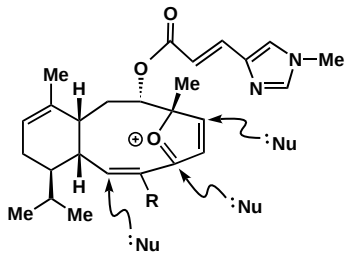


Figure 2. Alkylating properties of sarcodictyins and eleutherobins.

The chemistry and biological activity presented here shows the sarcodictyins to be a new class of potential anticancer agents. Access to additional derivatives and closer investigation are now possible through the use of molecular design and chemical synthesis. The first structure – activity information on sarcodictyins, reported here, should provide valuable guidelines for further chemical and biological studies.[14]

Received: January 22, 1998 [Z 11393 IE]
German version: *Angew. Chem.* **1998**, *110*, 1484 – 1487

Keywords: antitumor agents · sarcodictyins · structure – activity relationships · total synthesis · tubulin polymerizations

[1] a) M. D'Ambrosio, A. Guerriero, F. Pietra, *Helv. Chim. Acta* **1987**, *70*, 2019 – 2027; b) *ibid.*, **1988**, *71*, 964 – 976.
[2] M. Ciomei, C. Albanese, W. Pastori, M. Grandi, F. Pietra, M. D'Ambrosio, A. Guerriero, C. Battistini, Abstract 30, *Proc. Amer. Ass. Canc. Res.* **1997**, *38*, 5.
[3] W.-H. Fenical, P. R. Jensen, T. Lindel (University of California), US-A 5,473,057, **1995** [*Chem Abstr.* **1996**, *124*, 194297z].
[4] (a) T. Lindel, P. R. Jensen, W. Fenical, B. H. Long, A. M. Casazza, J. Carboni, C. R. Fairchild, *J. Am. Chem. Soc.* **1997**, *119*, 8744 – 8745; (b) B. H. Long, A. M. Casazza, J. Carboni, C. R. Fairchild, T. Lindel, P. R. Jensen, W. Fenical, *Cancer Res.*, submitted.
[5] S. Ketzinel, A. Rudi, M. Schleyer, Y. Benayahu, Y. Kashman, *J. Nat. Prod.* **1987**, *59*, 873 – 875.
[6] P. B. Schiff, J. Fant, S. B. Horwitz, *Nature* **1979**, *277*, 665 – 667.
[7] K. C. Nicolaou, J. Y. Xu, S. Kim, T. Ohshima, S. Hosokawa, J. Pfefferkorn, *J. Am. Chem. Soc.* **1997**, *119*, 11353 – 11354.
[8] K. C. Nicolaou, F. van Delft, T. Oshima, D. Vourloumis, J.-Y. Xu, S. Hosokawa, J. Pfefferkorn, S. Kim, T. Li, *Angew. Chem.* **1997**, *109*, 2630 – 2634; *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 2520 – 2524.
[9] D. B. Dess, J. C. Martin, *J. Org. Chem.* **1983**, *48*, 4155 – 4156.
[10] R. R. Schrock, J. Osborn, *J. Am. Chem. Soc.* **1976**, *98*, 2143 – 2144.
[11] D. M. Bollag, P. A. McQueney, J. Zhu, O. Hensens, L. Koupal, J. Liesch, M. Goetz, E. Lazarides, C. M. Woods, *Cancer Res.* **1995**, *55*, 2325 – 2333.
[12] K. C. Nicolaou, D. Vourloumis, T. Li, N. Winssinger, Y. He, S. Ninkovic, F. Sarabia, H. Vallberg, F. Roschangar, N. P. King, M. R. V. Finlay, Z. Yang, T. Li, P. Giannakakou, P. Verdier-Pinard, E. Hamel, *Angew. Chem.* **1997**, *109*, 2181 – 2187; *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 2097 – 2103.
[13] P. Giannakakou, D. L. Sackett, Y.-K. Kang, Z. Zhan, J. T. M. Buters, M. S. Fojo, M. S. Poruchynsky, *J. Biol. Chem.* **1997**, *272*, 17118 – 17125.
[14] For a recently reported total synthesis of the related natural product eleutherobin, see a) X.-T. Chen, C. E. Gutteridge, S. K. Bhattacharya, B. Zhou, T. R. R. Pettus, T. Hascall, S. J. Danishefsky, *Angew. Chem.* **1998**, *110*, 195 – 197; *Angew. Chem. Int. Ed.* **1998**, *37*, 185 – 187; b) X.-T. Chen, B. Zhou, S. K. Bhattacharya, C. E. Gutteridge, T. R. R. Pettus, S. J. Danishefsky, *Angew. Chem.* **1998**, *110*, 835 – 838; *Angew. Chem. Int. Ed.* **1998**, *37*, 789 – 792.

