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Templated photochemical synthesis of a uracil *vs*. thymine receptor

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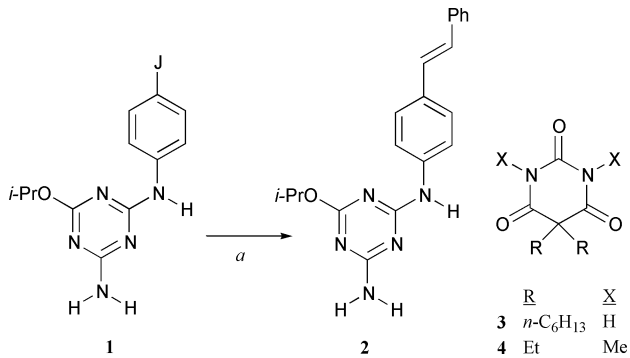
The first templated photochemical synthesis of a receptor capable of differentiating between thymine and uracil is described.

Supramolecular self-assembly can be used to build structures of controlled size, shape, and functionality.[1] Of particular interest is the application of such processes to construct molecular receptors by moulding small fragments containing molecular recognition elements around a given substrate.[2] However, a means by which the reversibly-bound adduct can be captured to allow the isolation and identification of the receptor (lock-in) is invariably required. We have recently described a system in which the photoinduced dimerisation of cinnamates appended with molecular recognition units was affected by the presence of a template molecule.[3] A salient feature appeared to be the possibility to consolidate supramolecular assemblies *via* light-triggered photoreactions through a process analogous to the topochemical control achieved in the solid.[4] It therefore seemed interesting to further explore the use of light to trigger the covalent capture of reversibly-formed supramolecular receptors. The binding properties of this new class of hydrogen-bonding receptors, incorporating a diphenylcyclobutane scaffold, were investigated. In particular, their ability to discriminate between analogues of the naturally-occurring pyrimidine bases thymine and uracil makes them potential candidates in the development of RNA *vs*. DNA targeting agents.

Compound **2** was synthesised by Heck coupling between styrene and **1**(Scheme 1), prepared by sequential substitution of cyanuric chloride† according to literature procedures.[5] The binding of **2** to the template molecule, 5,5-dihexylbarbituric acid (**3**), was investigated[6] by ^{1}H NMR spectroscopy. In principle, one may expect the formation of five distinct (two 1 : 1 and three 2 : 1) complexes (Fig. 1), only one of which places the stilbene chromophores in close proximity. Because the interconversion between the hydrogen-bonded complexes is fast on the NMR timescale, the observed binding isotherm only allows determination of the overall 1 : 1 and 2 : 1 association constants. Binding of **2** to **3** in $CDCl_3$ is accompanied by a 2 ppm downfield shift of the N–H resonance of **3**, from which association constants $K_1 = 1200 \pm 60$ M^{-1} and $K_2 = 250 \pm 12$ M^{-1} can be extracted. A good fit to the experimental data was obtained only for a model comprised of sequential 1 : 1 and 2 : 1 complex formation.



Scheme 1 (a) Styrene, Et_3N, $Pd_2(dba)_3$ (5 mol%), acetonitrile, 85 °C, 24 h (71%).

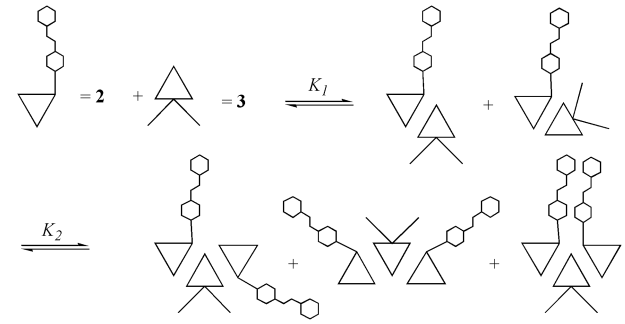


Fig. 1 Various hydrogen-bonded assemblies may be formed between **2** and **3**, only one of which places two stilbene chromophores in close proximity, favouring the formation of *syn* photodimers.

