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Reciprocal Template Effects in Bisubstrate Systems: A Replication Cycle

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Abstract: We report the use of high affinity synthetic receptors for adenine derivatives as a basis for designed templated reactions. The rate of the coupling of two adenine derivatives, an active ester and an amine, is enhanced in the presence of a template bearing two receptor sites. In complementary synthetic reactions, the formation of the template, itself the coupling product from an active ester and an amine, was shown to be enhanced by the product of the first reaction. Thus reciprocal template effects are observed. Rate enhancements due to the formation of termolecular complexes were up to 13 fold. Experimentally observed rate enhancements correlated well with calculated concentrations of the termolecular complex. In addition, large rate accelerations (10^3) were observed due to bimolecular complexes in the coupling of two components, one of which contains a receptor for the other.

The enhancement of chemical reactions by complementary surfaces - template effects - is widespread in biological and chemical processes.[1] Nucleic acid replication is the paradigm example: one strand acts as a template for the other.[2] The complementarity is both physical -size and shape- and chemical. The chemical effects result from several intermolecular forces as hydrogen bonding, $\pi-\pi$ interactions or metal ligand binding. Examples of all of these in synthetic systems have been reported.[3] The enhancement caused by a template can result in selectivity: the formation of one product at the expense of another. Template effects have made cyclizations possible which are otherwise hard to achieve. Stoddart and Sauvage provide excellent examples in the synthesis of interlocked molecules.[3d,3i] We have earlier reported the use of hydrogen bonds and π-stacking as the source of (self)-complementarity in replication systems.[4] Here we report reciprocal templating effects in bisubstrate coupling reactions. The reciprocal nature arises from the fact that in a set of two reactions, the product of one template enhanced reaction is a template for the other, and the choice of template and substrate is arbitrary. The role the templates play in a single templated reaction is schematically depicted in Figure 1, where the template binds two reagents simultaneously. This promotes an otherwise bimolecular reaction to a unimolecular (or intracomplex) one and leads to enhanced coupling rates by reducing activation entropies.

Figure 1.

The complementarity on which the current work relies is the binding of adenine derivatives by previously reported scorpion shaped receptors. The association involves the chelation of the purine nucleus of adenine by two imides attached to a carbazole surface. The imides provide simultaneous Watson-Crick and Hoogsteen base pairing while the carbazole stacks to the purine (Figure 2). High binding affinities between these two components (K_a = 10^4 - 10^5 M^{-1}) are observed in organic solvents,[5] and precise positionning of the adenine in the diimide pocket is expected.

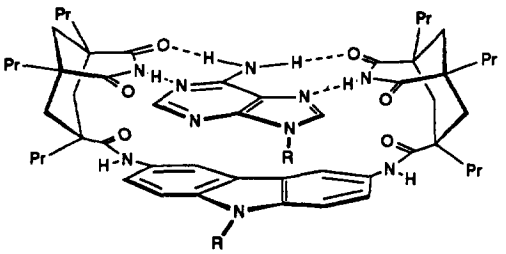


Figure 2.

Both the carbazole portion of the receptor and the ribose portion of adenosine are well suited for synthetic elaboration. They can be outfitted with complementary chemically reactive functions - amine nucleophiles and active ester electrophiles - for covalent coupling reactions. Two amines and two active esters were prepared (Figure 3) and the template effects of their coupling products were systematically evaluated.

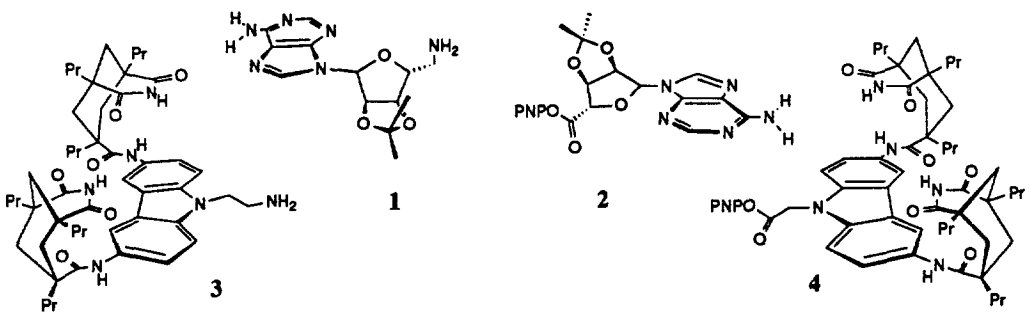
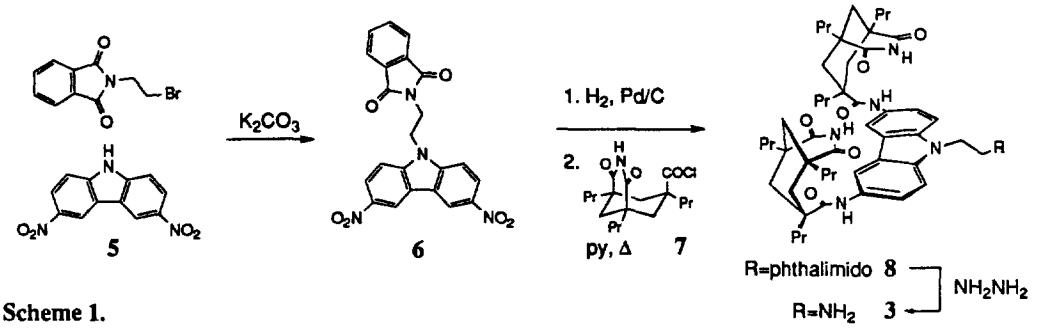


Figure 3.

