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Cleavage of Phosphodiesters and of DNA by a Bis(guanidinium)naphthol Acting as a Metal-Free Anion Receptor

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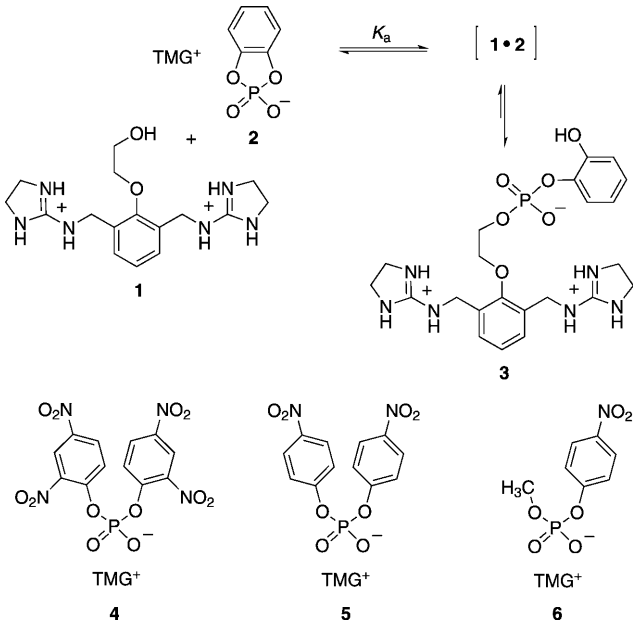
Phosphoric acid diesters form anions at neutral pH. As a result of charge repulsion they are notoriously resistant to hydrolysis. Nucleophilic attack, however, can be promoted by different types of electrophilic catalysts that bind to the anions and reduce their negative charge density. Although in most cases phosphodiester-cleaving enzymes and synthetic catalysts rely on Lewis acidic metal ions, some exploit the guanidinium residues of arginine as metal-free electrophiles. Here we report that a combination of two guanidines and a hydroxy group yields highly reactive receptor molecules that can attack a broad range of phosphodiester substrates by nucleophilic displacement at phosphorus in a single-turnover mode. Some stable *O*-phosphates were isolated and characterized further by NMR spectroscopy. The bis(guanidinium)naphthols also cleave plasmid DNA, presumably by a transphosphorylation mechanism.

Introduction

The remarkable kinetic stability of phosphoric acid diesters[1] presents a major challenge for the design of biomimetic artificial nucleases.[2–6] Most enzymes involved in phosphoryl transfer reactions[7] (and many synthetic analogues) rely on Lewis acidic metal ions. These can simultaneously bind and polarize substrates, together with the attacking nucleophiles, thus enhancing their intrinsic reactivity. Some phosphoryl transfer enzymes, on the other hand, do not require metal ions. Human DNA topoisomerase I,[8] as an example, activates the phosphate groups of DNA by forming ion pairs with two arginine residues. Reversible strand cleavage is then achieved by nucleophilic attack by a tyrosine hydroxy group. The bis(guanidinium) motif, also known from staphylococcal nuclease,[9] has guided chemists to the design of several synthetic anion receptors.[10] Some of them induce large rate effects on phosphoryl transfer reactions.[11] As an example, the bis(guanidinium) alcohol **1** forms ion pairs with catechol cyclic phosphate **2** in DMF (K_a = 2900 M^{-1} at 30 °C; see Scheme 1). In the presence of 0.25 M diisopropylethylamine, a fast and reversible reaction occurs, thus leading to **3**. Compared to simple uncharged alcohols, the guanidinium ions of **1** accelerate the phosphorylation by six to seven orders of magnitude.[11a] Compound **1**, however, fails to cleave less activated substrates such as phosphodiesters **5**–**6**. The reaction is sluggish even with substrate **4**, and ring nitrogens (instead of oxygens) are the preferred phosphorylation sites. It has been observed previously that the impressive rate effects in "enzyme models" are often restricted to artificial substrates that exceed, by far, the reactivity of their natural counterparts.[12] To broaden the application of phosphodiester cleavage by bis(guanidinium) alcohols, our strategy was to optimize the structure of the nucleophilic side chains.



Scheme 1. Top: Guanidinium alcohol **1** is phosphorylated in a supramolecular process involving ion-pair complexes [**1**·**2**]. Bottom: Phosphodiester substrates used in this study (TMG$^+$: Tetramethylguanidinium; anion: picrate).

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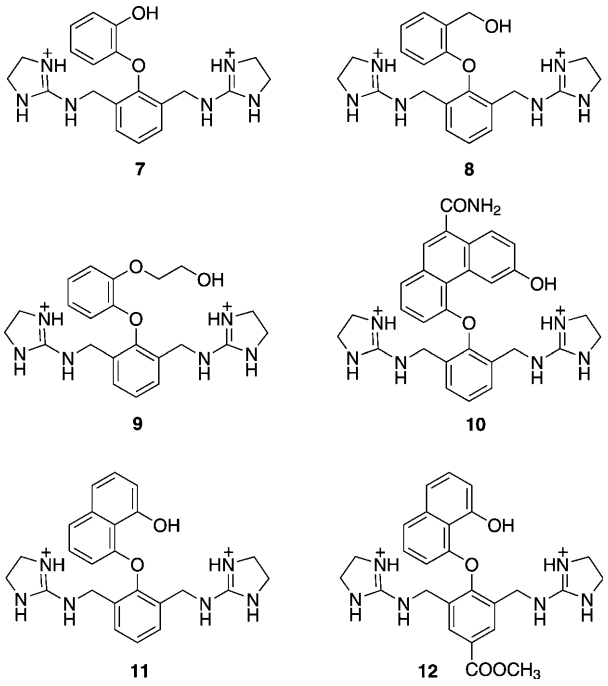
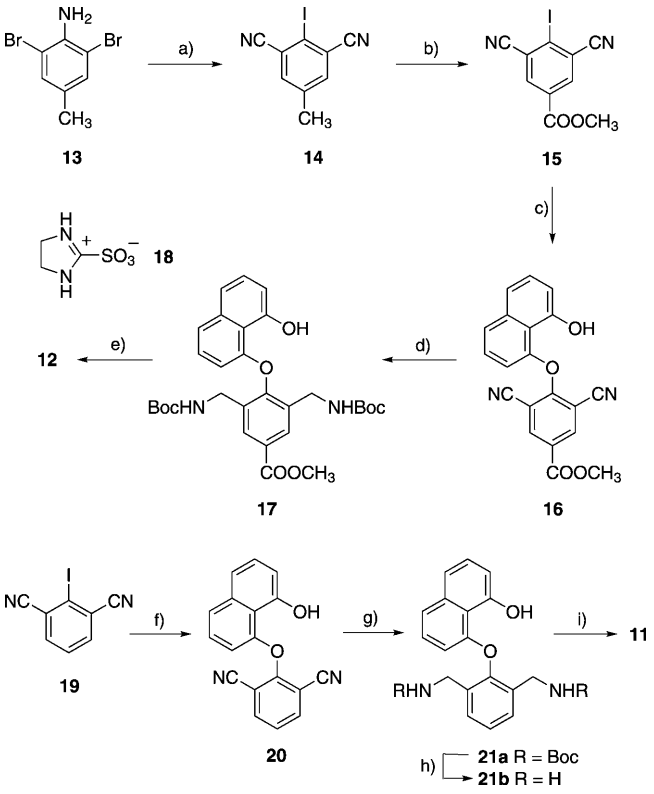
Results and Discussion

