[1] a) G. Schick, M. Levitus, L. Kvetko, B. A. Johnson, I. Lamparth, R. Lunkwitz, B. Ma, S. I. Khan, M. Garcia-Garibay, Y. Rubin, *J. Am. Chem. Soc.* **1999**, *121*, 3246 – 3247; b) K. Hutchison, J. Gao, G. Schick, Y. Rubin, F. Wudl, *J. Am. Chem. Soc.* **1999**, *121*, 5611 – 5612.
[2] a) M. Hetzer, H. Clausen-Schaumann, S. Bayerl, T. M. Bayerl, X. Camps, O. Vostrowsky, A. Hirsch, *Angew. Chem.* **1999**, *111*, 2103 – 2106; *Angew. Chem. Int. Ed.* **1999**, *38*, 1962 – 1965; b) M. Brettreich, S. Burghardt, C. Böttcher, T. Bayerl, S. Bayerl, A. Hirsch, *Angew. Chem.* **2000**, *112*, 1915 – 1918; *Angew. Chem. Int. Ed.* **2000**, *39*, 1845 – 1848.
[3] P. J. Fagan, J. C. Calabrese, B. Malone, *J. Am. Chem. Soc.* **1991**, *113*, 9408 – 9409.
[4] a) A. Hirsch, I. Lamparth, T. Grösser, *J. Am. Chem. Soc.* **1994**, *116*, 9385 – 9386; b) I. Lamparth, C. Maichle-Mössmer, A. Hirsch, *Angew. Chem.* **1995**, *107*, 1755 – 1757; *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 1607 – 1609; c) I. Lamparth, A. Herzog, A. Hirsch, *Tetrahedron*, **1996**, *52*, 5065 – 5075; d) X. Camps, A. Hirsch, *J. Chem. Soc. Perkin Trans. 1* **1997**, 1595 – 1596.
[5] a) B. Kräutler, J. Maynollo, *Angew. Chem.* **1995**, *107*, 69 – 70; *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 87 – 88; b) B. Kräutler, J. Maynollo, *Tetrahedron*, **1996**, *52*, 5033 – 5042; c) R. Schwenniger, T. Müller, B. Kräutler, *J. Am. Chem. Soc.* **1997**, *119*, 9317 – 9318.
[6] a) L. Isaacs, R. F. Haldimann, F. Diederich, *Angew. Chem.* **1994**, *106*, 2434 – 2437; *Angew. Chem. Int. Ed.* **1994**, *33*, 2339 – 2342; b) L. Isaacs, P. Seiler, F. Diederich, *Angew. Chem.* **1995**, *107*, 1636 – 1639; *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 1466 – 1469; c) P. Timmerman, L. E. Witschel, F. Diederich, C. Boudon, J.-P. Gisselbrecht, M. Gross, *Helv. Chim. Acta*. **1996**, *79*, 6 – 20; d) P. Seiler, L. Isaacs, F. Diederich, *Helv. Chim. Acta*. **1996**, *79*, 1047 – 1058; e) R. F. Haldimann, F.-G. Klärner, F. Diederich, *Chem. Commun.* **1997**, 237 – 238; f) L. Isaacs, F. Diederich, R. F. Haldimann, *Helv. Chim. Acta*. **1997**, *80*, 317 – 342.
[7] W. Qian, Y. Rubin, *Angew. Chem.* **1999**, *111*, 2505 – 2508; *Angew. Chem. Int. Ed.* **1999**, *38*, 2356 – 2360.
[8] a) F. Diederich, R. Kessinger, *Acc. Chem. Res.* **1999**, *32*, 537 – 545; b) F. Diederich, R. Kessinger in *Templated Organic Synthesis* (Eds.: F. Diederich, P. J. Stang), Wiley-VCH, Weinheim, **1999**, pp. 189 – 218.
[9] a) J.-F. Nierengarten, V. Gramlich, F. Cardullo, F. Diederich, *Angew. Chem.* **1996**, *108*, 2242 – 2244; *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 2101 – 2103; b) J.-F. Nierengarten, T. Habicher, R. Kessinger, F. Cardullo, F. Diederich, V. Gramlich, J.-P. Gisselbrecht, C. Boudon, M. Gross, *Helv. Chim. Acta*. **1997**, *80*, 2238 – 2276.