RESULTS AND DISCUSSION

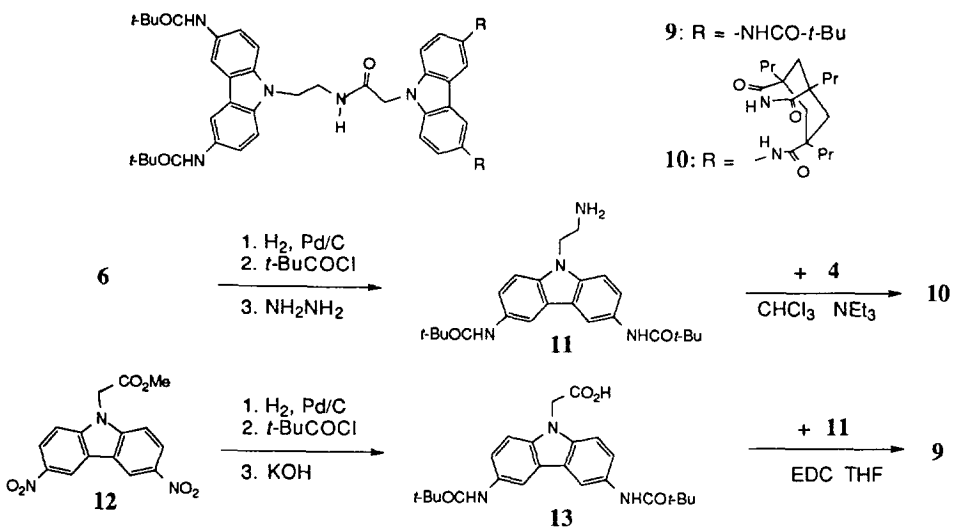
Synthesis

The two p-nitrophenylesters **2** and **4** (Figure 3) were prepared from from their corresponding carboxylic acids[5a] using p-nitrophenol and the coupling reagent EDC. Amine **1** was prepared according to a published procedure,[6] and amine **3** was made from 3,6-dinitrocarbazole in four steps (Scheme 1). This involved coupling **5** to N-(2-bromoethyl)-phthalimide to give **6**. Hydrogenation of the nitro groups, followed by acylation of the resulting amines with Kemp's imide acid chloride **7** gave **8**. Cleavage of the phthalimide with hydrazine resulted in **3** in 52 % overall yield.



Scheme 1.

Two reference templates **9** and **10**, containing many of the structural features of template **15** (vide infra) but lacking two recognition sites and one recognition site, respectively, were prepared according to Scheme 2. Hydrogenation of dinitro compound **6**, followed by acylation of the diamine with pivaloyl chloride and subsequent hydrazinolysis gave amine **11**. Coupling of this amine to active ester **4** gave **10**. Hydrogenation of dinitro compound **12**[5a] followed by acylation as before, then by saponification of the ester resulted in acid **13**. This acid was coupled to amine **11** with EDC to give **9**.



Scheme 2.

Template Effects

Coupling reactions in $CHCl_3$ were carried out at 25°C, and monitored spectrophotometrically following the release of p-nitrophenol. The results are summarized in Table 1. The first reaction studied was the coupling between **1** and **2**. At equimolar concentration of 0.05 mM in $CHCl_3$, in the presence of triethylamine, the coupling proceeds with an initial rate of 1.5×10^{-8} M•min^{-1} (Figure 5) to give the product amide **14** (Figure 4). The reaction was then run in the presence of 1 eq. of **15**, which is the product of the reaction between **3** and **4** (vide infra). Compound **15** accelerated the coupling reaction between **1** and **2** by a factor of ten. This

acceleration is reduced when competitive binders are present: the product **14** (1 eq.), which can be bound by **15** at both ends, reduces the acceleration to three fold whereas a large amount of 9-ethyladenine (10 eq.), which can only bind to a single end, lowers the acceleration to two fold. The rate of the uncatalyzed reaction is unaffected by these compounds. The reference compound **9**, which lacks both receptor sites, had no effect on the rate of the reaction. Compound **10**, having one receptor site, slightly *reduces* the initial coupling rate (by about 25%). This is presumably due to the fact that its bound reagents are less accessible for reaction with reagents in solution.

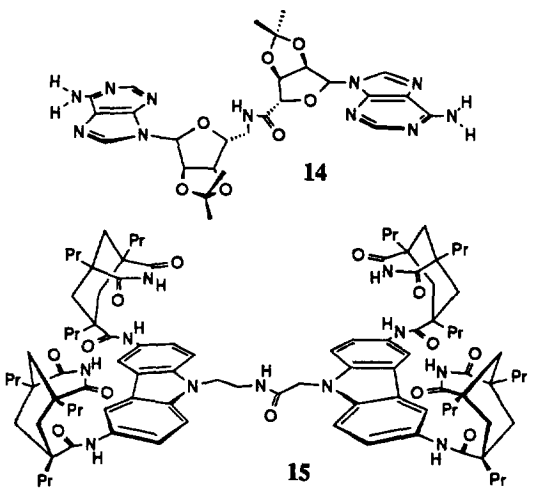


Figure 4.

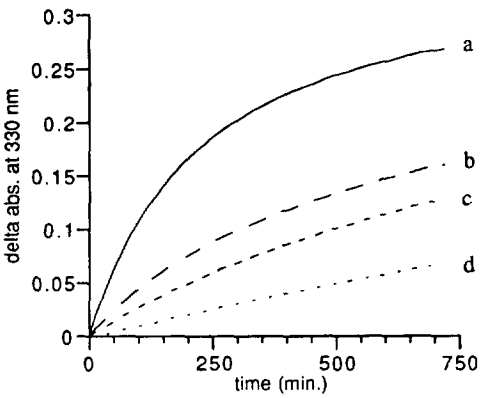


Figure 5. Reaction between **1** and **2** (both at 0.05 mM) in $CHCl_3$ at 25°C with 4 mM N_3Et and with (a) 1 eq. of **15**; (b) 1 eq. of **15** + 1 eq. of **14**; 1 eq. of **15** + 10 eq. of 9-ethyladenine; (d) no additives (background).

