823; e) 1996 yearbook of the Deutsche Akademie der Naturforscher Leopoldina: M. Regitz, A. Mack, *LEOPOLDINA* **1997**, *42*, 407; f) T. W. Mackewitz, M. Regitz, *Synthesis* **1998**, 125; short-lived phosphaalkynes: A. C. Gaumont, J.-M. Denis, *Chem. Rev.* **1994**, *94*, 1413.

[2] a) J. F. Nixon, *Chem. Rev.* **1988**, *88*, 1327; b) P. Binger in *Multiple Bonds and Low Coordination in Phosphorus Chemistry* (Eds.: M. Regitz, O. J. Scherer), Thieme, Stuttgart, **1990**, p. 90; c) J. F. Nixon, *Chem. Ind.* **1993**, 404; d) J. F. Nixon, *Coord. Chem. Rev.* **1995**, *145*, 201; e) J. F. Nixon, *Chem. Soc. Rev.* **1995**, 319.

[3] G. Becker, G. Gresser, W. Uhl, *Z. Naturforsch. B* **1981**, *36*, 16.

[4] G. Becker, W. Becker, R. Knebl, H. Schmidt, U. Weber, M. Westerhausen, *Nova Acta Leopold.* **1985**, *59*, 55.

[5] T. Allspach, M. Regitz, G. Becker, W. Becker, *Synthesis* **1986**, 31.

[6] A. Mack, E. Pierron, T. Allspach, U. Bergsträßer, M. Regitz, *Synthesis* **1998**, 1305.

[7] a) P. Binger, R. Milczarek, R. Mynott, M. Regitz, W. Rösch, *Angew. Chem.* **1986**, *98*, 645; *Angew. Chem. Int. Ed. Engl.* **1986**, *25*, 644; b) P. Binger, R. Milczarek, R. Mynott, C. Krüger, H. Tsay, E. Raabe, M. Regitz, *Chem. Ber.* **1988**, *121*, 637.

[8] P. B. Hitchcock, M. J. Maah, J. F. Nixon, *J. Chem. Soc. Chem Commun.* **1986**, 737.

[9] P. Binger, B. Biedenbach, R. Schneider, M. Regitz, *Synthesis* **1989**, 960.

[10] P. Binger, S. Leininger, J. Stannek, B. Gabor, R. Mynott, J. Bruckmann, C. Krüger, *Angew. Chem.* **1995**, *107*, 2411; *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 2227.

[11] P. Binger, G. Glaser, S. Albus, C. Krüger, *Chem. Ber.* **1995**, *128*, 1261.

[12] P. Binger, S. Leininger, K. Günther, U. Bergsträßer, *Chem. Ber.* **1997**, *130*, 1491.

[13] R. Milczarek, W. Rüssler, P. Binger, K. Jonas, K. Angermund, C. Krüger, M. Regitz, *Angew. Chem.* **1987**, *99*, 957; *Angew. Chem. Int. Ed. Engl.* **1987**, *26*, 908.

[14] P. Binger, S. Stutzmann, J. Stannek, K. Günther, P. Phillips, R. Mynott, J. Bruckmann, C. Krüger, *Eur. J. Inorg. Chem.* **1999**, 763.

[15] P. Binger, G. Glaser, B. Gabor, R. Mynott, *Angew. Chem.* **1995**, *107*, 114; *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 81.

[16] A metal-assisted pentamerization of *t*BuC≡P to a metal-free pentamer starting from ($\eta^4$-1-methylnaphthaline)($\eta^6$-toluene)iron was described by U. Zenneck et al.: D. Hu, H. Schäufele, H. Pritzkow, U. Zenneck, *Angew. Chem.* **1989**, *101*, 929; *Angew. Chem. Int. Ed. Engl.* **1989**, *28*, 900; D. Böhm, D. Hu, U. Zenneck, *Phosphorus Sulfur Silicon* **1993**, *77*, 5.

[17] a) M. Scheer, K. Schuster, T. A. Budzichowski, M. H. Chisholm, W. E. Streib, *J. Chem. Soc. Chem. Commun.* **1995**, 1671; b) M. Scheer, P. Kramkowski, K. Schuster, *Organometallics* **1999**, *18*, 2874; c) P. Kramkowski, G. Baum, U. Radius, M. Kaupp, M. Scheer, *Chem. Eur. J.* **1999**, *5*, 2890.

