paper have been deposited with the Cambridge Crystallographic Data Center as supplementary publication no. CCDC-103076. 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).

[10] a) A. W. Johnson in *Ylides and imines of phosphorus* (Ed.: A. W. Johnson), Wiley-Interscience, New York, **1993**; b) S. M. Bachrach, *J. Org. Chem.* **1992**, *57*, 4367 – 4373.

[11] a) P. von R. Schleyer, T. Clark, A. J. Kos, G. W. Spitznagel, C. Rohde, D. Arad, K. N. Houk, N. G. Rondan, *J. Am. Chem. Soc.* **1984**, *106*, 6467 – 6475; b) B. Römer, G. G. Gater, M. Zhong, J. I. Brauman., *J. Am. Chem. Soc.* **1998**, *120*, 2919 – 2924.

[12] a) R. S. McDowell, A. Streitwieser, Jr., *J. Am. Chem. Soc.* **1984**, *106*, 4047 – 4048; b) H. J. Bestmann, A. J. Kos, K. Witzgall, P. von R. Schleyer, *Chem. Ber.* **1986**, *119*, 1331 – 1349.

[13] G. M. Sheldrick, *Acta Crystallogr. Sect. A* **1990**, *46*, 467 – 473.

[14] SHELXL; Program for Crystal Structure Refinement; G. M. Sheldrick; University of Göttingen **1997**.

Opposite Orientation of Backbone Inclination in Pyranosyl-RNA and Homo-DNA Correlates with Opposite Directionality of Duplex Properties**

Ronald Micura, Rene Kudick, Stefan Pitsch, and Albert Eschenmoser*

In preceding publications on pyranosyl-RNA ("p-RNA")[1a–f] we have emphasized the special importance of interstrand (as opposed to intrastrand) base stacking for the properties of this oligonucleotide base pairing system. Among the properties concerned are the sequence dependence of p-RNA duplex stability,[1a,e,f] the regioselectivity of the influence of dangling bases on duplex stability,[1d] and the sequence dependence of the efficiency and selectivity of template-controlled ligation reactions in replication[1f] and autocatalytic oligomerization.[1e] The dominance of interstrand over intrastrand base stacking in this pairing system is a consequence of the pronounced inclination between the (approximated) backbone axes relative to the axes of Watson – Crick base pairs in p-RNA duplexes. The orientation and approximate degree of this inclination can be easily inferred from a p-RNA strand's (idealized) pairing conformation (Figure 1 a, b). This