Solvent Effects.

The acceleration of the coupling between **1** and **2** as caused by **15** was shown to be solvent dependent. At the same concentrations and temperatures, the ten fold acceleration in $CHCl_3$ increased to 13-fold when CCl_4 was used. In chlorobenzene a nine fold acceleration was measured, whereas in THF and CH_3CN no rate enhancements were observed. These results parallel the binding affinity of the adenine receptors in these solvents. Although the receptors bind adenine derivatives in e.g. THF, the binding constant is too low to result in significant association under the dilute reaction conditions. In CCl_4 the adenine affinity is expected to be even higher than in the weakly competitive $CHCl_3$. The observed acceleration probably reflects this enhanced affinity.

Molecular Modeling.

All the results above are consistent with a termolecular complex that is responsible for the observed rate enhancements. To evaluate (qualitatively) the viability of such a complex, a computer minimization was carried out. The three components were docked together and the complex was minimized using the AMBER*[7] force field and GB/SA $CHCl_3$ solvation[8] in MacroModel v. 3.5.[9] A minimized structure is shown in Figure 6.

The modeling suggests that both adenosine derivatives can be accommodated simultaneously by **15**, and that the binding permits, in a C-shape backbone conformation, a close proximity of the two reactive groups.

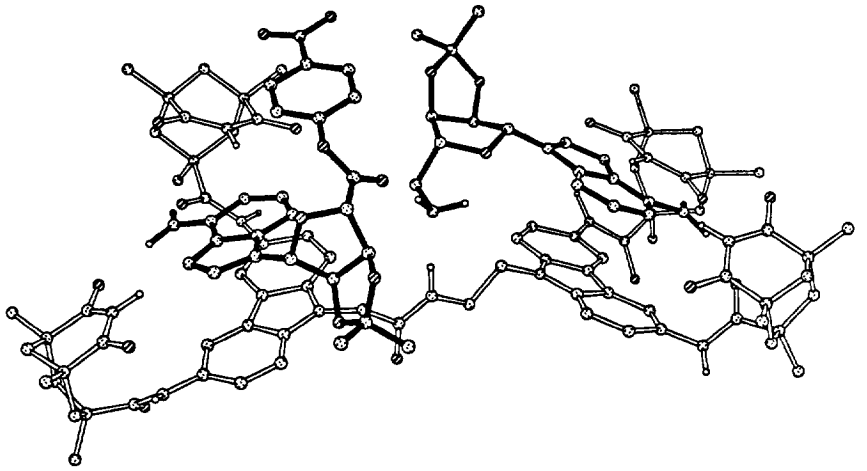


Figure 6. Minimized structure of the termolecular complex **1-2-15**. Carbon-bound hydrogens are not represented for clarity. The minimization was carried out with the trimethyl version of the Kemp's imide amide modules.

Simulation vs. Experiment.

More support for the mechanism came from experiments in which the concentration of added template was varied. Increasing the amount of **15** also increased the initial coupling rates to a maximum at 2 eq. Further addition resulted in lower coupling rates (Figure 7); the two reactive components become increasingly separated as complexes on different template molecules. In support of this theory, some simulations were carried out to see how the concentration of the productive termolecular complex varies with the amount of added template. The concentration of the complex should be proportional to the rate enhancements.

Calculations were based on the assumption that the template binds each substrate independently and with the same intrinsic affinity K. This is reasonable for an S-shaped overall conformation of the template, and perhaps less likely for the C-shaped conformation required for reaction. If $[T]_{tot.}$ represents the total concentration of Template, $[S]_{tot.}$ the sum of the total concentrations of the Substrates and [R], [RS] and [S] the concentrations of free and occupied Receptor sites and free Substrate respectively, combining the three following equations:

$$[R]+[RS]=2[T]_{tot.} \qquad [S]+[RS]=[S]_{tot.} \qquad K=\frac{[RS]}{[R][S]}$$

gives [S] as a solution of:

$$[S]^2+\left(2[T]_{tot.}-[S]_{tot.}+\frac{1}{K}\right)[S]-\frac{[S]_{tot.}}{K}=0$$

The fraction of occupied receptor sites is:

$$\frac{[RS]}{2[T]_{tot.}} = \frac{K[S]}{1+K[S]}$$

And therefore, the concentration of termolecular complex is calculated by substitution of [S] into:

$$[TS_2] = [T]_{tot.}\left(\frac{K[S]}{1+K[S]}\right)^2$$

When the concentrations of amine and esters are equal, the concentration of productive complex (containing one of each reagent) is $[TS_2]/2$. Using these calculations the solid line in Figure 7 was produced. The line shows that the calculated concentration of productive termolecular complex varies in the same way with template concentration as does the experimentally observed rate acceleration, supporting the proposed mechanism. The best fit was obtained when a K_a of 14000 M^{-1} was used in the calculation. This number is in good agreement with the experimentally observed association constants for similar receptor systems.[5a]

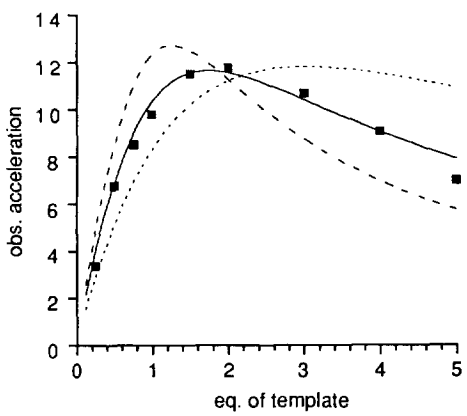


Figure 7. Observed acceleration of **1**+**2** vs. the amount of template **15** (■), (0.05 mM of each reagent, 4 mM NEt_3 in $CHCl_3$ at 25°C).
Calculated concentration of productive complex (**1-2-15**) (solid line) for an affinity receptor - adenine of 14000 M^{-1} under the same conditions.
Calculated concentration for K=5000 M^{-1} (dotted line), and for K=45000 M^{-1} (dashed line). Note than no vertical scale is represented for the calculated data, as the scale is different for each line.