[18] X-ray crystal structure data for $\mathbf{2}\cdot C_7H_8$: Stoe IPDS diffractometer, $Mo_{K\alpha}$ ($\lambda = 0.71069$ Å), structure solution with direct methods, least-squares method, all non-hydrogen atoms were anisotropically refined. The hydrogen atoms were fixed in idealized positions and refined isotropically as riding models. $C_{63}H_{55}O_{13}P_5W_3\cdot C_7H_8$, $M_r = 1818.60$, crystal dimensions $0.45\times0.19\times0.08$ mm$^3$, triclinic, space group $P\bar{1}$ (no. 2); $a = 15.985(3)$, $b = 16.797(3)$, $c = 17.358(4)$ Å, $\alpha = 90.99(3)$, $\beta = 111.35(3)$, $\gamma = 116.62(3)^\circ$, $T = 200(1)$ K, $Z = 2$, $V = 3788.5(13)$ Å$^3$, $\rho_{calcd} = 1.594$ Mg m$^{-3}$, $\mu(Mo_{K\alpha}) = 47.05$ cm$^{-1}$; of 13638 independent reflections ($2\theta_{max} = 53^\circ$), 12004 were observed with $F_o = 4\sigma(F_o)$; 900 parameters, $R_1 = 0.0361$, $wR_2 = 0.1069$; structure solution and refinement with the programs SHELXS-86[28a] and SHELXL-93.[28b] Crystallographic data (excluding structure factors) for the structure reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-125116. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: (+44) 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).

[19] For seven-coordinate W atoms, see also K. W. Chiu, D. Lyons, G. Wilkinson, M. Thornton-Pett, M. B. Hursthouse, *Polyhedron* **1983**, *2*, 803; R. M. Laine, R. E. Moriarty, R. Bau, *J. Am. Chem. Soc.* **1972**, *94*, 1402; G. Huttner, J. Borm, L. Zsolnai, *J. Organomet. Chem.* **1986**, *304*, 309.

[20] M. J. Aroney, I. E. Buys, M. S. Davies, T. W. Hambley, *J. Chem. Soc. Dalton Trans.* **1994**, 2827.

[21] R. Appel in *Multiple Bonds and Low Coordination in Phosphorus Chemistry* (Eds.: M. Regitz, O. J. Scherer), Thieme, Stuttgart **1990**, p. 157.

[22] J. E. Huheey, E. A. Keiter, R. L. Keiter in *Anorganische Chemie: Prinzipien von Struktur und Reaktivität*, de Gruyter, Berlin, **1995**, p. 1164.

[23] A. Simon, H. Borrmann, J. Horakh, *Chem. Ber.* **1997**, *130*, 1235.

[24] P. Kramkowski, M. Scheer, G. Baum, *J. Organomet. Chem.* **1998**, *553*, 511.

[25] M. Scheer, K. Schuster, U. Becker, *Phosphorus Sulfur Silicon* **1996**, *109–110*, 141.

[26] J. Grobe, D. Le Van, B. Lüth, M. Hegemann, *Chem. Ber.* **1990**, *123*, 2317.

[27] G. Becker, W. Schwarz, N. Seidler, M. Westerhausen, *Z. Anorg. Allg. Chem.* **1992**, *612*, 72.

[28] a) G. M. Sheldrick, *SHELXS-86*, Universität Göttingen, **1986**; b) G. M. Sheldrick, *SHELXL-93*, Universität Göttingen, **1993**.

# Facile, Novel Methodology for the Synthesis of Spiro[pyrrolidin-3,3′-oxindoles]: Catalyzed Ring Expansion Reactions of Cyclopropanes by Aldimines**

Phil B. Alper, Christiane Meyers, Andreas Lerchner, Dionicio R. Siegel, and Erick M. Carreira*

The spiro[pyrrolidin-3,3′-indole] ring system is a recurring structural motif in a number of natural products such as vinblastine and vincristine that function as cytostatics and are of prime importance in cancer chemotherapy.[1] The related spiro[pyrrolidin-3,3′-oxindole] ring system has been identified in a number of other cytostatic alkaloids, exemplified by spirotryprostatin A and strychnophylline.[2] Moreover, these structures embody stereochemical and structural complexities that continue to challenge the synthetic chemist.[3, 4] In combination, these factors have driven the development of novel, versatile, and efficient methods aimed at the synthesis of the spiro[pyrrolidin-3,3′-oxindole] moiety and related structures, as exemplified recently in the elegant total synthesis of spirotryprostatin by Danishefsky et al. and of aspidophytine by Corey et al.[5] We have been interested in the development of a variety of methods for the stereocontrolled synthesis of alkaloids.[6] Herein we report a novel