Among other analogues, guanidinium alcohols **7**–**10** were synthesized and tested for their ability to cleave substrates **4** and **5** (BDNPP and BNPP, Scheme 2). To summarize a large number of experiments, out of **7**–**10** the slowest reaction was observed with glycol ether **9**. Phenanthrene **10** can be regarded as a rigid, cyclic version of **9**. Both molecules have side chains of identical lengths, but the rigid analogue was much more effective in terms of rates. However, reaction at oxygen atoms was still accompanied by *N*-phosphorylation. Catechol **7** comes close to **10** in its reactivity in DMF, but declines in DMF-water mixtures, and also shows high levels of *N*-phosphorylation. Compared to **7** and **9**, benzylic alcohol **8** has a side chain of intermediate length, and is also much less acidic. In spite of the distinct pK_a difference, **8** did not react much slower than phenanthrene **10,** and it showed clean *O*-phosphorylation. The overall geometry of **8** therefore looked promising, and subsequent research was focused on the rigid naphthol analogues **11** and **12**.



Scheme 2. Structures of guanidinium alcohols **7**–**12** (counter ion: picrate).

The synthesis of compound **12** is depicted in Scheme 3. Starting from dibromotoluidine **13**,[13] the cyano and iodo substituents of intermediate **14** were introduced in a Rosenmund-von-Braun reaction,[14] followed by Sandmeyer replacement. Oxidation and esterification then led to **15**, a compound activated for nucleophilic displacement of iodide by the naphthol[15] moiety. Boc-protected diamine **17** was obtained by catalytic hydrogenation of the nitriles. Removal of Boc then allowed the diamine to be converted into the target compound **12** by reagent **18**.[11a] Analogous methods transformed 2-iodo-1,3-dicyanobenzene **19**[16] via **20** into diamines **21 a**, **21 b,** and finally into target compound **11**.



Scheme 3. a) 1: CuCN, DMF, Py, Δ, 8 h, 73%; 2: HNO_2, KI, 81%. b) 1: $Na_2Cr_2O_7$, H_2O, H_2SO_4, 0 °C, 3 h, RT, 1 h, 70%; 2: MeOH, H_2SO_4, Δ, 8 h, 94%. c) 1,8-dihydroxynaphthalene, NaH, DMF, 60 °C, 30 min, 70%. d) H_2, Pd/C, PtO_2, Boc_2O, MeOH, 45 bar, 55 °C, 24 h, 69%. e) 1: AcCl, MeOH; 2: **18**, MeOH, Et_3N, RT, 1 h; 3: picric acid, 41%. f) 1,8-dihydroxynaphthalene, NaH, DMF, 90 °C, 6 h, 62%. g) H_2, Pd/C, PtO_2, Boc_2O, MeOH, 40 bar, 55 °C, 42 h, 73%. h) AcCl, MeOH. i) 1: **18**, MeOH, H_2O, Et_3N, RT, 12 h; 2: picric acid, 70%.

The crystal structure of the picrate salt of **21 b** (Figure 1) shows how steric constraints limit the conformational space accessible to this molecule, thus forcing the amino and hydroxyl residues into the same space region.[17] Hydrogen bonds between the protonated nitrogens and the ether oxygen might further stabilize this arrangement. Analogous factors are expected to govern the conformation of guanidinium alcohols **11** and **12** as well.

The phosphorylation of **11** with the most reactive substrate, **4**, was studied first by ^{31}P NMR (250 mM diisopropylethylamine in [D_7]DMF, 30 °C). Apart from the substrate peak (−13.42 ppm), a new signal at −11.40 ppm appeared; this was assigned to the phosphorylated naphthol **22** (Scheme 4). When the experiment was repeated with substrate **5** (−11.83 ppm), a single product peak was formed at −11.29 ppm. ^{31}P NMR shifts unfortunately do not allow a clear distinction between *O*-phosphorylated and *N*-phosphorylated products. We therefore isolated **24** by HPLC. The ^{1}H NMR signal of all eight imidazoline C–H groups appeared as a broad singlet at 3.36 ppm, thus ruling out *N*-phosphorylated asymmetric structures.[18] Direct evidence for the structure of **24** comes from 2D NMR as shown in Figure 2.

