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Communications to the Editor

Functional Group Convergency in a Binuclear Dephosphorylation Reagent[†]

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There is substantial interest in learning how to effect the abiotic cleavage of phosphate esters hydrolytically, as accomplished by DNA and RNA phosphatases. Many metals provide acceleration in phosphate ester hydrolysis,[1] but cleavage by Co(III) complexes bearing substitutable *cis*-coordination sites is amongst the fastest.[2] While phosphodiesters require only one Co(III) to effect hydrolysis, phosphomonoesters require two: one for charge neutralization and one to deliver a metal hydroxide.[2w] Accordingly, the 1:1 trpn–Co(III) complex with 5′-AMP is hydrolytically stable; addition of a second equivalent of Co(III) results in rapid, quantitative dephosphorylation to afford adenosine and the bidentate μ^4-phosphato complex.[2v] Perhaps not surprisingly, many phosphatases converge two and even three metal centers at their active sites.[3] We have considered whether or not a kinetic advantage might be gained by converging two Co(III) centers onto a phosphate-sized "pocket",[4] but rigidly so as to avoid μ-oxo dimer formation. We now report the synthesis of such a binuclear complex that demonstrates cooperativity in the hydrolysis of phosphomonoesters.

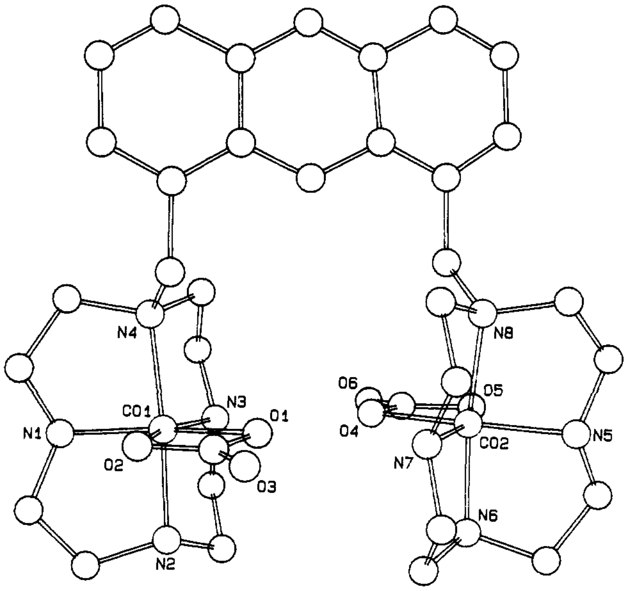

Figure 1. X-ray diffraction structure of compound **3**. The hydrogen atoms and counterions are not shown for ease of viewing the heavy atom framework.

The trpn·Co(III) complex sometimes demonstrates higher hydrolytic reactivity than does the cyclen·Co(III) complex,[2u] but we have been unable to prepare *N*-substituted trpn·Co(III) complexes as needed to bring the two together. However, *N*-substituted cyclen·Co(III) complexes can be made.[5] Thus, we synthesized binuclear complex **4** using the sequence shown in Scheme I.[6] Both models and MM calculations support the prediction that **4** fulfills two important design elements: (i) inorganic phosphate can "fit" into the internuclear pocket, and (ii) the complex is geometrically prohibited from forming an intramolecular μ-oxo dimer. Models likewise predicted that nonbonded interactions between cyclen and anthracene hydrogens would favor the cofacial conformation depicted in **4**. X-ray analysis of **3** (Figure 1), which could be obtained in crystalline form, supports this hypothesis.[7] The addition of inorganic phosphate **5a** to a D_2O solution (pD 7) of **4** yielded a set of ^{31}P NMR lines centered about 28 ppm; the spectrum is consistent with formation of a series of diastereomeric phosphate chelates, each with three P–O coordinations to Co(III) centers.[2p]

We conducted reactions of various Co(III) complexes with activated phosphate esters at 25 °C in pH 7.0 collidine buffer

