Organometallics **1990**, *9*, 66–74; c) M. Etienne, F. Biasotto, R. Mathieu, J. L. Templeton, *Organometallics* **1996**, *15*, 1106–1112; d) W. Hirpo, M. D. Curtis, *Organometallics* **1994**, *13*, 2706–2712.

[22] J. P. Collman, L. S. Hegedus, J. R. Norton, R. G. Finke, *Principles and Applications of Organotransition Metal Chemistry*, University Science, Mill Valley, CA, **1987**, chap. 9, pp. 459–520, and references therein.

[23] See, for example, a) M. D. Curtis, K. Shiu, W. M. Butler, *J. Am. Chem. Soc.* **1986**, *108*, 1550; b) M. D. Curtis, K. Shiu, *Inorg. Chem.* **1985**, *24*, 1213; c) M. D. Curtis, K. Shiu, W. M. Butler, *J. Am. Chem. Soc.* **1986**, *108*, 3335.

[24] a) S. E. Bromberg, H. Yang, M. C. Asplund, T. Lian, B. K. McNamara, K. T. Kotz, J. S. Yeston, M. Wilkens, H. Frei, R. G. Bergman, C. B. Harris, *Science* **1997**, *278*, 260; b) for related chemistry, see D. D. Wick, K. I. Goldberg *J. Am. Chem. Soc.* **1997**, *119*, 10235.

[25] a) C. A. Rusik, M. A. Collins, A. S. Gamble, T. L. Tonker, J. L. Templeton, *J. Am. Chem. Soc.* **1989**, *111*, 2550–2560; b) L. W. Francisco, P. S. White, J. L. Templeton, *Organometallics* **1997**, *16*, 2547.

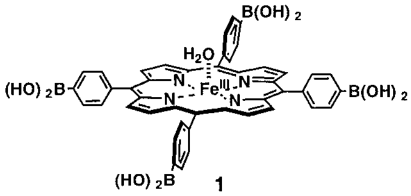
[26] a) H. G. Alt, H. E. Engelhardt, *J. Organomet. Chem.* **1985**, *288*, 165–177; b) P. L. Watson, R. G. Bergman, *J. Am. Chem. Soc.* **1979**, *101*, 2055–2062.

[27] Crystal structure analyses: a) **1**: $C_{61}H_{52}B_2F_{24}O_2N_6W$, $M_r = 1562.54$, triclinic, space group $P\bar{1}$, $a = 12.735(3)$, $b = 13.356(4)$, $c = 20.296(7)$ Å, $\alpha = 73.19(3)$, $\beta = 76.68(3)$, $\gamma = 82.63(2)°$, $Z = 2$, $V = 3208.7(17)$ A^3, $\rho_{calcd} = 1.617$ g cm^{-3}, blue plates, crystal dimensions: $0.45 \times 0.40 \times 0.06$ mm^3, Rigaku diffractometer, monochromated (graphite) $Mo_{K\alpha}$ radiation ($\lambda = 0.71073$ Å), $T = 173$ K, $5.00 < 2\theta < 46.00°$. Of 8884 independent reflections measured, 7060 with $I > 2.5\sigma(I)$ were used in the refinement, $R_1 = 0.045$, $R_2 = 0.041$. b) **2**: $C_{58}H_{44}B_2Cl_2F_{24}ON_6W$, $M_r = 1573.35$, monoclinic, space group $C2/c$, $a = 38.016(7)$, $b = 13.562(2)$, $c = 26.329(4)$, $Z = 8$, $V = 12\,856(4)$ A^3, $\rho_{calcd} = 1.626$ g cm^{-3}, blue plates, crystal dimensions: $0.25 \times 0.20 \times 0.20$ mm^3, Rigaku diffractometer, monochromated (graphite) $Mo_{K\alpha}$ radiation ($\lambda = 0.71073$ Å), $T = 295$ K, $5.00 < 2\theta < 45.00°$. Of 8396 independent reflections measured, 4661 with $I > 2.5\sigma(I)$ were used in the refinement, $R_1 = 0.048$, $R_2 = 0.052$. c) Crystallographic data (excluding structure factors) for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-101794 (**1**) and CCDC-101793 (**2**). Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB21EZ, UK (fax: (+44)1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).

