Keywords: carboxylic acids · hydrogen bonds · protonations · solid-state structures

[1] Crystal growth by miniature zone-melting[10] with the samples of stoichiometric composition in glass capillaries of 0.3 mm inner diameter. $C_5H_5N \cdot HCOOH$: monoclinic, space group $P2_1/n$, $a = 10.954(6)$, $b = 3.817(3)$, $c = 15.842(7)$ Å, $\beta = 104.96(5)^\circ$, $V = 639.9(7)$ Å^3, $Z = 4$, $\rho_{calcd} = 1.30$ g cm^{-3}, $\mu = 0.10$ mm^{-1}; $2\theta_{max} = 50^\circ$, 1120 independent reflections with $F_o^2 > -3\sigma_{F^2}$, 986 of them with $|F_o| > 4\sigma_F$ observed; direct methods, 111 variables refined on F^2, $R(F)(obsd) = 0.047$, $wR(F^2)(all) = 0.167$, residual electron density between -0.22 and $+0.16$ e Å^{-3}. $C_5H_5N \cdot 4HCOOH$: orthorhombic, $Pca2_1$, $a = 16.35(1)$, $b = 3.702(3)$, $c = 20.23(1)$ Å, $V = 1225(1)$ Å^3, $Z = 4$, $\rho_{calcd} = 1.43$ g cm^{-3}, $\mu = 0.13$ mm^{-1}; $2\theta_{max} = 60^\circ$, 1835 independent reflections with $F_o^2 > -3\sigma_{F^2}$, 1551 of them with $|F_o| > 4\sigma_F$ observed; direct methods, 216 variables refined on F^2, $R(F)(obsd) = 0.041$, $wR(F^2)(all) = 0.114$, residual electron density between -0.24 and $+0.22$ e Å^{-3}. Siemens Stoe AED 2 diffractometer adapted for low-temperature work, graphite-monochromated $Mo_{K\alpha}$ radiation ($\lambda = 0.71073$ Å); computer programs SHELXS-86, SHELXL-93 and SHELXTL PLUS.[11] Crystallographic data (excluding structure factors) for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication nos. CCDC-112269 and CCDC-112270. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ (fax: (+44) 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).

[2] For a recent discussion of C-H ··· O hydrogen bonding see: T. Steiner, *Chem. Commun.* **1997**, 727 – 734, and references therein.

[3] D. Boenigk, D. Mootz, *J. Am. Chem. Soc.* **1988**, *110*, 2135 – 2139.

[4] D. Wiechert, D. Mootz, T. Dahlems, *J. Am. Chem. Soc.* **1997**, *119*, 12665 – 12666.

[5] D. Mootz, D. Boenigk, *Z. Naturforsch. B* **1984**, *39*, 298 – 304.

[6] D. Mootz, M. Schilling, *J. Am. Chem. Soc.* **1992**, *114*, 7435 – 7439.

[7] T. Dahlems, D. Mootz, M. Schilling, *Z. Naturforsch. B* **1996**, *51*, 536 – 544.

[8] D. Mootz, W. Poll, *Z. Anorg. Allg. Chem.* **1982**, *484*, 158 – 164; D. Mootz, U. Ohms, W. Poll, *Z. Anorg. Allg. Chem.* **1981**, *479*, 75 – 83.

[9] W. Poll, M. Lohmeyer, D. Mootz, *Z. Naturforsch. B* **1989**, *44*, 1359 – 1364.

[10] D. Brodalla, D. Mootz, R. Boese, W. Osswald, *J. Appl. Crystallogr.* **1985**, *18*, 316 – 319.

[11] G. M. Sheldrick, *Acta Crystallogr. Sect. A* **1990**, *46*, 467 – 473; G. M. Sheldrick, Program for the Refinement of Crystal Structures, Universität Göttingen, **1993**; SHELXTL PLUS, Structure Determination System Revision 4.21/V, Siemens Analytical X-Ray Instruments, Inc., Madison, WI, **1990**.