[*] Prof. Dr. E. M. Carreira, Dr. P. B. Alper, C. Meyers, A. Lerchner
Laboratorium für Organische Chemie, ETH-Zentrum
Universitätstrasse 16, CH-8092 Zürich (Switzerland)
Fax: (+41) 1-632-13-28
E-mail: carreira@org.chem.ethz.ch

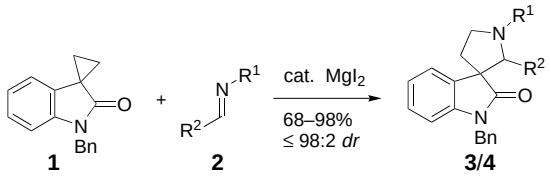
D. R. Siegel
Arnold and Mabel Beckman Laboratory for Chemical Synthesis
California Institute of Technology
Pasadena, CA 91125 (USA)

[**] We thank the ETH, Kontaktgruppe für Forschungsfragen (KGF), and Hoffmann-La Roche AG for their generous support.

Supporting information for this article is available on the WWW under http://www.wiley-vch.de/home/angewandte/ or from the author.

  1433-7851/99/3821-3186 $ 17.50+.50/0 

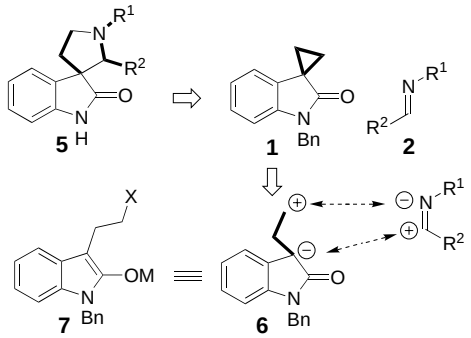
approach to the construction of the spiro[pyrrolidin-3,3′-oxindole] ring system from the reaction of spiro[cyclopropan-1,3′-oxindole] **1** and *N*-alkyl as well as *N*-arylsulfonyl aldimines **2** [Eq. (1)]. The successful implementation of the




(1)

unprecedented ring-expansion we report is made possible by careful selection of a catalyst ($MgI_2$) in which the Lewis acidity of the metal center ($Mg^{II}$) and nucleophilicity of the counterion ($I^-$) appear to operate in synergy.

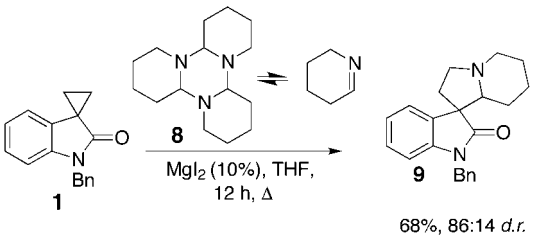
The most common approaches to spiro[pyrrolidin-3,3′-oxindoles] commence with tryptamine derivatives and assemble a dihydro- or tetrahydro- carboline by Bischler–Napieralski or Pictet–Spengler annulation reactions.[7, 8] Oxidative rearrangements of the resulting carbolines then furnish spirocyclic pyrrolidine-3,3′-oxindoles.[4, 8] We envisioned a more direct, alternate bond-construction strategy to these ring systems. In our retrosynthetic analysis, **5** is disconnected to cyclopropane **1** and aldimine **2** (Scheme 1). As depicted for




Scheme 1. Retrosynthetic and charge affinity analysis of the spiro[pyrrolidin-3,3′-oxindoles] ring system.