Reciprocal Template Effects.

The availability of the four molecules of Figure 3 allowed us to evaluate several other reactions involving template effects. The first reaction was the coupling of **3** and **4** to give **15**. For this reaction an initial rate of 4.3 x 10^{-9} M•min^{-1} was measured (Table 1). Addition of one equivalent of **14** increased the initial coupling rate five fold. Both product (**15**) and 9-ethyladenine acted as competitive inhibitors; both reduced the acceleration while having no significant effect on the background reaction rate. The acceleration is again likely due to a termolecular complex complementary in structure to the one shown in Figure 6, but this time the "outside" amide is formed instead of the "inside" one. This system is related to the previous one in a special way, since the product of one reaction is a template for the other: *These reciprocally templated reactions form a replication cycle.*

Table 1. Initial Rates of Amide formation.

reaction*	Concentration (mM)					Init. rate
	[14]	**[15]**	[9-Et-Ad.]	**[9]**	**[10]**	(10^{-9} M•min^{-1})**
1+2	-	-	-	-	-	15
1+2	0.05	-	-	-	-	16
1+2	-	0.05	-	-	-	150
1+2	0.05	0.05	-	-	-	42
1+2	-	0.05	0.5	-	-	30
1+2	-	-	0.5	-	-	15
1+2	-	-	-	0.05	-	15
1+2	-	-	-	-	0.05	11
3+4	-	-	-	-	-	4.3
3+4	0.05	-	-	-	-	23
3+4	-	0.05	-	-	-	4.3
3+4	0.05	0.05	-	-	-	13
3+4	0.05	-	0.5	-	-	15
3+4	-	-	0.5	-	-	4.8
3+2	-	-	-	-	-	53000
3+2	-	-	0.5	-	-	14000
1+4	-	-	-	-	-	2200
1+4	-	-	0.5	-	-	600

*Both components are present at 0.05 mM in $CHCl_3$ + 4 mM NEt_3 at 25 °C. **Values are averaged from multiple independent runs. Standard deviations are ±15%.

Bimolecular Coupling Reactions.

The remaining coupling combinations of the starting materials (Figure 3) are the reactions between **1** and **4** and between **2** and **3**. These reactions involve complementary bimolecular components and are both about three orders of magnitude faster than those previously discussed (Table 1). The high rates are due to the association of the two reaction partners, resulting in complexes in which the reactive groups are almost always in close proximity[10] (see Fig. 8 for **2-3**). This conclusion is supported by the observation, in both cases, of saturation kinetics, i.e. increasing the concentration of the amine results eventually in a maximum velocity (see Fig. 9 for **1** and **4**). The products of these coupling reactions are "folded shut" as intramolecular complexes. In the ^1H NMR spectrum ($CDCl_3$, room temperature), the two imide protons give separate signals around 13-ppm; one results from base pairing to the adenine in the Watson -Crick mode and the other binds in the Hoogsteen fashion. Dilution studies on the coupling product of **1** and **4** showed that the chemical shift of these bound protons is concentration independent. Additional support for intramolecular association came from Vapor Pressure Osmometry (VPO). The apparent molecular weight of observed for a 3 mM solution in $CHCl_3$ is 833, 72% of the actual value (1154.6), using a related receptor as a reference.[11]

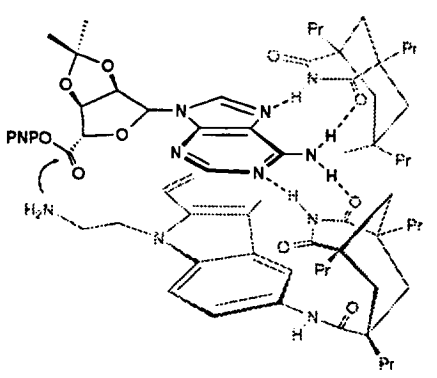


Figure 8. Bimolecular complex proposed for the fast reaction between **2** and **3**.

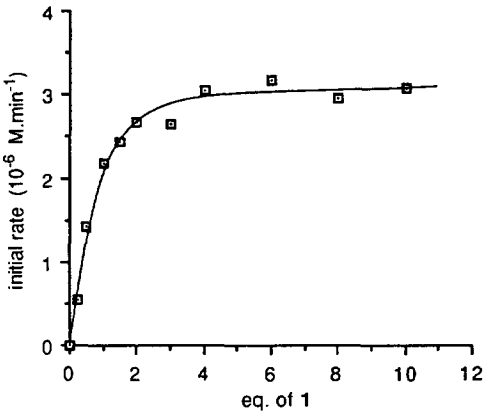


Figure 9 The initial rate of the reaction between **1** and **4** vs. the amount of **1**. [**4**] = $5 \cdot 10^{-5}$ M, [NEt_3] = $4 \cdot 10^{-3}$ M in $CHCl_3$ at 25°C. Line is drawn for visual guidance only.