Cooperative Hairpin Dimers for Recognition of DNA by Pyrrole – Imidazole Polyamides**

John W. Trauger, Eldon E. Baird, and Peter B. Dervan*

Small molecules which permeate cells and bind to specific DNA sequences can potentially control the expression of specific genes.[1, 2] Recently, a polyamide with eight heterocyclic units which binds to a target site consisting of six base pairs was shown to inhibit gene transcription in a cell culture.[2] Polyamides that recognize longer DNA sequences should provide more specific biological activity,[3] which could be achieved by synthesizing larger polyamides.[4] However, the upper limit of polyamide size with regard to efficient cell permeation is not known.

Alternatively, a more biomimetic approach is to bind larger DNA sequences while maintaining the size of the polyamide. Natural transcription factors often bind large DNA sequences by formation of cooperative protein dimers at adjacent half-sites.[5] In cooperatively binding extended pyrrole – imidazole (Py – Im) polyamide dimers, the two ligands can slip sideways with respect to one another to allow recognition of other sequences.[6] Polyamides containing the turn-specific γ-aminobutyric acid linker[7] adopt a hairpin conformation in which the DNA binding sites are fully overlapped and the slipped-motif option is precluded. Here we report a cooperative six-ring extended hairpin polyamide which dimerizes to specifically bind a predetermined sequence of ten base pairs.

As target site, we chose a sequence contained in the regulatory region of the HIV-1 genome.[8] To design the ligand we considered the polyamide ring-pairing rules,[9–13] the need to incorporate β-alanine (β) to relax the ligand curvature,[6, 13] and the preference of γ-aminobutyric acid (γ) for a hairpin-turn conformation in polyamide – DNA complexes.[6, 7a,e] This analysis suggested that the six-ring polyamide having the core sequence ImPyβImPyγImPy might bind the target sequence 5′-AGCAGCTGCT-3′ by formation of a cooperative hairpin dimer (Figure 1). To avoid a collision between the N-terminal end of one ligand and the C-terminal end of the other in the complex, the positively charged β-alaninedimethylaminopropylamide C terminus used in standard polyamides was replaced with the shorter, uncharged $(CH_2)_2OH$ group (C_2 – OH). The cationic turn residue (*R*)-2,4-diaminobutyric acid ($(R)^{H_2N}\gamma$)[14] maintains the overall positive charge needed for optimal solubility in water.

Polyamide ImPyβImPy$(R)^{H_2N}\gamma$ImPyC_2-OH (**1**) was synthesized by solid-phase methods[15] on glycine – PAM resin,[16] reductively cleaved from the solid support with $LiBH_4$,[17]

[*] Prof. P. B. Dervan, J. W. Trauger, E. E. Baird
Arnold and Mabel Beckman Laboratories of Chemical Synthesis
California Institute of Technology
Pasadena, CA 91101 (USA)
Fax: (+1) 626-568-8824
E-mail: dervan@cco.caltech.edu

[**] We are grateful to the National Institutes of Health for support (GM-27681), to the National Science Foundation and the Ralph M. Parsons Foundation for predoctoral fellowships to J.W.T., and to the Howard Hughes Medical Institute for a predoctoral fellowship to E.E.B. Supporting information for this article is available on the WWW under http://www.wiley-vch.de/home/angewandte/ or from the author.

