exchange of components **1**. The rate constant for the former process is in the order of 0.5–3.0 s^{-1} in [^{2}H$_8$]toluene at 303 K as determined by 2D EXSY NMR. The latter process is much slower and can only be measured at the 'laboratory timescale'.

§ The chemical shifts of the NH protons are strongly influenced ($\Delta\delta_{max}$ = 0.15) by the starting concentrations of **1a** and **1b**.

¶ The peak intensity of assembly [**2b**]$_3$ in the MALDI-TOF spectrum of the mixture is significantly lower than that for assembly [**2a**]$_3$ most probably due to the strongly decreased affinity of this assembly for Ag^+ ions as a result of the electron-withdrawing effect of the six NO_2 groups. Evidence comes from the low mass region of the spectrum, which exclusively displays a signal for the [**2a**]$\cdot^{107}Ag^+$ complex and not the [**2b**]$\cdot^{107}Ag^+$ complex even when [**2b**] is present in four-fold excess.

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