Conclusion.

Covalent coupling reactions have been accelerated by up to 13 fold with a bisubstrate template. The presence of a termolecular complex responsible for the enhancement was supported by the observation of 1) competitive inhibition, 2) the lack of activity of "no recognition templates", 3) solvent dependence, 4) molecular modeling and 5) the correlation between experiment and simulation. The rate enhancements here are comparable to those observed by Kelly[3b,c] in reaction templates for bimolecular S_N2 reactions and are considerably larger than those observed in template effects involving self-complementary structures.[4] The reciprocal templating effects of **14** and **15** represent a replication cycle. Whether these cycles are generally more efficient than the minimalist self-complementary replicators is the subject of ongoing research. The large

accelerations in bimolecular systems due to association of both reaction components shows the potential of molecular recognition in catalysis as well as stoichiometric reactions.

Acknowledgments.

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EXPERIMENTAL SECTION

General.

Infrared spectra were obtained from a Perkin Elmer 1600 FT IR spectrophotometer. ^{1}H NMR spectra were measured with Bruker WM-250 (250-MHz), AC-250 (250 MHz) and Varian XL-300 (300 MHz), Unity 300 (300MHz). UV measurements were taken on a Perkin Elmer Lambda 2 spectrophotometer. High-resolution mass spectra were obtained with a Finnegan Mat 8200 instrument. Melting points were taken on a Electrothermal 9100 melting point apparatus. Commercial-grade solvents were used without further purification

Adenosine active ester 2

In 5 mL of anhydrous THF were mixed: 2',3'-O-isopropylidene-adenosine-5'-carboxylic acid[12] (100 mg, 0.311 mmol), 4-nitrophenol (169 mg, 4 eq), EDC (265 mg, 4.5 eq) and DMAP (11 mg, 0.3 eq). The initial suspension slowly turned into a homogeneous solution and a sticky white solid separated on the flask walls (EDC-urea). The solution was stirred for 48 h at room temperature. It was then decanted, and the solvent was removed. The residue was dissolved in CH_2Cl_2 and subjected to a flash chromatography on a (short) silica gel column. The eluting solvent (EtOAc) was pushed through quickly to avoid product decomposition on silica gel. The residue was redissolved in a minimal EtOAc and Et_2O was added until no more precipitation occurred. The white solid was filtered off and dried. Yield 102 mg (74%); m.p. 118 - 122 °C. IR (KBr) 3322, 3164, 1756, 1639, 1594, 1526, 1349, 1207, 1099, 1074 and 864 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.20 (d, 2H, J = 9.3 Hz), 8.19 (s, 1H), 7.00 (d, 2H, J = 9.0 Hz), 6.28 (s, 1H), 6.09 (br.s., 2H), 5.90 (d, 1H, J = 5.4 Hz), 5.57 (d, 1H, J = 6.0 Hz), 5.10 (s, 1H), 1.66 (s, 3H), 1.47 (s, 3H). HRMS (EI) m/e calcd for $C_{19}H_{18}N_6O_8$: 442.1237; found 442.1234.

Carbazole diimide active ester 4

In 2 mL of anhydrous THF were mixed: the appropriate acid[5a] (50 mg, 0.058 mmol), 4-nitrophenol (32 mg, 4 eq), EDC (50 mg, 4.5 eq) and DMAP (2 mg, 0.3 eq). The initial suspension slowly turned into a homogeneous solution and a sticky white solid adhered to the flask (EDC-urea). The solution was stirred for 48 h at room temperature. It was then decanted off, and the solvent was removed. The residue was dissolved in CH_2Cl_2 and subjected to a flash chromatography on a (short) silica gel column. The eluting solvent (EtOAc) was pushed through quickly to avoid product decomposition on silica gel. The residue was redissolved in a little ether and hexane was added until total precipitation of a white solid which was filtered

off and dried. Yield 38 mg (67%) of pure product; m.p. 235°C (dec.). IR (KBr) 3380, 3225, 3959, 2933, 2871, 1698, 1526, 1490, 1466, 1346 and 1203 cm^{-1}. ^1H NMR (300 MHz, DMSO-d6) δ 10.37 (s, 2H), 9.17 (s, 2H), 8.31 (d, 2H, J = 7.0 Hz), 8.13 (s, 2H), 7.59 (d, 2H, J = 8.8 Hz), 7.54 - 7.45 (m, 4H), 5.66 (s, 2H), 2.67 (d, 4H, J = 14.0), 2.02 (d, 2H, J = 12.7 Hz), 1.90 - 1.70 (m, 4H), 1.55 -1.00 (m, 26H), 0.97 - 0.75 (m, 18H).

Phthalimide 6

N-(2-bromoethyl)-phthalimide (1.482 g, 4.77 mmol), 3,6-dinitrocarbazole **5** (300 mg, 1.17 mmol), and K_2CO_3 (1.612 g, 11.66 mmol), were mixed in DMF (7mL) and heated at 110°C. A yellow precipitate formed slowly, and after 1 h the mixture was poured into H_2O (100 mL). The solid was filtered off, washed with H_2O, EtOH and dried to give 437 mg (87%) of a yellowish solid; mp . 330°C. IR (KBr) 1706, 1602, 1586, 1509, 1475, 1336, 1313, 1225, 1131, 1103, 719 cm^{-1}; ^1H NMR (300 MHz, DMSO-d6) δ 9.45 (d, 2H, J = 2.2 Hz), 8.28 (dd, 2H, J = 9.2 and 2.3 Hz), 7.78 (d, 2H, J = 9.2 Hz), 7.80 - 7.65 (m, 4H), 4.85 (t, 2H, J = 5.3 Hz), 4.03 (t, 2H, J = 5.3 Hz). HRMS (EI) m/e calcd for $C_{22}H_{14}N_4O_6$: 430.0913; found: 430.0912.

