Keywords: aggregation · crystal growth · polymethines · solid-state spectroscopy · thin films

[1] a) J. Fabian, H. Nakazumi, M. Matsuoka, *Chem. Rev.* **1992**, *92*, 1197 – 1226; b) J.-M. Lehn, *Angew. Chem.* **1990**, *102*, 1347 – 1362; *Angew. Chem. Int. Ed. Engl.* **1990**, *29*, 1304 – 1319.
[2] T. Tani, *J. Imag. Sci*. **1990**, *34*, 143 – 151.
[3] a) K. Y. Law, *Chem. Rev.* **1993**, *93*, 449 – 486; b) P. Ball, *Nature* **1993**, *362*, 123 – 125; c) C. Liu, H. Pan, M. A. Fox, A. J. Bard, *Science* **1993**, *261*, 897 – 899.
[4] S. Dähne, *Science* **1978**, *199*, 1163 – 1167.
[5] M. Klessinger, J. Michl, *Lichtabsorption und Photochemie organischer Moleküle*, VCH, Weinheim, **1989**, pp. 117 – 126.
[6] V. Czikkely, H. D. Försterling, H. Kuhn, *Chem. Phys. Lett.* **1970**, *6*, 11 – 14.
[7] A. S. Davydov, *Theory of Molecular Excitons*, McGraw-Hill, New York, **1962**, pp. 23 – 47.
[8] a) D. L. Mills, E. Burstein, *Rep. Prog. Phys*. **1974**, *37*, 817 – 925; b) G. J. Small, M. A. Connolly, S. H. Stevenson, *Chem. Phys.* **1988**, *128*, 157 – 168.
[9] a) H. J. Hesse, F. Fuhs, G. Weiser, L. v. Szentpaly, *Phys. Stat. Sol. b* **1976,** *76*, 817 – 826; b) R. K. Ahrenkiel, *J. Opt. Soc. Am.* **1971**, *61*, 1651 – 1655.
[10] L. Dähne, G. Reck, A. Horvath, G. Weiser, *Adv. Mater.* **1996**, *8*, 486 – 490; L. Dähne, Habilitation thesis, Berlin, **1997**.
[11] L. Dähne, *J. Am. Chem. Soc.* **1995**, *117*, 12855 – 12860.
[12] E. E. Wahlstrom, *Optical Crystallography*, 5th ed., Wiley, New York, **1979**, pp. 232 – 234.
[13] L. Dähne, A. Horvath, G. Weiser, *Chem. Phys.* **1995,** *196*, 307 – 316.
[14] Z. Bao, A. J. Lovinger, A. Dodalapur, *Adv. Mater.* **1997**, *9*, 42 – 44.
[15] L. Dähne, G. Reck, *Z. Kristallogr.* **1995**, *210*, 40 – 43.
[16] L. Dähne, J. Tao, G. Mao, *Langmuir* **1998**, *14*, 565 – 569
[17] W. C. McCrone in *Physics and Chemistry of the Organic Solid State*, *Vol. 2* (Eds.: D. Fox, M. M. Labes, A. Weissberger), Wiley, New York, **1965**, pp. 726 – 766.
[18] a) H. Böttcher, *J. Prakt. Chem.* **1992**, *334*, 14 – 24; b) S. Jäger, H. Böttcher, *Adv. Mater.* **1996**, *8*, 93 – 97.

A Chemosensor for Citrate in Beverages**

Axel Metzger and Eric V. Anslyn*

The high specificity of antibodies and enzymes makes them the current tools of choice for sensing and quantitating structurally complex molecules in a mixture of analytes. However, the sophistication of rationally designed and synthetic receptors is now high enough that their use as sensors is realistic.[1] Neutral and cationic analytes such as sugars,[2] metal ions,[3] creatinine,[4] and arenes,[5] as well as a few anions,[6] have been targeted.[7] Until now, the vast majority of synthetic sensors have not demonstrated the sensitivity and selectivity necessary to analyze solutions in which contaminates are competitive. For example, almost all past studies focused upon the analysis of solutions of analyte alone. Herein, we demonstrate that a chemosensor, a synthetic receptor coupled with a signaling element,[8] can be used to analyze for a specific compound in a multicomponent aqueous solution in a manner similar to that of antibody-based biosensors in immunoassays.

Immunoassays are facile because a simple competition approach is most often employed.[9] When the solution to be analyzed, which contains an unlabeled antigen, is added to the receptor, a labeled antigen is released and hence a signal change results. Such competition assays have not been widely exploited with synthetic receptors. As long as the chemosensor has useful selectivity for its analyte within an application, results comparable to those obtained with antibodies would be expected. Further, immunoassays typically rely on fluorescence spectroscopy.[10] Fluorescence emission appears at longer wavelengths than excitation, and hence the background signal is typically low, resulting in very high sensitivity.[11]

Considering antibody analysis methods and the current state of the art using synthetic receptors, we had two goals in mind. The first was to demonstrate that synthetic receptors can be selective enough for their analytes to sense and quantitate them in competitive media. We defined competitive media as water that is relatively high in ionic strength and other contaminants. Our second goal was to show that a competition assay could be quite readily applied to any synthetic receptor. For accomplishing these goals, citrate seemed to be an ideal analyte.[12]

Since at neutral pH citrate has a charge of minus three, it is quite distinctive from other possible interfering species. Hence, a receptor complementary to citrate in both charge and hydrogen bonding ability would likely not suffer interference from many other competing analytes, such as salts, mono- and dicarboxylic acids, and sugars.