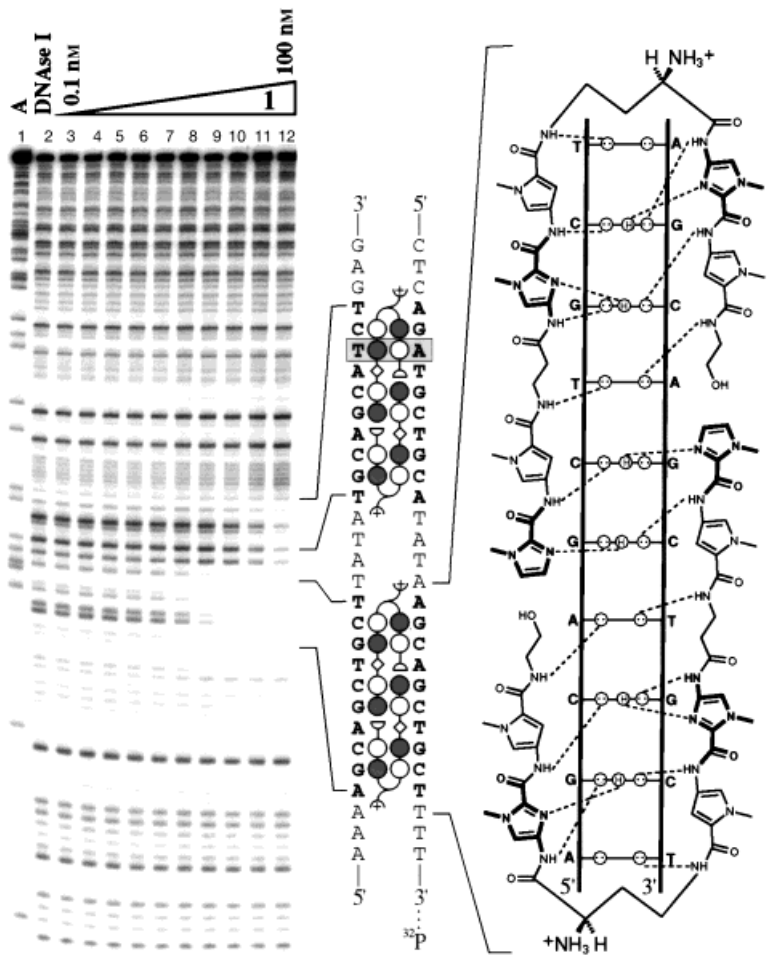
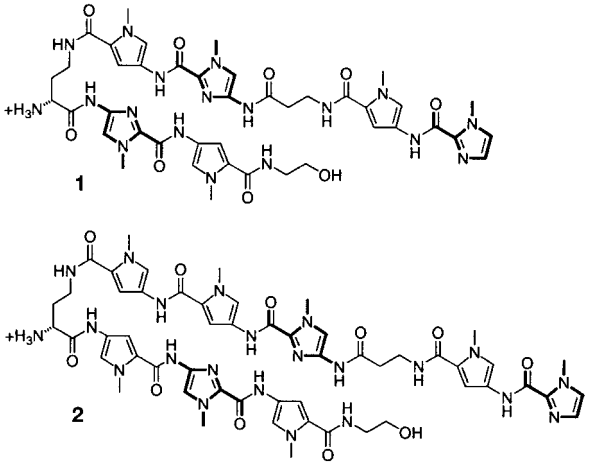


Figure 1. Right: Model of the $(\mathbf{1})_2 \cdot$5′-AGCAGCTGCT-3′ complex. The symbol ⊙ represents lone pairs on the N3 atoms of purine bases or on the O2 atoms of pyrimidine bases, and the symbol Ⓗ represents the hydrogen atom bound to the N2 atom of guanine. Postulated hydrogen bonds are illustrated by dashed lines. Middle: Binding models for complexes of $(\mathbf{1})_2$ at a ten base pair match site and at a site with one mismatched base pair (the mismatched base pair is highlighted by shading). The shaded and open circles represent imidazole and pyrrole rings, respectively, diamonds represent β-alanine, half-circles represent $(CH_2)_2OH$ groups, and curved lines represent (*R*)-2,4-diaminobutyric acid. Left: Storage ^{32}P autoradiogram of the denaturing 8% polyacrylamide gel used to separate the fragments generated by DNase I digestion in a quantitative footprint titration experiment[21] with polyamide **1**: lane 1: A lane; lane 2: DNase I digestion products obtained in the absence of polyamide; lanes 3–12, DNase I digestion products obtained in the presence of 0.1, 0.2, 0.5, 1, 2, 5, 10, 20, 50, and 100 nM polyamide **1**, respectively.

and purified by reverse-phase HPLC.[18] Quantitative DNase I footprinting[19] on a 3′-^{32}P-end-labeled restriction fragment with 245 base pairs showed that **1** binds its match site 5′-AGCAGCTGCT-3′ at nanomolar concentrations (apparent monomeric association constant $K_a = (1.9 \pm 0.3) \times 10^8\,M^{-1}$) and also binds a site with one mismatched base pair (5′-AG**A**TGCTGCA-3′) with ninefold lower affinity ($K_a = (2.2 \pm 0.5) \times 10^7\,M^{-1}$, Figure 1).[20] The binding data for match and single base pair mismatch sites were well fit by cooperative binding isotherms, consistent with formation of cooperative 2:1 polyamide–DNA complexes.[5] A site with two mismatched base pairs, 5′-AGCTGCA**T**C**C**-3′, was bound with 65-fold lower affinity. The fact that this mismatch site, which contains part of the target site (5′-AGCTGCA-3′), is not effectively bound indicates that recognition of the match site occurs through cooperative dimerization and not by formation of 1:1 hairpin complexes.