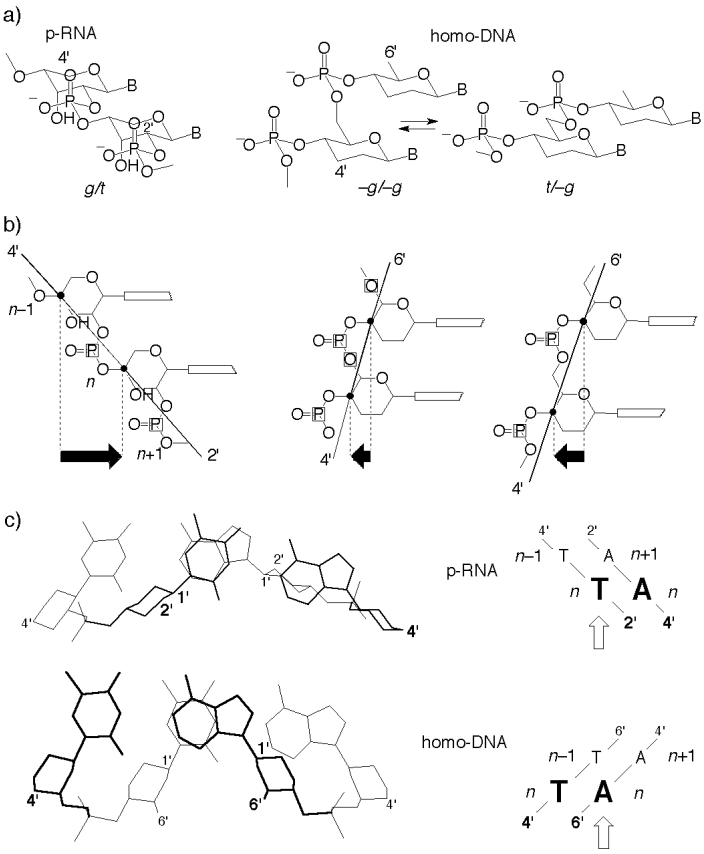


Figure 1. a) Idealized pairing conformations of p-RNA[1a,c] and homo-DNA.[2a,c,d] b) Projections of pairing conformations along an axis perpendicular to the mean planes of pyranose chairs, indicating sense and approximate degree of backbone inclination; nucleosidic torsion angle $-120°$. c) Projections perpendicular to the plane of a selected base pair of the p-RNA duplex $[pr(CGAATTCG)]_2$[1c] and the homo-DNA duplex $[ddGlc(A_5T_5)]_2$[2d] showing upstream interstrand stacking between a pyrimidine and a purine in p-RNA and corresponding downstream interstrand stacking in homo-DNA (taken from references [1c] and [2d]).

conformation has been derived by conformational analysis on the basis of steric repulsion criteria[1a] and confirmed in a NMR structure determination of the p-RNA duplex $[pr(CGAATTCG)]_2$.[1c] In accordance with this analysis, molecular mechanics based modeling of the same duplex shows[1c] that interstrand base stacking is expected to be effective between purines and purines as well as between purines and pyrimidines, but not between pyrimidines and pyrimidines (Figure 1 c).

The previously studied homo-DNA[2] is another pairing system with a pronounced backbone inclination. Compared to p-RNA, however, the inclination in homo-DNA is of opposite orientation, as can be deduced from homo-DNA's two (idealized) pairing conformations[2a,c] (Figure 1 a, b). The NMR structure analysis of the homo-DNA duplex $[ddGlc(A_5T_5)]_2$ by Otting et al.[2d] indicates that in at least one of the two pairing conformations, namely $t/-g$, interstrand base stacking should dominate over intrastrand stacking to the same extent as it does in p-RNA (Figure 1c). Whereas in p-RNA interstrand stacking acts in the upstream direction (base n stacks with base $n+1$ of the complementary strand), in homo-DNA it does so in the downstream direction

[*] Prof. Dr. A. Eschenmoser, Dr. R. Micura, Dr. R. Kudick, Dr. S. Pitsch
Laboratorium für Organische Chemie der
Eidgenössischen Technischen Hochschule
Universitätsstrasse 16, CH-8092 Zürich (Switzerland)
Fax: (+41) 1-632-1043
and
The Skaggs Institute for Chemical Biology at The Scripps Research Institute
10550 North Torrey Pines Road, La Jolla, CA, 92037 (USA)

[**] Chemistry of Pyranosyl-RNA, Part 8. Chemistry of α-Aminonitriles, Part 24. This work was supported by the Ciba-Geigy AG, Basel, and the Schulleitung of the ETH Zürich, as well as the Skaggs Foundation, La Jolla, CA. We thank Dr. H.-V. Hoppe, Dr. N. Windhab, and Dr. C. Miculka, previously Hoechst AG, Frankfurt, for providing us with p-RNA starting materials. R. M. thanks the Austrian Science Fund for a Schrödinger fellowship. Parts 7 and 23: Ref. [1e].

  1433-7851/99/3805-0680 $ 17.50+.50/0

(n stacks with $n-1$, see Figure 1c). If the postulate is correct that interstrand base stacking is the determinant for the sequence dependence of p-RNA duplex properties, then the sequence dependence of the same type of duplex properties in the homo-DNA series should possess opposite directionality. Therefore, a comparison of relevant properties of selected p-RNA and homo-DNA duplexes offers an opportunity for testing the thesis of the predominant role of interstrand base stacking in oligonucleotide systems with large backbone inclination. With this in mind, we have prepared missing base sequences (particularly of the homo-DNA series) that were required for such a comparison. We find indeed that the sequence dependence of duplex stability in these two pairing systems is, without exception, of opposite directionality. Moreover, we observe the remarkable phenomenon of a quasi-enantiomorphism in the CD spectra of duplexes of a given base sequence in the two systems, even though the sense of chirality of the corresponding sugar units is in both systems the same.

