# Selective product amplification of thymine photodimer by recognition-directed supramolecular assistance

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Two symmetric ditopic supramolecular templates (**1** and **2**) each presenting two hydrogen bonding recognition subunits were synthesized. Each such subunit comprises the same donor and acceptor pattern, capable of binding a substrate molecule with complementary hydrogen bonding groups to form a supramolecular complex. Substrate molecules, such as thymine or uracil derivatives, yield 2 : 1 complexes with the acceptors involving two hydrogen bonds to each subunit with ideal orientation for subsequent [2 + 2] dimerization upon photoirradiation. Selective *syn* photoproduct formation and concomitant suppression of the *trans* isomer are favored by orientation of the two guest nucleobases within the template cleft. Complementary donor and acceptor hydrogen bonding induced positioning of the two substrates and steric hindrance within the template clefts are responsible for the selective product formation.

## Introduction

The [2 + 2] photodimerizations[1,2] of thymine and uracil have been subject to much research effort as their products exhibit high biotoxicity.[3–6] Upon UV irradiation, these cellular nucleobases are known to undergo dimerization, giving products that are directly responsible for cell death *via* mutagenic action, suppression of DNA transformation and/or activation of carcinogenic pathways by basal cell and squamous cell tumors. The mechanism by which these toxic photodimers (as well as their derivatives) are produced, has been extensively investigated in order to suppress their formation or to repair the site by photochemical or enzymatic reversible cleavage.[7,8] Direct or sensitized photoirradiation of [2 + 2] precursors, including thymine and uracil, and similar photodimerization reactions potentially lead to four cycloaddition products; *cis–syn*, *trans–syn*, *cis–trans*, *anti–trans*, and *anti–cis* (**A**, **B**, **C**, **D**, respectively, Fig. 1).[6,9–11] The distribution of such products cannot be readily controlled so as to afford a given photodimer. Typically, specific reaction conditions, such as the use of various reagents or additives, changes in solvent polarity,[4,5,12,13] micelles,[14] solid-state irradiation,[15–21] additives,[22] host–guest inclusion complexes,[23–26] dipolar interactions,[27–30] and precursor tethering[31–38] give only moderate success in controlling the distribution of photoproducts. Recently, stoichiometric additives, forming 1 : 1 complexes through hydrogen donor/acceptor sites suitable for complementary hydrogen bonding with the precursors, have been investigated in the hope of influencing the photoproduct outcome through molecular recognition.[39–43]

Here we present two such hosts **1** and **2** (Fig. 2) displaying two recognition subunits that contain each a hydrogen bond donor N–H and a hydrogen bond acceptor –N= site. These receptors are capable of binding two complementary guests of the uracil (**4A**) or thymine (**4B**) type (Fig. 3) within their cleft through hydrogen bonds, thus providing supramolecular assistance[44,45] to the generation of photoproducts upon irradiation as well as to induction of product distribution by preorganization. Owing to our previous success of controlled photodimerization with **2**,[46] we investigated the use of this molecule as a host for biologically-relevant bases. We describe selective photoproduct amplification and suppression of normally produced photoproducts for the biologically relevant thymine nucleobase through formation of supramolecular hydrogen-bonded complexes of two thymine derived substrates **4C** with the molecular receptors **1** and **2**. Compound **3** was used as scaffold for control experiments.

## Results and discussion

Nucleobases of the uracil and thymine type are ideal candidates for studying selective photoproduct formation through supramolecular control of [2 + 2] photodimerization. They possess two sites capable of sustaining two hydrogen bonds with complementary receptors, leading to the formation of hydrogen-bonded complexes of specific orientation and positioning. Moreover, they readily undergo [2 + 2] photodimerization with varying distributions of the possible products **A**–**D** (Fig. 1). In view of enforcing photoproduct selectivity, we investigated whether the molecular receptors **1** and **2** were capable of forming a specifically oriented supramolecular adduct with two dimer precursors (Fig. 4). This, ultimately, would promote selective photoproduct amplification through geometric control arising from the supramolecular architecture and steric confinement. The inter-substrate distance in the 2 : 1 supramolecular adducts is suitable for the photodimerization.

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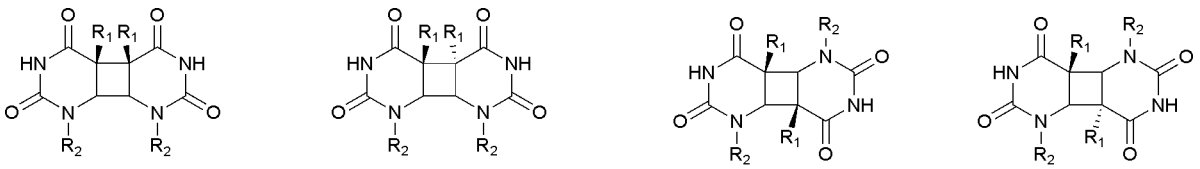
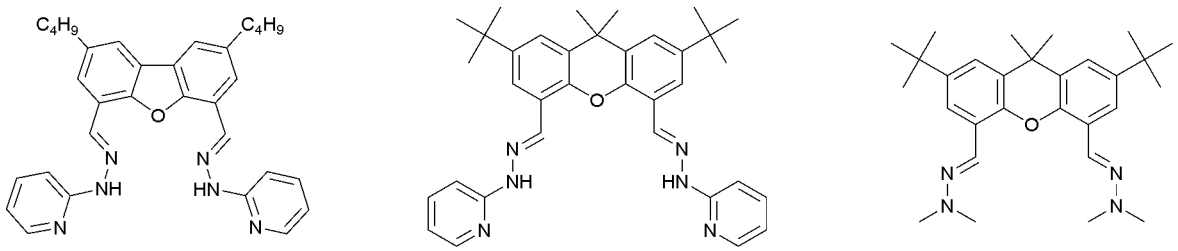


XA XB XC XD

X = **5** $R_1 = R_2 = H$
**6** $R_1 = CH_3$; $R_2 = H$
**7** $R_1 = CH_3$; $R_2 = -CH_2CO_2CH_2Ph$
**8** $R_1 = H$; $R_2 = p$Bz-$^t$Bu
**9** $R_1 = H$; $R_2 = -CH_2CO_2CH_2Ph$

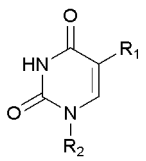
**Fig. 1** Potential dimers resulting from [2 + 2] photodimerization of nucleic bases.



**1** **2** **3**

**Fig. 2** Molecular receptor scaffolds **1** and **2** for selective [2 + 2] photodimerization of thymine derivatives through 2 : 1 supramolecular complex formation, and control scaffold **3**.

The symmetric structure of the 2 : 1 adduct (Fig. 4) is expected to favor the formation of *syn* (*cis* and *trans*) dimers upon irradiation. Normally produced *anti* dimers would be suppressed, since the corresponding orientation is not possible due to the donor–acceptor pattern and to steric hindrance within the template cleft.



**4A** $R_1 = R_2 = H$
**4B** $R_1 = CH_3$; $R_2 = H$
**4C** $R_1 = CH_3$; $R_2 = CH_2CO_2CH_2Ph$
**4D** $R_1 = H$; $R_2 = Bz$-$t$Bu
**4E** $R_1 = H$; $R_2 = CH_2CO_2CH_2Ph$

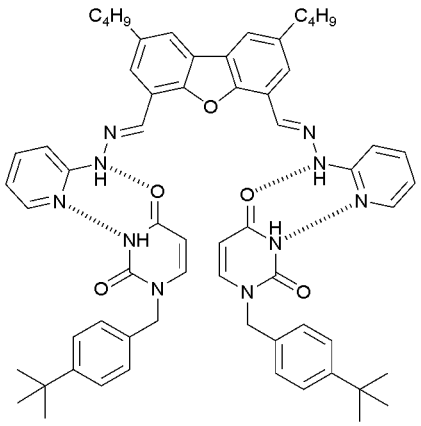
**Fig. 3** Thymine and uracil derivatives.