Further study of the generality and sequence specificity of this binding motif is in progress. For example, we found that the eight-ring polyamide ImPyβImPyPy(*R*)$^{H_2N}\gamma$PyImPyC$_2$-OH (**2**) binds the twelve-base-pair match site 5′-AAGCAGCTGCTT-3′ with tenfold higher affinity than **1**. The binding affinity of **2** to this sequence is approximately 100 times higher than to a site with two mismatched base pairs (5′-**C**AG**A**TGCTGCAT-3′).



It is noteworthy that the DNA-binding affinity and specificity of the six-ring polyamide **1** for its ten base pair binding site are comparable to those of standard six-ring hairpins that recognize five base pairs.[7] Thus, use of the cooperative hairpin dimer motif doubles the size of the binding site relative to the standard hairpin binding pattern without sacrificing affinity or specificity, and without increasing the molecular weight of the ligand. Our results show that by using a novel cooperative hairpin dimer motif, relatively low molecular weight pyrrole–imidazole polyamides ($M_r \approx 950$–1200) can specifically recognize 10–12 base pairs of DNA. Such polyamides will be useful in determining the optimal ligand size and optimal binding site size required for specific biological activity.

Received: January 8, 1998 [Z11344IE]
German version: *Angew. Chem.* **1998**, *110*, 1489–1492

Keywords: cooperative effects · DNA recognition · polyamides

[1] J. W. Trauger, E. E. Baird, P. B. Dervan, *Nature* **1996**, *382*, 559–561.
[2] J. M. Gottesfeld, L. Neely, J. W. Trauger, E. E. Baird, P. B. Dervan, *Nature* **1997**, *387*, 202–205.
[3] P. B. Dervan, *Science* **1986**, *232*, 464.
[4] J. M. Turner, E. E. Baird, P. B. Dervan, *J. Am. Chem. Soc.* **1997**, *119*, 7636–7644.
[5] a) M. Ptashne, *A Genetic Switch*, Blackwell and Cell Press, Palo Alto, CA, **1986**; b) C. O. Pabo, R. T. Sauer, *Annu. Rev. Biochem.* **1992**, *61*,

  1433-7851/98/3710-1422 $ 17.50+.50/0

1053 – 1095; c) R. Marmorstein, M. Carey, M. Ptashne, S. C. Harrison, *Nature* **1992**, *356*, 408 – 414; d) J. D. Klemm, M. A. Rould, R. Aurora, W. Herr, C. O. Pabo, *Cell* **1994**, *77*, 21 – 32; e) S. F. Bellon, K. K. Rodgers, D. G. Schatz, J. E. Coleman, T. A. Steitz, *Nature Struct. Biol.* **1997**, *4*, 586 – 591.

[6] a) J. W. Trauger, E. E. Baird, P. B. Dervan, *J. Am. Chem. Soc.* **1996**, *118*, 6160 – 6166; b) S. E. Swalley, E. E. Baird, P. B. Dervan, *Chem. Eur. J.* **1997**, *3*, 1600 – 1607.

[7] a) M. Mrksich, M. E. Parks, P. B. Dervan, *J. Am. Chem. Soc.* **1994**, *116*, 7983 – 7988; b) M. E. Parks, E. E. Baird, P. B. Dervan, *ibid.* **1996**, *118*, 6153 – 6159; c) S. E. Swalley, E. E. Baird, P. B. Dervan, *ibid.* **1996**, *118*, 8198 – 8206; d) *ibid.* **1997**, *119*, 6953 – 6961; e) J. W. Trauger, E. E. Baird, P. B. Dervan, *Chem. Biol.* **1996**, *3*, 369 – 377; f) R. P. L. Declairac, B. H. Geierstanger, M. Mrksich, P. B. Dervan, D. E. Wemmer, *J. Am. Chem. Soc.* **1997**, *119*, 7909 – 7916.