Table 1 gives experimental data concerning the thermal and thermodynamic stabilities of the duplexes investigated, each consisting of antiparallel self-complementary strands. In the p-RNA series, the sequence motif $(py)_n-(PU)_n$ gives rise to higher duplex stability than the inverse motif $(PU)_n-(py)_n$; in the homo-DNA series, the opposite holds (No. 1–6). Analogous behavior is found for the alternating sequence motifs $(py-PU)_n$ and $(PU-py)_n$, provided the base sequences do not allow for frameshifting (No. 13 and 14).[3] Dangling bases stabilize a duplex in the p-RNA series when located at the 2′-end (and not at the 4′-end);[1d] again, the opposite is true for the homo-DNA system (No. 13–18). Figure 2 illustrates our working hypothesis for interpreting and predicting relative stabilities of isomeric duplexes based on the type and number of interstrand base stackings.[4] The coherence observed in this comparison between prediction, based on a single discrimination criterion, and experimental observations in the two different oligonucleotide systems with diametrically opposed sequence dependence of duplex stabilities leads us to propose that backbone inclination is a useful new parameter for correlating structure and properties in oligonucleotide systems.[5]

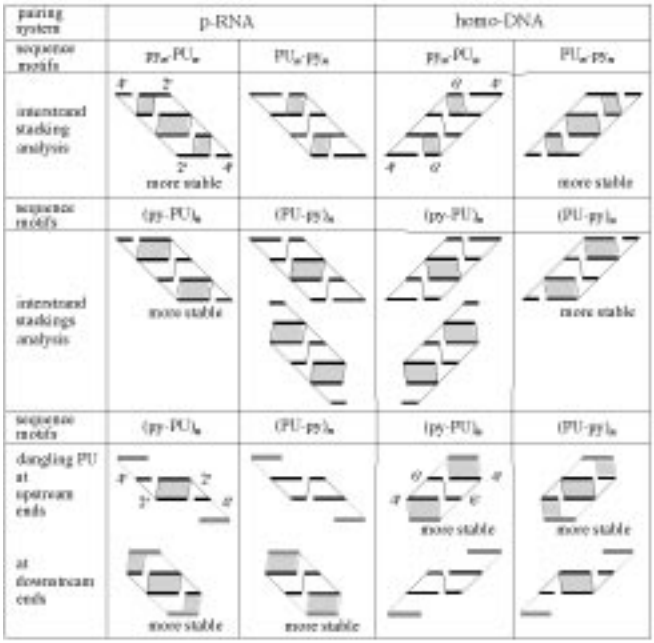


Figure 2. Formal analysis of the relationship between sequence motifs, positions of dangling bases and interstrand stacking in p-RNA and homo-DNA, and correlation with duplex stability. PU = purine, py = pyrimidine.

p-RNA and homo-DNA duplexes with related base sequences show quasi-enantiomorphic CD spectra although the sense of chirality of their pento- and hexopyranosyl sugar units, respectively, is the same. An especially illustrative

Table 1. Melting temperatures T_m (in °C) and thermodynamic parameters (in kcal mol^{-1}) of p-RNA and homo-DNA duplexes.[a–c]

No.	Self-complementary sequences	p-RNA duplexes				homo-DNA duplexes			
		T_m	$-\Delta G^0$	$-\Delta H^0$	$-T\Delta S^0_{25\,°C}$	T_m	$-\Delta G^0$	$-\Delta H^0$	$-T\Delta S^0_{25\,°C}$
1	TTTTAAAA	**40**	**9.8**	59.9	50.1	34	8.2	46.9	38.7
2	AAAATTTT	27	7.3	48.1	40.8	**38**	**8.6**	43.1	34.5
3	TTTTTAAAAA	**54**	**12.8**	67.6	54.8	45	10.3	55.1	44.8
4	AAAAATTTTT	43	10.8	71.9	61.1	**50**	**11.4**	58.3	46.9
5	CCCGGG	**68**	**13.0**	48.5	35.5	49	9.7	39.8	30.1
6	GGGCCC	58	10.8	41.3	30.4	**53**	**10.4**	40.9	30.5
7	TATATATA	40	9.3	51.6	42.3	38	8.8	45.4	36.6
8	ATATATAT	38	9.2	58.7	49.5	39	8.7	41.0	32.3
9	ATATATA	38	9.1	54.2	45.1	34	8.0	45.5	37.5
10	TATATAT	29	7.4	43.6	36.2	25	6.8	30.9	24.2
11	CGCGCG	65	12.5	47.9	35.4	55	10.9	40.7	29.8
12	GCGCGC	62	11.3	40.5	29.2	53	10.9	42.7	31.8
13	TACGTA	**39**	**8.7**	40.9	32.2	27	7.0	33.6	26.6
14	ATGCAT	29	7.3	36.0	28.7	**37**	**8.1**	34.2	26.1
15	TACGTAG	**46**	**10.3**	53.1	42.8	23	6.9	34.0	27.1
16	GTACGTA	37	8.3	39.0	30.7	**47**	**10.9**	52.0	41.1
17	ATGCATG	**51**	**10.9**	51.0	40.1	32	8.0	32.8	24.8
18	GATGCAT	28	7.1	36.6	29.5	**43**	**10.2**	54.7	44.5