A Strong Positive Allosteric Effect in the Molecular Recognition of Dicarboxylic Acids by a Cerium(IV) Bis[tetrakis(4-pyridyl)porphyrinate] Double Decker**

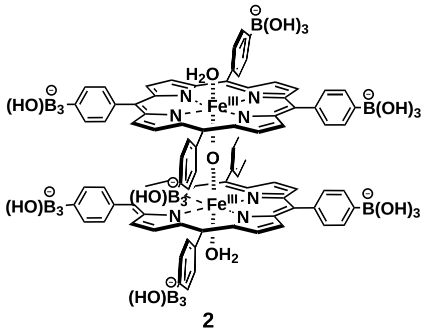
Masayuki Takeuchi, Tomoyuki Imada, and Seiji Shinkai*

Positive and negative allosteric effects are ubiquitous in nature where biological events must be efficiently regulated in response to chemical or physical signals from the outside world. Typical examples[1–4] are the cooperative binding of dioxygen to hemoglobin,[1] the hexamerization of the arginine repressor,[2] and a cooperative effect that depends on the concentration of arachidonate-containing phospholipids in cytosolic phospholipase A$_2$.[3] The biomimetic design of such allosteric systems is of great significance for regulating the complexation properties or catalytic activity of artificial receptors by means of allosteric effects.[5] Furthermore, the methodology is very useful for amplifying weak chemical or physical signals and converting them into forms that can be more conveniently measured. Several allosteric systems have been reproduced.[5–7] To the best of our knowledge, however, there is only one precedent for a positive allosteric effect with a large Hill coefficient n which involves cooperative binding of saccharides to a cyclic tetrameric resorcinol derivative ($n = 4$).[6]



1

We previously synthesized a porphyrinatoiron(III) complex bearing four boronic acid groups (**1**).[7] The μ-oxo dimer **2** is formed from **1** at alkaline pH values and shows extraordinarily high affinity and selectivity for glucose and galactose. However, only one pair of boronic acid groups is used to form 1:1 complexes with saccharides, and the remaining three pairs of boronic acids do not bind saccharides.[7] The strong negative allosterism was attributed to an inclination of the two porphyrin planes, which was induced by the binding of the first saccharide guest. Hence, if the first guest could suppress the rotation of the two porphyrin planes and maintain their parallel arrangement, the second guest should be bound more efficiently, and the system should exhibit positive allosterism.



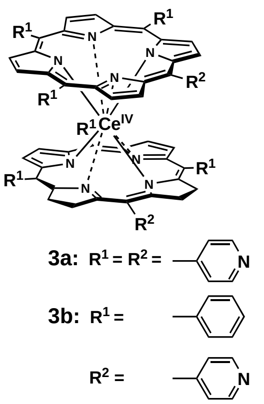
2

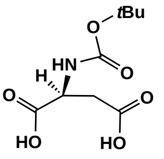
To construct such a porphyrin-based positively allosteric system, we chose a cerium(IV) bis(porphyrinate) double decker,[8, 9] namely, the tetrakis(4-pyridyl)porphyrin derivative **3a**.[8a, f–h] This molecule satisfies our requirements: First, slow rotation of the two porphyrin planes with respect to one another should be possible at room temperature, in analogy to similar cerium(IV) bis(diarylporphyrin) and bis(tetraarylporphyrin) complexes studied by Aida et al.;[9, 10] second, tilting of the two porphyrin planes is more difficult than in **2**; and third, the four pairs of 4-pyridyl groups are available as hydrogen-bond acceptor sites for diols, hydroxycarboxylic acids, and dicarboxylic acids. Compound **3b**, which has only one pair of pyridyl groups, was used as a reference. Compound

---

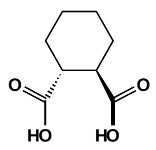
[*] Prof. S. Shinkai, Dr. M. Takeuchi, Dr. T. Imada
Department of Chemical Science and Technology
Faculty of Engineering, Kyushu University
Fukuoka 812 (Japan)
Fax: (+81) 92-642-3611
E-mail: seijitcm@mbox.nc.kyushu-u.ac.jp

[**] This work was supported by a Grant-in-Aid for COE Research "Design and Control of Advanced Molecular Assembly Systems" from the Ministry of Education, Science and Culture, Japan (#08CE2005).