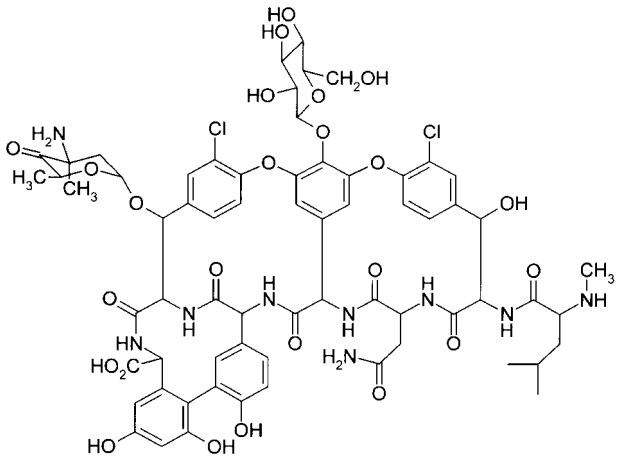
New Advances in the Biosynthesis of Glycopeptide Antibiotics of the Vancomycin Type from *Amycolatopsis mediterranei***

Roderich D. Süssmuth, Stefan Pelzer, Graeme Nicholson, Tilmann Walk, Wolfgang Wohlleben, and Günther Jung*

The glycopeptide antibiotic vancomycin was isolated in the mid 1950s,[1] and its structure was conclusively determined with spectroscopic methods and by means of crystal structure analysis.[2] As a drug of last resort, vancomycin is the most important agent after penicillin against Gram-positive bacteria, such as methicillin-resistant staphylococci (MRS).[3] It is employed in enantiomer analytics as a chiral selector.[4] The total synthesis of vancomycin is especially challenging owing to the synthetically demanding biphenyl ether and biphenyl bridges.[5] Although vancomycin has been known for more than 40 years, little is known about the biosynthesis and the intermediates of the aglycon.

Recently the DNA sequence of a gene cluster of the chloroeremomycin producer was described[6] which is assumed to encode enzymes for glycopeptide biosynthesis. Functional proof by means of expression studies or mutant analysis has so far not been reported. However, an understanding of the biosynthesis on a genetic and on a structural level is important for the development of novel glycopeptide analogs by combinatorial biosynthesis.

Amycolatopsis mediterranei, the producer of balhimycin (Scheme 1),[7] a glycopeptide antibiotic identical with vanco-



Scheme 1. Structure of balhimycin, an antibiotic of the vancomycin type.

[*] Prof. Dr. G. Jung, Dipl.-Chem. R. D. Süssmuth, G. Nicholson, Dipl.-Chem. T. Walk
Institut für Organische Chemie der Universität
Auf der Morgenstelle 18, D-72076 Tübingen (Germany)
Fax: (+49) 7071-29-5560
E-mail: guenther.jung@uni-tuebingen.de
Dr. S. Pelzer, Prof. Dr. W. Wohlleben
Lehrstuhl Mikrobiologie/Biotechnologie der Universität
Auf der Morgenstelle 28, D-72076 Tübingen (Germany)

[**] This work was supported by the Deutsche Forschungsgemeinschaft (SFB 323). Parts of this work were presented at the 25th European Peptide Symposium (1998) in Budapest. We thank Mr. G. Grewe and Prof. Dr. Hans-Peter Fiedler for the fermentation.

  1433-7851/99/3813-1976 $ 17.50+.50/0

mycin with regard to the aglycon, was used as a model organism. Through use of a reverse genetic approach the balhimycin biosynthesis cluster was isolated. To prove the involvement of the isolated genes in the biosynthesis of balhimycin, gene inactivation experiments were carried out in *Amycolatopsis mediterranei*.[8] The null-mutant SP1-1 was generated by integration mutagenesis in the region of the oxygenase genes of the balhimycin biosynthesis gene cluster (Figure 1). From the culture filtrate of this null-mutant, two new biosynthetically accumulated compounds (relative mono-

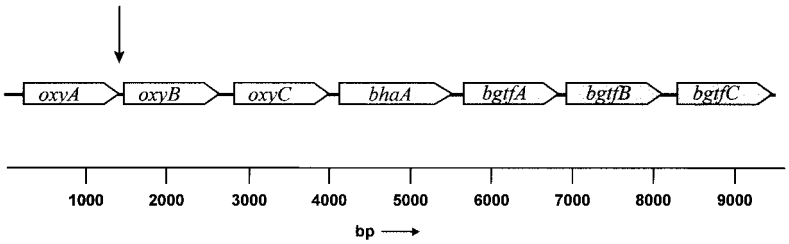


Figure 1. Schematic representation of part of the balhimycin biosynthesis gene cluster from *A. mediterranei* DSM5908. The deduced gene products show significant similarities to the following enzymes: Oxy A – C (cytochrome P450-dependent monooxygenases), BhaA (halogenases), Bgtf A – C (glycosyl transferases). The arrow symbolizes the targeted integration mutagenesis between the oxygenase genes *oxyA* and *oxyB*.