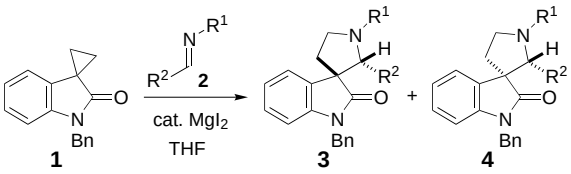
synthon **6**, the charge-affinity pattern of the cyclopropane complements that of an aldimine.[9] The dissonant charge-affinity pattern of cyclopropanes is manifest in their well-known reactivity, when substituted with electron-withdrawing groups, to serve as homo-Michael acceptors.[10] Pioneering work by Danishefsky et al. had established the participation of *doubly* activated cyclopropanes in tandem reactions resulting in ring formation.[11] By contrast, the strategy depicted in Scheme 1 necessitates nucleophilic ring opening of a *singly* activated ring system by a weakly nucleophilic aldimine. We speculated that the use of a catalyst exhibiting dual electrophilic and nucleophilic activation would enable the desired reaction. In this regard the selection of a Lewis acidic metal possessing nucleophilic counterions could efficiently lead to ring-opened products **7** possessing the same reactivity pattern of synthon **6**.[12] As a caveat, the application of dual nucleophilic/electrophilic catalysts to the reaction of imines and spiro-fused cyclopropanes would lead to the desired spiro[pyrrolidin-3,3′-oxindoles] provided competitive intramolecular cyclization (by O-alkylation) is precluded.

The implementation of the plan depicted in Scheme 1 as a practical alternative to existing strategies to the title ring systems, in part, rests on the accessibility of the starting material (**1**). This oxindole was easily prepared on multigram scale from the known *N*-benzyloxindole and 1,2-dibromoethane (NaH, DMF, 23 °C, 73 % yield).[13] In our initial study, **1** and **8**[14] were allowed to react in the presence of a number of metal catalysts (e.g. $MgBr_2$, $Mg(OTf)_2$, $ZnI_2$, $Zn(OTf)_2$, LiI (Tf = trifluoromethansulfonate)), whereupon the use of $MgI_2$ was identified as optimal [Eq. (2)].[15] Thus, treatment of **1** and **8** in THF at 125 °C in the presence of 5 % $MgI_2$ gave tetracyclic adduct **9** in 68 % yield as an 86:14 mixture of diastereomers.




(2)

The scope of this reaction was explored subsequently by examining aliphatic and aromatic *N*-allyl aldimines along with *N*-*p*-arylsulfonyl aldimines [Eq. (3), (Table 1)]. Treatment of **1** with catalytic amounts of $MgI_2$ (10 mol %) in THF at 60–80 °C afforded spiro[pyrrolidin-3,3′-oxindoles] in good yields




(3)

Table 1. Reaction of spiro[cyclopropan-1,3′-oxindole] **1** with aldimines.[a]

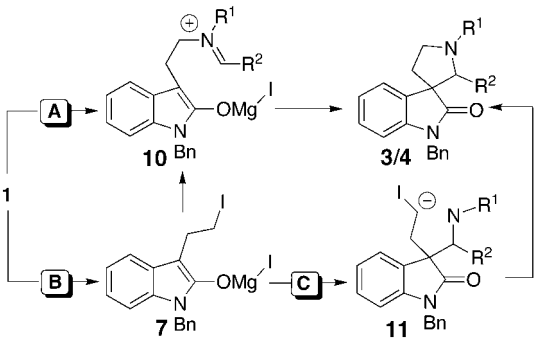
| Entry | $R^1$ | $R^2$ | Yield [%] | **3**:**4** |
|---|---|---|---|---|
| 1 | $-CH_2CH_2CH_2CH_2-$ | | 68 | 86:14 |
| 2 | Allyl | Et | 55 | 91:1 |
| 3 | Allyl | $Me_2CH$ | 83 | 80:20 |
| 4 | Allyl | Ph | 99 | 79:21 |
| 5 | Bu | Ph | 97 | 81:19 |
| 6 | *p*-$TolSO_2$ | Ph | 97 | 91:9 |
| 7 | *p*-$TolSO_2$ | 2-Me-$C_6H_4$ | 89 | 98:2 |
| 8 | *p*-$TolSO_2$ | 4-Me-$C_6H_4$ | 96 | 64:36 |
| 9 | *p*-$TolSO_2$ | 2-Br-$C_6H_4$ | 82 | 98:2 |
| 10 | *p*-$TolSO_2$ | 4-Br-$C_6H_4$ | 92 | 82:18 |
| 11 | *p*-$TolSO_2$ | 4-$CF_3$-$C_6H_4$ | 97 | 84:16 |
| 12 | *p*-$TolSO_2$ | 4-OMe-$C_6H_4$ | 75 | 67:33 |
| 13 | *p*-$TolSO_2$ | Furyl | 97 | 85:15 |
| 14 | *p*-$TolSO_2$ | PhCH=CH | 62 | 74:16 |
| 15 | *p*-$TolSO_2$ | PhCH=C(Me) | 55 | 52:48 |
| 16 | *p*-$TolSO_2$ | $iPr_3SiC\equiv C$ | 77 | 98:2 |