[10] a) J.-P. Bourgeois, L. Echegoyen, M. Fibbioli, E. Pretsch, F. Diederich, *Angew. Chem.* **1998**, *110*, 2203 – 2207; *Angew. Chem. Int. Ed.* **1998**, *37*, 2118 – 2121; b) J.-P. Bourgeois, L. Echegoyen, M. Fibbioli, E. Pretsch, F. Diederich, *Helv. Chim. Acta* **1999**, *82*, 1572 – 1595.
[11] A. G. Myers, P. S. Dragovich, E. L. Kuo, *J. Am. Chem. Soc.* **1992**, *114*, 9369 – 9386.
[12] New compounds were fully characterized by ^{1}H and ^{13}C NMR, FT-IR, UV/Vis, and high resolution MALDI-TOF MS.
[13] C. Bingel, *Chem. Ber.* **1993**, *126*, 1957 – 1959.
[14] a) J.-F. Nierengarten, D. Felder, J.-F. Nicoud, *Tetrahedron Lett.* **1999**, *40*, 273 – 276; b) J.-F. Nierengarten, C. Schall, J.-F. Nicoud, *Angew. Chem.* **1998**, *110*, 2037 – 2040; *Angew. Chem. Int. Ed.* **1998**, *37*, 1934 – 1936.
[15] X-ray crystal data of (±)-**1** ($C_{118}H_{52}O_{24}\cdot\approx 3.5\,CH_2Cl_2$, $M_r = 2150.8$): monoclinic, space group $P2_1/c$ (no. 14), $\rho = 1.495\ g\,cm^{-3}$, $Z = 4$, $a = 20.332(3)$, $b = 19.860(3)$, $c = 23.696(3)$ Å, $\beta = 93.02(1)^\circ$, $V = 9555(2)$ Å^3, $T = 228$ K. Nonius-CAD4 diffractometer, $Cu_{K\alpha}$ radiation, $\lambda = 1.5418$ Å. One red-black, slightly twinned crystal (linear dimensions approximately $0.3 \times 0.2 \times 0.1$ mm) was obtained by liquid – liquid diffusion of hexane into a CH_2Cl_2/C_6H_6 solution of (±)-**1**. The crystal was mounted at low temperature to prevent evaporation of the enclosed solvents. The twinning led to asymmetric reflection profiles for many reflections; an appropriate background correction was made to compensate for this effect. In addition, a semiempirical absorption correction, based on psi-scans, was applied to the data ($T_{max} = 0.99$, $T_{min} = 0.61$). The structure was solved by direct methods (SIR92: A. Altomare, G. Cascarano, C. Giacovazzo, A. Guagliardi, M. C. Burla, G. Polidori, M. Camalli, *J. Appl. Crystallogr.* **1994**, *27*, 435) and refined by full-matrix least-squares analysis (SHELXL-97, G. M. Sheldrick, University of Göttingen, Germany, **1997**) with the use of an isotropic extinction correction and $w = 1/[\sigma^2(F_0^2) + (0.125\,P)^2 + 25.85\,P]$, where $P = (F_0^2 + 2F_c^2)/3$. It consists of one ordered molecule of (±)-**1** and five disordered CH_2Cl_2 molecules with population parameters between about 0.5 and 0.75. All heavy atoms were refined anisotropically (hydrogen atoms of the ordered fullerene isotropically, in which hydrogen atomic positions are based on stereochemical considerations). Final $R(F) = 0.085$, $wR(F^2) = 0.235$ for 1365 parameters and 8593 reflections with $I > 2\sigma(I)$ and $2.2 < \theta < 57.0^\circ$ (corresponding R values based on all 12832 reflections are 0.125 and 0.274, respectively). 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-145416. 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).
[16] Sybyl force field from Spartan V.5.0 (Wavefunction, Irvine, CA, **1997**).
[17] C. Thilgen, I. Hosse, F. Diederich, *Top. Stereochem.*, in press.