isotopic masses 969 and 1134) were detected by means of HPLC-ES-MS coupling by comparison with the wild type, whereas the parent ion of balhimycin was no longer detectable.

In addition to an exact mass determination, ES-FT-ICR measurements yielded characteristic chlorine isotope distribution patterns for both compounds (Figure 2). As balhimycin is doubly chlorinated, these compounds had to be

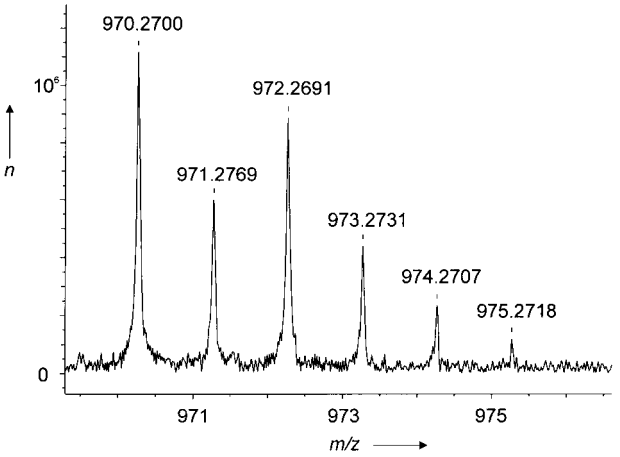


Figure 2. High-resolution ES-FT-ICR mass spectrum of the metabolite SP-969. The isotope distribution is characteristic for compounds of the vancomycin type with two chlorine atoms.

biosynthetic intermediates. The ES-MS daughter ion spectra for the mass signals *m*/*z* 970 and 1135 showed a multitude of fragment ion peaks (Figure 3), which made the presence of linear biosynthetic intermediates feasible.

The compounds SP-969 (5.0 mg) and SP-1134 (3.5 mg), named after their molecular masses, were isolated from the culture filtrate with a high degree of purity by means of preparative HPLC. The amino acid analysis with capillary GC-MS on Chirasil-Val showed that L-aspartic acid, D-leucine, and D-4-hydroxyphenylglycine (Hpg) are present in both compounds. In addition, L-3,5-dihydroxyphenylglycine (Dpg) was identified in the hydrolysate of SP-1134; the expected chlorine-containing amino acids could not be detected. The established configurations of the amino acids agreed with those of the vancomycin aglycon. From the daughter ion spectra together with the results of amino acid analytics and knowing the biosynthetic target, the partial sequences Leu-Xxx-Asn-Hpg-Hpg-Yyy (SP-969) and Leu-Xxx-Asn-Hpg-Hpg-Yyy-Dpg (SP-1134) could be deduced (Scheme 2).

The noncyclized structures of the peptides SP-969 and SP-1134 were furthermore established with the Edman degradation procedure. To be able to identify the expected unusual phenylthiohydantoine (PTH) amino acid derivatives, the gas-phase sequencer was directly connected to the ES mass spectrometer.[9] The number of degradation