[a] The assignment of the configuration of the products was established by $^1H$ HMR spectroscopy and correlation to the major product of entry 5, for which an X-ray structure was obtained (see Supporting Information). The diastereomeric ratios for entries 1–5 were determined from the mass of the separated, purified products. The diastereomeric ratios reported for entries 6–16 were obtained by HPLC analysis.

  1433-7851/99/3821-3187 $ 17.50+.50/0 

and useful diastereoselectivities for both classes of electron-rich and electron-deficient aldimines. The *N*-arylsulfonyl imines reacted smoothly at 60 °C, while the more electron-rich *N*-allyl imines required reaction temperatures of 80 °C for product formation. We have observed that the stereoselectivity could be improved by appropriate selection of the *N*-alkyl protecting group on the oxindole or the *N*-arylsulfonyl group of the aldimine. In this regard, the diastereoselectivity of the reaction of the *N*-tosyl imine of *p*-tolualdehyde and **1** (entry 8, 64:36 *dr*) could be improved to 71:29 by utilizing the *N*-benzhydril rather than a *N*-benzyl protected oxindole. Moreover, the use of the *N*-*β*-napthylsulfonyl aldimine derivative of *p*-tolualdehyde with **1** affords adduct in 89:11 *dr*.

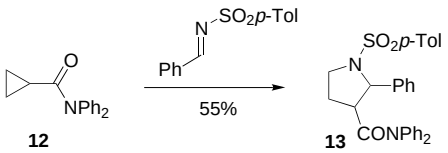
For the process we have delineated, three mechanistic pathways may be postulated that differ in the sequence of C–N and C–C bond-formation and in the nucleophilic ring-opening step (Scheme 2). In the first pathway (**A**, Scheme 2), the imine nitrogen participates directly in the nucleophilic



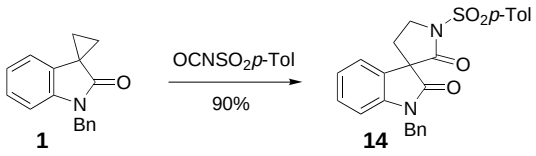

Scheme 2. Potential mechanistic pathways leading to the formation of the pyrrolidine ring.

cleavage of the cyclopropyl ring, generating iminium ion **10** which can undergo ring closure (**1** → **10** → **3/4**). In the second pathway (**B**, Scheme 2), iodide effects ring opening of the cyclopropane furnishing enolate **7**. The reactivity of this ambiphilic species may be manifest in two distinct ways. *N*-Alkylation of the aldimine furnishes **10** in a route that subsequently converges with **A** (**1** → **7** → **10** → **3/4**). Alternatively, reaction of enolate **7** with the starting imine furnishes **11** which can in turn undergo alkylative cyclization **C** (**1** → **7** → **11** → **3/4**). Although a mechanistic study of the process is ongoing, a number of observations lead us to prefer **B** and **C** for *N*-allyl and *N*-arylsulfonyl imines. In this regard, the fact that both *N*-alkyl and *N*-arylsulfonyl aldimines are substrates in the reaction argues against the nitrogen moiety functioning directly as a nucleophile as depicted for **A**. Moreover, when $Mg(OTf)_2$ was employed the starting materials were recovered unchanged and no product formation could be observed. These results strongly implicate a critical role for the halide in the course of the reaction (cf. **7** and **11**).