### Receptor synthesis (Scheme 1)

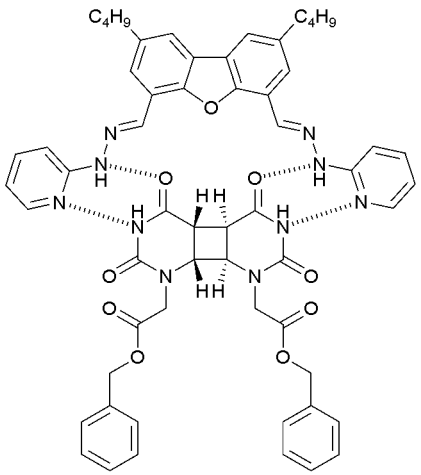
The synthesis of receptor **2** was achieved in an overall 50% yield from the combined steps from commercial reagents. The selected route provided nearly quantitative yield for each step with the advantage of easy purification of the desired intermediate dialdehyde **16**. Subsequent coupling of **16** according to our previous method, afforded template **2**. In addition, a more rigid template **21** was synthesized which comprised a dibenzofuran consisting of a planar backbone. It was unfortunately insoluble in most organic solvents, in particular those commonly used to promote intermolecular hydrogen bonding. To circumvent this short fall, dialkylation in positions 2 and 8 of the dibenzofuran moiety was pursued, leading to a much more soluble alternative template **1**. The methodology for the synthesis of **1** was similar to that used for **2**, resulting in approximately the same overall yield.

### Binding of uracil and thymine derivatives to templates 1 and 2

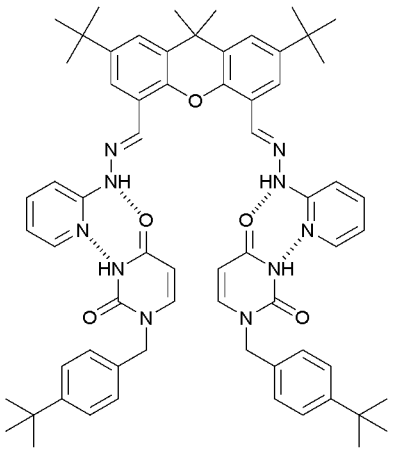
Previous work has shown the capability of bis-receptors such as **1** and **2** to efficiently bind substrate molecules *via* N–H···N hydrogen bonds between the pyridine nitrogen and the adjacent NH site and complementary donor and acceptor sites.[46–48] The association constants for the formation of such supramolecular adducts with one ($K_1$) and two ($K_2$) monomeric substrates or with a dimer was determined by NMR binding isotherms. These were obtained by titrating 2 mM host concentrations in $CDCl_3$ with the corresponding nucleobases and monitoring the resulting chemical shift changes of the =C–H and the –NH proton of



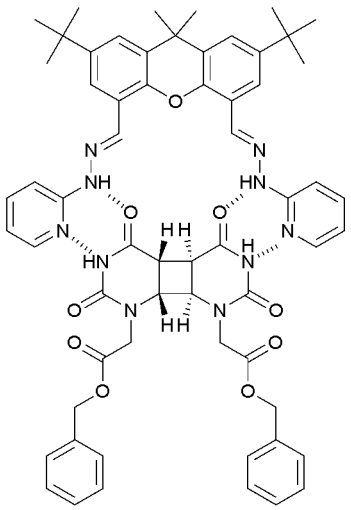
**10**



**11**



**12**



**13**

**Fig. 4** Supramolecular complexes arising from multiple hydrogen bonding between receptors **1** and **2** and uracil and photodimer substrates.

the templates. Subsequent treatment of the NMR data with the ChemEqui program[49] provides the association constants (Table 1) corresponding to the binding through hydrogen bond formation between complementary O, N and N–H sites, as represented by the structures shown in Fig. 4. The NH signal undergoes a chemical shift change of $\delta$ 1.6 ppm and the isotherm measured correlated well with that determined for the =CH proton signal. The binding isotherms provide the association constants for 1 : 1 and 2 : 1 complexes, which undergo formation of a weak 1 : 1 complex followed by a 2 : 1 complex upon further substrate addition (Table 1). The values measured are within the range of complementary donor/acceptor interactions of the $-NH\cdots N(=R_1)R_2$ type.[41,42] For template **1**, the photodimer complex **11** exhibits a stronger binding affinity than the monomer form **10**. The donor/acceptor sites are consequently blocked upon photoproduct formation and cannot undergo further monomer binding. Conversely, the binding affinities for the monomer complex **12** are greater than the photodimer **13**. The greater preference for the monomer over the photoproduct demonstrated for template **2** implies it ultimately can be used as a photocatalyst for dimerization.

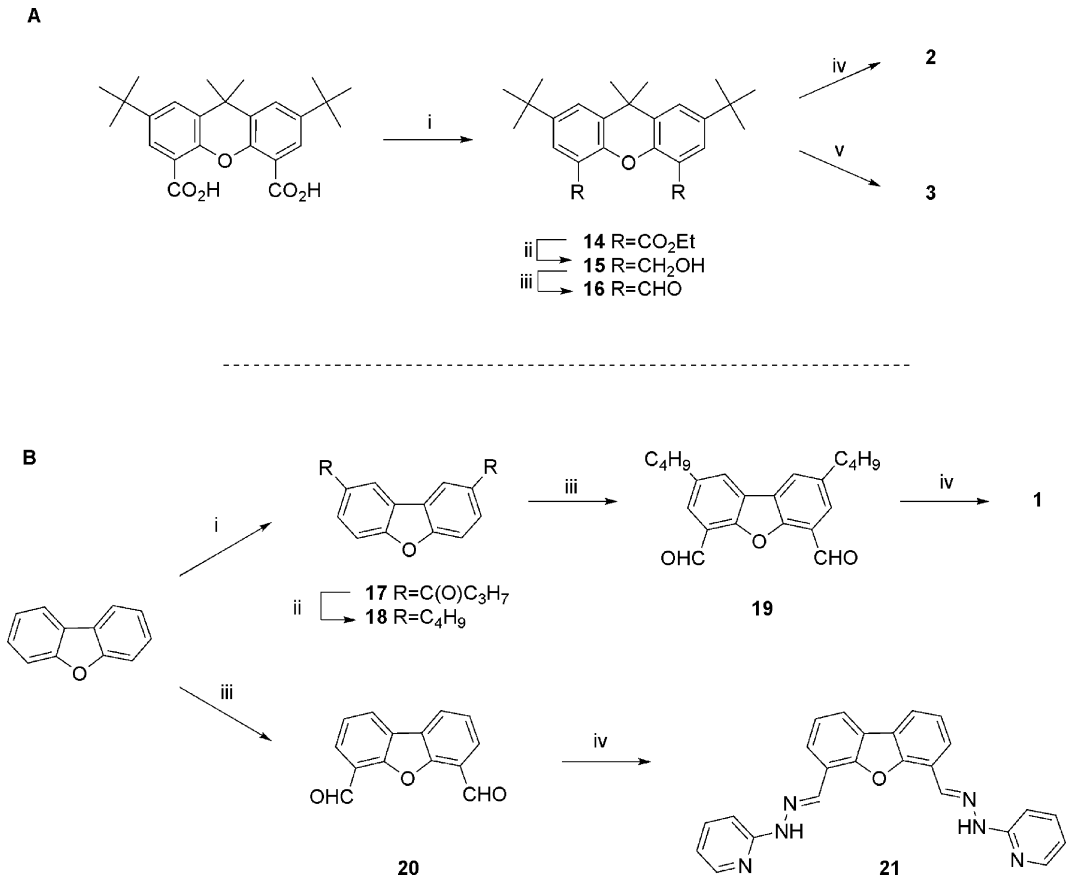
**Table 1** Association constants determined by NMR titration in $CDCl_3$ from isotherms using ChemEqui[49]

| Complex | $K_1/M^{-1a}$ | $K_2/M^{-1a}$ |
|---|---|---|
| **10** | 30 ± 5 | 100 ± 10 |
| **11** | 20 000 ± 700 | — |
| **12** | 180 ± 10 | 1 100 ± 110 |
| **13** | 240 ± 5 | — |

[a] $K_1$ and $K_2$ are the ChemEqui β derived values for the A + B → AB and the A + 2B → $AB_2$ reactions, respectively.