Irradiation of dilute (10^{-2} M) CH_2Cl_2 solutions of **2** results in rapid *E*, *Z* photoisomerisation of the stilbene chromophore leading to a photostationary equilibrium mixture enriched in the *Z* isomer (*Z*:*E* = 5.5), known to be unreactive towards cyclodimerisation.‡ Upon prolonged irradiation, slow grow-in of five additional products is observed by HPLC (Fig. 2). Isolation of four photoproducts by preparative HPLC allowed their characterisation by ^{1}H NMR and mass spectroscopy, which identified them as [2 + 2] cycloadducts. The structures of the isolated photoproducts **5a**–**5d** were assigned on the basis of their spectral properties by comparison with known stilbene dimers described in the literature.[7] The major cycloadducts are the head-to-head and head-to-tail dimers (**5a** and **5d**), accounting for roughly two thirds of the total dimer formation.

Under identical irradiation conditions, the presence of 0.5 eq. of **3** was found to enhance the formation of three of the photoproducts (**5b**, **5c**, and **5d**), while inhibiting the formation of **5a**. This difference in reactivity is attributed to the formation of a ternary supramolecular complex in which the stilbene moieties are held in a face-to-face geometry that is favourable to dimerisation. The preference for photodimers in which the

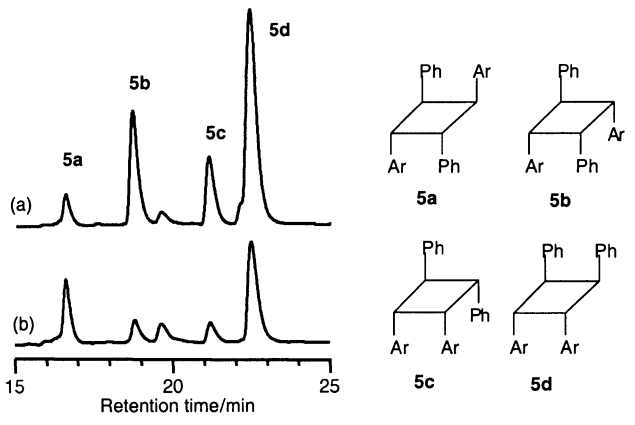


Fig. 2 Portion of the HPLC chromatograms showing the formation of photodimers in the presence (a) and absence (b) of **3** upon irradiation, and the proposed structures of dimers **5a**–**5d**.

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Table 1 Quantum yields[a] of photodimers × 10^3

Dimer	**2** Alone	0.5 eq. **3**	0.5 eq. **4**
5a	0.7	0.5	0.7
5b	0.1	1.6	0.1
5c	0.1	0.6	0.1
5d	0.6	2.4	0.6

[a] In degassed dichloromethane, [**2**] = 0.01 M.

triazine units are oriented *syn* is in agreement with the involvement of **3** as a template during the dimerisation reaction. This is further supported by the lack of activity of **4**, in which the hydrogen bonding sites are blocked by methylation. The reduced yield of **5a** is consistent with the inability of **3** to promote structures that are not suitable receptors for barbiturate derivatives. Quantum yields for the formation of dimers **5a**–**5d** in the presence and absence of **3** or **4** are given in Table 1. From the measured association constants, and assuming a statistical distribution of 1:1 and 1:2 complexes, one can estimate the quantum yield for the formation of dimer **5d** *within* the supramolecular assembly to be 0.1, approximately a 170-fold increase with respect to solution. A rationalization of the catalysis and product distribution for an analogous cinnamate derivative has already been described,[3] and will therefore not be discussed further.

