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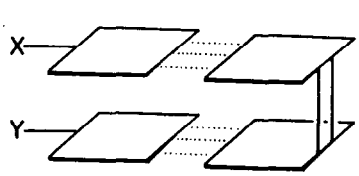
MOLECULAR RECOGNITION: CYCLOBUTANE THYMINE DIMERS AS RIGID TWO-SITE RECEPTORS

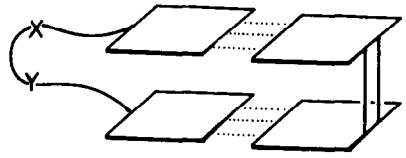
Simon C. Hirst and Andrew D. Hamilton*
Department of Chemistry, University of Pittsburgh
Pittsburgh, PA 15260

Abstract: Cyclobutane photodimers of thymine were synthesized and shown, in organic solvent, to bind to mono-and bis-diamidopyridine derivatives.

The hydrogen bond is a critical interaction in both biological and artificial molecular recognition.[1] The precise positioning of several hydrogen bonding groups within a cavity can lead to highly selective complexation of those substrates with complementary binding groups. In recent years we[2] and others[3] have reported a number of synthetic receptors based on this strategy of directed hydrogen bonding interactions. By careful choice of the number and nature of binding interactions selective hosts for such key biomolecules as nucleotides,[2a,b,g,3a,b] peptides,[2c] barbiturates[2d] and uric acid[3d] have been prepared. However, an important step in the development of this field involves the construction of hosts containing two discrete binding regions.[4] These double receptors will permit the complexation of two substrates (fig. 1a) or alternatively can bind in a constrained manner to a single substrate containing two binding regions (fig. 1b). We herein report a series of novel two site receptors based on the rigid framework provided by cyclobutane pyrimidine dimers.



a



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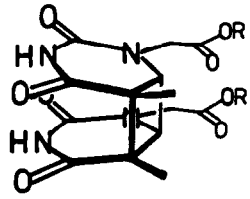


1

Fig. 1

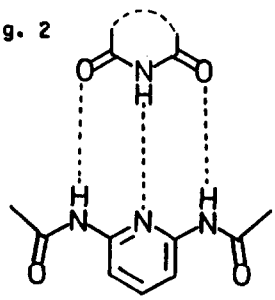
4 R=*n*-Butyl

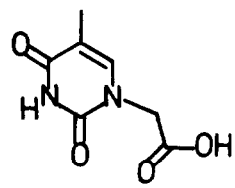
Tricyclic diimides of type (*1*) are readily formed by the photolysis of bis-thymine derivatives. *In vivo*, this is the principal mechanism of UV-damage in DNA and necessitates repair by a class of photoactivated enzymes, called photolyases.[5] Despite the

intense interest in these derivatives there has been little work on the binding properties of thymine dimers. The structure of the *syn,cis*-dimer (*1*) places two imide groups in close proximity at an angle of ~45° and a closest distance (4-CO to 4-CO) of 2.7 Å to each other. We have previously demonstrated[2a] the triple hydrogen bonding complementarity that exists between the imide functional group and 2,6-diamidopyridine (DAP) derivatives (Fig. 2). Thus, thymine dimer (*1*) can potentially bind two DAP substrates or a single substrate containing two DAP groups in a favorable arrangement. In polar solvents these interactions are expected to be weak and so an important design consideration involves confering organic solubility on the dimer.

Organic soluble thymine dimers were synthesized by two routes. 1-Carboxymethylthymine[6] (*2*) was converted to dimeric diester (*3*) with ethane-1,2-diol and carbonyl diimidazole and photolysed (at 300 nm) in a pyrex reaction vessel. The short linking chain[7] enforces a *syn,cis* stereochemistry in the product which formed in 77% yield. Hydrolysis of the esters (LiOH, MeOH) and reesterification with *n*-butanol (CDI) gave thymine dimer *4* in 45% yield. In a more direct route carboxymethylthymine (*2*) was esterified with dibutyl L-tartrate to diester *5* followed by photolysis in 9:1 CH_2Cl_2-acetone to form organic soluble dimer *6*[8] in 65% overall yield from *2*. A series of bis-DAP substrates *7-9* were prepared by mono-acylation of 2,6-diaminopyridine with propionyl chloride, followed by reaction with the corresponding diacid chloride.