High-Throughput Structure Verification of a Substituted 4-Phenylbenzopyran Library by Using 2D NMR Techniques**

Harald Schröder,* Peter Neidig, and Gérard Rossé

Innovative technologies for combinatorial chemistry and automated synthesis make possible the synthesis of large collections of compounds as potential sources of lead structures in medicinal chemistry. While the synthesis, purification, and biological screening of combinatorial libraries can be performed automatically, purity control and structure verification remain bottlenecks. Insufficient purity or ambiguous structures of screened samples hinder the exploitation of structure – activity relationships, which are critical elements for the further design of libraries. The HPLC, MS, and liquid chromatography mass spectrometry (LC-MS) techniques are generally accepted as the most appropriate means of characterization.[1] These analytical methods are fast and easy to automate, but they do not provide sufficient structural and quantitative data on the desired product. The existing automated methods based on ^{13}C and ^{1}H NMR spectroscopy[2] are not routinely applied due to the intrinsic low sensitivity of ^{13}C NMR spectroscopy and the lack of reliable proton-based automated structure verification methods. We report here a novel approach for the automated structure verification of compound libraries by using the experimental data from 2D

[*] Dr. H. Schröder, Dr. G. Rossé[+]
Pharma Division, Preclinical Research
F. Hoffmann-La Roche Ltd., 4070 Basel (Switzerland)
Fax: (+41) 61-688-7408
E-mail: harald.schroeder@roche.com
Dr. P. Neidig
Bruker Analytik GmbH
Silberstreifen, 76287 Rheinstetten (Germany)
[+] Present Address:
Selectide Corporation, A subsidiary of Aventis Pharmaceuticals, Inc.
1580 E. Hanley Blvd., Tucson, AZ 85737 (USA)
[**] We thank W. Meister for the electrospray-ionization mass spectrometry (ESIMS) analysis and Dr. H. Senn for fruitful discussions and critical reading of the manuscript.

  1433-7851/00/3921-3816 $ 17.50+.50/0

^{1}H,^{13}C-correlated (HSQC) NMR spectra. The method is designated as the automated definition and recognition of patterns (AutoDROP).[3] AutoDROP was applied to the rapid structure verification of all members of a library of ninety-six substituted 4-phenylbenzopyrans **1**. The automated analysis of the 2D HSQC NMR spectra gave the structural classification true, false, or unclear.

Structures from a library of compounds can be formally decomposed into a central core common to all compounds and a few variable modules (A_x, B_y, C_z,...), which are varied systematically with a limited number of structural fragments within the library (Figure 1). Molecules are thus regarded as a

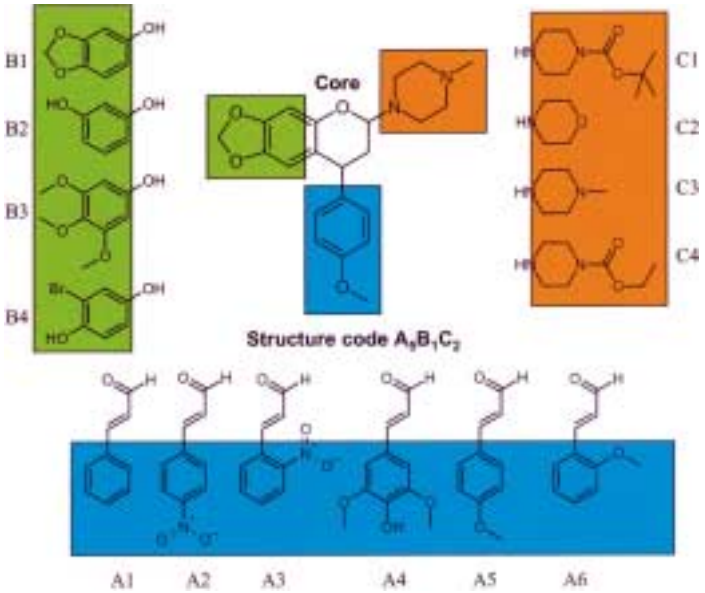
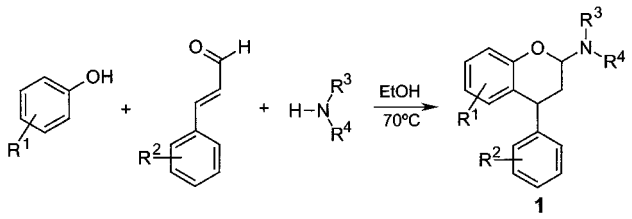


Figure 1. Redundant information in compounds array $A_xB_yC_z$. For $x=6$, $y=4$, and $z=4$, $x\times y\times z=96$ different compounds with the structure codes $A_xB_yC_z$ are obtained. This library is characterized by $x+y+z+\text{core}=15$ different structural fragments, and six of the ninety-six compounds contain all structural fragments (i.e., $A_1B_1C_1$, $A_2B_2C_2$, $A_3B_3C_3$, $A_4B_4C_4$, $A_5B_1C_3$, and $A_6B_2C_4$).

combination of different substructures, and a structure code $A_xB_yC_z$, defined by its synthesis, is assigned to each. Correspondingly, 2D HSQC NMR spectra can be regarded as a sum of spectra of substructures. A 4-phenylbenzopyran[4] library **1** was used to validate the automated structure–verification procedure (Scheme 1). The ^{1}H and 2D HSQC NMR spectra were acquired for each of the ninety-six substituted 4-phenylbenzopyrans and interpreted by using AutoDROP. In addition, compounds were analyzed by ESIMS (electrospray-ionization mass spectrometry) and HPLC.