L-4



(1*R*, 2*R*)-5

3a has a sharp positive allosteric effect with $n = 4$ and shows high selectivity for the enantiomers of BOC-aspartic acid (**4**; BOC = *tert*-butoxycarbonyl) and of 1,2-cyclohexanedicarboxylic acid (**5**).

Compounds **3a** and **3b** were synthesized from 5,10,15,20-tetrapyridylporphyrin[11] and 5,10,15-triphenyl-20-pyridylporphyrin,[11] respectively, according to the method reported by Buchler and Nawra.[8a] The products were identified by ^{1}H NMR spectroscopy, mass spectrometry, and elemental analyses (see Experimental Section). The ^{1}H NMR spectra were recorded in dichloromethane/ethyl acetate (30/1) at 25 °C. As chiral guest molecules, we chose five α-amino acid derivatives,[12] L-tartaric acid, dimethyl L-tartrate, and **5**.

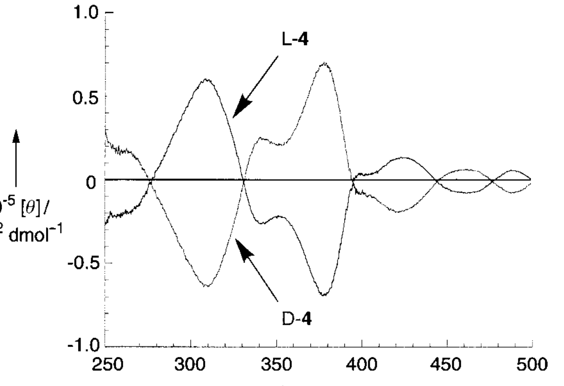


Figure 1. CD spectra of **3a** (0.1 mM) in the presence of **4** (10 mM) at 25 °C in dichloromethane/ethyl acetate (30/1). Similar CD spectra were obtained in the presence of the enantiomers of **5**. The circular dichroism of (1*R*,2*R*)-**5** has the same sign as that of that of L-**4**. [θ] = molar ellipticity.

First, circular dichroism (CD) spectra of **3a** and **3b** were recorded in the presence of each of the eight guest molecules. As shown in Figure 1, exciton-coupling CD bands were clearly observed for **3a** in the presence of **4** or **5**. Compound **3a** was CD-inactive in the presence of the other six guest molecules. Compound **3b** was CD-inactive in the presence of all guest molecules. Therefore, the strong CD bands in Figure 1 can be observed for a only a portion of the host – guest combinations. In Figure 2, $[\theta]_{max}$ at 310 nm is plotted against the guest concentration. The sigmoidal curvature indicates that the binding of the guest to **3a** is "self-accelerating". This cooperative guest binding can be analyzed with the Hill equation[13] [Eq. (1)] where [G] is the concentration of the

$$\lg(y/(1-y)) = n\lg[\mathrm{G}] + \lg K \tag{1}$$

guest, K the association constant, and n the Hill coefficient, and $y = K/([\mathrm{G}]^{-n} + K)$. From the slope and the intercept of the linear plots we obtained $K = 2.63 \times 10^{11}$ (mol dm^{-3})$^{-4}$ and $n = 3.9$ for L-**4** (correlation coefficient 0.988), and $K = 2.75 \times 10^9$ (mol dm^{-3})$^{-4}$ and $n = 4.0$ for (1*R*,2*R*)-**5** (correlation coefficient 0.995). The 1:4 composition of the CD-active complexes was further corroborated by a Job diagram.[14] As shown in Figure 3, a plot of $[\theta]_{max}$ at 310 nm against [**3a**]/([**3a**] + [L-**4**]) has a maximum at 0.2. This supports the view that the complex consists of one **3a** host and four L-**4** guests. These findings consistently indicate that four pairs of pyridyl groups in **3a** cooperatively bind these chiral guest molecules and that the two porphyrin planes are immobilized in a chiral conformation to give the CD-active complexes. Hence, this is a rare example of an artificial system with a strong positive allosteric effect and $n = 4$.