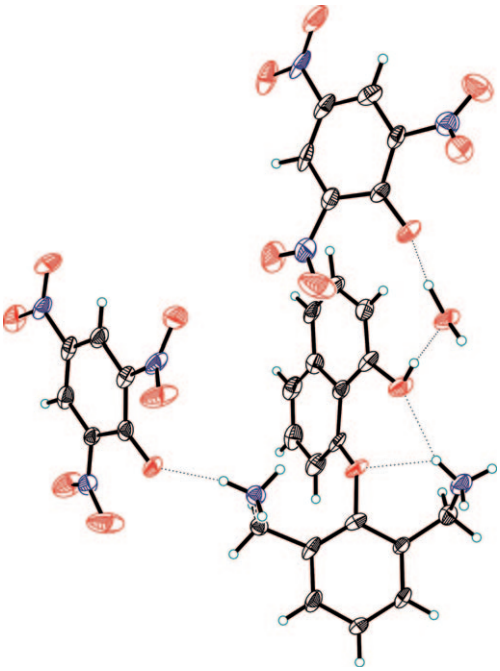
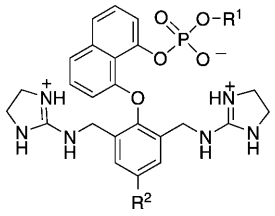


Figure 1. Crystal structure of the picrate salt of diamine **21 b**. One of two disordered conformations is shown (see also the Supporting Information).[17]



22 R^1 = 2,4-dinitrophenyl, R^2 = H
23 R^1 = R^2 = H
24 R^1 = 4-nitrophenyl, R^2 = H
25 R^1 = methyl, R^2 = H
26 R^1 = 4-nitrophenyl, R^2 = $COOCH_3$

Scheme 4. Reaction products observed in phosphorylation studies with guanidinium naphthols **11** and **12**.

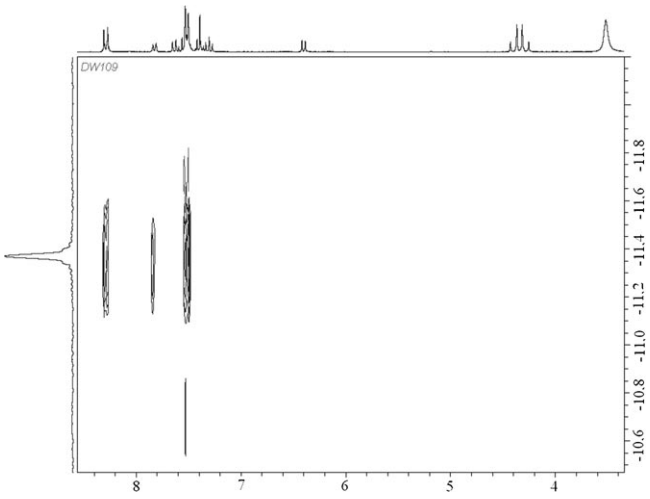


Figure 2. ^{31}P,^{1}H HMBC spectrum of **24** ([D_3]acetonitrile + $CDCl_3$ + [D_1]TFA, 250 MHz). Crosspeaks from ^{31}P to the ^{1}H signals of 4-nitrophenyl (8.28 ppm) and H(7) of the naphthol (7.85 ppm) but not to the CH_2 signal of the guanidines at 3.52 ppm demonstrate the attachment of the phosphate ester to the naphthol moiety.

Pseudo-first-order rates of phosphorylation were then determined by HPLC (10 mM **11** as picrate salt, 30 mM substrate, 250 mM diisopropylethylamine in DMF, 30 °C). At the chosen concentrations, almost complete complexation of guanidinium naphthols by phosphate substrates can be expected.[11a] For substrate **4**, the value of k_{obs} (3.6×10^{-2} min^{-1}) corresponds to a half life of only 19 min. Bis-4-nitrophenyl phosphate **5** showed an initial rate of $k_{obs}=2.2\times10^{-4}$ min^{-1} ($t_{1/2}=53$ h). The same rates were observed for the reaction of substrate **5** with compound **12 a** (chloride salt) to yield **26**. Experimental errors of rate determinations were ±10%. At longer reaction times some deviation from first order kinetics occur in all cases, presumably due to competitive binding of nitrophenolate ions. Even the least reactive substrate, **6**, slowly phosphorylated **11** to form **25** ($k_{obs}=1.4\times10^{-5}$ min^{-1}; $t_{1/2}=830$ h).

The stability of ion pair complexes is distinctly reduced by the addition of polar and protic solvents. Nevertheless, when the reaction of **11** with substrate **4** was tested in DMF/aqueous TRIS-HCl buffer (2:1; pH 10.7) an initial rate of $k_{obs}=2.8\times10^{-3}$ min^{-1} was observed. In the presence of water, **22** was slowly hydrolyzed to form phosphomonoester **23** and dephosphorylated **11**. Compared to the value found in DMF/diisopropylethylamine, the reaction in the DMF/water mixture was reduced just 13-fold.

We therefore tested the ability of guanidinium alcohols **11** and **12** to cleave supercoiled pUC19 plasmid DNA. Each cyclic strand consists of 2686 nucleotides. If at least one of the nucleotides gets disrupted, the supercoiled DNA (form I) uncoils, and the nicked but still cyclic form II results. These forms can be separated by electrophoresis on agarose gels. The assay, therefore, produces an overlay of all processes that occurred at the 5372 individual nucleotides. In addition to superhelical strain, this statistical factor makes plasmid DNA sensitive against cleavage. Good preparations of pUC19 contain less than 5% of form II. After 20 h not much additional degradation was observed in TRIS or HEPES buffers at pH 7.0 or above. However, while stable at pH 6.0 or 6.5 in TRIS, almost complete conversion into form II and even linear DNA (form III) was observed in acidic HEPES buffers (a cationic alcohol!). When incubated for 20 h at 37 °C, 10 mM of either **11** or **12** converted about 37% of the plasmid into form II (HEPES, pH 7.0). Linear DNA was not observed. Significant effects above background occurred at cleaver concentrations down to 1 mM. DNA cleavage, however, might result from different mechanisms. To exclude general acid catalyzed depurination—potentially exerted by the naphthol or guanidinium moieties—the interdependence of pH and cleavage yields was tested (Figure 3 and Table 1).