Scheme 1. Synthesis of 4-phenylbenzopyran library **1**.

The key idea of AutoDROP is to systematically examine representative 2D HSQC NMR spectra and to derive from them individual subspectra of the individual substructures A_x, B_y, C_z. The subspectra are managed as spectral patterns in AutoDROP. Once the spectral patterns of all individual substructures have been defined, all available spectra can be tested for the presence of particular substructures in the synthesized compounds.

In the first step, an automated procedure was applied to define spectral patterns. The 2D HSQC spectra are peak-picked, and all peaks are given a normalized intensity of unity. Linear combinations of spectra were applied in a systematic way to automatically isolate and define the spectral patterns of specific substructures (Table 1). An addition and subtraction procedure affected thereby all peaks within a given search radius. Peak areas were finally obtained by an unsupervised direct cluster analysis, which detects groups of peaks. Rectangular envelopes around such groups were then compiled into spectral patterns (Figure 2). Alternatively, the spectral patterns could be obtained by manual interpretation of the representative 2D HSQC spectra.

Table 1. Linear combination of spectra to obtain the patterns for the substructure A_1.[a]

	A1	A2	B1	B2	C1	C2	C3	C4
+ A1B1C1	1	0	1	0	1	0	0	0
+ A1B1C2	1	0	1	0	0	1	0	0
+ A1B2C3	1	0	0	1	0	0	1	0
+ A1B2C4	1	0	0	1	0	0	0	1
− A2B1C1	0	1	1	0	1	0	0	0
− A2B1C2	0	1	1	0	0	1	0	0
− A2B2C3	0	1	0	1	0	0	1	0
− A2B2C4	0	1	0	1	0	0	0	1
sum:	4	−4	0	0	0	0	0	0

[a] The threshold is adjusted so that only peak areas of the substructure remain. A threshold of two would discriminate between A_1 and all other substructures. Integral boxes are then defined for the remaining peak areas.

In the second step of AutoDROP, the compound structures were validated by applying the defined spectral patterns to the NMR spectra. This automated operation was based on the integration of spectral patterns. The peak integral of the central core shared by all members of library **1** was used as an internal reference integral. Suitable reference spectra for each structural fragment were selected, and the ratio of each integral to the reference integral (ratio of core integral to single integral) was calculated for all spectra. This ratio was set to 100% for the reference spectra, and integral values for all other spectra were rescaled accordingly. Comparison of the ratio of the spectrum to be analyzed with the ratio of the corresponding reference spectrum allowed discrimination between correct and incorrect structures. A given structural fragment was verified when the threshold was above a predetermined value. For library **1** a threshold of 35% was selected. The proposed structure was assigned the attribute true when the patterns of all expected structural fragments of the compound were found in the 2D HSQC spectra (Table 2). The ratio of core integral to single integral or the signal-to-noise ratio was used to identify samples containing very small