Phthalimide 8

Dinitro compound **6** (400 mg, 0.930 mmol) was dissolved in DMF (150 mL). Pd/C (10%, 350 mg) was added and a H_2 balloon was connected. After stirring at room temperature for 14 h the catalyst was filtered off through celite and the solvent was removed, to give 315 mg (92%) of the cream-colored amine. ^1H NMR (300 MHz, DMSO-d6) δ 7.78 (s, 4H), 7.05 (d, 2H, J = 8.9 Hz), 7.03 (d, 2H, J = 2.1 Hz), 6.62 (dd, 2H, J = 8.5 and 2.0 Hz), 4.62 (br.s., 4H), 4.39 (t, 2H, J = 6.2 Hz), 3.89 (t, 2H, J = 6.1 Hz).

The diamine (312 mg, 0.843 mmol), imide acid chloride **7** (605 mg, 1.77 mmol) and a catalytic amount of DMAP were dissolved in pyridine (12 mL) and heated at reflux for 3 h. The solvent was removed and the residue was dissolved in CH_2Cl_2, washed with a saturated $NaHCO_3$ solution (30 mL), dried on $MgSO_4$, filtered and concentrated. The product was purified through column chromatography over silica gel (2% MeOH in CH_2Cl_2) to give 543 mg (60% from **6**) of a beige solid; m.p. 197 °C. IR (KBr) 3380, 2958, 1713, 1489, 1466, 1396, 1310, 1191, 803, 719 cm^{-1}. ^1H NMR (300 MHz, DMSO-d6) δ 10.38 (s, 2H), 9.12 (s, 2H), 8.09 (s, 2H), 7.78 (s, 4H), 7.37 (2, 4H), 4.65 - 4.55 (m, 2H), 4.00 - 3.90 (m, 2H), 2.65 (d, 4H, J = 13.9 Hz), 2.02 (d, 2H, J = 12.5 Hz), 1.86 - 1.70 (m, 4H), 1.55 - 1.00 (m, 26 H), 0.95 - 0.72 (m, 18H). HRMS (FAB) m/e calcd for $C_{58}H_{73}N_6O_8$ (M + H): 981.5490; found: 981.5486.

Amine 3

Phthalimide **8** (540 mg, 0.550 mmol) was dissolved in MeOH (25 mL). Hydrazine (520 μL, 16.6 mmol) was added and the mixture was stirred at room temperature for 14 h. The solvent was removed and CH_2Cl_2 (50 mL) was added to the residue. The insoluble part was filtered off and the filtrate was concentrated to give 462 mg (99%) of an off-white solid; m.p. 196 °C (dec.). IR (KBr) 3378, 2958, 1699, 1488, 1466, 1200 and 803 cm^{-1}. ^1H NMR (300 MHz, DMSO-d6) δ 10.36 (s, 2H), 9.13 (s, 2H), 8.12 (s, 2H), 7.50 - 7.36 (m, 4H), 4.35 - 4.25 (m, 2H), 2.91 - 2.82 (m, 2H), 2.66 (d, 4H, J = 13.4 Hz), 2.02 (d, 2H, J = 12.7 Hz), 1.85 - 1.70 (m, 4H0, 1.57 - 1.00 (m, 26 H), 0.95 - 0.73 (m, 18 H). HRMS (FAB) m/e calcd for $C_{50}H_{71}N_6O_6$ (M + H); 851.5435 found: 851.5428.

Amine 11

Dinitro compound **6** (158 mg, 0.367 mmol) was dissolved in DMF (100 mL). Pd/C (10%, 150 mg) was added and a H_2 balloon was connected. After stirring at room temperature for 14 h the catalyst was filtered off through celite and the solvent was removed. The diamine was dissolved in CH_2Cl_2 (25 mL). Trimethylacetyl chloride (271 µl, 2.20 mmol) and NEt_3 (307 µL, 2.20 mmol) were added and the mixture was stirred for 1 h. CH_2Cl_2 (40 mL) was added, and the mixture was washed with a saturated $NaHCO_3$ solution (30 mL). After drying on $MgSO_4$, filtration and removal of the solvent, the diamide was purified using column chromatography over silica gel (5 % MeOH in CH_2Cl_2), to give 135 mg (68%) of a beige solid; ^1H NMR (300 MHz, $CDCl_3$) δ 8.23 (d, 2H, J = 2.0 Hz), 7.82 - 7.77 (m, 2H), 7.71 - 7.66 (m, 2H), 7.48 (dd, 2H, J = 8.6 and 2.0 Hz), 7.41 (s, 2H), 7.39 (d, 2H, J = 8.6 Hz), 4.53 (t, 2H, J = 6.8 Hz), 4.08 (t, 2H, J = 6.7 Hz), 1.36 (s, 18 H). This diamide (122 mg, 0.227 mmol) was dissolved in 5 mL MeOH and 20 mL THF. Hydrazine (214 µL, 6.8 mmol) was added and the mixture was stirred for 14 h. The solvent was removed and the residue treated with CH_2Cl_2(25 mL). The resulting suspension was filtered and the filtrate concentrated to obtain 76 mg (56 % from **6**) of an off-white powder; m.p. 227 - 229 °C. IR (KBr) 3346, 3277, 2957, 1650, 1536, 1488, 1317, 1216 and 791 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ 8.23 (d, 2H, J = 2.2 Hz), 7.54 (dd, 2H, J = 8.9 and 2.2 Hz), 7.45 (br. s, 2H), 7.37 (d, 2H, J = 8.8 Hz), 4.35 (t, J = 6.0 Hz), 3.18 (t, J = 6.0 Hz). 1.37 (2, 18 H). HRMS (EI) m/e calcd for $C_{24}H_{32}N_4O_2$: 408.2525; found: 408.2527.