The binding differences between the two templates can be ascribed to the different cleft sizes and the intramolecular donor–acceptor distances. Crystallographic studies (see Fig. 5 and 6)




**Scheme 1** Receptor synthesis (A) (i) ethanol, catalytic $H_2SO_4$, reflux; (ii) $LiAlH_4$–$Et_2O$ (anhydrous); (iii) $MnO_2$–toluene–$CH_2Cl_2$, room temperature (1 : 1); (iv) 2-hydrazinopyridine–$CHCl_3$, room temperature; (v) *N*,*N*-dimethyl hydrazine–EtOH, reflux. (B) (i) $AlCl_3$, butyryl chloride, 1,2-dichloroethane, reflux; (ii) hydrazine hydrate, KOH, triglycol reflux; (iii) TMEDA, *n*-BuLi in $Et_2O$ (anhydrous) −78 °C, DMF; (iv) 2-hydrazino pyridine–$CHCl_3$, room temperature.

show that the $sp^3$ bridgehead of **2** gives it a non-planar shape with a tighter bite angle. Conversely, the rigid and planar structure of **1** has a large bite angle. These factors, affecting inter-substrate distances and orientation, are ultimately responsible for the observed differences in binding affinities. The hydrogen bonding capabilities of these two receptors is further evidenced by their crystal state structures. In the solid state, the two receptors self-associate through their complementary receptor acceptor sites forming supramolecular networks. The differences in the receptor orientation and backbone rigidity affect the resulting supramolecular structures. The non-planar shape of receptor **2**, combined with the small bite angle, allows the complementary donor–acceptor sites to align so that the thermodynamically stable structure (Fig. 7 and 8) is a dimer resulting from hydrogen bonding between the ditopic sites. Conversely, the rigid dibenzofuran backbone of **1** forces the receptor sites to point outwards. There is also a slight twist of the dibenzofuran backbone that further orients the receptor sites out of the plane of the aromatic unit. The resulting large receptor cleft can accommodate two receptors side arms through complementary hydrogen bonding, resulting in a supramolecular ribbon architecture (Fig. 9).

### Photodimerization of thymine 4C

Samples of authentic photodimers were obtained by photosensitized irradiation of thymine derivative **4C** in acetone and were isolated through a sequence of column chromatography, preparative thin layer chromatography and semi-preparative HPLC. NOE and COSY 2-dimensional NMR, in addition to analytical HPLC, allowed for differentiation and subsequent identification of the three main photoproducts, which were **7A**, **7B** and **7D**. The latter two were formed in majority. The overall results are consistent with those described for uracil and thymine derivatives.[50–52] Isolation of sufficient quantities of photodimerization product of the thymine **4C** can only be done by sensitized irradiation with acetone. Direct irradiation at *ca.* 254 nm is possible, however the dimerization reaction is photoreversible in the UV region leading to photoproduct decomposition, which reverts back to the starting material. Furthermore, under direct irradiation, the templates examined act as light screens and undergo severe photodecomposition, which does not occur *via* the triplet-sensitized method.

### Photodimerization of 4C with templates 1 and 2

Photosensitized irradiation of a mixture of **2** and two equivalents of thymine derivative **4C** led to the complete suppression of the *anti* products illustrated in Fig. 10 (**7C** and **7D**). To confirm that the formation of the supramolecular complex between the template and **4C** was responsible for the suppression of these products, trace amounts of acetic acid were added to the reaction mixture, in order to disrupt the hydrogen bonding and displace the