  1433-7851/00/3921-3817 $ 17.50+.50/0

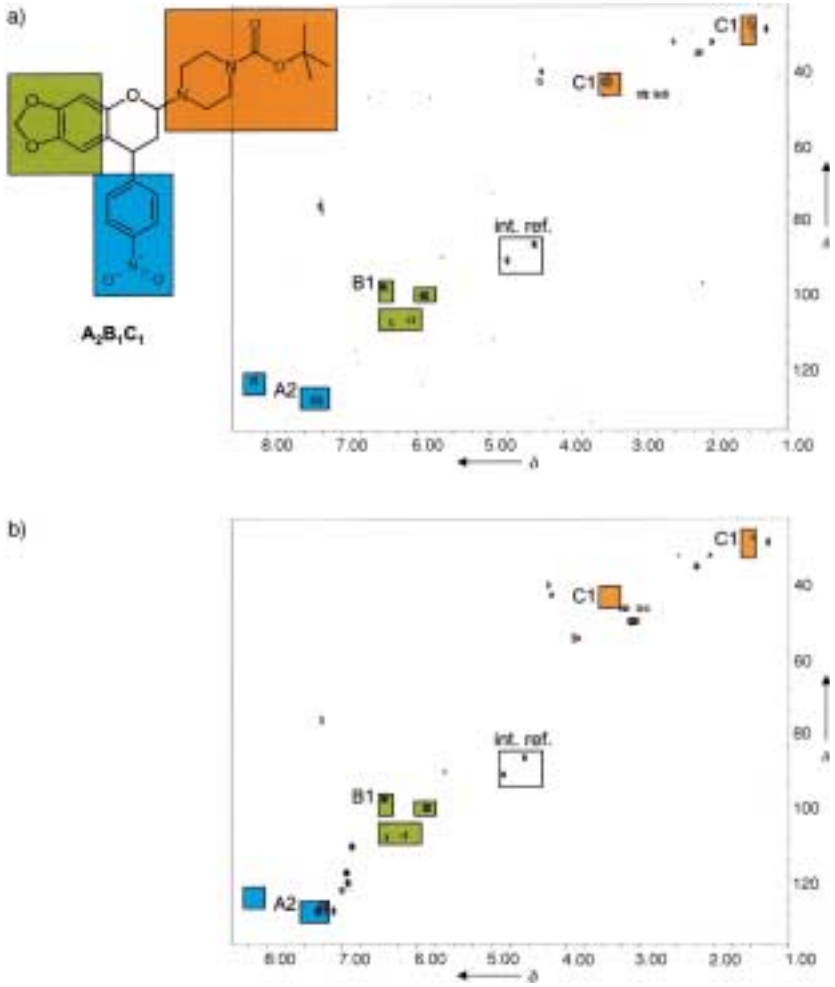
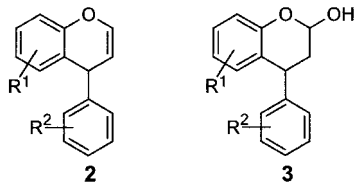


Figure 2. Decomposition of a 2D HSQC spectrum of a compound into the spectral patterns (color-coded). For example, the spectral pattern of B1 is composed of three signal regions (rectangular envelopes). a) The spectral patterns of each A_2, B_1, C_1 were found, and the structure of the expected compound $A_2B_1C_1$ is therefore validated. b) Structure of compound $A_2B_1C_1$ was not verified because the spectral patterns of both A2 and C1 are missing.

amounts of compound, as well as samples with large contents of impurities. The attribute "unclear" was assigned to these particular samples.

The ^{1}H NMR and 2D HSQC experiments established that the 4-phenylbenzopyrans were obtained as mixtures of diastereomers in an approximately 1.8:1 (*syn*:*anti*) ratio. Signals of two side products **2** and **3** overlap with signals of the compound in the spectrum. The spectra of the ninety-six 4-phenylbenzopyrans were measured in 16 h. The automated calculation steps to interpretate all spectra were performed in less than 5 min.



Of the ninety-six compounds analyzed with AutoDROP, sixty-eight proposed structures were found to be true (one false positive), eleven were proved false (one false negative), and seventeen designated "unclear". Subsequent manual analysis of the ^{1}H NMR spectra of these seventeen unclear structures found nine to be true (A6, C4, C10, D2, E6, E10, G5, G6, G10; see Figure 3) and eight false (B10, C12, D1, D8, D9, F12, H7, H8). These analyses showed that eighteen compounds were not obtained by the synthetic procedure. Comparison of the results of the NMR method with those of an automated ESIMS analysis (Figure 3) showed that the structures of four samples out of the ninety-six were not validated by either NMR or ESIMS analysis, and four analyses were in contradiction. These four samples were classified by interpretation of the ^{1}H NMR spectra. Erroneous automated NMR results were obtained for compounds in wells A12 (true structure) and D04 (false structure) but were easily corrected by means of the ^{1}H NMR spectra.