Acid 13

Dinitro compound **12**[5a] (247 mg, 0.88 mmol) was dissolved in DMF (30 mL). Pd/C (10%, 30 mg) was added and a H_2 balloon was connected. After strirring at room temperature for 15 h. the mixture was filtered through celite and the filtrate was concentrated. Trituration with Et_2O solidified the diamine which was dried under vacuum. The solid and NEt_3 (3 eq) were dissolved in anhydrous CH_2Cl_2 (5 mL) at 0°C and trimethylacetyl chloride (0.32 mL, 3 eq) was added dropwise. After 3 h at room temperature, a sat. $NaHCO_3$ solution (10 mL) was added and the mixture was stirred for 1h. The layers were separated and the organic phase was concentrated. The residue was dissolved in a mixture of THF (30 mL), EtOH (90 mL). and aqueous NaOH (1 *N*, 30 mL) and stirred at room temperature for 30 min. The mixture was acidified to pH 1 with conc. HCl and the solvent was removed. The crude material was triturated with water, filtered, washed with water and dried under vacuum to yield **13** as an off-white solid (339 mg, 91% from **12**). m.p. 176 - 178 °C. IR (KBr) 3296, 2960, 1722, 1662, 1523, 1491, 1201 and 792 cm^{-1}. ^1H NMR (300 MHz, DMSO-d6) δ 12.48 (broad s, 1H), 9.20 (s, 2H), 8.33 (d, J = 1.8 Hz, 2H), 7.54 (dd, J = 8.8 and 1.8 Hz, 2H), 7.43 (d, J = 8.7 Hz, 2H), 5.14 (s, 2H), 1.26 (s, 18H). HRMS (EI) m/e calcd for $C_{24}H_{29}N_3O_4$: 423.2158; found: 423.2157.

Amide 9

Amine **11** (25 mg, 0.061 mmol), acid **13** (26 mg, 0.061 mg), EDC (23 mg, 0.123 mmol) and NEt_3 (9 µL, 0.06 mmol) were mixed together in THF (10 mL) and stirred for 14 h. After filtration and concentration of the filtrate the residue was purified using radial chromatography over silica gel (10 % MeOH in CH_2Cl_2). Obtained was 39 mg (78%) of a white solid; m.p. 230 °C (dec.). IR (KBr) 3349, 2960, 1659, 1530, 1488, 1310, 1202 and 798 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ 8.21 (s, 2H), 8.17 (s, 2H), 8.02 (d, 2H, J = 2.2 Hz), 7.99 (d, 2H, J = 1.8 Hz), 7.21 (dd, 4H, J = 8.4 and 1.7 Hz), 6.68 (d, 2H, J = 8.8 Hz), 6.59 (d, 2H, J = 8.9 Hz),

4.74 (br. t, 1H), 4.55 (s, 2H), 4.19 (t, 2H, J = 6.2 Hz), 3.77 - 3.66 (m, 2H), 1.51 (s, 18 H), 1.50 (s, 18 H). HRMS (EI) m/e calcd for $C_{48}H_{59}N_7O_5$: 813.4578; found:813.4581.

Aminolysis products

Equimolar amounts of amine and PNP ester were mixed in $CHCl_3$ in the presence of NEt_3 (80 eq.) at room temperature. After completion of the reaction the solvent and NEt_3 were removed and the product was purified using radial chromatography over silica gel.

Amide 14: m.p. 161 - 165 °C. IR (KBr) 3425, 1644, 1599, 1384, 1212 and 1088 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ 8.17 (s, 1H), 8.03 (s, 1H), 7.95 - 7.85 (m, 1H), 7.74 (s, 1H), 7.69 (s, 1H), 6.43 (br. s, 2H), 6.39 (br. s, 2H), 5.98 (d, 1H, J = 3.3 Hz), 5.84 (d, 1H, J = 2.4 Hz), 5.33 (dd, 1H, J = 6.2 and 2.0 Hz), 5.24 - 5.15 (m, 2H), 4.89 (dd, 1H, J = 6.3 and 4.8 Hz), 4.80 (d, 1H, J = 1.6 Hz), 4.04 (dd, 1H, 8.0 and 4.2 Hz), 3.84 - 3.72 (m, 1H), 3.37 - 3.25 (m, 1H), 1.63 (s, 3H), 1.59 (s, 3H), 1.36 (s, 3H), 1.32 (s, 3H). HRMS (EI) m/e calcd for $C_{26}H_{31}N_{11}O_7$: 609.2408; found: 609.2410.

Amide 15: m.p. 245 °C (dec). IR (KBr) 3380, 2958, 1700, 1528, 1489, 1466 and 1197 cm^{-1}. ^1H NMR (300 MHz, DMSO-d6) δ 10.37 (s, 2H), 10.35 (s, 2H), 9.16 (s, 2H), 9.14 (s, 2H), 8.42 - 8.35 (m, 1H), 8.16 (s, 4H), 7.53 - 7.42 (m, 6H), 7.31 (d, 2H, J = 8.8 Hz), 4.86 (s, 2H), 4.41 - 4.30 (m, 2H), 3.48 - 3.37 (m, 2H), 2.67 (d, 8H, J = 13.3 Hz), 2.03 (d, 4H, J = 12.5 Hz), 1.86 - 1.70 (m, 8H), 1.60 - 1.05 (m, 52H), 0.95 - 0.75 (m, 36H). HRMS (FAB) m/e calcd for $C_{100}H_{136}N_{11}O_{13}$ (M + H): 1699.0319; found: 1699.0310.