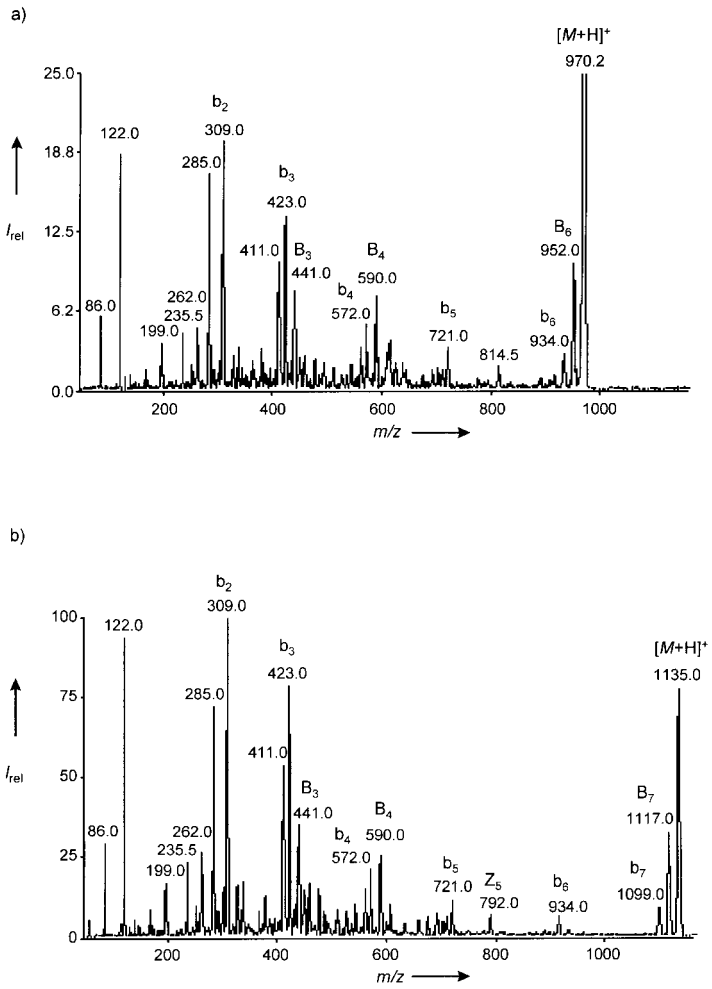


Figure 3. Daughter ion spectra of SP-969 (a) and SP-1134 (b), which show mainly fragments of the B series. The b series marks B fragment ions that emerge after loss of water.

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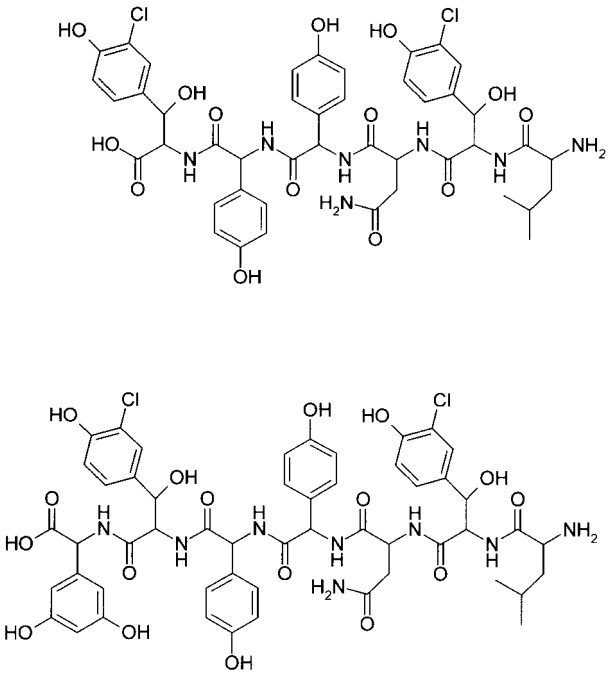

Scheme 2. Structures of SP-969 (above) and SP-1134 (below).

cycles of SP-969 corresponded to six, that of SP-1134 to seven amino acids. For the degradation cycles 1 and 3 of compounds SP-969 and SP-1134, Leu and Asn were detected. The PTH derivatives of the degradation cycles 2 and 6 both agree with the amino acid 3-chloro-β-hydroxytyrosine (Cht) of the aglycon structure with respect to their molecular masses. The occurrence of the PTH derivatives of Gly and 3-chlorodehydrotyrosine, which were also detected, indicates a retroaldol reaction of the β-hydroxyamino acid to glycine and the corresponding aldehyde and an elimination of water to furnish the 2,3-didehydroamino acid, respectively. The degradation cycles 4 and 5 correspond to Hpg, which was confirmed by an Hpg standard. For SP-1134, Dpg was additionally identified in degradation cycle 7.