A novel procedure based on 2D NMR experiments was used for the rapid automated structure validation of all members of a 4-phenylbenzopyran library. The throughput of the AutoDROP NMR method is comparable to that of

Table 2. Graphical output of the NMR automated structure verification.[a]

	Result	A1	A2	A3	A4	A5	A6	B1	B2	B3	B4	C1	C2	C3	C4
A1B1C1	+	+	−	−	−	−	−	+	−	−	−	+	−	−	−
A1B2C1	+	+	−	−	−	−	−	−	+	−	−	+	−	−	−
A1B3C1	−	+	−	−	−	−	−	−	−	−	−	+	−	−	−
A1B4C1	?	+	−	−	−	−	−	−	−	−	+	−	−	−	−
A1B1C2	+	+	−	−	−	−	−	+	−	−	−	−	+	−	−
A1B2C2	+	+	−	−	−	−	−	−	+	−	−	−	+	−	−
A1B3C2	−	+	−	−	−	−	−	−	+	−	−	−	+	−	−
A1B4C2	−	+	−	−	−	−	−	−	−	−	+	−	−	−	−
A2B1C1	+	−	+	−	−	−	−	+	−	−	−	+	−	−	−
A2B2C1	+	−	+	−	−	−	−	−	+	−	−	+	−	−	−

[a] All 2D HSQC spectra were tested for the presence of each of the possible substructures by using the integration procedure. The substructure was considered to be present in the molecule (+) when all integrals of one substructure exceeded a defined threshold. When all substructures of the sample were present, the structure was assigned the category true (+). When some of the expected substructures were not found, the structure was given the category false (−). If the signal-to-noise ratio or the ratio of the core integral to the single integral was below a certain limit, the category unclear (?) was assigned.

  1433-7851/00/3921-3818 $ 17.50+.50/0

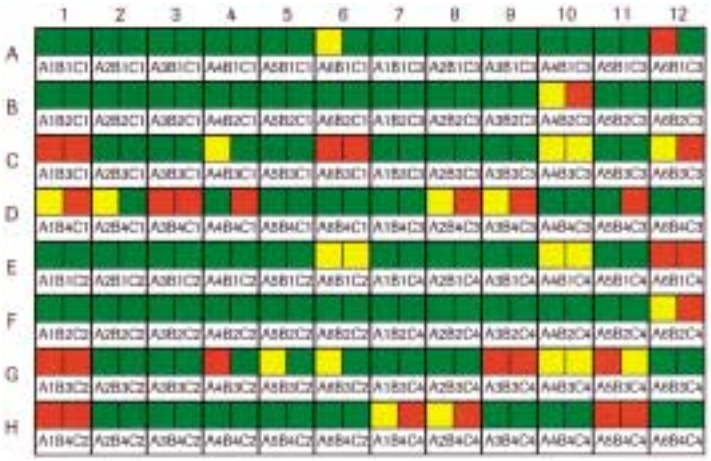


Figure 3. Combined results of the automated NMR and ESIMS analysis are summarized. Each cell contains the expected structure code and the data for NMR (top left), ESIMS (top right). Green means that the proposed structure is true in NMR spectroscopy and gives the expected molecular ion in ESIMS. Yellow indicates unclear results in both NMR spectroscopy and ESIMS. Red means that the proposed structure is false by NMR spectroscopy or does not give a diagnostic molecular ion in ESIMS.

current LC-MS methods. An automated qualitative and relative quantitative analysis of the compounds by using AutoDROP would be possible if the exact amount of sample were known. AutoDROP can be used in combination with ESIMS to analyze compound arrays from combinatorial chemistry and automated synthesis programs, and a wide application of this method is expected.

Experimental Section

The samples (from <1 to 5 mg) were dissolved in [D_6]DMSO (600 μL). NMR spectra were acquired in standard NMR tubes (5 mm) on a 400 MHz Bruker DRX 400 spectrometer equipped with a 120-sample changer. Tetramethylsilane was used as internal standard. For ^{1}H NMR 16 experiments were performed; two scans per increment and 128 experiments were performed for 2D HSQC. Cycle time to acquire ^{1}H and 2D HSQC NMR spectra and change the sample was 10 min per sample. AutoDROP is implemented in AMIX software (Bruker) and can be applied to 1D or 2D NMR spectra. ESIMS spectra were acquired on a PE Sciex API 300. Gradient of acetonitrile and H_2O/0.05 % trifluoroacetic acid were used for HPLC. Analytical HPLC was performed on an YMC Pack Pro C_{18} column (5 μm, 75 × 4.6 mm), flow rate 2.5 mL min^{-1}, detection at 254 nm.