Molecular modelling of **5d** indicates that it has a tweezer-like geometry, with both the aminotriazine groups oriented in the same direction. Concomitant binding of a substrate to both triazines is therefore restricted both by the hydrogen-bonding pattern of triazine and by the size and shape of the cavity. Titration of **5d** with Barbital (5,5-diethylbarbituric acid, **6**) in $CDCl_3$ was monitored using NMR spectroscopy by observing the barbiturate N–H protons, which underwent a large downfield shift (> 4 ppm) upon complexation. The association constant was found to be 2400 M^{-1}, much higher than the binding of **2** to **3**, for which the microscopic binding constant is calculated to be 300 M^{-1}.[8] The increase in the association constant is attributed to the binding of **6** within the cleft formed by the triazine groups in **5d**, resulting in the formation of multiple hydrogen bonds. *In this respect, **5d** is an example of substrate-induced receptor synthesis*. The magnitude of the binding constant between **6** and **5d** is very similar to that of an analogous recently synthesised Barbital receptor containing a ferrocene unit,[9] though smaller than those previously reported by Hamilton and co-workers.[10] Thus, the preparation of receptors *via* light-induced capture of supramolecular assemblies can lead to functional receptors, of similar binding affinity as those obtained by conventional synthetic methodologies.

The rigid structure of **5d** is the basis for the observed selectivity in the binding of uracil *vs*. thymine or adenine. NMR titration ($CDCl_3$) of **5d** by 5-(4-*tert*-butylbenzyl)uracil (**7**) results in a binding isotherm indicating the formation of a 1:1 complex with a binding constant of 960 ± 120 M^{-1}.§ Molecular modelling using semi-empirical PM3 calculations (Fig. 3) reveals that uracil can bind within the diaminotriazine cleft with formation of 4 hydrogen bonds. In contrast, the presence of the methyl substituent in thymine is expected to prevent it from entering the binding cleft, and should therefore result in a lower binding affinity. This is indeed the case, and the binding of 5-(4-*tert*-butylbenzyl)thymine (**8**) to dimer **5d** can only be fitted to a model comprised of sequential 1:1 and 1:2 complex formation. This is rationalised by the binding of a thymine molecule to **5d** following partial rotation about the C–C bond connecting the diaminotriazine unit to the cyclobutane scaffold. The binding constants (K_1 = 1980 ± 65 M^{-1}, K_2 = 150 ± 5 M^{-1}) reflect a more favourable statistical weighting for binding the first thymine molecule, and a modest anticoopertive effect towards binding of the second thymine, presumably due to steric interactions. To adequately compare the binding of **7** or **8** to **5d**, one must take into consideration that whereas in the case of thymine four distinct 1:1 complexes may be formed, only one complex can be formed between **5d** and uracil. Thus, the microscopic binding constant of thymine is actually only one half that of uracil (500 *vs*. 960 M^{-1}). An upper limit of 50 M^{-1} was estimated for the association constant between **5d** and 9-ethyladenine, suggesting the formation of a rather labile complex. This is consistent with the binding of adenine in a fashion similar to that of thymine, but involving only two hydrogen bonds.

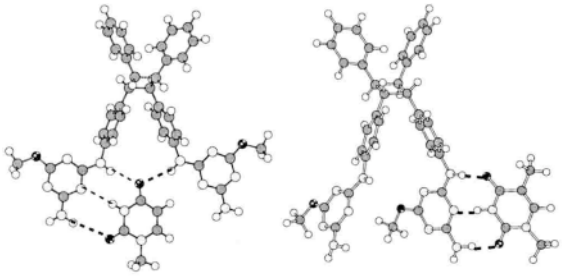

Fig. 3 Energy minimised (PM3) structures of complexes formed between **5d** and uracil (left) and thymine (right).

The methyl group in thymine has been recently recognized to play an important role in the recognition and suppression of DNA sequences by certain bacteria,[11] and receptors capable of mimicking this form of recognition would be of interest. The ability to differentiate between uracil and thymine may be further enhanced by preventing rotation of the binding site in **5d**, and this may open new possibilities for the selective recognition of RNA *vs*. DNA fragments.

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