The definite structures of the peptides SP-969 and SP-1134 were confirmed by NMR spectroscopy[10] (Tables 1 and 2). In the TOCSY spectra (TOCSY = total correlation spectroscopy), five and six spin systems, respectively, were identified in the range of the amide protons. The assignment of aromatic protons in the TOCSY experiment was carried out with two AB spin systems from Hpg (SP-969), three AB spin systems from Hpg and Dpg (SP-1134), and two ABC spin systems each from Cht. In the TOCSY spectrum of the aliphatic region, Leu and Asn were detected. The presence of Asn was verified by a spin system in the TOCSY spectrum for which no ^{13}C contacts could be established in the HSQC experiment (HSQC = heteronuclear single quantum coherence), but for which ROE contacts (ROESY = rotating frame Overhauser effect spectroscopy) to the Asn spin system were proved. In accordance with the signals in the TOCSY spectrum, all further $^{1}H-^{13}C$ contacts of the HSQC spectrum were assigned. The connections of the aromatic rings to the peptide backbone were elucidated with the HMBC spectrum (HMBC = heteronuclear multiple bond correlation). The complete amino acid sequences for SP-969 and SP-1134 could be determined with the ROESY spectra consulted for sequence information.

Peptides SP-969 and SP-1134 are the first known, linear biosynthetic intermediates of antibiotics of the vancomycin type to be isolated. Accordingly, the halogenation of ^{2}Cht and ^{6}Cht already takes place before the oxidative ring closures to the biaryl ethers (positions 2, 4, and 6) and the biphenyl

Table 1. ^{1}H and ^{13}C NMR shifts of the peptide SP-969 ([D$_6$]DMSO, $c_{SP\text{-}969} = 8.3\ mg\,mL^{-1}$, 305 K).

	NH	C_α	H_α	C_β	H_β	$\delta(^{13}C)_n/\delta(^{1}H)_n$
^{1}Leu	–	50.8	3.63	39.7	1.43	23.2/1.45, 21.0/0.80, 21.0/0.80
^{2}Cht	8.51	58.3	4.58	71.7	4.64	5.64 (β-OH), 133.6 (a), 127.9/7.34 (b), 118.8 (c), 152.0 (d), 115.3/6.86 (e), 126.4/7.10 (f), 10.09 (OH)
^{3}Asn	8.39	49.0	4.66	37.0	2.38/2.48	6.98 (NH), 7.40 (NH)
^{4}Hpg	8.00	54.7	5.54	–	–	128.9, 127.5/7.17, 114.7/6.68, 156.3, 9.35 (OH)
^{5}Hpg	8.77	54.7	5.54	–	–	128.0, 127.7/6.99, 114.4/6.62, 156.4, 9.33 (OH)
^{6}Cht	8.09	57.7	4.32	70.8	4.91	– (β-OH), 133.7 (a), 127.2/7.21 (b), 118.8 (c), 151.6 (d), 114.6/6.70 (e), 125.3/6.78 (f), 9.98 (OH)

Table 2. ^{1}H and ^{13}C NMR shifts of the peptide SP-1134 ([D$_6$]DMSO, $c_{SP\text{-}1134} = 5.8\ mg\,mL^{-1}$, 305 K).

	NH	C_α	H_α	C_β	H_β	$\delta(^{13}C)_n/\delta(^{1}H)_n$
^{1}Leu	–	50.6	3.64	39.6	1.43	23.2/1.49, 21.9/0.80, 21.9/0.80
^{2}Cht	8.56	58.2	4.52	71.6	4.64	5.64 (β-OH), 133.4 (a), 127.9/7.34 (b), 118.5 (c), 151.7 (d), 115.4/6.84 (e), 126.5/7.08 (f), 10.08 (OH)
^{3}Asn	8.34	49.0	4.62	37.1	2.41/2.41	6.96 (NH), 7.37 (NH)
^{4}Hpg	8.01	54.8	5.49	–	–	(–), 127.4/7.17, 114.5/6.65, 156.3, 9.36 (OH)
^{5}Hpg	8.73	55.1	5.46	–	–	(–), 128.1/6.98, 114.5/6.65, 156.4, 9.40 (OH)
^{6}Cht	7.82	57.7	4.56	71.1	4.84	5.61 (β-OH), – (a), 127.4/7.16 (b), 118.5 (c), 151.1 (d), 114.5/6.65 (e), 125.2/6.70 (f), 9.93 (OH)
^{7}Dpg	8.57	56.0	5.10	–	–	138.3, 105.3/6.27, 158.0, 101.5/6.16, 9.36 (OH)

  1433-7851/99/3813-1978 $ 17.50+.50/0 *Angew. Chem. Int. Ed.* **1999**, *38*, No. 13/14

(positions 5 and 7). The N-methylation of Leu and the O-glycosylations presumably are later biosynthetic steps. With knowledge of the linear precursor peptides, the synthesis of varied linear peptides which could possibly serve as substrates for the cyclization is now being pursued in a combinatorial approach. Thus the way towards new, semi-synthetic glycopeptide antibiotics would be opened.