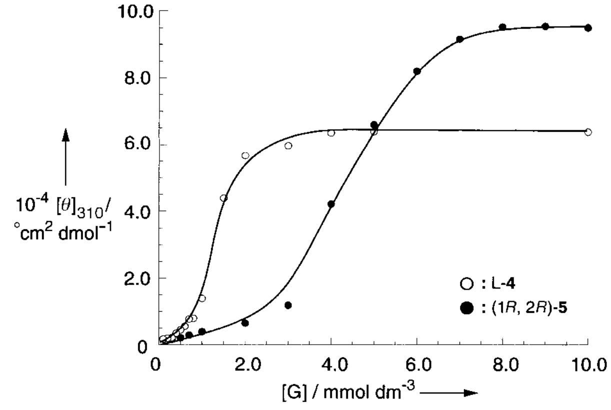


Figure 2. Plots of $[\theta]_{max}$ at 310 nm for **3a** versus the concentration of the guest compound [G] (measurement conditions as in Figure 1).

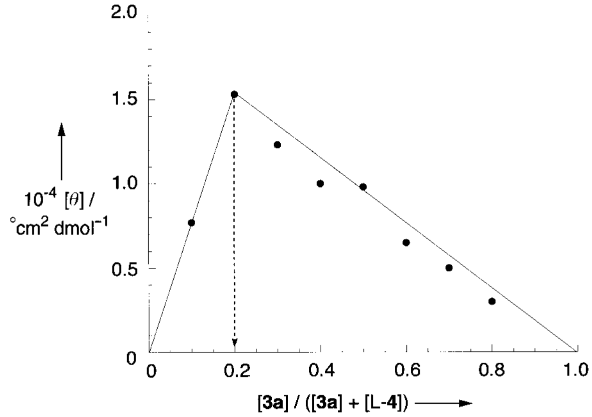


Figure 3. Job diagram. The sum [**3a**] + [L-**4**] was kept constant (1 mM).

At this point, two important questions come to mind: First, why can **3a** bind dicarboxylic acids, whereas **3b** cannot? Second, why do only **4** and **5** form CD-active complexes with

3a? To clarify the first question, we recorded the ^{1}H NMR spectra [25 °C, $CD_2Cl_2/CD_3CO_2C_2D_5$ (30/1)] of L-**4** in the presence of **3a** (1.00 mM). The resonances of the NH, α-CH, and β-CH_2 protons of L-**4** were shifted to lower field (from $\delta = 5.59$ to 5.70 for NH, from $\delta = 4.56$ to 4.62 for α-CH, and from $\delta = 2.95$ to 3.06 for β-CH_2; [L-**4**] = 1.00 mM) owing to the anisotropic effect of the porphyrin rings of **3a**, and the plots of $\Delta\delta$ versus [L-**4**] showed sigmoidal curvature. In contrast, **3b** does not induce such a downfield shift for these protons of L-**4**. The difference reveals the mechanism that is responsible for the positive allosteric effect in this system. Although host **3b** has a pair of pyridyl groups and could therefore potentially bind **4** or **5** by hydrogen bonding, the unchanged chemical shifts in the ^{1}H NMR spectrum suggest that this site exhibits at most a very weak interaction with dicarboxylic acids. In other words, the first association constant K_1 for binding the first guest is very small for **3b**. Conceivably, the gain in Gibbs free energy from the pyridine/carboxylic acid interaction is overcompensated by the loss of Gibbs free energy associated with suppression of the rotation of the porphyrin rings. This is also the case in **3a** for the binding of the first guest. In **3a**, however, once the rotation of the porphyrin rings has been suppressed by the first guest, successive binding of the second, third, and fourth guests can occur without such a loss of Gibbs free energy. Thus, as the number of the bound guests increases, rotation of the porphyrin rings becomes more strongly suppressed, and guest binding becomes increasingly favorable (Figure 4). This type of guest binding is possible only in the 1:4 complex.