[a] T_m values refer to an oligomer concentration of 10 μM, 150 mM NaCl, 10 mM Tris · HCl, pH 7.0; thermodynamic parameters determined from plots of T_m^{-1} vs. ln c; for method see reference [9]; estimated error on ΔH^0 ± 5%. [b] For preparation of p-RNA duplexes see reference [1a,b]; for preparation of homo DNA-duplexes see reference [2b,e]. [c] p-RNA data of No. 1, 2, 8 from reference [1b,f], of No. 5, 6 from ref. [1f], of No. 11, 12 from reference [1b]; homo-DNA data:[10] for No. 12 see also ref. [2c]. Tris = Tris(hydroxymethyl)aminomethane.

  1433-7851/99/3805-0681 $ 17.50+.50/0

example is depicted in Figure 3a, which shows the CD spectra of the self-complementary base sequences TACGTA and ATGCAT, comprising the sequence motifs $(py-PU)_3$ and $(PU-py)_3$. In both pairing systems, interstrand base stacking

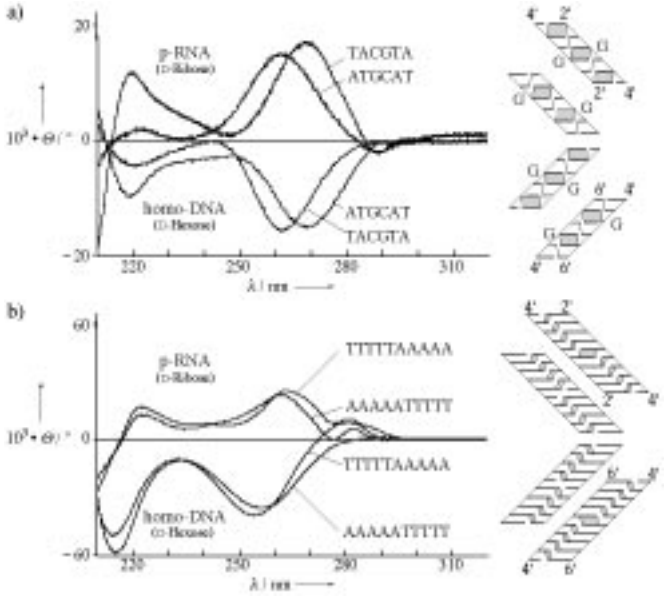


Figure 3. CD spectra of p-RNA and homo-DNA duplexes containing the sequence motifs a) $(py-PU)_3$ and $(PU-py)_3$ and b) $(py)_5-(PU)_5$ and $(PU)_5-(py)_5$ ($c \approx 10$ μM; in 150 mM NaCl, 10 mM Tris · HCl, pH 7.0, at 20 °C) with illustration of interstrand base stacking analyses.

operates between purines exclusively. The quasi mirror-image-like correspondence of the CD profiles in the two series does not primarily refer to the duplexes of identical base sequence, but rather to the duplexes with identical interstrand base stacking pattern. Thus, the CD profile of the p-RNA duplex TACGTA corresponds to that of the homo-DNA duplex ATGCAT; the base stacking pattern common to both is A/A, G/G, A/A. The spectrum with the bathochromically shifted maximum belongs in both series to the duplex with an additional G/G stacking in the duplex center (Figure 3a, right), which is considered to be responsible for the higher duplex stability. Mirror-image character of the CD spectra was observed in all p-RNA/homo-DNA-duplex pairs investigated thus far; Figure 3b depicts an example (duplex No. 3) which is about average with respect to the degree to which duplexes of the two pairing systems show quasi-enantiomorphism of their CD spectra.