Experimental Section

The SP1-1-oxygenase mutant from *Amycolatopsis mediterranei* DSM5908 was fermented in a liquid culture[11] with 50 μg mL^{-1} of erythromycin (output: 3.1 mg (SP-969) and 2.2 mg (SP-1134) per L of culture medium). After separation on XAD-16 adsorber resin, the peptides were purified by means of preparative HPLC (Nucleosil C18, 5 μm, 250 × 20 mm, Grom, Herrenberg, Germany), eluent: water (0.1% trifluoroacetic acid) and acetonitrile (0.1% trifluoroacetic acid).

The ES-MS and ES-MS/MS experiments were carried out with a triple quadrupole mass spectrometer (Perkin-Elmer Sciex, Thornhill, Canada) using a pneumatically supported electrospray source and argon collision gas.

The high-resolution mass spectra were recorded with the APEX-II-FT-ICR mass spectrometer 4.7 T (Bruker-Franzen, Bremen, Germany) with electrospray ionization in the positive-ion mode. The calibration took place externally with PPG-1020. The resolution achieved amounts to 17000 (SP-969) and 30000 (SP-1134), respectively, with accuracies of mass of 9 and 16 ppm, respectively. The NMR experiments were carried out on a WM-400 spectrometer console (Bruker, Karlsruhe, Germany).

For the GC-MS investigation (Carlo Erba 2900/Varian MAT112S; MAT, Bremen), the peptides were hydrolyzed under a vacuum (6N HCl, 110 °C, 24 h) and derivatized with methanol/HCl (110 °C, 15 min) and trifluoroacetic anhydride (110 °C, 10 min), respectively. Fused silica capillaries (25 m × 0.25 mm covered with L-Chirasil-Val and Lipodex E (30%), respectively, in PS255, film thickness 0.13 μm) were used as columns.

Received: December 29, 1998 [Z12848]
German version: *Angew. Chem.* **1999**, *111*, 2096 – 2099

Keywords: balhimycin · biosynthesis · glycopeptides · structure elucidation · vancomycin