Although significant variation occurred in each individual experiment, the data clearly shows an increase of cleavage yield with increasing pH, thus arguing against acid-induced depurination. Up to 2 mM of EDTA did not significantly alter reaction rates, whereas a slight reduction in cleavage yield was seen at 5 and 10 mM. In this concentration range, the polyanionic EDTA is expected to interfere with DNA binding of the cationic naphthols **11** and **12**.[19] Only minor gains in plasmid cleavage resulted from the addition of 1–10 mM Mg^{2+} ions. The reac-

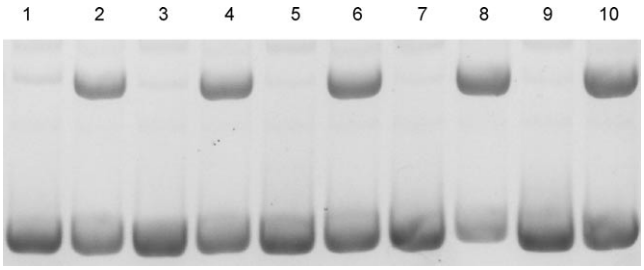


Figure 3. Cleavage of pUC19 plasmid DNA by guanidinium naphthol **12** as a function of pH. Bottom: superhelical DNA (form I); top: nicked circular DNA (form II). Lane 1 (from left to right): control, pH 7.0; lane 2: 5 mM **12**, pH 7.0; lane 3: control, pH 7.5; lane 4: 5 mM **12**, pH 7.5; lane 5: control, pH 8.0; lane 6: 5 mM **12**, pH 8.0; lane 7: control, pH 8.5; lane 8: 5 mM **12**, pH 8.5; lane 9: control, pH 9.0; lane 10: 5 mM **12**, pH 9.0 (22 nM DNA, HEPES buffer, 37 °C, 20 h; electrophoresis on 1% agarose gel, ethidium staining; inverted grayscale).

Table 1. Yields of plasmid cleavage as a function of pH (conditions as given in Figure 3).

pH	exp.1 [%]	exp.2 [%]	exp.3 [%]	Ø[a] [%]
7.0	32.1	33.2	30.0	31.8
7.5	31.0	32.0	31.1	31.4
8.0	35.3	41.9	42.1	39.8
8.5	49.8	51.6	45.4	48.9
9.0	50.4	39.8	48.8	46.3
9.4[b]	59.3	65.4	66.1	63.6
10.2[b]	67.5	55.8	65.1	62.8

[a] Mean value of the three independent experiments. For details of quantification see the Supporting Information. [b] Separate experiments not shown in Figure 3.

tions of **11** and **12** with DNA, therefore, do not depend on metal ions (data not shown).

Conclusions

Compounds **11** and **12** resulted from a long-term attempt to "synthesize" chemical reactivity by a proper assembly of simple functional groups. Even though the bis(guanidinium) naphthols cannot compete at present with advanced metal-based phosphate cleavers, they exhibit remarkable rates, and even degrade DNA. What is the mechanism of plasmid cleavage? Experimental evidence is in accord with nucleophilic substitution at phosphorus atoms. However, we cannot yet exclude pathways that involve naphthoxy radicals. Further studies on reaction mechanisms and sequence specific DNA cleavers are planned.

Experimental Section

Syntheses

*2-Iodo-5-methylisophthalonitrile (**14**):* 2-Amino-5-methylisophthalonitrile[14] (2.00 g, 12.72 mmol) was suspended in H_2SO_4 (40 mL of a 50% aqueous solution), cooled to −5 °C, and stirred for 60 min. A solution of $NaNO_2$ (1.31 g, 19.09 mmol) in water (5 mL) was added slowly, and the mixture stirred for 30 min at 0 °C. The pale yellow solution was then slowly added to an ice-cooled solution of KI (21.12 g, 127.3 mmol) in water (300 mL). The ice bath was then removed, and the foamy suspension was stirred for 2 h at ambient temperature. CH_2Cl_2 (100 mL) was added, followed by a saturated $Na_2S_2O_3$ solution (50 mL). The organic layer was separated, and the aqueous layer was extracted with CH_2Cl_2 (2×50 mL). The combined CH_2Cl_2 layer was dried over $MgSO_4$, the solvent was evaporated, and the crude product was purified by column chromatography (200 g silica gel, CH_2Cl_2/hexane/EtOAc 30:30:1) to yield **14** as colorless crystals (2.75 g, 81%). m.p. 200–201 °C; ^{1}H NMR (250 MHz, [D$_6$]DMSO): δ = 7.98 (s, 2H; 2Ar-*H*), 2.32 (s, 3H; Ar-C*H*$_3$); ^{13}C NMR (60 MHz, [D$_6$]DMSO): δ = 140.2, 138.7, 121.1, 118.7, 102.2, 19.7; IR (KBr): ν = 3056, 2236, 1815, 1560, 1448, 1418, 1409, 1380, 1286, 1231, 1030, 886, 706, 678, 626, 565 cm^{-1}; elemental analysis: calcd (%) for $C_9H_5N_2I$: C 40.33, H 1.88, N 10.45; found: C 40.53, H 2.08, N 10.45.

*Methyl 3,5-dicyano-4-iodobenzoate (**15**):* Water (2.5 mL) was carefully added to a solution of **14** (2.00 g, 7.46 mmol) in conc. H_2SO_4 (30 mL), and the resulting mixture cooled to 0 °C. $Na_2Cr_2O_7 \cdot 2H_2O$ (5.55 g, 18.65 mmol) was dissolved in water (20 mL) and added dropwise over 3 h to the cooled solution of **14**. After complete addition the cooling was removed, water (10 mL) added, and the solution was stirred at ambient temperature for another 60 min. The deep green mixture was poured slowly into ice–water (50 mL). EtOAc (50 mL) was added, and the mixture was stirred vigorously for 30 min. The organic layer was separated, and the aqueous layer was extracted with EtOAc (4×30 mL). The combined organic layer was washed twice with 20% H_2SO_4, the solvent was evaporated, and the residue was purified by column chromatography (200 g silica gel, CH_2Cl_2/MeOH 4:1) to yield 3,5-dicyano-4-iodobenzoic acid **14a** as a colorless solid (1.55 g, 70%). m.p. 254–255 °C; ^{1}H NMR (250 MHz, [D$_6$]DMSO): δ = 14.05 (br, 1H; COO*H*), 8.43 (s, 2H; 2Ar-*H*); ^{13}C NMR (60 MHz, [D$_6$]DMSO): δ = 164.1, 137.7, 132.4, 122.3, 118.2, 112.7; IR (KBr): ν = 3238, 3076, 2246, 1734, 1624, 1586, 1412, 1393, 1377, 1265, 1224, 1182, 1138, 1126, 1027, 936, 910, 777, 744, 708, 687, 646 cm^{-1}; ESI$^-$ MS: *m/z*: calcd for $C_9H_3IN_2O_2$-H$^+$: 296.9; found 296.8 [*M*−H]$^-$; elemental analysis calcd (%) for $C_9H_3IN_2O_2$: C 36.27, H 1.01, N 9.40; found: C 36.65, H 0.83, N 9.67.