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(1) See, for example: (a) Benkovic, S. J.; Dunikoski, L. K. *J. Am. Chem. Soc.* **1971,** *93,* 1526. (b) Eichhorn, G. L.; Tarien, E.; Butzow, J. J. *J. Biochem.* **1971,** *10,* 2014. (c) Murakami, Y. *Bull. Chem. Soc. Jpn.* **1971,** *44,* 1827. (d) Steffens, J. J.; Siewers, I. J.; Benkovic, S. J. *J. Biochem.* **1975,** *14,* 2431. (e) Butzow, J. J.; Eichhorn, G. L. *Nature (London)* **1975,** *254,* 358. (f) Haake, P.; Atwood, L. *Bioorg. Chem.* **1976,** *5,* 373. (g)Sigel, H.; Hofstetter, F.; Martin, R. B.; Milburn, R. M.; Scheller-Krattiger, V.; Scheller, K. H. *J. Am. Chem. Soc.* **1984,** *106,* 7935. (h) Hendry, P.; Sargeson, A. M. *J. Chem. Soc., Chem. Commun.* **1984,** 164. (i) Gutsche, C. D.; Mei, G. C. *J. Am. Chem. Soc.* **1985,** *107,* 7965. (j) Bose, R. W.; Cornelius, R. D.; Viola, R. E. *Inorg. Chem.* **1985,** *24,* 3989. (k) Hay, R. W.; Basak, A. K.; Purjari, M. P.; Perotti, A. *J. Chem. Soc., Dalton Trans.* **1986,** 2029. (l) Gellman, S. H.; Petter, R. Breslow, R. *J. Am. Chem. Soc.* **1986,** *108,* 2388. (m) Sheller-Krattiger, V.; Siegel, H. *Inorg. Chem.* **1986,** *25,* 2628. (n) Hendry, P.; Sargeson, A. M. *Aust. J. Chem.* **1986,** *39,* 1177. (o) Bastille, L. A.; Barton, J. K. *J. Am. Chem. Soc.* **1987,** *109,* 7550. (p) Herschlag, D.; Jencks, W. P. *J. Am. Chem. Soc.* **1987,** *109,* 4665. (q) Menger, F. M.; Gan, L. H.; Johnson, E.; Durst, D. H. *J. Am. Chem. Soc.* **1987,** *109,* 2800. (r) Morrow, J. R.; Trogler, W. C. *Inorg. Chem.* **1988,** *27,* 3387. (s) Fife, T. H.; Pujari, M. P. *J. Am. Chem. Soc.* **1988,** *110,* 7790. (t) Hendry, P.; Sargeson, A. M. *J. Am. Chem. Soc.* **1989,** *111,* 2521. (u) Clewley, R. G.; Ślebocka-Tilk, H.; Brown, R. S. *Inorg. Chim. Acta* **1989,** *157,* 233. (v) Breslow, R.; Huang, D.-L.; Anslyn, E. *PNAS* **1989,** *86,* 1746. (w) Komiyama, M.; Matsumoto, Y. *Chem. Lett.* **1989,** 719. (x) Nielsen, P. E.; Hiort, C.; Sönnichsen, S. H.; Buchardt, O.; Dahl; Nordén *J. Am. Chem. Soc.* **1992,** *114,* 4967. (y) Browne, K. A.; Bruice, T. C. *J. Am. Chem. Soc.* **1992,** *114,* 4951.