Library **1**[4] was synthesized by a multicomponent reaction[5]. A 0.5 M stock solution of all reagents was prepared in ethanol. A solution of a phenol (400 μL, 0.2 mmol) was added to the reactor, and a solution of the corresponding unsaturated aldehyde (400 μL, 0.2 mmol) followed by a solution of the appropriate secondary amine (400 μL, 0.2 mmol) were dispensed. The reactors were closed and heated to 70 °C for 3 h. After cooling to room temperature, twenty-four compounds were collected by decantation, and the remaining seventy-two compounds were purified by preparative HPLC on a YMC Pack Pro C_{18} column (5 μm, 120 Å, 50 × 20 mm).

Received: May 26, 2000 [Z15177]

[1] a) *Combinatorial Chemistry* (Ed.: G. Jung), Wiley-VCH, Weinheim, **1999** and references therein; b) N. Sepetov, O. Issakova, *Comb. Chem. Technol.* **1999**, 169 – 203.

[2] a) A. Pretsch, *Nachr. Chem. Tech. Lab.* **1998**, *46*, 71 – 73, Suppl. 4; b) R. Bürgin Schaller, M. E. Munk, E. Pretsch, *J. Chem. Inf. Comput. Sci.* **1996**, *36*, 239 – 243; c) A. Williams, D. Mityushev, V. Shilay, M. Kvasha, Presentation at Eastern Analytical Symposium, Somerset, NJ, **1999**; d) M. Will, W. Fachinger, J. R. Richert, *J. Chem. Inf. Comput. Sci.* **1996**, *32*, 221 – 227.

[3] a) H. Schröder, P. Neidig, DE-19849231-C2, **1998**; b) H. Schröder, G. Rossé, P. Neidig, *Automated Structure Verification of Combinatorial Library Members using 2D NMR Techniques*, 37th IUPAC Congress/ 27th GDCh General Meeting, Berlin, **1999**; c) Technical components of AutoDROP are described in: H. Schröder, P. Neidig, *Bruker Report* **1999**, *147*, 18 – 21.

[4] L. Jurd, *J. Heterocycl. Chem.* **1991**, *28*, 983 – 986.

[5] a) I. Ugi, A. Dömling, B. Ebert in *Combinatorial Chemistry* (Ed.: G. Jung), Wiley-VCH, Weinheim, **1999**, pp. 125 – 165; b) L. Weber, K. Illgen, M. Alsmstter, *Synlett* **1999**, *3*, 366 – 374.

Molecular Topology: Easy Self-Assembly of an Organometallic Doubly Braided [2]Catenane**

Christopher P. McArdle, Jagadese J. Vittal, and Richard J. Puddephatt*

Molecular topology[1–5] is in a period of remarkable growth as the advent of new synthetic strategies, based on ideas such as metal-ion templating[6–8] and self-assembly through non-covalent interactions,[9–15] has allowed the design and isolation of supermolecules such as catenanes, rotaxanes, and knots.[16–18] Today, with the synthesis of increasingly intricate molecular topologies,[19–22] these supermolecules attract continued attention for their potential application in the development of molecular devices.[23] In this context, we report the discovery of an elegant example of molecular self-assembly which has led to the isolation and first structural characterization of a doubly braided [2]catenane. This complex organometallic structure, assembled in one step from eight components, exhibits evidence of a fast "rocking" motion of the two 50-membered rings. Fine tuning the organic backbone results in the formation of topologically distinct complexes; a simple ring and a single braid [2]catenane.

In a system comprising two rings, the formation of mechanical bonds (nonbonded interconnections) can lead to topologically isomeric molecules (Figure 1). While simple rings (**A**) and [2]catenanes (**B**) are both well recognized at the molecular level, the doubly braided [2]catenane (**C**) has

[*] Prof. R. J. Puddephatt, Dr. C. P. McArdle
Department of Chemistry
University of Western Ontario
London, ON, N6A 5B7 (Canada)
Fax: (+1) 519-661-3022
E-mail: pudd@uwo.ca
Prof. J. J. Vittal
Department of Chemistry
National University of Singapore
3 Science Drive 3, Singapore 117543 (Singapore)

[**] We thank Dr. C. Kirby for assistance with low-temperature NMR studies, Dr. M. C. Jennings for the X-ray structure determination of **3**, and the NSERC (Canada) for financial support. Funding from the National University of Singapore for the purchase of a Bruker AXS CCD diffractometer is also greatefully acknowledged.

  1433-7851/00/3921-3819 $ 17.50+.50/0