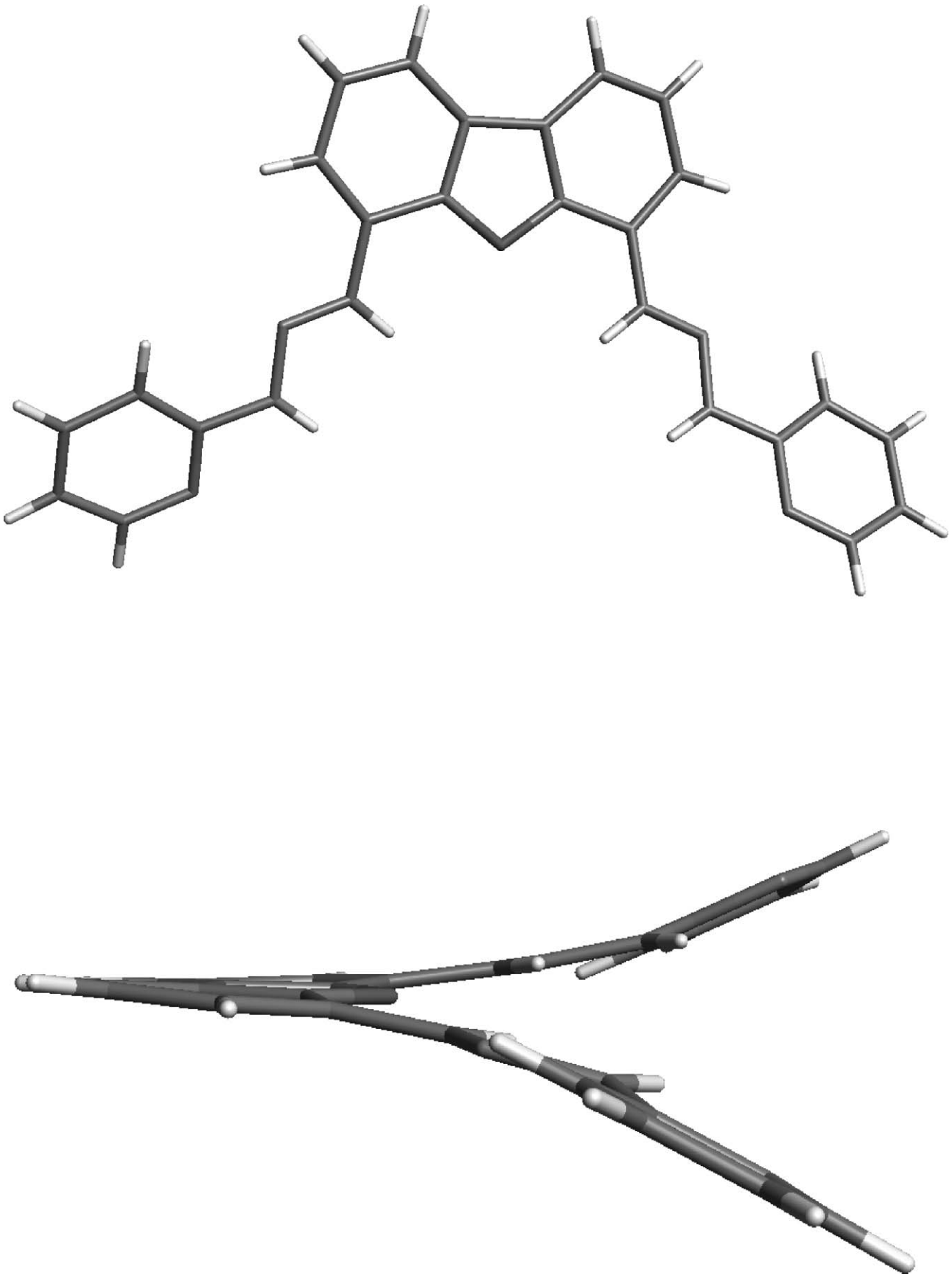


**Fig. 5** Crystal structure of receptor **1**.

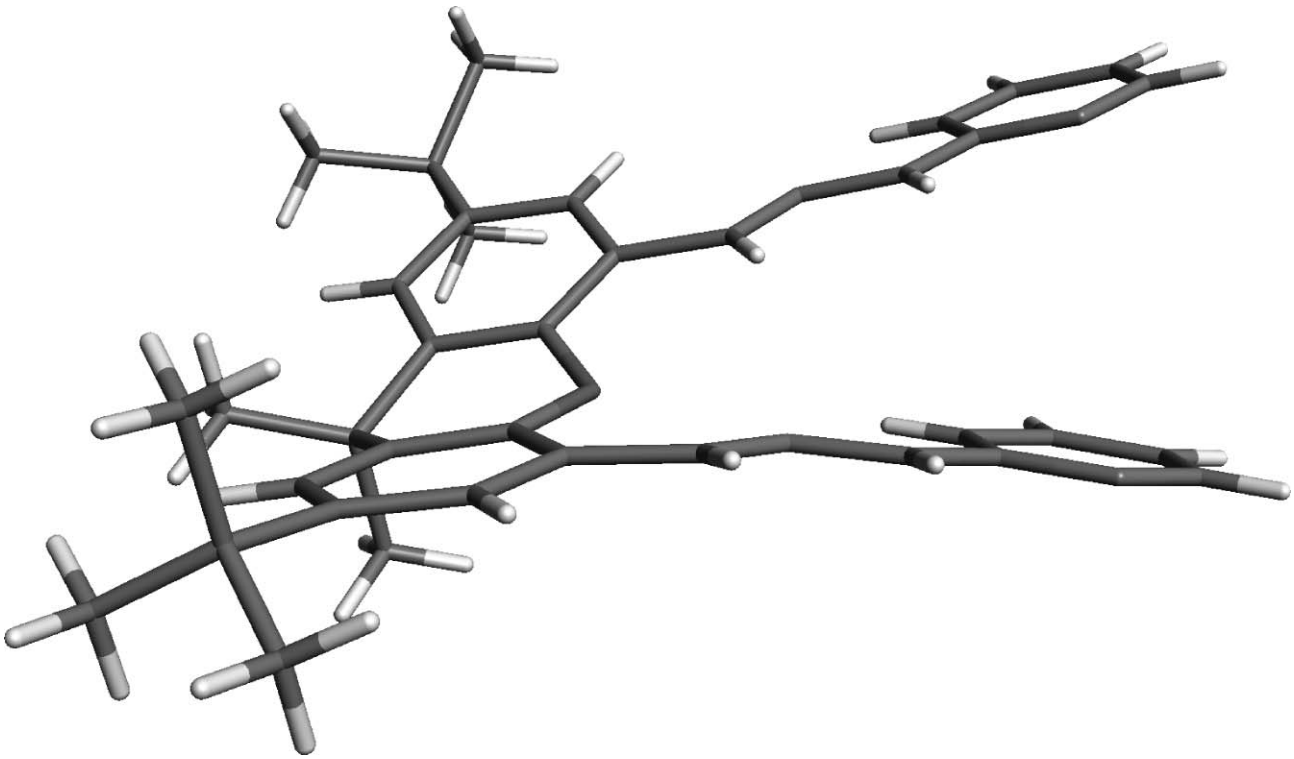


**Fig. 6** Crystal structure of receptor **2**.

bound thymine substrate. Photoirradiation gave a photoproduct distribution similar to that in the absence of the template. This result, coupled with the strong binding constant, demonstrates the capacity of the bis-receptor **2** to amplify the formation of photoproducts **7A** and **7B**. This arises through formation of a hydrogen bonded 2 : 1 supramolecular complex, with parallel positioning of the two substrate molecules consistent with *syn* [2 + 2] photodimers. The two *anti* products are suppressed because the antiparallel precursor alignment required for their formation is incompatible with the hydrogen bond donor–acceptor site arrangement and the steric hindrance within the template cleft.

Template **1** also exhibited photoproduct control similar to that observed with **2**. Due to its poor solubility in acetone, a mixture of acetone–chloroform was used for the photoirradiations. A control experiment under the same conditions indicated again photodimerization control with selective product formation through the ground state supramolecular complex.

### Photoirradiation control experiments

Experiments with the pseudo-template **3** were undertaken to confirm the role of the hydrogen bonding pattern and the steric effects for controlled photodimerization.[53] This analogue lacks the NH donor sites, but has a cleft similar to **2** into which two monomers could potentially be accommodated, on steric grounds, but without hydrogen bond formation. The photoproduct distribution obtained upon photoirradiation of **4C** in the presence of **3** was identical to that observed in the absence of this pseudo template. The effect of 2-aminopyridine itself was also examined, because it is capable of forming a supramolecular complex with a single substrate *via* its donor and acceptor motifs. It cannot promote molecular organization by positioning two substrates within the optimal 6 Å distance required for photodimerization. No effect on photoproduct distribution was therefore expected and, indeed, none was observed. The selective photoproduct amplification caused by the receptors **1** and **2** may, therefore, be ascribed to the formation of a 2 : 1 supramolecular adduct with two substrate molecules **4C**. The hydrogen bonding recognition positions the two thymine monomers in a favourable pre-photodimer fashion. The parallel orientation of the two substrates ensures that only the *syn* products are formed upon photoirradiation. The intramolecular distance of the N–H donor sites within the cleft of **2** was found to be 4.6 Å, according to the crystal structure data.[54] This corresponds well with the amplification of the *syn* dimers, the intramolecular O acceptor site distances of which are 3.9 Å.[3] The pocket could potentially transversally incorporate a single monomer forming a 1 : 1 complex. Under stoichiometric control, such an arrangement would block the receptor, preventing selective *syn* amplification, while the residual monomer would undergo conventional unselective photodimerization. Given the templated dimerization data, the 2 : 1 complex predominates and is responsible for the results observed.

## Experimental

### Materials and methods

Unless otherwise stated, all chemicals were purchased from Aldrich and were used as received. HLPC-spectroscopic grade solvents were used for HPLC analyses run on a HP 1100 series HPLC equipped with a diode array detector and an Eclipse™ XDB-C18 reverse phase column. The mobile phase used for