(2) (a) Spiro, T. G.; Farrell, F. J.; Kjellstrom, W. A. *Science* **1969,** *164,* 320. (b) Anderson, B.; Milburn, R. M.; Harrowfield, J. M.; Robertson, G. B.; Sargeson, A. M. *J. Am. Chem. Soc.* **1977,** *99,* 2652. (c) Suzuki, S.; Higashiyama, T.; Nakahara, A. *Bioinorg. Chem.* **1978,** *8,* 277. (d) Hubner, P. W.; Milburn, R. M. *Inorg. Chem.* **1980,** *19,* 1267; (e) Cornelius, R. D. *Inorg. Chem.* **1980,** *19,* 1286. (f) Harrowfield, J. M.; Jones, D. R.; Lindoy, L. F.; Sargeson, A. M. *J. Am. Chem. Soc.* **1980,** *102,* 7733. (g) Hediger, M.; Milburn, R. M. *J. Inorg. Biochem.* **1982,** *16,* 165. (h) Norman, P. R.; Cornelius, R. D. *J. Am. Chem. Soc.* **1982,** *104,* 2356. (i) Jones, D. R.; Lindoy, L. F.; Sargeson, A. M. *J. Am. Chem. Soc.* **1983,** *105,* 7327. (j) Milburn, R. M.; Rawji, G.; Hediger, M. *Inorg. Chim. Acta* **1983,** *79,* 248. (k) Jones, D. R.; Lindoy, L. F.; Sargeson, A. M. *J. Am. Chem. Soc.* **1984,** *106,* 7807. (l) Kenley, R. A.; Fleming, R. H.; Laine, R. M.; Tse, D. S.; Winterle, J. S. *Inorg. Chem.* **1984,** *23,* 1870. (m) Milburn, R. M.; Gautem-Basak, M.; Tribolet, R.; Sigel, H. *J. Am. Chem. Soc.* **1985,** *107,* 3315. (n) Milburn, R. M.; Massoud, S. S.; Tafesse, F. *Inorg. Chem.* **1985,** *24,* 2591. (o) Milburn, R. M.; Tafesse, F. *Inorg. Chim. Acta* **1987,** *135,* 119. (p) Haight, G. P., Jr. *Coord. Chem. Rev.* **1987,** *79,* 293. (q) Wijesekera, R.; Hendry, P.; Sargeson, A. M. *Aust. J. Chem.* **1992,** *45,* 1187. (r) Chin, J.; Zou, X. *Can. J. Chem.* **1987,** *65,* 1882. (s) Chin, J.; Banaszczyk, M.; Jubian, V. *J. Chem. Soc., Chem. Commun.* **1988,** 735. (t) Chin, J.; Zou, X. *J. Am. Chem. Soc.* **1988,** *110,* 223. (u) Chin, J.; Banaszczyk, M.; Jubian, V.; Zou, X. *J. Am. Chem. Soc.* **1989,** *111,* 186. (v) Chin, J.; Banaszczyk, M. *J. Am. Chem. Soc.* **1989,** *111,* 4103. (w) Chin, J. *Acc. Chem. Res.* **1991,** *24,* 145. (x) Kim, J. H.; Chin, J. *J. Am. Chem. Soc.* **1992,** *114,* 9792.

(3) Included are purple acid phosphatase, which is binuclear, and alkaline phosphatase, which is trinuclear.

(4) We have reported previously the synthesis of a flexible binuclear Co(III) complex that did *not* demonstrate cooperative phosphomonoester hydrolysis: Chung, Y. S.; Akkaya, E. A.; Venkatachalam, T. K.; Czarnik, A. W. *Tetrahedron Lett.* **1990,** *31,* 5413.

(5) (a) Akkaya, E.; Czarnik, A. W. *J. Am. Chem. Soc.* **1988,** *110,* 8553. (b) Akkaya, E. U.; Czarnik, A. W. *J. Phys. Org. Chem.* **1992,** *5,* 540.

(6) Synthetic and characterization information for **2-4** are included in the supplementary material.

(7) The carbonyl oxygens of **3** cannot occupy the same region of space, resulting in the skew conformation observed in the X-ray picture. We anticipate that this is not the case in aquo complex **4**, which unfortunately could not be crystallized.