Fig. 2





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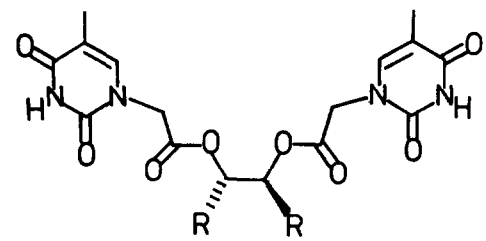
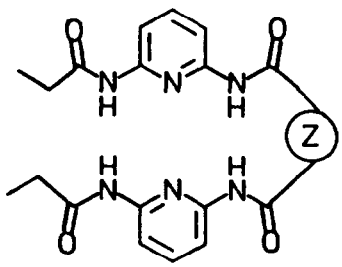


3 R=H

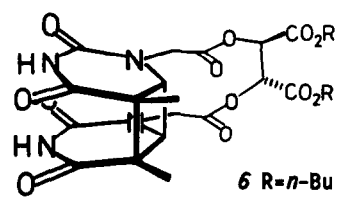
5 R=CO_2*n*-Bu



7 Z=$(CH_2)_6$

8 Z=$(CH_2)_5$

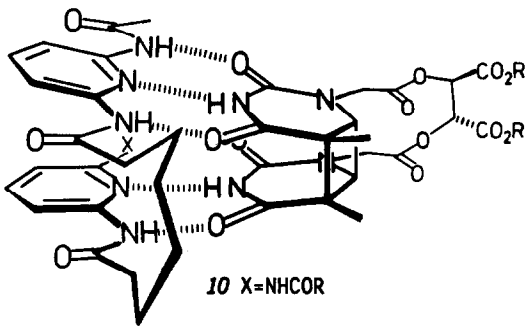
9 Z=CH_2OCH_2



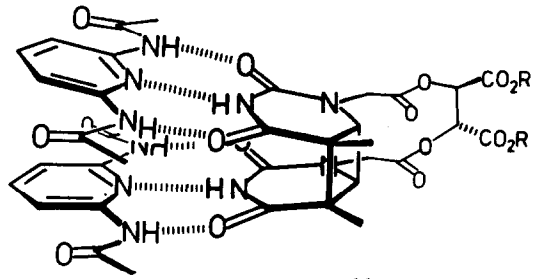
6 R=*n*-Bu

Addition of one equivalent of *7* to a $CDCl_3$ solution of *6* led to large downfield shifts of the amide-NHs (1.51 and 1.71 ppm) on the substrate and the imide-NH (2.4 ppm) on the receptor. These changes are consistent with a complex of type *10*[10] in which the bis-DAP substrate takes up a U-turn conformation to form six hydrogen bonds with the bis-imide receptor. Similar results were also seen with dimer *4*. The 1:1 stoichiometry

of the complexes was confirmed using Job's method of continuous variance.[11] Continuing the addition of substrate into receptor leads to a binding curve that can be analyzed by non-linear regression methods[12] to determine association constants for the interaction. These values for dimer *6* are collected in table 1 and show a steady increase with the length of the linking chain. The strongest complex is formed with the suberoyl-linked substrate and corresponds to a binding free energy of -4.6 kcal mol^{-1} which is a little less than twice that of 2,6-dibutyramidopyridine and thymine (-2.7 kcal mol^{-1}).[2a] The linking chain presumably takes up a chair conformation with the acylpyridine groups occupying adjacent equatorial positions. Binding is weaker between *6* and the pimeloyl- and glycolyl-linked substrates reflecting greater strain in the bound conformation and, in the latter case, intramolecular hydrogen bonding. Simple 2,6-dibutyramidopyridine derivatives give binding isotherms with *4* or *6* that indicate 2:1 stoichiometries as shown in *11*. This was confirmed by a Job's plot (Fig. 3) that contained a maximum at a mole ratio of 0.33.



10 X=NHCOR



11

Table 1

Spacer-Z	K_a (M^{-1})
$-(CH_2)_6-$	2200(±400)
$-(CH_2)_5-$	1700(±250)
$-CH_2OCH_2-$	520(±50)

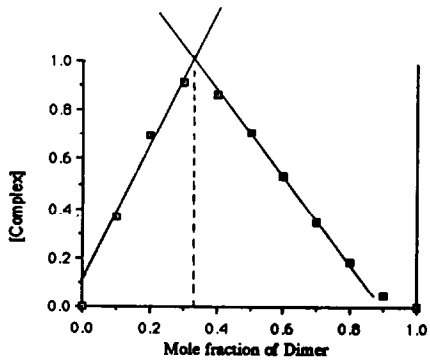


Fig. 3

In summary, we have shown that organic soluble cyclobutane thymine dimers form 2:1 and 1:1 complexes with mono- and bis-2,6-diamidopyridine derivatives, respectively. We are currently exploiting this property to influence bimolecular reactions between bound DAP derivatives or unimolecular processes within the constrained linking chain of a bound bis-DAP molecule.

Acknowledgment. We thank the National Institutes of Health (GM35208) for financial support of this work.

References

1. A. R. Fersht, Trends in Biochemical Science, 1987, 301. R. Taylor and O. Kennard, Acc. Chem. Res. 1984, *17*, 320.

2. a) A. D. Hamilton and D. Van Engen, J. Am. Chem. Soc. 1987, *109*, 5035; b) A. D. Hamilton and N. Pant, J. Chem. Soc. Chem. Commun. 1988, 765; c) N. Pant and A. D. Hamilton, J. Am. Chem. Soc. 1988, *110*, 2002; d) S. K. Chang and A. D. Hamilton, J. Am. Chem. Soc. 1988, *110*, 1318; e) S. Goswami, A. D. Hamilton and D. Van Engen, J. Am. Chem. Soc. 1989, *111*, 3425.