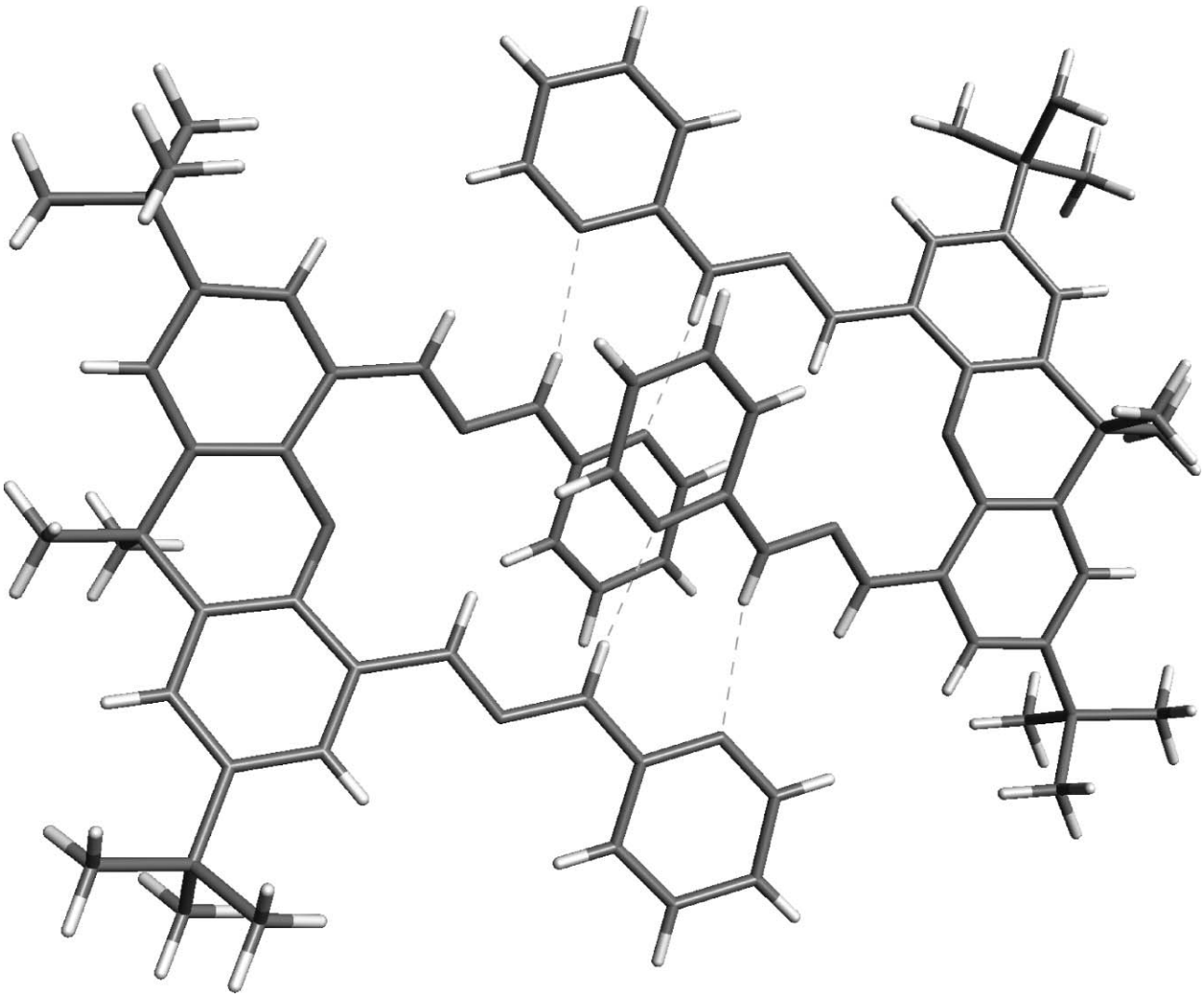


**Fig. 7** Top view of the supramolecular dimer network of receptor **2**.

**Fig. 8** Side view of the supramolecular dimer network of receptor **2**.

separation was 50% methanol–50% water and the retention times monitored at 220 nm and absorption maxima were compared to authentic samples (*vide infra*). The ChemEqui program was used to determine the binding constants from the isotherm data obtained by NMR titration in $CDCl_3$.[49] NOE and COSY 2D NMR spectra were recorded on a Bruker 300 MHz spectrometer.

## Synthesis

**2,7-Di-*tert*-butyl-9,9-dimethyl-9*H*-xanthene-4,5-dicarboxylic acid diethyl ester (14).** 2,7-Di-*tert*-butyl-9,9-dimethyl-9*H*-xanthene-4,5-dicarboxylic acid (1 g, 2.44 mmol) was dissolved in ethanol (50 mL). Concentrated sulfuric acid (2 mL) was added

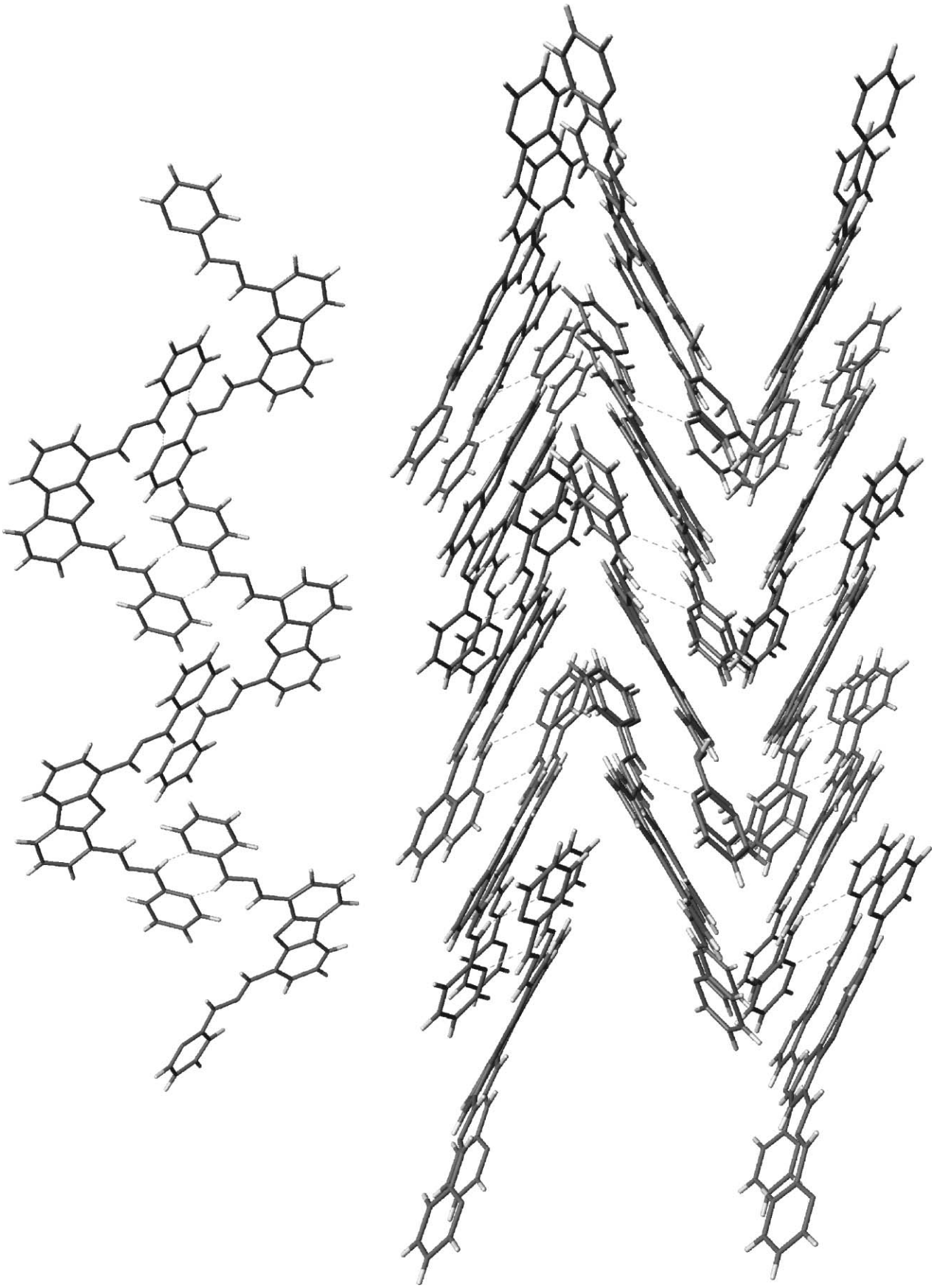


**Fig. 9** Solid-state supramolecular bonding network of receptor **1**.

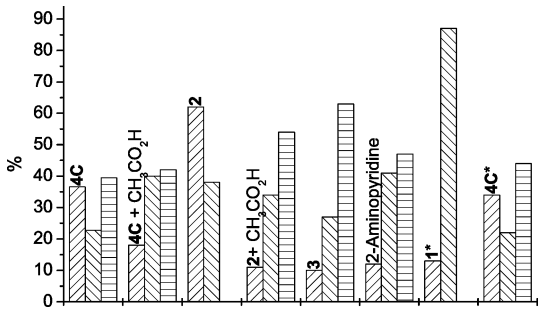



**Fig. 10** Distribution of *trans–syn* **7B** (///), *cis–syn* **7A** (\ \ \) and *trans–anti* **7D** (≡), isomers, formed by photoirradiation of **4C** for 12 h performed in acetone under in the presence and absence of various additives. *Denotes reaction undertaken in 2 : 1 (vol%) chloroform–acetone mixture.