Preliminary observations suggest that the catalyzed ring expansion may be general, extending in scope beyond the substrates described above. Carboxamide **12** undergoes ring expansion in the presence of $MgI_2$ to give **13** [Eq. (4)]. Additionally, the reaction of **1** and *N*-*p*-tolylsulfonyl isocyanate furnishes **14** [Eq. (5)].[16]




(4)




(5)

We have described novel methodology which provides direct access to spiro[pyrrolidin-3,3′-oxindoles], a ring system found in many natural products with intriguing biological activity. The reaction process employs readily available spiro[cyclopropan-1,3′-oxindoles] and *N*-alkyl or *N*-arylsulfonyl imines. The successful implementation of the ring expansion strategy involving moderately activated cyclopropanes and aldimines is enabled by selection of $MgI_2$ as a catalyst possessing dual nucleophilic and electrophilic activity. The observation that simple, singly activated cyclopropanes also participate suggests that the process may be considerably generalized. Further extensions to other reacting partners as well as the use of optically active Mg complexes for the asymmetric synthesis of chiral spiro[pyrrolidin-3,3′-oxindoles] are topics of current investigation and will be reported in due course.

Received: June 30, 1999 [Z13659IE]
German version: *Angew. Chem.* **1999**, *111*, 3379 – 3381

**Keywords:** alkaloids · cyclopropanes · nucleophilic additions · ring expansions · spiro compounds

[1] *The Alkaloids: Chemistry and Biology*, *Vol. 5* (Ed: G. A. Cordell), Academic, San Diego, **1998**.

[2] a) J. Leclercq, M.-C. De Pauw-Gillet, R. Bassleer, L. Angenot, *J. Ethnopharmocol.* **1986**, *15*, 305; b) P. L. Dupont, J. Lamotte-Brasseur, O. Dideberg, H. Campsteyn, M. Vermeire, L. Angenot, *Acta. Crystallogr. Sect. B* **1977**, *33*, 1801.

[3] Overman et al. have developed a novel approach to the catalytic, asymmetric synthesis of spiro oxindoles utilizing Heck reactions of unsaturated haloanilides; for a leading reference, see: A. Ashimori, B. Bachand, L. Overman, D. J. Poon, *J. Am. Chem. Soc.* **1998**, *120*, 6477; b) T. Matsuura, L. E. Overman, D. J. Poon, *J. Am. Chem. Soc.* **1998**, *120*, 6500.

[4] a) T. Fukuyama, G. Liu, *J. Am. Chem. Soc.* **1996**, *118*, 7426; b) M. E. Kuehne, T. Wang, P. J. Seaton, *J. Org. Chem.* **1996**, *61*, 6001; c) T. D. Cushing, J. F. Sanz-Cervera, R. M. Williams, *J. Am. Chem. Soc.* **1996**, *118*, 557; d) S. Atarashi, J.-K. Choi, D.-C. Ha, D. J. Hart, D. Kuzmich, C.-S. Lee, S. Ramesh, S. C. Wu, *J. Am. Chem. Soc.* **1997**, *119*, 6226; e) T. Gan, R. Liu, P. Yu, S. Zhao, J. M. Cook, *J. Org. Chem.* **1997**, *62*, 9298; f) S. Kozmin, V. H. Rawal, *J. Am. Chem. Soc.* **1998**, *120*, 13523; g) M. E. Kuehne, F. Xu, *J. Org. Chem.* **1998**, *63*, 9434.

[5] a) S. D. Edmonson, S. J. Danishefsky, *Angew. Chem.* **1998**, *110*, 1190; *Angew. Chem. Int. Ed.* **1998**, *37*, 1138; b) S. Edmonson, S. J. Danishefsky, L. Sepp-Lorenzino, N. Rosen, *J. Am. Chem. Soc.* **1999**, *121*, 2147; c) F. He, Y. Bo, J. D. Altom, E. J. Corey, *J. Am. Chem. Soc.* **1999**, *121*, 6771.

[6] a) J. Du Bois, C. S. Tomooka, J. Hong, E. M. Carreira, *Acc. Chem. Res.* **1997**, *30*, 364; b) M. R. Mish, F. Guerra-Martínez, E. M. Carreira, *J. Am. Chem. Soc.* **1997**, *119*, 8379; c) G. A. Whitlock, E. M. Carreira, *J. Org. Chem.* **1997**, *62*, 7916; d) J. Du Bois, J. Hong, E. M. Carreira,

  1433-7851/99/3821-3188 $ 17.50+.50/0 

M. W. Day, *J. Am. Chem. Soc.* **1996**, *118*, 915; e) J. Du Bois, C. S. Tomooka, J. Hong, E. M. Carreira, *J. Am. Chem. Soc.* **1997**, *119*, 3179; f) C. S. Tomooka, D. D. LeCloux, H. Sasaki, E. M. Carreira, *Org. Lett.* **1999**, *1*, 149.