[8] a) K. A. Jones, B. M. Peterlin, *Annu. Rev. Biochem.* **1994**, *63*, 717 – 743; b) K. Frech, R. Brack-Werner, T. Werner, *Virology* **1996**, *224*, 256 – 267.

[9] a) W. S. Wade, M. Mrksich, P. B. Dervan, *J. Am. Chem. Soc.* **1992**, *114*, 8783 – 8794; b) M. Mrksich, W. S. Wade, T. J. Dwyer, B. H. Geierstanger, D. E. Wemmer, P. B. Dervan, *Proc. Natl. Acad. Sci. USA* **1992**, *89*, 7586 – 7590; c) W. S. Wade, M. Mrksich, P. B. Dervan, *Biochemistry* **1993**, *32*, 11385 – 11389.

[10] a) J. G. Pelton, D. E. Wemmer, *Proc. Natl. Acad. Sci. USA* **1989**, *86*, 5723 – 5727; b) J. G. Pelton, D. E. Wemmer, *J. Am. Chem. Soc.* **1990**, *112*, 1393 – 1399; c) X. Chen, B. Ramakrishnan, S. T. Rao, M. Sundaralingam, *Nature Struct. Biol.* **1994**, *1*, 169 – 175; d) S. White, E. E. Baird, P. B. Dervan, *Biochemistry* **1996**, *35*, 12532 – 12537.

[11] a) M. Mrksich, P. B. Dervan, *J. Am. Chem. Soc.* **1993**, *115*, 2572 – 2576; b) B. H. Geierstanger, T. J. Dwyer, Y. Bathini, J. W. Lown, D. E. Wemmer, *ibid.* **1993**, *115*, 4474; c) B. H. Geierstanger, J. P. Jacobsen, M. Mrksich, P. B. Dervan, D. E. Wemmer, *Biochemistry* **1994**, *33*, 3055; d) S. White, E. E. Baird, *Chem. Biol.* **1997**, *4*, 569 – 578.

[12] a) B. H. Geierstanger, M. Mrksich, P. B. Dervan, D. E. Wemmer, *Science* **1994**, *266*, 646 – 650; b) M. Mrksich, P. B. Dervan, *J. Am. Chem. Soc.* **1995**, *117*, 3325 – 3332.

[13] C. L. Keilkopf, E. E. Baird, P. B. Dervan, D. C. Rees, *Nature Struct. Biol.* **1998**, *5*, 104 – 109.

[14] D. M. Herman, E. E. Baird, P. B. Dervan, *J. Am. Chem. Soc.* **1998**, *120*, 1382 – 1391.

[15] E. E. Baird, P. B. Dervan, *J. Am. Chem. Soc.* **1996**, *118*, 6141 – 6146.

[16] Available from Peptides International, Louisville, KY, with degree of substitution of 0.3 mmol/g.

[17] a) A. R. Mitchell, S. B. Kent, M. Engelhard, R. B. J. Merrifield, *J. Org. Chem.* **1978**, *43*, 2845; b) J. M. Stewart, J. D. Young, *Solid Phase Peptide Synthesis*, Pierce Chemical Company, Rockford, IL, **1984**.

[18] The identity and purity of polyamides **1** and **2** was confirmed by ^{1}H NMR spectroscopy, analytical HPLC, and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). MALDI-TOF MS (monoisotopic): **1**: found 953.3 [*M*+H], calcd for $C_{42}H_{53}N_{18}O_9$ 953.4; **2**: found 1197.5 [*M*+H], calcd for $C_{54}H_{65}N_{22}O_{11}$ 1197.5.

[19] a) M. Brenowitz, D. F. Senear, M. A. Shea, G. K. Ackers, *Methods Enzymol.* **1986**, *130*, 132 – 181; b) M. Brenowitz, D. F. Senear, M. A. Shea, G. K. Ackers, *Proc. Natl. Acad. Sci. USA* **1986**, *83*, 8462 – 8466; c) D. F. Senear, M. Brenowitz, M. A. Shea, G. K. Ackers, *Biochemistry* **1986**, *25*, 7344 – 7354.