The carboxylic acid **14a** (2.00 g, 6.71 mmol) was dissolved in MeOH (75 mL), conc H_2SO_4 (10 mL) was added and the mixture heated to reflux for 8 h. The solution was cooled to ambient temperature and carefully diluted with water (50 mL), then the pH was adjusted to ~3 with saturated $NaHCO_3$. The aqueous layer was extracted with EtOAc (3×30 mL), the organic layers were combined, and the solvent was evaporated. The crude product was purified by column chromatography (200 g silica gel, hexane/EtOAc 2:1) to yield **15** as a colorless solid (1.98 g, 94%). m.p. 189 °C; ^{1}H NMR (250 MHz, [D$_6$]DMSO): δ = 8.45 (s, 2H; 2Ar-*H*), 3.90 (s, 3H; OC*H*$_3$); ^{13}C NMR (60 MHz, [D$_6$]DMSO): δ = 163.3, 140.1, 136.8, 131.8, 116.3, 113.5, 53.0; IR (KBr): ν = 3058, 2950, 2236, 1718, 1588, 1439, 1394, 1313, 1233, 1132, 1030, 992, 951, 891, 772, 762, 712, 639 cm^{-1}; elemental analysis calcd (%) for $C_{10}H_5IN_2O_2$: C 38.49, H 1.61, N 8.98; found: C 38.76, H 1.73, N 9.18.

*Methyl 3,5-dicyano-4-(8-hydroxynaphthalen-1-yloxy)benzoate (**16**):* A solution of 1,8-dihydroxynaphthalene (0.77 g, 4.81 mmol) in anhydrous DMF (8 mL) was added dropwise to a suspension of NaH (0.18 g of a 60% dispersion in mineral oil, 4.49 mmol) in anhydrous DMF (10 mL) under argon. When the addition was complete, the resulting yellow solution was stirred at ambient temperature for 30 min. Subsequently, a solution of **15** (1.00 g, 3.20 mmol) in anhydrous DMF (10 mL) was added, and the mixture was stirred for 30 min at 60 °C. The red solution was allowed to cool to room tem-

perature, poured into acidic water (50 mL of 2 N HCl) and extracted with EtOAc (2×20 mL). The combined organic layer was dried over $MgSO_4$ and concentrated in vacuo, and the crude product was purified by column chromatography (65 g silica gel, hexane/EtOAc 3:1) to yield **16** as a slightly yellow solid (0.77 g, 70%). m.p. 207 °C; ^{1}H NMR (250 MHz, [D$_6$]DMSO): δ = 10.06 (br, 1H; Ar-O*H*), 8.56 (s, 2H; phenyl-*H*), 7.86 (dd, *J* = 0.8, 8.3 Hz, 1H; naphthyl-*H*), 7.48–7.33 (m, 4H; naphthyl-*H*), 6.84 (dd, *J* = 0.9, 7.6 Hz, 1H; naphthyl-*H*), 3.88 (s, 3H; O*CH*$_3$); ^{13}C NMR (60 MHz, [D$_6$]DMSO): δ = 163.5, 163.0, 152.2, 149.1, 140.0, 136.7, 127.4, 125.6, 124.3, 119.0, 118.0, 117.5, 113.5, 110.7, 103.3, 52.8; IR (KBr): ν = 3510, 3084, 2957, 2238, 1733, 1712, 1629, 1610, 1597, 1578, 1458, 1433, 1395, 1352, 1315, 1285, 1234, 1213, 1195, 1107, 1021, 987, 933, 903, 821, 760, 724, 614 cm^{-1}; ESI$^-$ MS: *m/z*: calcd for $C_{20}H_{12}N_2O_4$-H$^+$: 343.1; found 343.1 [*M*−H]$^-$; elemental analysis calcd (%) for $C_{20}H_{12}N_2O_4$: C 69.76, H 3.51, N 8.14; found: C 69.53, H 3.62, N 7.95.

*Methyl 3,5-bis((*tert*-butoxycarbonylamino)methyl)-4-(8-hydroxynaphthalen-1-yloxy)benzoate (**17**):* A solution of **16** (200 mg, 0.581 mmol) and Boc_2O (500 mg, 2.29 mmol) in anhydrous MeOH (10 mL) was added to a suspension of Pd/C (73 mg, 10% on active charcoal) and PtO_2 (34 mg) in anhydrous MeOH (10 mL). The mixture was stirred in a steel autoclave under H_2 (45 bar) for 24 h at 55 °C. The catalyst was removed by filtration through Celite, the solvent was evaporated, and the crude product was purified by column chromatography (60 g silica gel, hexane/EtOAc 4:1) to yield **17** as colorless solid (220 mg, 69%). m.p. 80 °C; ^{1}H NMR (250 MHz, [D$_6$]DMSO): δ = 9.50 (s, 1H; O*H*), 7.89 (s, 2H; 2Ar-*H*), 7.51 (dd, *J* = 0.8, 8.3 Hz, 1H; naphthyl-*H*), 7.42–7.35 (m, 4H; 2naphthyl-*H*, 2N*H*), 7.21 (t, *J* = 7.9 Hz, 1H; naphthyl-*H*), 6.94 (m, 1H; naphthyl-*H*), 6.22 (d, *J* = 7.5 Hz, 1H; naphthyl-*H*), 4.21 (dd, *J* = 5.4/16.6 Hz, 2H; Ar-C*H*$_2$), 3.93–3.84 (m, 5H; COOC*H*$_3$, Ar-C*H*$_2$), 1.36 (s, 18H; 2OC(C*H*$_3$)$_3$); ^{13}C NMR (60 MHz, [D$_6$]DMSO): δ = 165.6, 155.6, 153.8, 153.4, 152.3, 137.0, 133.4, 127.5, 126.5, 125.8, 122.3, 118.6, 114.7, 110.8, 106.8, 78.0, 52.1, 38.2, 28.0; IR (KBr): ν = 3467, 3373, 3058, 2977, 2931, 1721, 1634, 1606, 1581, 1514, 1456, 1437, 1394, 1367, 1314, 1298, 1249, 1221, 1169, 1117, 1078, 1049, 1026, 1001, 968, 941, 901, 862, 834, 818, 773, 756 cm^{-1}; ESI$^+$ MS: *m/z*: calcd for $C_{30}H_{36}N_2O_8$+H$^+$: 553.3; found 553.4 [*M*+H]$^+$; elemental analysis calcd (%) for $C_{30}H_{36}N_2O_8$: C 65.20, H 6.57, N 5.07; found: C 65.28, H 6.78, N 4.87.