[7] For an excellent review, see: a) E. D. Cox, J. Cook, *Chem. Rev.* **1995**, *95*, 1797; b) J. E. Saxton, *Nat. Prod. Rep.* **1997**, 559.

[8] J. P. Kutney in *The Total Synthesis of Natural Products*, *Vol. 3* (Ed.: J. ApSimon), Wiley, New York, **1977**, p. 273.

[9] For a discussion of charge affinity patterns and retrosynthetic analysis, see: D. A. Evans, G. C. Andrews, *Acc. Chem. Res.* **1974**, *7*, 147.

[10] a) H. N. C. Wong, M.-Y. Hon, C.-W. Tse, Y.-C. Yip, J. Tanko, T. Hudlicky, *Chem. Rev.* **1989**, 89, 165; b) for an excellent review see: S. Danishefsky, *Acc. Chem. Res.* **1977**, *12*, 66.

[11] The reactivity cyclopropanes towards nucleophiles is typically limited to those activated by two electron-withdrawing groups or those that are highly strained. Exceptions to this generalization occur with strong nucleophiles such as metal selenides and Ni-catalyzed additions of organolaluminum compounds, see: a) A. B. Smith, R. M. Scarborough, Jr., *Tetrahedron Lett.* **1978**, 1649; b) L. Bagnell, A. Meisters, T. Mole, *Aust. J. Chem.* **1975**, *28*, 821.

[12] $MgI_2$ has been observed to function as a uniquely effective catalyst in asymmetric Diels–Alder and aldol addition reactions. Its superiority has been attributed to the facile dissociation of iodide from $L_2MgI_2$, see: E. J. Corey, W. Li, G. A. Reichard, *J. Am. Chem. Soc.* **1998**, *120*, 2330.

[13] a) C. Crestini, R. Saladino, *Syn. Commun.* **1994**, *24*, 2835; b) the sulfonylimines were prepared using known methods, see: R. Albrecht, G. Kresze, B. Mlagar, *Chem. Ber.* **1963**, *97*, 483.

[14] G. P. Claxton, L. Allen, J. M. Grisar, *Org. Synth. Coll. Vol. VI* **1988**, 968.

[15] It is important to note that in the absence of added metal salts, no reaction was observed.

[16] In this context, we have shown that the *p*-tolylsulfonyl protecting group could be removed in good yields (Na/naphthalene, THF, $-100\,^{\circ}C$, 68% yield).

## Specific RNA Dinucleotide Cleavage by a Synthetic Calix[4]arene-Based Trinuclear Metallo(II)-phosphodiesterase**

Peter Molenveld, Johan F. J. Engbersen,* and David N. Reinhoudt*

*Dedicated to Professor H. C. Beyerman on the occasion of his 80th birthday*

Phosphodiesterase enzymes such as nuclease P1 use three divalent metal ions (e.g. $Zn^{II}$) in the active site to catalyze the hydrolytic cleavage of phosphate diester bonds in nucleotides like RNA and DNA.[1] Synthetic catalysts that cleave RNA at specific sites are of interest, for example, for future application in gene technology.[2, 3] There are several mononuclear complexes of trivalent metal ions[3, 4] (e.g. lanthanide(III) and $Co^{III}$) that efficiently cleave RNA because they are strong Lewis acids. According to previous studies, mononuclear and even dinuclear $Zn^{II}$ complexes[2, 5, 6] generally exhibit only a moderate catalytic activity in RNA cleavage. Recently, we have shown that synthetic dinuclear and trinuclear[7] metallophosphodiesterases based on calix[4]arenes[8] exhibit a very high catalytic activity in the transesterification of the RNA model substrate 2-hydroxypropyl-*p*-nitrophenyl phosphate (HPNP).[6, 9, 10]

Here we report that **1**-$Zn_3$ efficiently catalyzes the cleavage of RNA dinucleotides (3′,5′-NpN) by the cooperative action of the $Zn^{II}$ centers, with high rate enhancement and significant nucleobase specificity. The heterotrinuclear complex **1**-$Zn_2Cu$ is even more active; it mimics phosphodiesterases with a heterotrinuclear metal cluster including a $Zn^{II}$ center in the active site.[1]