Mirror-image character of oligonucleotide CD profiles can, but does not necessarily need to, reflect opposite helicity of twisted duplex structures. In quasi-linear systems with dominating interstrand base stacking, quasi-enantiomorphism of CD spectra may also reflect the fact that in duplexes of opposite backbone inclination a structural subunit comprising two interstand-stacking bases possesses the opposite sense of chirality (compare Figure 1c).[7] Unfortunately, in the present case neither the sense nor the degree of backbone-twisting in duplexes of the p-RNA and homo-DNA series are really established thus far.[8] Therefore, we have to leave the problem of the origin of the remarkable quasi-enantiomorphism of p-RNA and homo-DNA duplexes for further study; a more detailed structural knowledge to be obtained by X-ray crystallography seems indispensable. We do conclude, however, that quasi-linear oligonucleotide systems with strongly inclined backbones represent promising objects of study for detecting and analyzing previously unknown structure–property relationships in nucleic acids.[5]

Received: October 5, 1998 [Z12484IE]
German version: *Angew. Chem.* **1999**, *111*, 715–718

Keywords: DNA structures · duplex stability · homo-DNA · oligonucleotides · pyranosyl-RNA

[1] a) S. Pitsch, S. Wendeborn, B. Jaun, A. Eschenmoser, *Helv. Chim. Acta* **1993**, *76*, 2161–2183; b) S. Pitsch, R. Krishnamurthy, M. Bolli, S. Wendeborn, A. Holzner, M. Minton, C. Lesueur, I. Schlönvogt, B. Jaun, A. Eschenmoser, *Helv. Chim. Acta* **1995**, *78*, 1621–1635; c) I. Schlönvogt, S. Pitsch, C. Lesueur, A. Eschenmoser, B. Jaun, R. M. Wolf, *Helv. Chim. Acta* **1996**, *79*, 2316–2345; d) R. Micura, M. Bolli, N. Windhab, A. Eschenmoser, *Angew. Chem.* **1997**, *109*, 899–902; *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 870–873; e) M. Bolli, R. Micura, A. Eschenmoser, *Chem. Biol.* **1997**, *4*, 309–320; f) M. Bolli, R. Micura, S. Pitsch, A. Eschenmoser, *Helv. Chim. Acta* **1997**, *80*, 1901–1951.

[2] a) A. Eschenmoser, M. Dobler, *Helv. Chim. Acta* **1992**, *75*, 218–259; b) M. Böhringer, H.-J. Roth, J. Hunziker, M. Göbel, R. Krishnan, A. Giger, B. Schweizer, J. Schreiber, C. Leumann, A. Eschenmoser, *Helv. Chim. Acta* **1992**, *75*, 1416–1477; c) J. Hunziker, H.-J. Roth, M. Böhringer, A. Giger, U. Diederichsen, M. Göbel, R. Krishnan, B. Jaun, C. Leumann, A. Eschenmoser, *Helv. Chim. Acta* **1993**, *76*, 259–352; d) G. Otting, M. Billeter, K. Wüthrich, H.-J. Roth, C. Leumann, A. Eschenmoser, *Helv. Chim. Acta* **1993**, *76*, 2701–2756; e) K. Groebke, J. Hunziker, W. Fraser, L. Peng, U. Diederichsen, K. Zimmermann, A. Holzner, C. Leumann, A. Eschenmoser, *Helv. Chim. Acta* **1998**, *81*, 375–474.

[3] Frameshifts in $(PU-py)_n$ duplexes can lead to the same number of purine–purine interstrand stackings as present in corresponding $(py-PU)_n$ duplexes. This could be the reason for the negligibly small stability differences between the motif pairs No. 7 and 8.[1f] Such an interpretation seems supported by the relative duplex stabilities of No. 9 and 10. In these sequences the (compulsory) frameshifts can occur upstream (in p-RNA) and downstream (in homo-DNA) so that duplex No. 9 with four, as opposed to three, adenine–adenine stackings is the more stable one in both systems (see Figure 2).