3. a) J. Rebek, Jr., Science (Washington, D.C.) 1987, *235*, 1478; b) J. Rebek, Jr., J. Mol. Recog. 1988, *1*, 1 and references therein; c) R. E. Sheridan and H. W. Whitlock, J. Am. Chem. Soc. 1986, *108*, 7120; d) T. R. Kelly, and M. P. Maguire, J. Am. Chem. Soc. 1987, *109*, 6549; e) V. M. L. J. Aarts, C. J. van Staveren, P. D. J. Grootenhuis, J. van Eerden, L. Kruise, S. Harkema, and D. N. Reinhoudt, J. Am. Chem. Soc. 1986, *108*, 5035; f) J. D. Kilburn, A. R. Mackenzie, and W. C. Still, J. Am. Chem. Soc. 1988, *110*, 1307; g) T. W. Bell, and J. Liu, J. Am. Chem. Soc. 1988, *110*, 3673.

4. a) M. Kim, and G. W. Gokel, J. Chem. Soc. Chem. Commun. 1987, 1687; b) D.T. Browne, J. Eisinger, and N. J. Leonard, J. Am. Chem. Soc. 1968, *90*, 7302; c) F. Kotzyba-Hibert, J. M. Lehn, and P. Vierling, Tetrahedron Lett. 1980, 941; d) M. R. Johnson, I. O. Sutherland, and R. F. Newton, J. Chem. Soc., Perkin I 1980, 586; e) T. R. Kelly, C. Zhao, and G. J. Bridger, J. Am. Chem. Soc., 1989, *111*, 3744.

5. B. M. Sutherland, Enzymes 1981, *24*, 481.

6. A. G. Cochran, R. Sugasawara and P. G. Schultz, J. Am. Chem. Soc. 1988, *110*, 7888.

7. *syn,cis*-Stereochemistry of *4* was confirmed by monobenzylation (BzBr, K_2CO_3) and analysis of coupling constants. $J_{6,6'}$ = 7.2 Hz compares to 7.5 Hz for literature *syn,cis* dimer see M. W. Logue and N. J. Leonard, J. Am. Chem. Soc. 1972, *94*, 2842.

8. M.p. 127-128°C. ^{1}H NMR δ($CDCl_3$), 0.94(6H, t, J=7.4 Hz, chain-CH_3), 1.35 (4H, m, CH_2CH_3), 1.47 (6H, s, ring-CH_3), 1.65 (4H, m, $CH_2CH_2CH_3$), 3.56 (2H, d, J=18.1 Hz, NCH_2CO), 3.80 (2H, s, CCH), 4.19 (4H, m, CO_2CH_2), 4.80 (2H, d, J=18.1 Hz, NCH_2CO), 5.76 (2H, s, $OCHCO_2$), 8.54 (2H, s, NH).

9. The chemical shifts of the amide-Hs in *7* and *8* (~7.6 ppm) were similar to that of 2,6-dibutylamidopyridine (constant at 7.52 ppm below 10^{-2} M concentrations), confirming the absence of intramolecular hydrogen bonding. In contrast, shorter linking chains (e.g. glycolic *9*) resulted in significant hydrogen bonding between the DAP groups (δ amide NH = 8.5 ppm).

10. Complex *6*:*7* (1:1) δ($CDCl_3$) 0.95 (6H, t, J=7.4 Hz, CH_3, *6*), 1.23 (6H, t, J=7.5 Hz, chain-CH_3, *7*), 1.37 (4H, m, CH_2CH_3, *6*), 1.54 (6H, s, ring-CH_3, *6*), 1.66 (4H, m, $CH_2CH_2CH_3$, *6*), 2.44 (4H, q, J=7.5 Hz, CH_2CH_3, *7*), 3.58 (2H, d, J=18.0 Hz, NCH_2CO, *6*), 3.84 (2H, s, CCH, *6*), 4.21 (4H, m, CO_2CH_2, *6*), 4.83 (2H, d, J=18.0 Hz, NCH_2CO, *6*), 5.80 (2H, s, $OCHCO_2$, *6*), 7.70 (2H, t, J=8.1 Hz, 4-pyr, *7*), 7.93 (4H, d, J=8.1 Hz, 3+5-pyrH, *7*), 8.29 (2H, br.s, NH, *7*), 8.42 (2H, br.s, NH, *7*) and 8.70 (2H, br.s, NH, *6*).

11. A. Job, Annales de Chim. 1928, *9*, 113.

12. At 298K, [Thy_2] varied from 2 x 10^{-4} to 7 x 10^{-2} M. [Guest] 8 x 10^{-4} M. M. Cowart, I. Sucholeiki, R. R. Bukownik and C. S. Wilcox, J. Am. Chem. Soc. 1988, *110*, 6204.

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