*Dihydrochloride of methyl 3,5-bis((4,5-dihydro-1*H*-imidazol-2-ylamino)methyl)-4-(8-hydroxynaphthalen-1-yloxy)benzoate (**12a**):* Dry MeOH (2 mL) was cooled to 0 °C, and acetyl chloride (1 mL) was slowly added dropwise, followed by stirring for 30 min. Compound **17** (50 mg, 90.5 μmol) was dissolved in anhydrous MeOH (0.5 mL), added dropwise to the acidic MeOH solution, which was stirred for 30 min at 0 °C, then 30 min at ambient temperature. When the reaction was complete, the solvent was evaporated, and the residue was dried in vacuo. The deprotected diamine was dissolved in MeOH/NEt_3 (2:1, 3 mL), **18** (41 mg, 0.27 mmol) was added, and the solution was stirred for 1 h at ambient temperature. The solvent was evaporated, and the residue was purified by column chromatography (20 g silica gel, EtOAc/EtOH/H_2O/HOAc 11:4:4:1). After the solvent had been removed, the residue was redissolved in MeOH, picric acid (138 mg, water content 40%; 0.36 mmol, 4 equiv) was added, followed by addition of water until the solution became turbid. This mixture was heated to reflux, slowly cooled to ambient temperature, and then stored at 4 °C overnight. The orange-brown solid was filtered and dried in vacuo. To replace the counterion of **12** with chloride, the residue was dissolved in a little MeOH, and filtered through a small column filled with Dowex 1×8 (Cl$^-$ form). Further elution of the column with MeOH and removal of the solvent yielded **12a** as a yellow solid (21 mg, 41%). M.p. 189–191 °C; ^{1}H NMR (250 MHz, [D$_6$]DMSO): δ = 9.91 (s, 1H; O*H*), 8.75–8.67 (m, 2H; 2benzyl-N*H*), 8.50–8.00 (br s, 6H; 4dihydroimidazole-N*H*, 2phenyl-*H*), 7.54 (d, *J* = 7.9 Hz, 1H; naphthyl-H), 7.41 (m, 2H; naphthyl-H), 7.22 (t, *J* = 8.0 Hz, 1H; naphthyl-H), 7.10–7.02 (m, 1H; naphthyl-H), 6.22 (dd, *J* = 1.4, 7.6 Hz, 1H; naphthyl-H), 4.44–4.25 (m, 4H; 2benzene-CH$_2$), 3.92 (s, 3H; COOCH$_3$), 3.43 (s, 8H; 4dihydroimidazole-CH$_2$); ^{13}C NMR (60 MHz, [D$_6$]DMSO): δ = 165.3, 159.4, 153.7, 153.3, 137.0, 131.5, 130.0, 127.5, 127.1, 125.9, 122.6, 118.7, 115.0, 111.4, 107.3, 52.4, 42.3, 40.8; IR (KBr): ν = 3411, 3158, 3053, 1723, 1670, 1603, 1578, 1490, 1456, 1436, 1386, 1355, 1317, 1289, 1222, 1197, 1070, 1025, 1004, 932, 902, 819, 758, 706, 632 cm^{-1}; ESI$^+$ MS: *m/z*: calcd for $C_{26}H_{28}N_6O_4$ + H$^+$: 489.2; found 489.3 [*M*+H]$^+$, 245.0 [*M*+2H]$^{2+}$.

*2-(8-Hydroxynaphthalen-1-yloxy)isophthalonitrile (**20**):* NaH (0.25 g of a 60% dispersion in mineral oil, 6.2 mmol) was suspended in dry DMF (3 mL) under argon. A solution of 1,8-dihydroxynaphthalene (1 g, 6.2 mmol) in dry DMF (8 mL) was added slowly, and the resulting mixture was stirred for an additional 30 min. A solution of **19**[15] (1.6 g, 6.2 mmol) in dry DMF (8 mL) was added dropwise, and the mixture was heated (90 °C, 6 h). The solution was cooled to room temperature and quenched by pouring into ice-cooled aqueous HCl (30 mL, 1 M). EtOAc (20 mL) was added, then the organic layer was separated and dried over $MgSO_4$. The solvent was removed in vacuo, the crude product was purified by flash chromatography (EtOAc/hexane 1:1) and recrystallized from EtOAc/hexane to yield **20** as a crystalline solid (1.1 g, 62%). m.p. 149–151 °C; ^{1}H NMR (250 MHz, [D$_6$]DMSO): δ = 9.96 (s, 1H; -O*H*), 8.16 (d, *J* = 7.8 Hz, 2H; 2Ar-*H*), 7.78 (d, *J* = 8.0 Hz, 1H; naphthyl-*H*), 7.44–7.34 (m, 4H; 4naphthyl-*H*), 7.11 (d, *J* = 7.2 Hz, 1H; naphthyl-*H*), 6.88 (d, *J* = 7.2 Hz, 1H; Ar-*H*); ^{13}C NMR (60 MHz, [D$_6$]DMSO): δ = 160.2, 152.8, 150.6, 139.4, 136.9, 127.4, 126.3, 125.6, 123.7, 118.8, 117.3, 115.9, 114.3, 110.6, 104.0; IR (KBr): ν = 3854, 3839, 3676, 3650, 3506, 3494, 3069, 2490, 2240, 1978, 1930, 1858, 1755, 1734, 1629, 1610, 1573, 1514, 1451, 1394, 1301, 1247, 1217, 1194, 1157, 1111, 1081, 1022, 968, 932, 866, 854, 819, 800, 780, 754, 669, 628, 612, 570 cm^{-1}; ESI$^-$ MS: *m/z*: calcd for $C_{18}H_{10}O_2N_2$-H$^+$: 285.1; found: 284,9 [*M*−H]$^-$; elemental analysis calcd (%) for $C_{18}H_{10}N_2O_2$: C 75.52, H 3.52, N 9.79; found: C 75.54, H 3.56, N 9.80.