According to the X-ray crystallographic studies of the cerium(IV) bis(porphyrinate) double decker, the distance between the two porphyrin planes is about 3.4 Å.[8c] This distance is comparable with that between two the carboxylic acid groups in **4** and **5**, which are separated by a C_2 spacer, and they can therefore quadruply bridge two porphyrin rings with eight hydrogen bonds. In these complexes, the two porphyrin rings are twisted in the right-or left-handed direction, depending on the configuration of the guest, but the distance between two porphyrin planes should not be changed by cooperative guest binding. The enantiomers of BOC-glutamic acid, which have a C_3 spacer, cannot satisfy these requirements and therefore do not result in CD-active species. It is surprising that a difference of one methylene unit can be recognized in an all-or-nothing manner. This is due to multiplication of the small difference by the allosteric binding process. On the other hand, L-tartaric acid, which has a C_2 spacer, satisfies the basic structural requirement as a guest molecule. When a solution of **3a** in dichloromethane and an solution of L-tartaric acid in ethyl acetate were mixed, a colored precipitate formed immediately. The elemental analysis of this precipitate established that it consists of one **3a** host and four L-tartaric acid guests.[15] These results answer our second question regarding host–guest selectivity.

In conclusion, we have demonstrated that the cerium(IV) bis[tetrakis(4-pyridyl)porphyrinate] double decker shows a high positive allosteric effect with a Hill coefficient of 4. Such strong allosteric effects are very rare in artificial systems.[6] The origin of the cooperative guest binding is attributable to the successive suppression of the rotation of the porphyrin rings without deformation of the basic structure of the cerium double decker.[10] Thus, the present system should be readily applicable to the regulation of association processes and catalytic activity: For example, **3a** is useful for the efficient release or capture of **4** and **5** in solution, and the catalytic activity of porphyrins can be regulated by means of **4** or **5**.

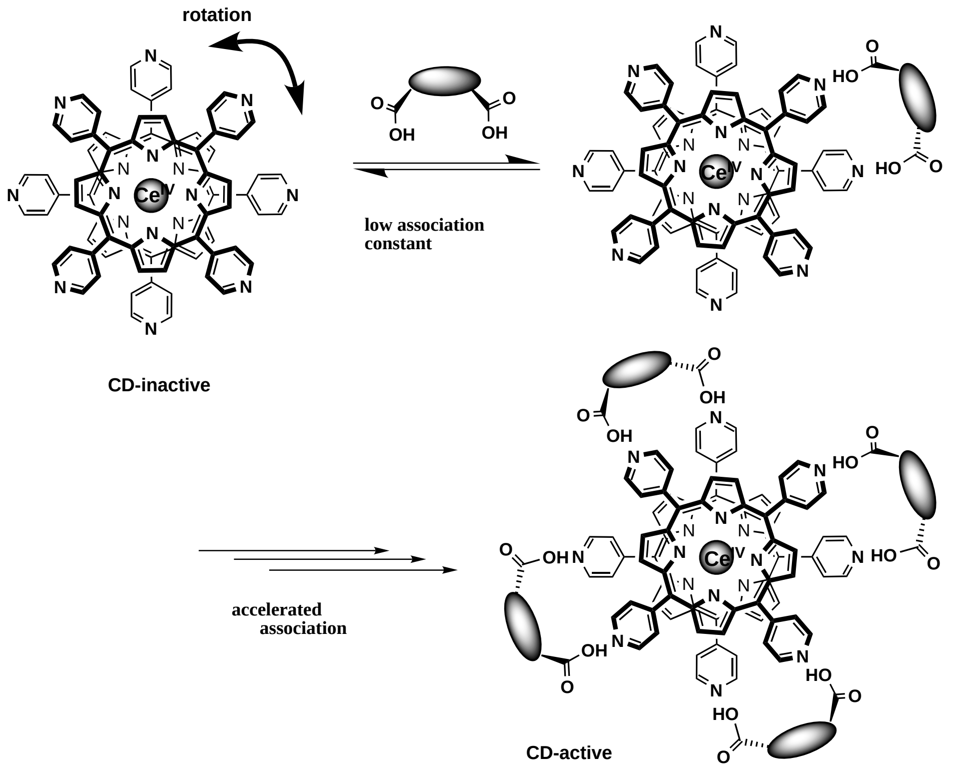


Figure 4. Schematic representation of the cooperative binding of **4** or **5** to **3a**.