[1] M. K. McCormick, W. M. Stark, G. E. Pittenger, R. C. Pittenger, G. M. McGuire, *Antibiot. Annu.* **1955 – 1956**, 606 – 611.
[2] a) G. M. Sheldrick, P. G. Jones, O. Kennard, D. H. Williams, G. A. Smith, *Nature* **1978**, *271*, 223 – 225; b) P. J. Loll, A. E. Bevivino, B. D. Korty, P. H. Axelson, *J. Am. Chem. Soc.* **1997**, *119*, 1516 – 1522; c) C. M. Harris, H. Kopecka, T. M. Harris, *J. Am. Chem. Soc.* **1983**, *105*, 6915 – 6922; d) S. G. Grdadolnik, P. Pristovsek, D. F. Mierke, *J. Med. Chem.* **1998**, *41*, 2090 – 2099.
[3] R. C. Yao, L. W. Crandall in *Glycopeptide Antibiotics* (Ed.: R. Nagarajan), Marcel Dekker, New York, **1994**, pp. 1 – 21.
[4] D. W. Armstrong, Y. Tang, S. Chen, Y. Zhou, C. Bagwill, J.-R. Chen, *Anal. Chem.* **1994**, *66*, 1473 – 1484.
[5] a) D. A. Evans, M. R. Wood, B. W. Trotter, T. I. Richardson, L. C. Barrow, J. L. Katz, *Angew. Chem.* **1998**, *110*, 2864 – 2868; *Angew. Chem. Int. Ed.* **1998**, *37*, 2700 – 2704; b) K. C. Nicolaou, S. Natarajan, H. Li, N. F. Jain, R. Hughes, M. Solomon, J. Ramanjulu, C. N. C. Boddy, M. Takayanagi, *Angew. Chem.* **1998**, *110*, 2872 – 2878; *Angew. Chem. Int. Ed.* **1998**, *37*, 2708 – 2714.
[6] A. M. A. van Wageningen, P. N. Kirkpatrick, D. H. Williams, B. R. Harris, J. K. Kershaw, N. J. Lennard, M. Jones, S. J. M. Jones, P. J. Solenberg, *Chem. Biol.* **1998**, *5*, 155 – 162.
[7] S. Chatterjee, E. K. S. Vijayakumar, S. R. Nadkarni, M. V. Patel, J. Blumbach, B. N. Ganguli, H.-W. Fehlhaber, H. Kogler, L. Vertesy, *J. Org. Chem.* **1994**, *59*, 3480 – 3484.
[8] S. Pelzer, R. Süssmuth, D. Heckmann, J. Recktenwald, P. Huber, G. Jung, W. Wohlleben, *Antimicrob. Agents Chemother.*, submitted.
[9] C. Kempter, D. Kaiser, S. Haag, G. Nicholson, V. Gnau, T. Walk, K. H. Gierling, H. Decker, H. Zähner, G. Jung, J. W. Metzger, *Angew. Chem.* **1997**, *109*, 510 – 513; *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 498 – 501.
[10] S. Braun, H.-O. Kalinowski, S. Berger, *150 and More Basic NMR Experiments*, 2nd Ed., WILEY-VCH, Weinheim, **1998**.
[11] S. Pelzer, W. Reichert, M. Huppert, D. Heckmann, W. Wohlleben, *J. Biotechnol.* **1997**, *56*, 115 – 128.

A Highly Luminescent Tetranuclear Silver(I) Cluster and Its Ligation-Induced Core Rearrangement**

Vincent J. Catalano,* Heidi M. Kar, and Joanna Garnas

Gold compounds, in particular gold – phosphane complexes, form inter- and intramolecular aggregates with Au–Au interactions shorter than twice the van der Waals radius.[1] Such aggregation produces numerous structural motifs that range from extended, linear chains and arrays to macromolecular clusters.[2] These assemblies often exhibit intense emissions, and the excited states are directly related to the Au ··· Au interaction. Recently, it was demonstrated that perturbation of this interaction forms the basis of a luminescent sensor by altering the intermolecular Au ··· Au separations and hence the emission properties.[3]

While the aurophilicity principle[4] has been used extensively to describe the strong, closed-shell metal – metal interactions of gold clusters, surprisingly few examples of argentophilic behavior of closely related silver analogues have been reported. In contrast, for silver compounds aggregation is not the rule, and high-nuclearity silver phosphane clusters are rarely formed.[5] The sparsity of homonuclear silver clusters may be attributed to the weaker metallophilic nature of silver compared to gold. For example, the association energy of Au ··· Au interactions is comparable to that of typical hydrogen bonds (ca. 5 – 10 kcal mol^{-1}). This energy is large enough to dictate the structure in solution and the solid state, but the weaker Ag ··· Ag interactions preclude such directed aggregation.

Here we report the synthesis and dynamic behavior of a tetranuclear silver cluster and its unusual core rearrangement upon further ligation. With their strong luminescence and high sensitivity to ligation, these compounds have the ability to act as solution-state sensors.

As shown in Scheme 1, reaction of two equivalents of $AgBF_4$ with 6,6-bis(diphenylphosphanyl)-2,2′-bipyridyl (P_2-bpy)[6] in trichloromethane produces a species tentatively identified as $[Ag_4(P_2\text{-bpy})_2](BF_4)_4$ on the basis of $^{31}P\{^1H\}$ NMR spectroscopy. At room temperature in $CDCl_3$, a

[*] Prof. V. J. Catalano, H. M. Kar, J. Garnas
Department of Chemistry
University of Nevada
Reno, NV 89557 (USA)
Fax: (+1) 775-784-6804
E-mail: vjc@unr.edu

[**] We thank the National Science Foundation (CHE-9624281) for their support of this work, and Prof. T. W. Bell for use of his fluorometer.

  1433-7851/99/3813-1979 $ 17.50+.50/0