and the solution stirred at reflux for 14 h. The solution was poured onto ice (100 g) and the resulting white precipitate was collected by filtration. It was washed with cold water until the wash water was neutral and then dried under vacuum to yield the product (1.10 g, 96%) as a white powder. Mp 149–150 °C. IR (thin film): $\nu$ = 2963, 2863, 1724, 1612, 1446, 1393, 1366, 1314, 1243, 1180, 1146, 1100, 1029, 857, 782 cm$^{-1}$. $^1$H NMR (200 MHz, [D] chloroform): $\delta$ = 7.58 (d, $J$ = 2.4 Hz, 2 H); 7.53 (d, $J$ = 2.4 Hz, 2 H), 4.42 (q, $J$ = 7.1 Hz, 4 H), 1.64 (s, 6 H), 1.40 (t, $J$ = 7.2 Hz, 6 H), 1.33 (s, 18 H). $^{13}$C NMR (125 MHz, [D] chloroform): $\delta$ = 166.9, 146.9, 145.4, 130.5, 125.8, 125.6, 120.6, 61.1, 34.8, 34.6, 32.1, 31.5, 14.4. FAB-MS: $m/z$ 467.2 ([M + H]$^+$, 100%). Anal. calcd for $C_{29}H_{38}O_5$ (466.61): C 74.65, H 8.21; found: C 74.55, H 8.36.

**(2,7-Di-*tert*-butyl-5-hydroxymethyl-9,9-dimethyl-9*H*-xanthen-4-yl)-1 methanol (15).** A solution of **14** (1.1 g, 2.35 mmol) in diethyl ether (10 mL) was added dropwise to a stirred suspension of lithium aluminum hydride (LAH, 0.15 g) in diethyl ether (25 mL). The mixture was subsequently refluxed for 1.5 h. Additional LAH was added (0.5 g) and the suspension heated to reflux for 1 h to drive the reaction to completion. The mixture was then cooled to 0 °C and ice water was added dropwise until the evolution of hydrogen ceased. 10% Sulfuric acid was added until the precipitate of $AI(OH)_3$ was dissolved. The ether layer was removed and the aqueous layer was washed twice with ether (50 mL). The combined organic extracts were dried over $MgSO_4$, evaporated to dryness and chromatographed ($SiO_2$) with 30% ethyl acetate–hexane to provide a white powder (0.89 g, 99%). Mp 199–200 °C. IR (thin film): $\nu$ = 3261, 2951, 2863, 1464, 1413, 1362, 1326, 1297, 1279, 1223, 1143, 1033, 903, 883, 852, 763, 664 cm$^{-1}$. $^1$H NMR (200 MHz, [D] chloroform): $\delta$ = 7.42 (d, $J$ = 2.2 Hz, 2 H), 7.20 (d, $J$ = 2.2 Hz, 2 H), 4.66 (s, 4 H), 4.49 (br. s, 2 H), 1.67 (s, 6 H), 1.36 (s, 18 H). $^{13}$C NMR (125 MHz, [D] chloroform): $\delta$ = 146.9, 145.3, 129.7, 127.2, 125.0, 122.5, 62.2, 34.6, 32.3, 31.7. FAB-MS: $m/z$ 382.2 ([M]$^+$, 57%). Anal. calcd for $C_{25}H_{34}O_3$ (382.54): C 78.49, H 8.96; found: C 78.46, H 9.18.

**(2,7-Di-*tert*-butyl-9,9-dimethyl)-9*H*-xanthene-4,5-dicarbaldehyde (16).** To a solution of **15** (1.00 g, 2.61 mmol) in toluene (150 mL) and dichloromethane (50 mL) was added $MnO_2$ (5 g). The reaction was followed by TLC and was complete after 24 h of stirring at room temperature followed by refluxing for 2 h. The finely dispersed $MnO_2$ suspension was filtered through a short plug of celite and silica, and then washed with dichloromethane. The organic layer was evaporated and pure compound (0.79 g, 80%) was obtained as a white powdery solid after recrystallization from dichloromethane–hexane and from hot toluene–hexane. Mp 248–249 °C. IR (thin film): $\nu$ = 2958, 2863, 1688, 1606, 1460, 1365, 1296, 1260, 1228, 1167, 930, 902, 868, 851, 748, 704, 646 cm$^{-1}$. $^1$H NMR (200 MHz, [D] chloroform): $\delta$ = 10.68 (s, 2 H), 7.83 (d, $J$ = 2.4 Hz, 2 H), 7.72 (d, $J$ = 2.4 Hz, 2 H), 1.70 (s, 6 H), 1.37 (s, 18 H). $^{13}$C NMR (125 MHz, [D] chloroform): $\delta$ = 188.9, 149.6, 146.7, 130.6, 129.5, 124.1, 123.5, 62.2, 34.8, 32.5, 31.4, 29.7. FAB-MS: $m/z$ 379.3 ([M + H]$^+$, 100%). Anal. calcd for $C_{25}H_{30}O_3$ (378.50): C 79.33, H 7.99; found: C 79.59, H 8.14.

**2,7-Di-*tert*-butyl-9,9-dimethyl-4,5-bis-(pyridin-2-ylhydrazonomethyl)-9*H*-xanthene (2).** Template **2** was synthesized from **16** as previously described.[46]

***N*′-[2,7-Di-*tert*-butyl-5-(dimethylhydrazonomethyl)-9,9-dimethyl-8*a*,10*a*-dihydro-9*H*-xanthen-4-yl methylene]-*N*,*N*-dimethylhydrazine (3).** The scaffold was prepared from **16** according to known methods.[46]

**(5-Methyl-2,4-dioxo-3,4-dihydro-2*H*-pyrimidin-1-yl)acetic acid benzyl ester (4C).** Thymine-1-acetic acid (1.21 g, 6.57 mmol) was dissolved in 30 DMF with sonication, to which was then added diimidazol-1-ylmethanone (1.23 g, 7.58 mmol). The slurry was stirred at room temperature for 10 min until the reagents were dissolved. Benzyl alcohol was then added (780 µl, 7.56 mmol) and the solution stirred at room temperature for 12 h. The solvent was removed under reduced pressure and the paste was suspended in water. The product was isolated as a white solid (1.67 g, 93%) upon filtration. Mp = 201–203 °C. $^1$H NMR (200 MHz, [D] DMSO): $\delta$ = 11.42 5 (s, 1 H), 7.65 (s, 1 H), 7.53 (s, 3 H), 5.57 (s, 2 H), 4.82 (s, 2 H), 1.44 (s, 3 H). $^{13}$C NMR (50 MHz, [D] chloroform): $\delta$ = 168.09, 164.16, 150.87, 141.41, 135.44, 128.36, 128.12, 127.83, 108.52, 90.18, 66.31, 48.36, 11.78. EI-MS: $m/z$ 574.2 ([M]$^+$, 20%). Anal. calcd for $C_{14}H_{14}N_2O_4$ (274.10): C 61.31, H 5.14, N, 10.21; found: C 60.90, H 5.12, N 10.39.

**1-(4-*tert*-Butylbenzyl)-1*H*-pyrimidine-2,4-dione (4D).** To a suspension of uracil (1.00 g, 9.00 mmol) in 20 mL anhydrous acetonitrile was added *N*,*O*-bis-trimethylsilyacetamide (5.6 g, 7.0 mL, 28 mmol). The mixture was stirred at room temperature until complete dissolution of the initial solid. To this was added *tert*-butylbenzyl bromide (2.04 g, 9 mmol) and the mixture was refluxed for 12 h, at which time the solvent was evaporated and the residue purified by column chromatography ($SiO_2$) with 5% methanol–dichloromethane. The product was obtained as a white solid. Mp = 165–166 °C. $^1$H NMR (200 MHz, [D] chloroform): $\delta$ = 9.65 (d, $J$ = 7.9 Hz, 1 H), 7.39 (d, $J$ = 8.3 Hz, 2 H), 7.22 (d, $J$ = 8,3 Hz, 2 H), 7.16 (d, $J$ = 7.9 Hz, 1 H), 5.69 (d, $J$ = 7.9 Hz, 1 H), 4.88 (s, 2 H), 1.31 (s, 9 H). $^{13}$C NMR (50 MHz, [D] chloroform): $\delta$ = 164.0, 151.8, 151.4, 144.1, 132,1. 128.0, 126.2, 102.7, 51.0, 34.7, 31.3. EI-MS: $m/z$ 258.2 ([M]$^+$, 60%). Anal. calcd for $C_{15}H_{18}N_2O_2$ (258.32): C 69.74, H 7.02, N 10.84; found: C 69.99, H 7.24, H 11.06.