Experimental Section

3a: This compound was synthesized from 5,10,15,20-tetrapyridylporphyrin according to the method of Buchler and Nawra.[8a] ^{1}H NMR (250 MHz, CD_2Cl_2, 27 °C, TMS): $\delta = 6.35$ (brs, 8H, ArH), 8.34 (brs, 16H, pyrrole β-H), 8.60 (brs, 8H, ArH), 9.45 (m, 16H, ArH); MS (positive SI-MS): m/z calcd for $C_{80}H_{48}N_{16}Ce$: 1372 [M^+]; found: 1372; elemental analysis calcd for $C_{80}H_{48}N_{16}Ce$: C 69.96, H 3.51, N 16.32; found: C 69.92, H 3.23, N 16.06.

3b: To a solution of 5,10,15-triphenyl-20-(4-pyridyl)porphyrin (128 mg, 0.2 mmol) in 1,2,4-trichlorobenzene (TCB, 60 mL, distilled) was added *t*BuLi (1.6 M in *n*-hexane, 1.5 mL, excess). The color of the solution changed from purple to green. The solution was stirred under N_2 for 30 min at room temperature, [Ce(acac)$_3$]·H_2O (270 mg, 0.6 mmol) added, and the mixture heated to reflux under N_2 for 5 h. After removal of TCB under reduced pressure, the crude product was dissolved in CH_2Cl_2 and purified by chromatography on a silica gel column with CH_2Cl_2/MeOH (95/5). Evaporation of the third fraction yielded 15 mg (11 %) of **3b** as a brown-violet powder. ^1H NMR (250 MHz, CD_2Cl_2, 27 °C, TMS): $\delta = 6.35$ (m, 8H, ArH), 8.34 (brs, 16H, pyrrole β-H), 8.60 (m, 8H, ArH), 9.45 (m, 22H, ArH); MS (positive SI-MS): m/z calcd for $C_{86}H_{54}N_{10}Ce$: 1368 [M^+]; found: 1368; elemental analysis calcd for $C_{80}H_{48}N_{16}Ce$: C 75.53, H 3.98, N 10.24; found: C 74.92, H 3.70, N 10.06; UV/Vis (CH_2Cl_2): λ_{max} ($\lg\varepsilon$) = 398 nm (5.22), 543 nm (4.20), 650 nm (3.57), 722 nm(3.48).

CD spectroscopy: A stock solution of dicarboxylic acid in ethyl acetate was added to a 0.1 mM solution of **3a** or **3b** in dichloromethane. The dichloromethane/ethyl acetate ratio of the sample was adjusted to 30/1. The CD spectra were recorded from 250 nm to 500 nm for different concentrations of guest molecules at 25 °C.

Instruments: Shimadzu UV-160A (absorption spectra), JASCO J-720WI (CD spectra), and JEOL 400 MHz FT-NMR (GSX-400) (^1H NMR spectra).

Received: November 14, 1997
Revised version: April 20, 1998 [Z11159IE]
German version: *Angew. Chem.* **1998**, *110*, 2242–2246

Keywords: allosterism · amino acids · chirality · molecular recognition · porphyrinoids

