Nucleotide Base Recognition: Synthesis of Artificial Receptors containing Two Distinct Binding Regions for the Complexation of Bis-thymine Derivatives

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Oxidative coupling of a propargyloxy-substituted, diamidopyridine-based receptor for thymine leads to a new dimeric receptor that shows strong binding to bis-thymine derivatives.

In recent publications we have reported a novel approach to the molecular recognition of nucleotide bases.[1] A family of macrocyclic receptors has been prepared combining both hydrogen bonding to the periphery and aromatic stacking to the plane of a single heterocyclic base [Figure 1(a)].[2] Changing the complementarity of the hydrogen bonding component has led to receptors with different specificities for thymine,[3] guanine,[4] and adenine,[5,6] while the orientation of the π-stacking is influenced by the electronic characteristics of the interacting groups.[7] An important step in this work involves covalently linking individual binding units to provide a multiple receptor for substrates containing several nucleotide base derivatives [*e.g.*, Figure 1(b)]. We herein report the synthesis of a double receptor that contains two *two-site* binding regions for thymine and forms strong complexes with bis-thymine derivatives.[8,9]

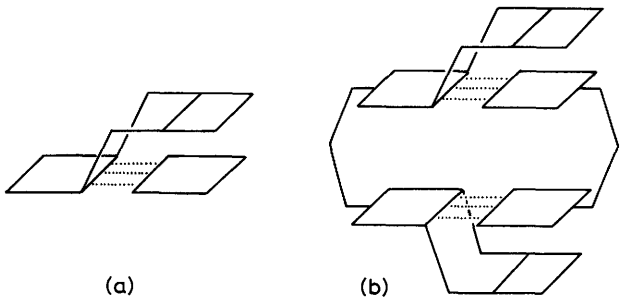
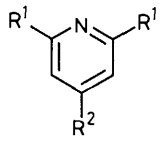


Figure 1

A similar stacking and hydrogen bonding strategy is seen in the DNA-binding antibiotics, triostin[10] and echinomycin,[11] which sandwich a two-base pair sequence between two intercalating quinoxalin rings. Self-association of the quinoxalin rings is inhibited by a bicyclic octapeptide backbone which also forms several hydrogen bonds to the minor groove side of the two-base pairs. In the design of our double receptor, collapse of the cavity will be prevented by positioning a rigid diyne spacer[9b,12] between the two binding regions. This also suggests a facile synthesis of the dimer *via* an acetylene oxidative coupling reaction.[13]

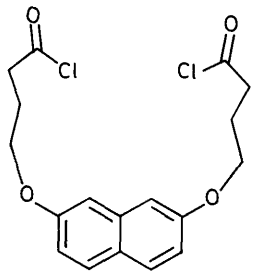
The key 4-propargyloxy-2,6-diaminopyridine (**3**) was prepared by propargylation of dimethyl chelidamate (**1**) (propargyl bromide, Bu^{t}OK) to diester (**2**) followed by conversion



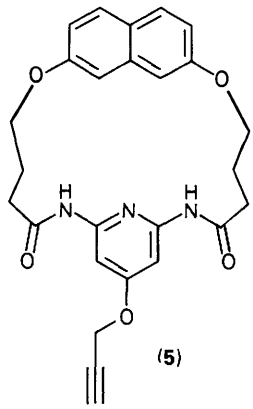
(**1**) $R^1 = CO_2Me$, $R^2 = OH$

(**2**) $R^1 = CO_2Me$, $R^2 = OCH_2CCH$

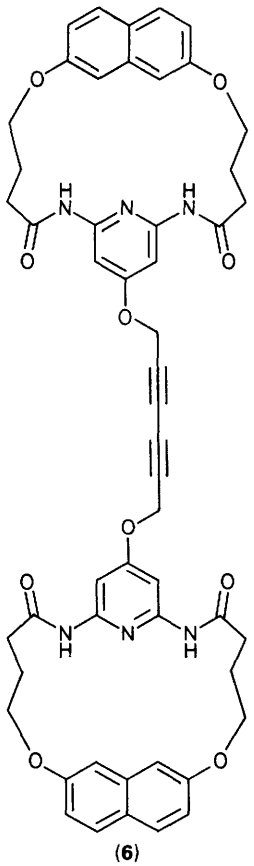
(**3**) $R^1 = NH_2$, $R^2 = OCH_2CCH$



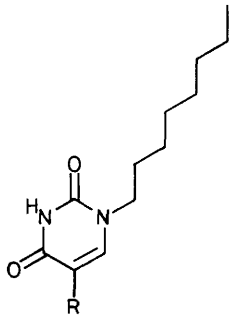
(**4**)



(**5**)