tert-*Butyl (2-(8-hydroxynaphthalen-1-yloxy)-1,3-phenylene)bis(methylene)dicarbamate (**21a**):* Pd/C (0.28 g, 10% on active charcoal) and PtO_2 (0.09 g) were suspended in dry MeOH (10 mL). A solution of di-*tert*-butyl dicarbonate (0.64 g, 2.9 mmol) and **20** (0.4 g, 1.4 mmol) in dry MeOH (20 mL) was added, and the mixture was stirred under hydrogen (40 bar) at 55 °C in a steel autoclave for 42 h. H_2 was replaced with argon, the catalyst was filtered off (Celite), and the solvent was evaporated. The crude product was purified by flash chromatography (EtOAc/hexane 1:1), and the solid was recrystallized from EtOAc/hexane (0.5 g, 73%). m.p. 90 °C; ^{1}H NMR: (250 MHz, [D$_6$]DMSO): δ = 9.39 (s, 1H; O*H*), 7.48 (d, *J* = 8.1 Hz, 1H; naphthyl-*H*), 7.39–7.21 (m, 8H; 3Ar-*H*, 2N*H*, 3naphthyl-*H*), 6.93–6.90 (m, 1H; naphthyl-*H*), 6.21 (d, *J* = 7.6 Hz, 1H; naphthyl-*H*), 4.16 (dd, *J* = 5.3, 16.2 Hz, 2H; C*H*$_2$), 3.88 (dd, *J* = 6.4, 16.1 Hz, 2H; C*H*$_2$), 1.34 (s, 18H; 2C(C*H*$_3$)$_3$); ^{13}C NMR (60 MHz, [D$_6$]DMSO): δ = 155.5, 153.9, 153.8, 148.1, 136.9, 132.6, 127.4, 126.6, 125.8, 125.6, 121.9, 118.6, 114.5, 110.7, 106.3, 77.8, 38.3, 28.0; IR (KBr): ν = 3854, 3822, 3802, 3752, 3736, 3712, 3690, 3676, 3650, 3630, 3461, 3366, 3056, 2979, 2932, 2345, 1924, 1683, 1630, 1609, 1580, 1528, 1457, 1392, 1366, 1299, 1277, 1251, 1223, 1166, 1088, 1047, 1028, 976, 895, 859, 851, 819, 775, 757, 636, 570 cm^{-1}; ESI$^+$ MS: *m/z*: calcd for $C_{28}H_{34}O_6N_2$ + H$^+$: 495.2; found: 495.5 [*M*+H]$^+$; elemental analysis calcd (%) for $C_{28}H_{34}N_2O_6$: C 68.00, H 6.93, N 5.66; found: C 67.96, H 7.01, N 5.36.

*Picrate of 1-(2,6-bis((4,5-dihydro-1H-imidazol-2-ylamino)methyl)phenoxy)naphthalen-8-ol (**11**):* Acetyl chloride (2 mL) was added to a solution of **21a** (0.27 g, 0.55 mmol) in MeOH (3 mL) and stirred at ambient temperature for 2 h. The solvent was evaporated, and the residue (**21b**) was dissolved in NEt_3/H_2O/MeOH (1:1:1, 6 mL). Compound **18** (0.18 g, 1.2 mmol) was added, and the reaction mixture was stirred at room temperature for another 12 h. H_2O (10 mL) and 2 N NaOH were added to a final pH of 13, and the mixture was extracted twice with CH_2Cl_2 to remove NEt_3. After evaporation of the aqueous phase to about 50%, it was acidified with 2 N HCl, evaporated completely, and dried in vacuo. Separation of the hydrochloride of **11** from NaCl was achieved by extracting the solid residue three times with hot, dry MeOH, and the solution was reduced to 10 mL. Picric acid (0.44 g; water content 40%, 2 equiv) in MeOH (5 mL) was added, then water was added until the mixture became turbid. The precipitate was redissolved by heating, and yellow crystals of **11** formed upon slow cooling (0.35 g, 70%). $R_f = 0.16$ (EtOAc/EtOH/H_2O/HOAc 15:5:4:1); m.p. 215–216 °C; ^{1}H NMR: (250 MHz, [D$_6$]DMSO); $\delta = 9.35$ (s, 1H; O*H*), 8.80–6.80 (br s, 4H; 4 ethylene-N*H*), 8.59 (s, 4H; 4 picrate-*H*), 8.52–8.38 (m, 2H; Ar-*H*), 7.54–7.41 (m, 6H; Ar-*H*, 3 naphthyl-*H*, 2N*H*), 7.32 (t, $J = 7.8$ Hz, 1H; naphthyl-*H*), 7.00 (t, $J = 3.8$, 1H; naphthyl-*H*), 6.18 (d, $J = 7.6$ Hz, 1H; naphthyl-*H*), 4.34 (dd, $J = 4.6$, 15.3 Hz, 2H; Ar-CH_2), 4.21 (dd, $J = 6.2$/15.5, 2H; Ar-CH_2), 3.39 (s, 8H; 4CH_2); ^{13}C NMR (60 MHz, [D$_6$]DMSO): $\delta = 160.7$, 159.0, 158.9, 153.9, 153.6, 148.8, 141.7, 136.9, 130.4, 129.3, 127.6, 125.9, 125.1, 124.1, 122.3, 118.8, 114.5, 110.7, 42.5, 42.3, 41.1, 40.9; IR (KBr): $\nu = 3903$, 3890, 3870, 3854, 3838, 3821, 3806, 3785, 3770, 3758, 3750, 3744, 3735, 3724, 2711, 2701, 3689, 3676, 3656, 3649, 3628, 2618, 3608, 3587, 3567, 3366, 3331, 3243, 3081, 2928, 2373, 2344, 2278, 1910, 1870, 1845, 1751, 1740, 1734, 1718, 1698, 1675, 1654, 1636, 1600, 1578, 1560, 1534, 1522, 1508, 1499, 1490, 1475, 1466, 1458, 1450, 1431, 1394, 1384, 1364, 1334, 1314, 1296, 1270, 1188, 1162, 1134, 1078, 1025, 942, 930, 910, 848, 818, 806, 788, 778, 746, 711, 682, 670, 662, 638, 620, 609, 584, 576, 559 cm^{-1}; elemental analysis calcd (%) for $C_{36}H_{32}N_{12}O_{16}\cdot 1H_2O$: C 47.68, H 3.75, N 18.53; found: C 47.74, H 3.88, N 18.57.