[4] For the assessment of sequence dependence of duplex stabilities in the DNA and RNA series, empirical nearest neighbor parameters are used which do not explicitly differentiate between intra- and interstrand base stacking; see N. Sugimoto, S. Nakano, M. Yoneyama, K. Honda, *Nucleic Acids Res.* **1996**, *24*, 4501–4505; J. SantaLucia, Jr., H. T. Allawi, P. A. Seneviratne, *Biochemistry* **1996**, *35*, 3555–3562, and references therein. On the influence of dangling bases in the DNA and RNA series see N. Sugimoto, R. Kierzek, D. H. Turner, *Biochemistry* **1987**, *26*, 4554–4558; M. Senior, R. A. Jones, K. J. Breslauer, *Biochemistry* **1988**, *27*, 3879–3885.

[5] In oligonucleotide systems without (or with small) backbone inclination, base stacking is predominately intrastrand; this is the case, for instance, for DNA duplexes of the B-type (but less so for the A-type). The backbone inclination in a strongly helical duplex is not an easily discernible parameter and it is therefore understandable that it has not been used for the classification of DNA and RNA structures (see R. E. Dickerson et. al., *Nucleic Acids Res.* **1989**, 17, 1797–1803). Backbone inclination may nevertheless become a useful parameter for natural nucleic acids when it comes to a detailed structural description of stacking-dependent biological processes such as replication, transcription and codon/anticodon recognition. Local alterations of backbone inclination can arise through corresponding local alterations of nucleosidic torsion angles. Since local strand dissociation

  1433-7851/99/3805-0682 $ 17.50+.50/0

processes may proceed with retention of intrastrand, but must occur with breakage of interstrand stacking, such local structural fluctuations can result in local fluctuations of pairing strength within a duplex. To what extent such effects are significant in biological processes of the type mentioned above is an open, yet interesting, question. The definition of backbone inclination for helical duplexes and the application of this parameter for differentiating DNA and RNA duplex structures will be described in a forthcoming paper together with M. Egli (Northwestern University, Illinois, USA).

[6] For CD spectroscopy of oligonucleotides of the natural series see W. C. Johnson, Jr. in *Circular Dichroism and the Conformational Analysis of Biomolecules* (Ed.: G. D. Fasman), Plenum Press, New York, **1996**, p. 433 – 468.

[7] See for example: I. Jodal, A. Kovacs, J. Ott, G. Snatzke, *Chem. Ber.* **1989**, *122*, 1207 – 1210.

[8] The NMR structure analysis of the p-RNA and homo-DNA duplexes described in references [1c, 2d] did not allow the determination of these data.

[9] L. Marky, K. J. Breslauer, *Biopolymers* **1987**, *26*, 1601 – 1620.

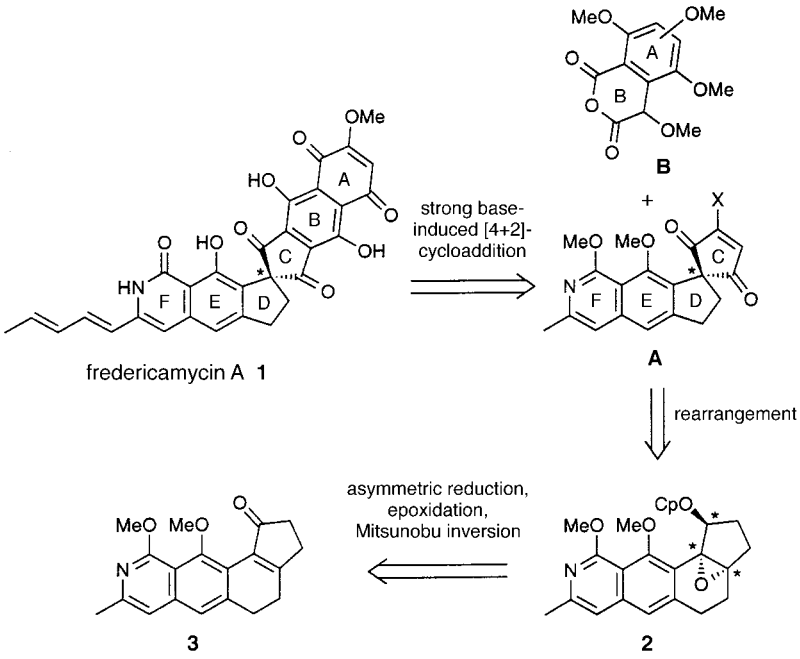
[10] Data of the homo-DNA duplexes No. 7, 9, and 10 were determined by S. Guntha (ETH, Zürich).