**1-(8-Butyryldibenzofuran-2-yl)-butan-1-one (17).** Dry aluminum chloride (21.42 g, 161 mmol) was added in small portions over 1 h to a well stirred solution of butyryl chloride (17.12 g, 161 mmol) in 1,2-dichloroethane (65 mL). A solution of

dibenzofuran (10.4 g, 61.8 mmol) in 1,2-dichloroethane (20 mL) was added dropwise to the mixture over 30 min while at 50 °C. The mixture was subsequently heated to reflux for 12 h, cooled to 30 °C, and poured slowly into ice water (150 mL). Concentrated hydrochloric acid (25 mL) was added and the mixture was further stirred for another hour. The organic layer was separated and the aqueous layer extracted with dichloromethane. The combined organic extracts were dried, evaporated and chromatographed ($SiO_2$; 10% dichloromethane–hexane) to give the title compound (9.9 g, 52%) as a slightly brown solid. Mp 125–127 °C. IR (thin film): $\nu$ = 2960, 2872, 1680, 1631, 1596, 1564, 1467, 1412, 1368, 1306, 1248, 1116, 1011, 997, 900, 845, 753 $cm^{-1}$. $^1H$ NMR (200 MHz, [D6] DMSO): $\delta$ = 9.00 (d, 41 = 1.4 Hz, 2 H); 8.15 (dd, 31 = 8.7 Hz, 41 = 0.9 Hz, 2 H); 7.81 (d, 31 = 8.7 Hz, 2 H); 3.13 (t 31 = 7.1 Hz, 4 H); 1.68 (sm, 4 H); 0.98 (t 31 = 7.4 Hz, 6 H). $^{13}C$ NMR (50 MHz, [D6] DMSO): $\delta$ = 198.9; 158.5; 132.7; 128.1; 123.5; 122.5; 111.9; 17.3; 13.6. FAB-MS: $m/z$ 309.1 ($[M + H]^+$, 100%). Anal. calcd for $C_{20}H_{20}0_3$ (308.37): C 77.90, H 6.54; found: C 77.80, H 6.52.

**2,8-Dibutyldibenzofuran (18).** To **17** (9.75 g, 31.6 mmol), hydrazine hydrate (9.5 g, 10 mL, 190 mmol) and finely ground potassium hydroxide (14.28 g, 254 mmol) were suspended in triglycol (150 mL) and heated to reflux for 2 h. The excess hydrazine and water were subsequently distilled off. After cooling to below 90 °C, water (300 mL) was added and the mixture was extracted with diethyl ether and dichloromethane (twice, 200 mL, portions). The combined organic extracts were evaporated and the residue applied to a column ($SiO_2$; 7% dichloromethane–hexane) to afford the product (2.6 g, 29%) as a highly viscous translucid oil. IR (thin film): $\nu$ = 3030, 2959, 2928, 2867, 1869, 1485, 1468, 1417, 1337, 1362, 1318, 1274, 1246, 1207, 1187, 1103, 1091, 1118, 1024, 931, 869, 811, 747 $cm^{-1}$. $^1H$ NMR (200 MHz, [D6] DMSO): $\delta$ = 7.91 (s, 2 H); 7.54 (d, $^3J$ = 8.4 Hz, 2 H); 7.30 (d, $^3J$ = 8.4 Hz, 2 H); 2.72 (t, $^3J$ = 7.5 Hz, 4 H); 1.63 (sm, 4 H); 1.35 (sm, 4 H); 0.91 (t, $^3J$ = 7.2 Hz, 6 H). $^{13}C$ NMR (50 MHz, [D6] DMSO): $\delta$ = 154.1; 136.9; 127.6; 123.5; 120.1; 111.0; 34.6; 33.5; 21.6; 13.7. FAB-MS: $m/z$ 280.1 ($[M]^+$, 100%). Anal. calcd for $C_{20}H_{24}0_3$ (280.40): C 85.67, H 8.63; found: C 85.73, H 8.90.

**2,8-Dibutyldibenzofuran-4,6-dicarbaldehyde (19).** To a solution of **18** (1 g, 3.56 mmol) and TMEDA (1.66 g, 14.3 mmol) in dry diethyl ether (25 mL) was added a hexane solution of *n*-butyllithium (1.6 M, 8.9 mL, 14.3 mmol). After refluxing for 16 h, DMF (1.25 mL, 16.1 mmol) was added over a period of 5 min while cooling at 0 °C, The solution was then warmed to room temperature and allowed to stir for 24 h. The mixture was then poured into cold 1 M hydrochloric acid (30 mL) and the acidic aqueous layer was extracted twice with dichloromethane. The combined organic extracts were washed with water and brine, dried over $MgSO_4$, and then purified by chromatography ($SiO_2$; 10% ethylacetate–hexane) to afford the product (0.54 g, 45%) as a white solid. $^1H$ NMR (200 MHz, [D] chloroform): $\delta$ = 10.53 (s, 2 H); 7.91 (d, $^4J$ = 1.6 Hz, 2 H); 7.73 (d, $^4J$ = 1.6 Hz, 2 H); 2.77 (t, $^3J$ = 7.7 Hz, 4 H); 1.66 (sm, 4 H); 1.36 (sm, 4 H); 0.94 (t, $^3J$ = 7.2 Hz, 6 H). $^{13}C$ NMR (50 MHz, [D] chloroform): $\delta$ = 187.6; 155.2; 138.6; 127.3; 126.2; 124.6; 120.8; 35.2; 33.8; 22.2; 13.8. FAB-MS: $m/z$ 336.2 ($[M]^+$, 100%).

**2,8-Dibutyl-4,6-bis-(pyridin-2-ylhydrazonomethyl)dibenzofuran (1).** To a flask containing **19** (0.24g, 0.713 mmol) in chloroform (4 mL) was added a solution of 2-hydrazinopyridine (0.18 g, 1.57 mmol) in chloroform (1 mL). The solution was stirred overnight at room temperature after which the product precipitated as a yellow–grey solid (0.35 g, 96%) which was collected by filtration, washed with small amounts of chloroform and hexane, and then dried under the high vacuum. Mp 248–249 °C. IR (thin film): $\nu$ = 3186, 2923, 2839, 1602, 1572, 1515, 1442, 1305, 1182, 1155, 1106, 1078, 990, 767 $cm^{-1}$.

**Dibenzofuran-4,6-dicarbaldehyde (20).** Dibenzofuran (5.77 g, 34.3 mmol) and TMEDA (11.96 g, 102.9 mmol) were dissolved in dry diethyl ether (200 mL). A 1.6 M solution of *n*-butyllithium (64.1 mL, 102.9 mmol) was added dropwise and the mixture refluxed for 16 h. DMF (7.9 mL, 102.9 mmol) was added over a period of 10 min at 0 °C, and then the mixture was stirred further for 24 h at room temperature. The mixture was poured into cold 1 M hydrochloric acid (150 mL) and the aqueous layer was extracted 3 times with dichloromethane. The combined organic extracts were washed with water and brine, dried over $MgSO_4$, and chromatographed ($SiO_2$; dichloromethane) to yield the product (4.63 g, 60%) as a white crystalline solid. As a side product, dibenzofuran-4-carbaldehyde could be obtained. Mp 224–225 °C. IR (thin film): $\nu$ = 2839, 1682, 125, 1601, 1479, 1435, 1422, 13393, 1336, 1259, 1227, 1186, 1131, 1056, 996, 844, 814, 782, 720 $cm^{-1}$. $^1H$ NMR (200 MHz, [D] chloroform): $\delta$ = 10.70 (s, 2 H); 8.25 (dd, $^3J$ = 7.7 Hz, $^4J$ = 1.0 Hz, 2 H); 8.05 (dd, $^3J$ = 7.7 Hz, $^4J$ = 1.0 Hz, 2 H); 7.56 (t, $^3J$ = 7.7 Hz, 2 H). FAB-MS: $m/z$ 225.0 ($[M + H]^+$, 100%).