*Isolation of phosphorylation product **24**:* Compound **11** (40 mg, 0.044 mmol) was dissolved in anhydrous DMF (2 mL) and di-isopropylethylamine (0.07 mL) under argon. Bis-4-nitrophenylphosphate **5** (0.05 g, 0.11 mmol) was added, and the resulting mixture was stirred at ambient temperature for one week. The solution was diluted with water (10 mL), NaOH (2 mL, 2 N) was added, and the mixture was extracted with CH_2Cl_2 (2×15 mL). The organic layers were combined, the solvent was evaporated, and the solid was dried in vacuo. To replace the counter ions with chloride, the residue was dissolved in MeOH and filtered through a small column filled with Dowex 1×8 (Cl^- form). The solvent was evaporated again, and the residue was purified by flash chromatography (EtOAc/EtOH/H_2O/HOAc 14:4:4:1) to yield **24** (15 mg, 50%). The analytical sample was purified further by HPLC (Maisch Reprosil-Pur C18-AQ, 10 μ, 250×20 mm; water/acetonitrile (10:5), +0.1% trifluoroacetic acid; 9 mL min^{-1}). $R_f = 0.20$ (EtOAc/EtOH/H_2O/HOAc 14:4:4:1); ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta = 8.80$ (t, $J = 5.3$ Hz, 3H; exchangeable with D_2O, N*H*), 8.25 (d, $J = 9.1$ Hz, 2H), 7.75 (d, $J = 8.3$ Hz, 1H), 7.61 (d, $J = 8.2$ Hz, 1H), 7.55–7.49 (m, 5H), 7.42–7.37 (m, 1H), 7.32–7.27 (m, 2H; 1 proton exchangeable with D_2O), 7.17 (d, $J = 7.6$ Hz, 1H), 7.10 (s, 1H; exchangeable with D_2O, N*H*), 6.93 (s, 1H; exchangeable with D_2O, N*H*), 6.30 (d, $J = 7.7$ Hz, 1H), 4.35–4.17 (m, 4H; 2 Ar-CH_2), 3.36 (s, 8H; 2CH_2CH_2); ^{31}P NMR (121.5 MHz, [D$_6$]DMSO): $\delta = -11.91$; ESI$^+$ MS: *m/z*: calcd for $C_{30}H_{31}N_7O_7P$: 632.2; found 632.7 $[M]^+$.

Phosphorylation kinetics: All reactants and substances were weighed as precisely as possible, and dissolved in the required amounts of solvent. An NMR tube (6 cm long) was loaded with the solution, and sealed with a standard cap. 1-Nitronaphthalene (recrystallized from *n*-hexane) was used as the internal standard. All kinetic experiments were carried out at 30 °C (±0.1 °C) in a Lauda RM6 thermostat (Lauda, Königshofen, Gernamy) . Samples (3 μL) were taken at defined times, the reaction was quenched with 57 μL of a 1:1 mixture of water and MeOH (containing 3% TFA), and samples stored in liquid nitrogen. Aliquotes (20 μL) were then analyzed by HPLC with UV detection at 275 nm on a *Merck* LiChrospher 100 RP-18e (5 μm) column. For details see the Supporting Information.

DNA cleavage assay: The pUC19 plasmid DNA, prepared from DH5α cells, was purified by using a HiSpeed Plasmid Purification Kit (Qiagen, Hilden, Germany). The assay mixture (10 μL) contained **11** or **12** (0.1–10 mM, as hydrochlorides) in a 50 mM HEPES-NaOH (pH 7.0, if not otherwise indicated) and pUC19 DNA (22.5 nM). Incubation was performed at 37 °C for 20 h. Prior to electrophoresis, gel loading buffer (2 μL, 0.2% crocein orange G, 40% sucrose) and 20% SDS (1 μL) were added to each sample. The addition of SDS, and replacement of the usual tracking dye (bromophenol blue) with crocein orange G is recommended to prevent aggregation of compound and DNA during sample loading. Aliquots (10 μL) prepared in TBE buffer (90 mM Tris, 90 mM boric acid, 2 mM EDTA) were loaded on a 1% agarose gel that contained 0.5 μg mL^{-1} ethidium bromide (EtBr). The same buffer was used as the running buffer. Subsequent to electrophoresis (120 V, 150–180 min), the gel was placed on a transilluminator (302 nm) and photographed through a yellow-orange filter (Etbr filter 540/640, P575; Biostep, Jahnsdorf, Germany). The digital photos were analyzed by using Phoretix 1D Quantifier software (Nonlinear dynamics, UK). The peak areas of the supercoiled form and the open circle form were determined, and the percentage open circle form was calculated. To normalize the decreased ability of EtBr to intercalate into form I DNA (relative to forms II and III) a factor of 1.22 was used.[20]

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[17] Salt of diamine **21b** with picric acid ($C_{18}H_{20}N_2O_2 \cdot 2C_6H_2N_3O_7 \cdot H_2O \cdot 0.5CH_3OH$). $M_r = 786.6$. Crystal size: $0.04 \times 0.45 \times 0.60$ mm. Crystal system: rriclinic. Space group $P\bar{1}$. Cell dimensions: $a = 10.344$ (3) Å, $b = 11.479$ (5) Å, $c = 14.960$ (9) Å, $\alpha = 79.08$ (5)°, $\beta = 70.99$ (4)°, $\gamma = 88.60$ (7)°, $V = 1647.8$ (13) Å^3, $Z = 2$, $\rho_{ber} = 1.585$ mg m^{-3}. $\mu = 0.133$ mm^{-1}. $Mo_{K\alpha}$ radiation, $\lambda = 0.71073$ Å. $T = 141$ K. $\theta = 1.47$–27.94°. 14284 reflections collected, 6915 independent ($R_{int} = 0.1739$). $R1 = 0.1226$, $wR2 = 0.2164$ ($I > 2\sigma(I)$). CCDC 743278 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif

[18] *O*-phosphorylation product of compound **7**: ^{1}H NMR signals of the dihydroimidazolium ions ([D$_6$]DMSO, 250 MHz): 3.43 ppm, *s*, 8H. *N*-phosphorylation product of compound **7**: 3.74 (*m*, 2H), 3.53 (*m*, 6H).

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