0002-7863/93/1515-12165$04.00/0

Scheme I

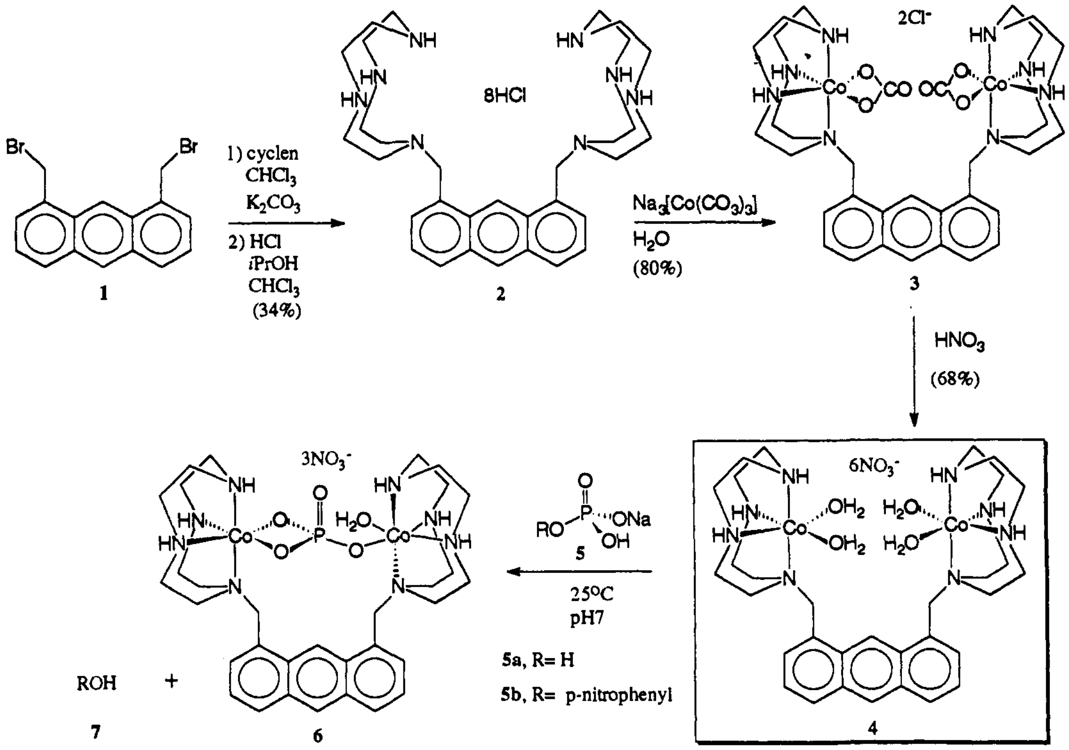

Table I. Phosphate Ester Hydrolysis Mediated by DiaquoCobalt(III) Complexes[a]

phosphate ester	**4**	cyclen·Co(III)	trpn·Co(III)	uncatalyzed	**4**/cyclen·Co(III)
p-nitrophenyl phosphate	1.33×10^{-2}	1.27×10^{-3}	9.25×10^{-4}	8.2×10^{-9} [b]	10
bis(*p*-nitrophenyl) phosphate	1.17×10^{-4}	1.66×10^{-4}	4.21×10^{-4} [d]	1.3×10^{-11} [c]	0.7

[a] Reaction conditions: 25 °C, 0.1 M collidine buffer, pH 7, 25 μM phosphate ester, 2.0 mM total Co(III), and monitored at 440 nm. All pseudo-first-order rate constants are reported in units of s^{-1}. [b] Reference 9, extrapolated from rate measurement at 39 °C, $\Delta S^\ddagger = 3.5$ eu. [c] Reference 10; extrapolated from rate measurement at 100 °C, $\Delta S^\ddagger = 25.5$ eu. [d] Trpn·Co(III) hydrolyzes bis(*p*-nitrophenyl) phosphate 2.5-fold faster than cyclen·Co(III). This rate increase agrees with the literature precedent[2u] observed at 50 °C.

(0.1 M)[8] under pseudo-first-order conditions. The reaction rateconstants using cyclen·Co(III), trpn·Co(III), and **4** (each total [Co(III)] = 2.0 mM) with *p*-nitrophenyl phosphate (PNPP; **5b**) and bis(*p*-nitrophenyl) phosphate (BPNPP) (each 25 μM) are provided in Table I. It is observed that the reaction of **4** under these conditions is 10 times greater than that of 2 equiv of cyclen·Co(III) and over 10^6 times greater than that of the uncatalyzed reaction. ^{31}P NMR analysis at the end of the reaction between **4** and PNPP reveals tricoordinated phosphate resonances exactly like those observed using inorganic phosphate. As expected from the mechanism of action, **4** does not show enhanced reactivity toward BPNPP, a phosphodiester.

In summary, we report: (1) the synthesis of a binuclear Co(III) complex structurally prohibited from forming a deactivating μ-oxo dimer; (2) X-ray analysis documenting cofacial orientation of the substitutable coordination sites; and (3) kinetic indication of cooperative phosphate hydrolysis by both Co(III) centers in the binuclear reagent.

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Supplementary Material Available: Synthetic details and characterization data for **2–4** (3 pages). Ordering information is given on any current masthead page.

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(8) Other investigators have utilized pH-stat conditions to avoid the use of buffers, which competitively react with and inactivate Co(III) complexes. We observe that use of collidine (2,4,6-trimethylpyridine; pK_a 7.43) permits buffering without rapid inactivation.

(9) Kirby, A. J.; Jencks, W. P. *J. Am. Chem. Soc.* **1965**, *87*, 3209.

(10) Kirby, A. J.; Younas, J. *J. Chem. Soc. B* **1970**, 510.