Coupling product of 2 and 3: IR (KBr) 3384, 2958, 2932, 2871, 1698, 1466, 1207, 869 and 798 cm^{-1}. ^1H NMR (300 MHz, DMSO-d6) δ 10.74 (s, 2H), 9.10 (s, 2H), 8.25 (s, 1H), 8.14 (d, 2H, J = 1.5 Hz), 7.84 (s, 1H), 7.73 - 7.66 (m, 1H), 7.40 (dd, 2H, J = 8.8 and 2.0 Hz), 7.33 (br. s, 2H), 7.27 (d, 2H, J = 8.8 Hz), 6.31 (d, 1H, J = 1.9 Hz), 5.32 - 5.22 (m, 2H), 4.47 (d, 1H, J = 1.9 Hz), 4.08 - 3.88 (m, 2H), 3.27 - 3.10 (m, 2H), 2.68 (d, 4H, J = 13.9 Hz), 2.04 (d, 2H, J = 12.4 Hz), 1.88 - 1.71 (m, 4H), 1.54 - 1.02 (m, 32H), 0.95 - 0.72 (m, 18 H). HRMS (FAB) m/e calcd for $C_{63}H_{84}N_{11}O_{10}$ (M + H): 1154.6403; found: 1154.6408.

Coupling product of 1 and 4 [13] : IR (KBr) 3377, 3214, 2959, 1700, 1696, 1647, 1533, 1468, 1201 cm^{-1}; ^1H NMR (300 MHz, DMSO-d6) δ 10.69 (s, 2 H), 9.12 (s, 2 H), 8.31 (s, 2 H), 8.13 (d, 2 H, J = 1.6 Hz), 8.05 (s, 1 H, amide), 7.39 (m, 4 H), 7.30 (s, 1 H), 7.27 (s, 1 H), 6.15 (d, 1 H, J = 2.9 Hz), 5.42 (m, 1 H), 4.95 (s, 2 H), 4.91 (m, 1 H), 4.21 (m, 1 H), 3.4 (m, 2 H), 2.68 (d, 4 H, J = 13.5 Hz), 2.04 (d, 2 H, J = 12.7 Hz), 1.8 (m, 4 H), 1.54 (s, 3 H), 1.51 (m, 4 H), 1.4-1.0 (m, 22 H), 1.31 (s, 3 H), 0.88 (t, 12 H, J = 6.9 Hz), 0.80 (t, 6 H, J = 7.2 Hz); HRMS (FAB) m/e calcd for $C_{63}H_{84}N_{11}O_{10}$ (M+H), 1154.6403; found, 1154.6408.

Amide 10

This compound was prepared according to the above mentioned aminolysis procedure from **4** and **11**. Isolated yield 94 %; m.p. 225 °C. IR (KBr) 3381, 2959, 2933, 2872, 1700, 1527, 1489, 1310, 1199 and 802 cm^{-1}. ^1H NMR (300 MHz, DMSO-d6) δ 10.36 (s, 2H), 9.21 (s, 2H), 9.15 (s, 2H), 8.38 (d, 2H, J = 1.8 Hz), 8.35 (m, 1H), 8.12 (d, 2H, J = 1.8 Hz), 7.60 (dd, 2H, J = 8.8 and 1.8 Hz), 7.45 (m, 4H), 7.23 (d, 2H, J = 8.8 Hz), 4.80 (s, 2H), 4.37 (m, 2H), 3.48 - 3.37 (m, 2H), 2.67 (d, 4H, J = 13.3 Hz), 2.03 (d, 2H, J = 12.5 Hz), 1.86 - 1.70 (m, 4H), 1.60 - 1.05 (m, 44H), 0.95 - 0.75 (m, 18H). HRMS (FAB) m/e calcd for $C_{74}H_{98}N_9O_9$ (M + H): 1239.7488; found: 1256.7492.

Aminolysis Kinetics

Typically, $CHCl_3$ solutions of the amine (125 μL, 0.4 mM), NEt_3 (125 μL, 32 mM) and other additives (template, inhibitors), were mixed together in a quartz cuvet (1 cm pathlength). The volume was adjusted to 875 μL with $CHCl_3$, and the solution of active ester was added (125 μL, 0.4 mM). The cuvet was closed with a teflon cap, shaken and quickly transferred to the temperature controlled (25°C ±0.1°C) compartment of the spectrometer. The reaction was periodically monitored at 330 nm. The data were collected using the PECSS software package v. 4.2 (Perkin-Elmer). The reactions were generally followed to at least 80% completion. Initial rates were determined from the first 10% of reaction. Reactions were run in duplicate or triplicate and numbers were averaged. Reactions were run with and without amine nucleophile to ensure the p-nitrophenol release was due to aminolysis rather than hydrolysis from residual water or undetected impurity.

Product analysis A product analysis was performed by taking two solutions with both 0.1 mM **1** and **2** (in $CHCl_3$ (20 mL) + 8 mM NEt_3). One of these also contained 0.1 mM **15**, which lead to an 8 fold initial rate acceleration as determined spectrophotometrically. After 72 h. the solvents and NEt_3 were removed and the residues were redissolved in DMSO-d6 and analyzed by ^1H NMR. In both cases **14** was the only observed product.

Vapor Pressure Osmometry.

The molecular weight determinations were performed on a Wescan Model 233 vapor pressure osmometer. Standards were measured at 1, 3, 6 and 10 mM and molecular weights determined at 3 mM in HPLC grade glass-distilled $CHCl_3$ (Aldrich). There is a ±20% margin of error for the measurements.

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