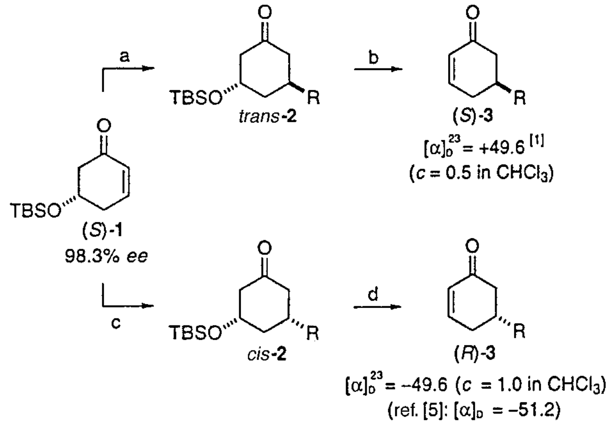
---

[1] a) M. F. Perutz, *Annu. Rev. Biochem.* **1979**, *48*, 327; b) J. Monod, J.-P. Changeux, F. Jacob, *J. Mol. Biol.* **1963**, *6*, 306; c) M. F. Perutz, G. Fermi, B. Luisi, B. Shaanan, R. C. Liddington, *Acc. Chem. Res.* **1987**, *20*, 309.
[2] R. Gramdori, T. A. Lavoie, M. Pflumm, G. Tian, H. Niersbach, W. K. Maas, R. Fairman, J. Carey, *J. Mol. Biol.* **1995**, *254*, 150.
[3] J. R. Burke, M. R. Witmer, J. Tredup, R. Micanovic, K. R. Gregor, J. Lahiri, K. M. Tramposch, J. J. Villaframca, *Biochemistry* **1995**, *34*, 15165.
[4] a) A. M. Filenko, V. M. Danilova, A. Sobieszek, *Biophys. J.* **1997**, *73*, 1593; b) S. Modi, D. E. Gilham, M. J. Sutcliffe, L.-Y. Lian, W. V. Primrose, C. R. Wolf, G. C. K. Roberts, *Biochemistry* **1997**, *36*, 4461; c) F. J. Bruzzese, P. R. Connelly, *Biochemistry* **1997**, *36*, 10428; d) J. A. Schetz, D. R. Sibley, *J. Neurochem.* **1997**, *68*, 1990.
[5] a) T. G. Traylor, M. J. Mitchell, J. P. Ciconene, S. Nelson, *J. Am. Chem. Soc.* **1982**, *104*, 4986; b) J. Rebek, Jr., *Acc. Chem. Res.* **1984**, *17*, 258; c) J. Rebek, Jr., T. Costello, L. Marshall, R. Wattley, R. C. Gadwood, K. Onan, *J. Am. Chem. Soc.* **1985**, *107*, 7481; d) I. Tabushi, S. Kugimiya, M. G. Kinnaird, T. Sasaki, *J. Am. Chem. Soc.* **1985**, *107*, 4129; e) I. Tabushi, S. Kugimiya, *J. Am. Chem. Soc.* **1986**, *108*, 6926; f) P. D. Beer, A. S. Rothin, *J. Chem. Soc. Chem. Commun.* **1988**, 52; g) R. C. Petter, J. S. Salek, C. T. Sikorski, G. Kumaravel, F.-T. Lin, *J. Am. Chem. Soc.* **1990**, *112*, 3860; h) H.-J. Schneider, D. Ruf, *Angew. Chem.* **1990**, *102*, 1192; *Angew. Chem. Int. Ed. Engl.* **1990**, *29*, 1159; i) R. P. Sijbesma, R. J. Nolte, *J. Am. Chem. Soc.* **1991**, *113*, 6695; j) Y. Kobuke, Y. Satoh, *J. Am. Chem. Soc.* **1992**, *114*, 789.
[6] K. Kobayashi, Y. Asakawa, Y. Kato, Y. Aoyama, *J. Am. Chem. Soc.* **1992**, *114*, 10307.
[7] M. Takeuchi, T. Imada, S. Shinkai, *J. Am. Chem. Soc.* **1996**, *118*, 10658.
[8] Syntheses of metal bis(porphyrinate) double deckers: a) J. W. Buchler, M. Nawra, *Inorg. Chem.* **1994**, *33*, 2830; b) J. W. Buchler, V. Eiermann, H. Hanssum, G. Heinz, H. Rüterjans, M. Schwarzkopf, *Chem. Ber.* **1994**, *127*, 589; c) J. W. Buchler, A. De Cian, J. Fischer, P. Hammerschmitt, J. Löffler, B. Scharbert, R. Weiss, *Chem. Ber.* **1989**, *122*, 2219; d) J. W. Buchler, G. Heinz, *Chem. Ber.* **1996**, *129*, 1073; e) J. W. Buchler, G. Heinz, *Chem. Ber.* **1996**, *129*, 201, and references therein; f) J. Jiang, K. Machida, E. Yamamoto, G. Adachi, *Chem. Lett.* **1991**, 2035; g) J. Jiang, K. Machida, G. Adachi, *Bull. Chem. Soc. Jpn.* **1992**, *65*, 1990; h) J. Jiang, K. Machida, G. Adachi, *J. Alloys Compd.* **1993**, *32*, 950, and references therein.
[9] K. Tashiro, K. Konishi, T. Aida, *Angew. Chem.* **1997**, *109*, 882; *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 856.
[10] The rate of rotation of the porphyrin rings in cerium bis(porphyrinate) double deckers is comparable to or slower than the NMR time scale.[9] However, the allosteric behavior is observable for this "static" equilibrium system as long as the porphyrin rings rotate.
[11] S. Takagi, T. Yamamura, M. Nakajima, K. Ishiguro, Y. Kawanishi, S. Nihojima, H. Tsuchiya, T. Saito, Y. Sasaki, *Bull. Chem. Soc. Jpn.* **1981**, *54*, 3879.
[12] The α-amino acid derivatives tested here were **4**, BOC-L-glutamic acid, BOC-L-serine, BOC-L-histidine, and di-BOC-L-cystine.
[13] a) J. Baldwin, C. Chothia, *J. Mol. Biol.* **1979**, *129*, 175; b) K. A. Connors, *Binding Constants*, Wiley, New York, **1987**.
[14] A. Job, *Ann. Chim. (Paris)* **1928**, *9*, 113.
[15] Elemental analysis of the precipitate: calcd for **3a** · (L-tartaric acid)$_{4.0}$: C 58.42, H 3.68, N 11.35; for **3a** · (L-tartaric acid)$_{3.8}$: C 58.83, H 3.67, N 11.53; for **3a** · (L-tartaric acid)$_{3.0}$: C 60.59, H 3.65, N 12.29; found: C 58.76, H 3.68, N 11.49. Thus, the experimental result is closest to **3a** · (L-tartaric acid)$_{3.8}$. The slight deviation from the 1:4 stoichiometry is due to immediate precipitation after mixing.