**4,6-Bis-(pyridin-2-ylhydrazonomethyl)dibenzofuran (21).** To **20** (0.24 g, 0.713 mmol) in chloroform (4 mL) was added a solution of 2-hydrazinopyridine (0.18 g, 1.57 mmol) in chloroform (1 mL). The solution was stirred overnight at room temperature and the product precipitated as a yellow–grey solid (0.35 g, 96%) that was collected by filtration, washed with small amounts of chloroform and hexane, and then dried under high vacuum. Mp >250 °C. IR (thin film): $\nu$ = 3198, 2982, 1594, 1570, 1510, 1423, 1331, 1277, 1175, 1115, 1083, 1143, 983, 770, 736 $cm^{-1}$. $^1H$ NMR (200 MHz, [D6] DMSO): $\delta$ = 11.19 (s, 2 H); 8.54 (s, 2 H); 8.15 (d, $^3J$ = 4.3 Hz, 2 H); 7.93 (d, $^4J$ = 1.6 Hz, 2 H); 7.80 (d, $^4J$ = 1.6 Hz, 2 H); 7.70 (td, $^3J$ = 7.9 Hz, $^4J$ = 1.7 Hz, 2 H); 7.37 (d, $^3J$ = 8.3 Hz, 2 H); 6.81 (t, $^3J$ = 7.9 Hz, 2 H). $^{13}C$ NMR (50 MHz, [D6] DMSO): $\delta$ = 159.0; 152.6; 147.8; 138.1; 132.2; 124.1; 123.6; 123.3; 120.9; 120.2; 106.6. FAB-MS: $m/z$ 407.1 ($[M + H]^+$, 100%). Anal. calcd for $C_{24}H_{18}N_{60}$ (406.44): C 70.92, H 4.46, N 20.68; found: C 73.34, H 4.40, N 20.62.

## Photodimerization

**Photodimers.** The photodimers were isolated according to the following procedure. In a Schlenk line was added 200 mg of **4C** which was dissolved in 60 ml acetone with sonication. The flask was subjected to four cycles of freeze–pump–thaw, sealed, and then irradiated for 12 h with a 400 W tungsten lamp. The solvent was subsequently removed and the yellow oil purified on silica with neat dichloromethane. The apolar fractions were collected and then purified by semi-preparative HPLC to yield **7A**. The polar fractions were collected, concentrated, and then isolated by

preparative TLC with 98% chloroform–methanol to afford **7B** and **7D**. Absolute assignment of the photoproducts was done by COSY and NOE $^1$H-NMR and compared to uracil dimer analogues.[55,56]

**[8-(Benzyloxycarbonylaminomethyl)-4*a*,4*b*-dimethyl-2,4,5,7-tetraoxodecahydro-1,3,6,8-tetraazabiphenylen-1-ylmethyl]carbamic acid benzyl ester (7A).** $^1$H NMR (300 MHz, [D6] DMSO): $\delta$ = 10.54 (s, 2 H), 7.36 (m, 10 H), 5.15 (d, $J$ = 6.1 Hz, 4 H), 4,48 (s, 2 H), 4.28 (d, $J$ = 17.8 Hz, 2 H), 4.1 (m, 2 H), 3.92 (d, $J$ = 16.9 Hz, 2 H), 1.23 (s, 6 H).

**[8-(Benzyloxycarbonylaminomethyl)-4*a*,4*b*-dimethyl-2,4,5,7-tetraoxodecahydro-1,3,6,8-tetraazabiphenylen-1-ylmethyl]carbamic acid benzyl ester (7B).** $^1$H NMR (300 MHz, [D6] DMSO): $\delta$ = 10.77 (s, 2 H), 7.37 (s, 10 H), 5.16 (s, 4 H), 4.44 (d, $J$ = 17.8 Hz, 2 H), 3.96 (s, 2 H), 3.47 (d, $J$ = 17.4 Hz, 2 H), 1.25 (s, 6 H).

**[5-(Benzyloxycarbonylaminomethyl)-4*a*,8*a*-dimethyl-2,4,6,8-tetraoxodecahydro-1,3,5,7-tetraazabiphenylen-1-ylmethyl]carbamic acid benzyl ester (7D).** $^1$H NMR (300 MHz, [D6] DMSO): $\delta$ = 10.64 (s, 2 H), 7.37 (s, 10 H), 5.13 (s, 4 H), 4.22 (d, $J$ = 17.9 Hz, 2 H), 4.09 (d, $J$ = 17.5 Hz, 2 H), 3.98 (s, 2 H), 1.22 (s, 6 H)

**Photodimerization of 4C mediated by template 2.** The template **2** ($1.3 \times 10^{-2}$ mmol) and **4C** ($2.6 \times 10^{-2}$ mmol) were added to a Pyrex NMR tube then dissolved with 2 mL acetone. Residual oxygen was removed from the homogeneous solution *via* four freeze–pump–thaw cycles. The sample was then irradiated with a 400 W lamp for 12 h and the product distribution was determined by HPLC analysis relative to authentic photodimers. Template **1** and analogue **3** were similarly irradiated.

## Conclusion

Selective amplification of *syn* [2 + 2] photoproducts **7A** and **7B** was achieved by photoirradiation of the molecular recognition directed 2 : 1 supramolecular adduct of the symmetric ditopic receptors **1** and **2** with thymine derivative **4C**. The results agree with a parallel pre-photoirradiation positioning of two thymine type substrate molecules within the cleft of the templates. The results obtained illustrate the possibility to control the regio- and/or stereoselectivity of chemical reactions between molecular species *via* the formation of well-defined supramolecular entities.

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53 A hydrazinophenyl analogue of **2** would be a better alternative for model studies, however the former is photochemically and thermally very unstable.
54 The crystal data and experimental information for **1** and **2** is included as electronic supplementary information. Compound **1**. $C_{24}H_{18}N_6O$, $M$ = 406.154, orthorhombic, $a$ = 13.111(3), $b$ = 10.123(2), $c$ = 14.928(3) Å, $U$ = 1981.3 Å$^3$, $T$ = 100(2) K, space group *Pbcn*, $Z$ = 7, $\mu$(Mo K) = 0.71703 nm$^{-1}$, 4066 reflections measured, 2265 unique ($R_{int}$ = 0.0217) which were used in all calculations. The final $R1$ and $wR2$ were 0.0682 and 0.1854 ($I>2\sigma(I)$). Compound **2**. $C_{36}H_{41}Cl_3N_6O_7$, $M$ = 680.10, monoclinic, $a$ = 15.280(3), $b$ = 22.540(5), $c$ = 33.477(3) Å, $U$ = 3619.8(13) Å$^3$, $T$ = 298 K, space group $P21/c$, $Z$ = 4, $\mu$(Mo K$\alpha$) = 71703 nm$^{-1}$, 14283 reflections measured, 8274 unique ($R_{int}$ = 0.0396) which were used in all calculations. The final $R1$ and $wR2$ were 0.1183 and 0.0688 ($I>2\sigma(I)$). CCDC reference numbers 604878–604879. For crystallographic data in CIF format see DOI: 10.1039/b605658j.
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