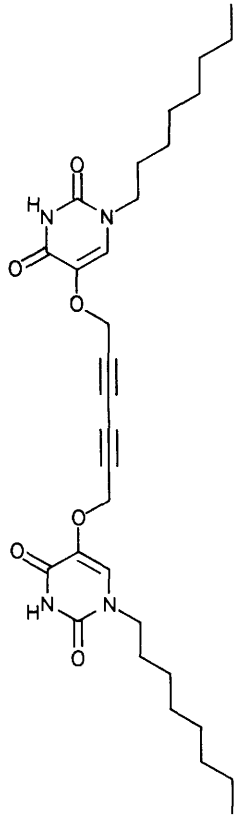
(**6**)



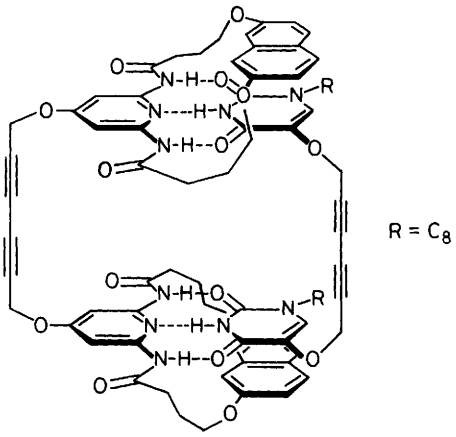
(**7**) R = H

(**8**) R = OH

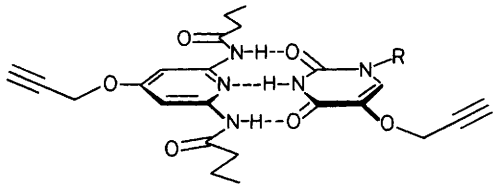
(**9**) R = OCH_2CCH



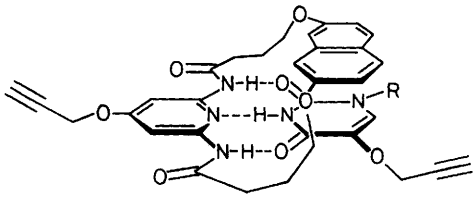
(**10**)



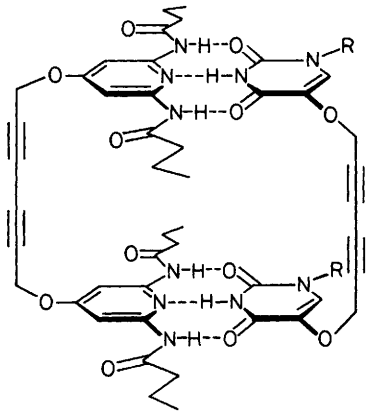
(11)



(12)



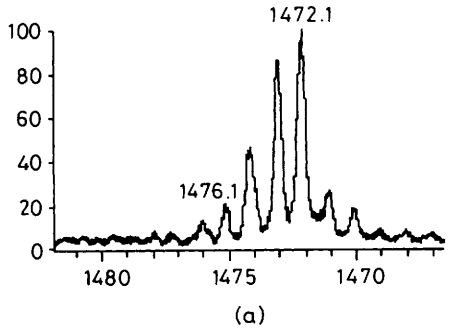
(13)



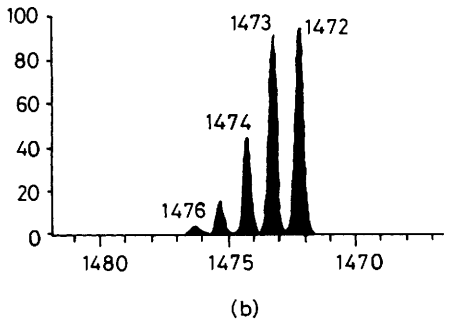
(14)

of the carboxymethyl groups to amines under Curtius conditions.[14] High dilution coupling of (**3**) and diacid chloride (**4**)[3] (CH_2Cl_2, Et_3N) gave macrocycle (**5**)[15] (11% yield) which was oxidatively dimerized [tetramethylethylenediamine (TMEDA), CuCl, O_2] to double receptor (**6**)† in 70% yield. In order to study the double binding properties of (**6**) we have constructed a $CDCl_3$-soluble, bis-thymine derivative containing an analogous diyne spacer. 1-Octyluracil (**7**)[15] was converted to its 5-hydroxy derivative (**8**) (i, Br_2, H_2O; ii, pyridine)[16] which was then propargylated (propargyl bromide, NaOH)[17] to (**9**) and oxidatively dimerized (TMEDA, CuCl, O_2) to bis-thymine (**10**)† in 59% yield from (**8**).

In spacer length as well as hydrogen bonding and aromatic stacking characteristics, double receptor (**6**) provides a



(a)



(b)

Figure 2. (a) $M + H^+$ peak from FAB MS of (**11**). (b) Computer simulation of isotope pattern for $C_{82}H_{91}N_{10}O_{16}$.