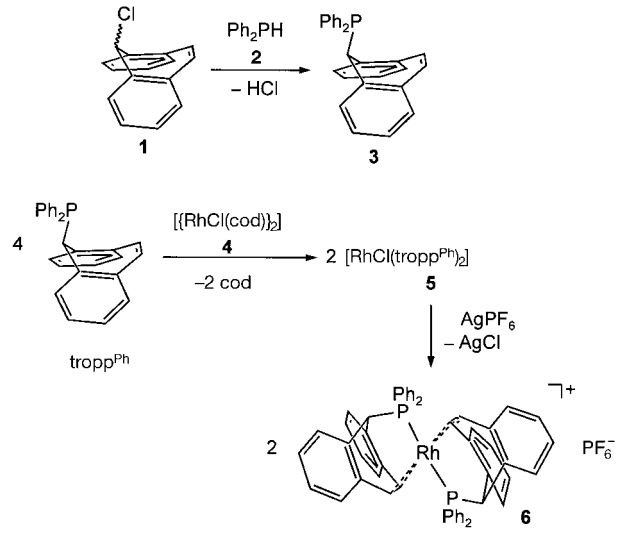
[20] For the evaluation of data on cooperative association of ligands, see C. R. Cantor, P. R. Schimmel, *Biophysical Chemistry, Part III: The Behavior of Biological Macromolecules*, W. H. Freeman, New York, **1980**, p. 863.

[21] All reactions contain a 3′-^{32}P-end-labeled (15000 cpm) *Eco*RI/*Hin*dIII restriction fragment of the plasmid pJT-LTR, 10 mM Tris · HCl, 10 mM KCl, 10 mM $MgCl_2$, and 5 mM $CaCl_2$ (pH 7.0, 24 °C). The plasmid pJT-LTR was prepared by ligating an insert having the sequence 5′-CCGGTAACCAGAGAGACCCAGTACAGGCAA-AAAG-CAGCTGCTTATATGCAGCATCTGAGGGACGCCACTCCC-CAGTCCCGCCCAGGCCACGCCTCCCTGGAAAGTCCC-CAGCGGAAAGTCCCTTGTAGAAAGCTCGATGTCAG-CAGTCTTTGTAGTACTCCGGATGCAGCTCTCGGGCCACGT-GATGAAATGCTAGGCGGCTGTCAATCGA-3′ to the large *Ava*I/*Sal*I fragment of pUC19.

A Monomeric d^9-Rhodium(0) Complex**

Hartmut Schönberg, Souad Boulmaâz, Michael Wörle, Lorenz Liesum, Arthur Schweiger,* and Hansjörg Grützmacher*

Complexes with rhodium centers in low formal oxidation states (0 or −1) are rare. They have been described as relatively short-lived intermediates in electrochemical reactions,[1] as polynuclear clusters,[2] or as complexes with strong π-acceptor ligands (such as $[Rh(CO)_4]^-$,[2] $[Rh(PF_3)_4]^-$[3]).[4] As far as we are aware, the isolation of a paramagnetic, monomeric d^9-rhodium(0) compound has not yet been achieved. We report here a new ligand that allows the easy synthesis and reversible transformation of rhodium complexes with formal oxidation states +1, 0, and −1. 5-Chloro-5*H*-dibenzo[*a*,*d*]cycloheptene (**1**)[5] reacts with diphenylphosphane (**2**) to form (5*H*-dibenzo[*a*,*d*]cycloheptene-5-yl)diphenylphosphane (**3**) ("dibenzo-tropylidenyldiphenylphosphane", tropp^{Ph}) in good yield. In contrast to the synthesis of simple cycloheptatrienylphosphanes,[6] only one isomer of **3** is obtained: the Ph_2P group is in the axial position of the seven-membered ring[7] (Scheme 1).



Scheme 1. Synthesis of the rhodium(I) complexes **5** and **6**.

Reaction of four equivalents of **3** with $[\{RhCl(cod)\}_2]$ (**4**) (cod = cyclooctadiene) leads in almost quantitative yield to the intense yellow rhodium(I) complex $[RhCl(tropp^{Ph})_2]$ (**5**).

[*] Prof. A. Schweiger, L. Liesum
Laboratorium für Physikalische Chemie, ETH-Zentrum
CH-8092 Zürich (Switzerland)
E-mail: schweiger@phys.chem.ethz.ch
Prof. H. Grützmacher, Dr. H. Schönberg, Dr. S. Boulmaâz, Dr. M. Wörle
ETH-Zentrum, Laboratorium für Anorganische Chemie
Universitätstrasse 6, CH-8092 Zürich (Switzerland)
Fax: (+41) 1-632-1090
E-mail: gruetz@inorg.chem.ethz.ch

[**] This work was supported by the Schweizerische Nationalfonds zur Förderung der wissenschaftlichen Forschung. We thank the reviewers for valuable comments.

  1433-7851/98/3710-1423 $ 17.50+.50/0