Catalytic cleavage of the RNA dinucleotides 3′,5′-NpN (0.09 mM) by the complexes **1**-$M_3$ (0.9 mM) was carried out in 35% EtOH/20 mM aqueous HEPES buffer[11] at $50\,^{\circ}C$ and monitored by HPLC. The formation of cyclic ribonucleoside monophosphates (2′,3′-cNMP) and the corresponding nucleosides show that the RNA dinucleotides are cleaved by intramolecular transesterification of the hydroxyl group at the 2′-position.[12] The catalytic activity of **1**-$Zn_3$ was measured for a series of RNA dinucleotides, namely, GpG, UpU, CpC, GpA, ApG, and ApA (Table 1). The trinuclear complex **1**-$Zn_3$ exhibits a very high catalytic activity; rate accelerations over the uncatalyzed reactions are on the order of $10^4$–$10^5$.[5b, 13] Moreover, **1**-$Zn_3$ is a genuine catalyst that exhibits turnover. A threefold excess of UpU is completely converted, while a reference solution of UpU without catalyst is unaffected. Surprisingly, for different nucleobases in the dinucleotides large differences in rate were observed: GpG ≫ UpU ≫ ApA (see below).

Table 1. Observed pseudo-first-order rate constants ($k_{obs}/10^5\ s^{-1}$) for the cleavage of RNA dinucleotides.[a]

| Substrate | **1**-$Zn_3$ | **1**-$Zn_2Cu$ | **1**-$Cu_3$ | **2**-$Zn_2$ | **3**-Zn |
|---|---|---|---|---|---|
| GpG | 72 | 88 | 28 | 0.45 | –[b] |
| UpU[c] | 8.5 | 13 | 1.2 | 0.45 | 0.56 |
| CpC | 6.1 | 7.1 | 1.9 | 0.58 | –[b] |
| GpA | 4.6 | 5.9 | –[b] | –[b] | –[b] |
| ApG | 2.7 | 2.4 | –[b] | –[b] | –[b] |
| ApA[d] | 0.44 | 0.46 | 0.47 | 0.28 | 0.31 |

[a] In 35% EtOH/20 mM HEPES (pH 8.0) at $50\,^{\circ}C$; [substrate] = 0.09 mM; [**1**-$Zn_3$] = [**1**-$Cu_3$] = [**2**-$Zn_2$] = 0.9 mM; **1**-$Zn_2Cu$ is a statistical mixture of [**1**] = 0.9 mM, [Zn] = 1.8 mM, and [Cu] = 0.9 mM;[15] [**3**-Zn] = 2.7 mM. [b] Not determined. [c] $k_{uncat} \approx 9.8 \times 10^{-9}\ s^{-1}$.[5b] [d] $k_{uncat} \approx 1.7 \times 10^{-9}\ s^{-1}$.[13]

The dependence of rate on the pH value for the **1**-$Zn_3$-catalyzed cleavage of UpU shows a bell-shaped curve with an optimum at pH 8. The apparent p$K_a$ of a $Zn^{II}$-bound water molecule in **3**-Zn is 7.9;[9] the value is lower for **1**-$Zn_3$ due to hydrophobic and cooperative effects.[9, 10] Therefore, it is likely that at pH 8 one or two $Zn^{II}$ centers in **1**-$Zn_3$ are coordinated by a hydroxide ion. Furthermore, the activity reaches a

[*] Prof. Dr. J. F. J. Engbersen, Prof. Dr. D. N. Reinhoudt, Dr. P. Molenveld
Laboratory of Supramolecular Chemistry and Technology
MESA+ Research Institute, University of Twente
P.O. Box 217, NL-7500 AE Enschede (The Netherlands)
Fax: (+31) 53-489-4645
E-mail: smct@ct.utwente.nl

[**] This work was supported by the Council for Chemical Sciences of the Netherlands Organization for Scientific Research (CW-NWO). We thank Dr. F. C. J. M. van Veggel for molecular modeling, and Dr. F. Venema and Dr. H. van Schijndel of Organon Teknika BV for their help with preliminary experiments with RNA oligonucleotides.

Supporting information for this article is available on the WWW under http://www.wiley-vch.de/home/angewandte/ or from the author.

  1433-7851/99/3821-3189 $ 17.50+.50/0 