† *Selected spectroscopic data* for (**6**): ^1H NMR ($CDCl_3$): δ 7.83 (4H, br. s, NH), 7.69 (4H, d, *J* 9 Hz, naphthalene H-4,5), 7.65 (4H, s, pyridine H), 7.05 (4H, d, *J* 2 Hz, naphthalene H-1,8), 7.01 (4H, dd, *J* 2, 9 Hz, naphthalene H-3,6), 4.88 (4H, s, pyridine OCH_2), 4.24 (8H, t, *J* 6 Hz, OCH_2CH_2), 2.50 (8H, m, CH_2CO), 2.23 (8H, m, OCH_2CH_2). (**10**): ^1H NMR ($CDCl_3$): δ 8.98 (2H, br. s, NH), 7.07 (2H, s, thymine H), 4.80 (4H, s, CH_2O), 3.71 (4H, t, *J* 7.5 Hz, CH_2N), 1.68 (4H, m, CH_2CH_2N), 1.30 [20H, m, $Me(CH_2)_5$], 0.88 (6H, t, *J* 6 Hz, CH_3). Complex (**10**:**6**): ^1H NMR ($CDCl_3$): δ 10.64 (2H, br. s, imide NH), 9.58 (4H, br. s, amide NH), 7.72 (4H, s, pyridine H), 7.33 (4H, d, *J* 9 Hz, naphthalene H-4,5), 6.74 (4H, dd, *J* 9, 2 Hz, naphthalene H-3,6), 6.69 (2H, s, thymine H), 6.58 (4H, d, *J* 2 Hz, naphthalene H-1,8), 4.94 (4H, s, pyridine OCH_2), 4.79 (4H, s, thymine OCH_2), 4.12 (8H, m, CH_2CH_2O), 3.39 (4H, t, *J* 6 Hz, CH_2N), 2.52 (8H, m, CH_2CO), 2.20 (8H, m, CH_2CH_2O), 1.70 (4H, m, CH_2CH_2N), 1.30 [20H, m, $Me(CH_2)_5$], 0.90 (6H, m, Me).

Table 1. Association constant (K_s) values.

Complex	K_s/mol^{-1} dm^3	Complex	K_s/mol^{-1} dm^3
(**12**)	5.21×10^2	(**14**)	4.50×10^3
(**13**)	1.60×10^3	(**11**)	2.03×10^4

matched fit to bis-thymine (**10**) and can readily form a complex of type (**11**).† Addition of one equivalent of (**10**) to a $CDCl_3$ solution of (**6**) gave ^{1}H NMR changes consistent with this formulation.† In particular, large downfield shifts of the amide and imide resonances (δ 1.66 and 1.75) and upfield shifts of the thymine ring–H and N–CH_2 (δ 0.38 and 0.32) and naphthalene–(H-1, -8), and –(H-4, -5) (δ 0.47, 0.27, and 0.36) resonances confirm the presence of both hydrogen bonding and aromatic stacking interactions in (**11**).[3] Further addition of (**10**) to (**6**) gave a saturation curve that established 1 : 1 stoicheiometry and after Foster–Fife[18] analysis yielded an association constant of 2.03×10^4 mol^{-1} dm^3. Evidence that (**11**) is a discrete 1 : 1 complex was obtained from mass spectrometry. Fast atom bombardment (FAB) ionization of a 1 : 1 mixture of (**6**) and (**10**) in *p*-nitrobenzyl alcohol gave an $M + H^+$ peak at $m/z = 1472$ (Figure 2) corresponding to (**11**), with no detectable peaks at higher mass.

The importance of dimerization of both hydrogen bonding and aromatic stacking components was assessed by studying a series of complexes based on monomeric or partial dimeric receptors (**12**—**14**). The association constants for these complexes are collected in Table 1 and show an expected increase in binding strength as the number of binding regions increases from 1 to 4. The K_s values for (**12**) and (**13**) are larger (~5 fold) than those for analogous, unsubstituted amidopyridine receptors[3] due to the increased basicity of the pyridine from the 4-propargyloxy substituent. However, the contribution from π-stacking [3—4 fold increase in K_s from (**12**—**13**)] remains the same.[3,4] Dimerization of the binding regions [(**12**—**14**), (**13**—**11**)] results in a ~10 fold increase in K_s. That the effect of dimerization is not larger may be due to some strain in forming the macrocyclic hydrogen bonded complex.[19]‡

In summary, we have shown that individual receptors for thymine can be dimerized to double receptors that show increased binding to bis-thymine derivatives. These complexes have important implications in construction of supramolecular assemblies held together by hydrogen bonds.[8a] The central cavity of (**11**) and (**14**) resembles that of a two base pair unit of duplex nucleic acids and its intercalation properties are presently being investigated.

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‡ An idealized geometry for the complex is shown by (**11**). In a large molecular aggregate of this type a number of conformations are possible. In particular, the relatively minor contribution of π-stacking to the overall binding free energy in (**13**) and (**11**) indicates that, in $CDCl_3$ solution, non-stacked conformations with the naphthalene away from the thymine ring will also be present. In addition, rotation of one of the thymine rings by 180° in (**11**) will lead to a diastereoisomeric complex. These different conformational forms are in fast exchange at room temperature.

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