Asymmetric Total Synthesis of Fredericamycin A**

Yasuyuki Kita,* Kazuhiro Higuchi, Yutaka Yoshida, Kiyosei Iio, Shinji Kitagaki, Shuji Akai, and Hiromichi Fujioka

Fredericamycin A (**1**), isolated from *Streptomyces griseus* in 1981, possesses potent antitumor activity against a variety of tumor models (in vivo) such as P388 leukemia, B16 melanoma, and CD8F mammary, and does not show mutagenicity in the Ames test.[1, 2] Its structure consists of two sets of *peri*-hydroxy tricyclic aromatic moieties connected through a spiro quaternary carbon center, which is made chiral by the presence of a single methoxy group at the farthest position on the A-ring. Its promising biological profile as well as its unprecedented unique structure has made **1** quite attractive as a lead compound for a novel type of chemotherapeutic drug for human cancers, and hence extensive attention is being focused on its total synthesis. In spite of the enormous efforts towards this goal, including the total syntheses of racemic **1** by five research groups[3–7] and a recently reported synthesis of optically pure **1** by HPLC separation of a racemic intermediate of **1** using a special chiral column,[8] no one has so far succeeded in the asymmetric total synthesis of **1**, and its absolute configuration still remains unknown. Most of the reported total syntheses and the related model studies involved the construction of the spiro CD-ring at their final stages, and the lack of sufficient methods for the enantiodifferentiation of the highly symmetrical AB-plane has been the major obstacle in these asymmetric approaches. We present here the first asymmetric total synthesis of **1** with definite absolute configuration of the spiro center, which elucidates the absolute configuration of natural **1** 17 years after its isolation.[9, 10]

Our synthetic strategy, outlined in Scheme 1, is based on the strong base-induced intermolecular [4+2] cycloaddition of a suitably functionalized homophthalic anhydride (**B**) to an optically pure dienophile (**A**) corresponding to the CDEF-moiety, in which the regiochemistry during the cycloaddition is known to be controlled by the substituent X on the dienophile.[11, 12] We envisaged that the cycloaddition of **A** having unambiguous absolute configuration would afford **1** with the retention of the chiral integrity. Since the absolute stereochemistry of **1** is unknown, any synthetic strategy to be developed should be planned in such a way that it allows the synthesis of both enantiomers readily. The dienophile **A** could be prepared from the optically pure *trans*-epoxy camphanate **2** through the stereospecific rearrangement which we have disclosed recently.[13] As per our previous study, **2** in turn could be prepared from the enone **3** by an asymmetric reduction of



Scheme 1. Retrosynthesis of fredericamycin A (**1**).

[*] Prof. Dr. Y. Kita, K. Higuchi, Y. Yoshida, K. Iio, Dr. S. Kitagaki, Dr. S. Akai, Dr. H. Fujioka
Graduate School of Pharmaceutical Sciences, Osaka University
1-6, Yamada-oka, Suita, Osaka 565 – 0871 (Japan)
Fax: (+81) 6-6879-8229
E-mail: kita@phs.osaka-u.ac.jp

[**] This research was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, and Culture, Japan, a Special Coordination Funds of the Science and Technology Agency, Japan, and Japan Research Foundation for Optically Active Compounds. We are also grateful to Mr. Keita Matsumoto, Taisho Pharmaceutical Co., Ltd., for X-ray crystallographic analysis and Dr. Hiroshi Hasegawa (SS Pharmaceutical Co., Ltd., Japan) for generously providing an authentic sample of fredericamycin A.

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

  1433-7851/99/3805-0683 $ 17.50+.50/0