Unexpected *cis*-Selective 1,4-Addition Reaction of Lower Order Cyanocuprates to Optically Active 5-(*tert*-Butyldimethylsiloxy)-2-cyclohexenone**

Georges Hareau-Vittini, Shinichi Hikichi, and Fumie Sato*

We have recently reported an efficient and practical synthesis of the 5-(*tert*-butyldimethylsiloxy)-2-cyclohexenone (**1**; 98.3 % *ee*) as a convenient chiral 2,5-cyclohexadienone synthon.[1, 2] The enone **1**, as expected, undergoes highly selective *anti*-1,4-additions with organocopper reagents such as [R_2CuLi] or [$R_2Cu(CN)Li_2$] to yield *trans*-**2** as the major products. These can be easily converted into the optically active 5-substituted-2-cyclohexenone **3** upon treatment with DBU or toluenesulfonic acid (Scheme 1).[1]



Scheme 1. Preparation of both enantiomers of **3** starting from *(S)*-**1**. a) [nBu$_2$Cu(CN)Li$_2$], 92 %, 98 % *dr*; b) DBU (3 equiv), DMF, 20 °C, 5 h, 93 %; c) [nBuCu(CN)Li], 91 %, 99 % *dr*; b) DBU (5 equiv), DMF 100 °C, 1 h, 74 %. DBU = 1,8-diazabicyclo[5.4.0]undec-7-ene, TBS = *tert*-butyldimethylsilyl.

---

[*] Prof. Dr. F. Sato, Dr. G. Hareau-Vittini, S. Hikichi
Department of Biomolecular Engineering
Tokyo Institute of Technology
4259 Nagatsuta-cho, Midori-ku, Yokohama,
Kanagawa 226-8501 (Japan)
Fax: (+81) 45-924-5826
E-mail: fsato@bio.titech.ac.jp

[**] This work was supported by the Japan Society for the Promotion of Science.