We recently reported that receptor **1** is selective for the recognition of citrate in water over di- and monocarboxylates, phosphates, sugars, and simple salts.[13a] The receptor binds citrate better than simple dicarboxylic acids and monocarboxylic acids by factors of approximately 35 and 700, respectively.[13b] The receptor consists of three guanidinium groups for hydrogen bonding and charge pairing[14] with citrate.[13c] The steric gearing imparted by ethyl groups on the 2-, 4-, and 6-positions[15] ensures that the guanidinium moieties are preorganized on the same face of the benzene ring. This conformation yields several hydrogen bonds and three sets of ionic interactions in the host – guest complex (as shown in Scheme 1), leading to good binding in water ($K_a = 6.8 \times 10^3\,\text{M}^{-1}$).

Our assay for citrate employed an ensemble of 5-carboxyfluorescein (**2**, a fluorescent probe) and **1**. Carboxyfluorescein **2** was chosen since it is commercially available, it has two carboxylates for binding with host **1**, and only very small quantities proved to be necessary (typically 5 to 18 μM). The absorbance/fluorescence of probe **2** is very sensitive to pH changes, and therefore a buffer to maintain constant pH was necessary in the sensing experiments.[16] It is well known that any charged buffer will act as a competitor for the binding

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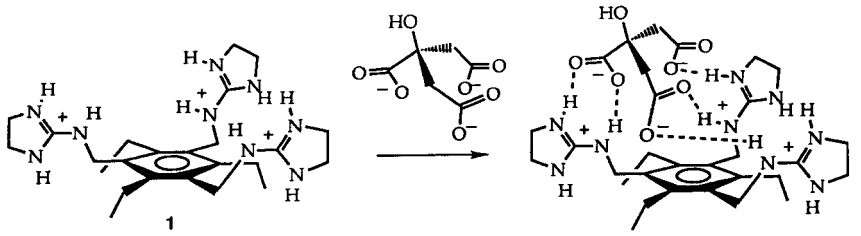
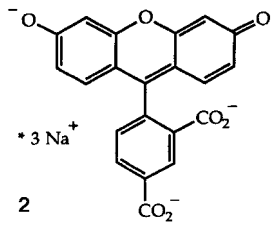


Scheme 1.



sites in charged receptors. To compensate for this effect and to enhance the affinity towards citrate, methanol was added to the buffered solutions. The most advantageous solvent system was 25% water in methanol by volume (5 mM HEPES buffer at pH 7.4). UV/Vis spectroscopy was employed to determine the binding constant of complex **1**·**2** ($K_a = 4.7 \times 10^3\,\text{M}^{-1}$) under the conditions of our sensing assay, using the Benesi–Hildebrand method.[17] In the same solvent system a binding constant of $2.9 \times 10^5\,\text{M}^{-1}$ was determined for citrate·**1**, by using a competition assay in which citrate was added to a solution of host **1** and probe **2**, and the change in absorbance of **2** was monitored.[18] This binding constant is certainly of a magnitude appropriate for sensing citrate at milli- and micromolar concentrations.

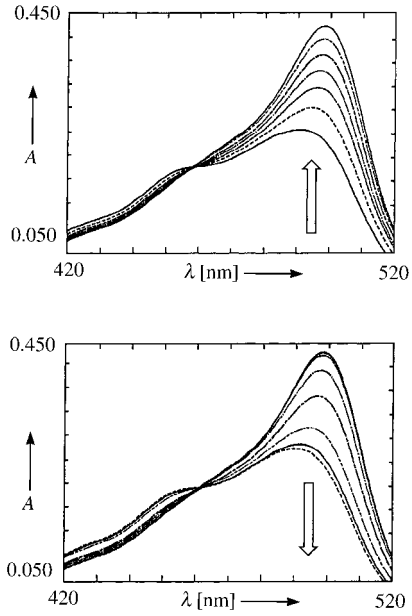


Figure 1. UV spectra of **2**. Top: Addition of **1** to a solution of **2** at constant concentration (14 μM) causes an increase of the absorption A. The curve with the lowest absorptivity was obtained with **2** alone. Bottom: Addition of citrate to a solution containing both **1** (74 μM) and **2** (14 μM) at constant concentrations causes a decrease of the absorption. The curve with the highest absorptivity was measured without citrate present.

We reasoned that binding between **1** and **2** would lower the pK_a of the phenol moiety of **2** due to the positively charged microenvironment presented by **1**. This shift in pK_a would cause the phenol moiety to be in a higher state of protonation when **2** is free in solution. The absorbance or fluorescence of **2** is known to decrease with higher protonation of the phenol.[16] Hence, we predicted that upon introduction of citrate to a mixture of **1** and **2** the absorbance and fluorescence of **2** would decrease. As shown in Figure 1, the intensity of the absorbance of **2** ($\lambda_{max} = 498$ nm) increases with addition of host **1** to a constant concentration of probe **2**, and as predicted the intensity decreases upon addition of citrate to the ensemble of **1** and **2**. The same effect was seen in the fluorescence spectrum ($\lambda_{max} = 525$). This cycling of absorbance and fluorescence could typically be repeated five times before any significant signal degradation occurred.

Calibration curves for the concentration of citrate were generated by incrementally adding citrate to a solution having constant concentrations of **1** and **2**. Addition of succinate to the sensing ensemble (**1**·**2**) resulted in small absorbance (fluorescence) changes, but the addition of acetate, salt, or sugars resulted in essentially no changes (Figure 2). Therefore common contaminants present in beverages (e.g. malate, ascorbate, lactate, benzoate, phosphate) should not effect our sensing assay. This was verified by the success of the method with soft drinks.

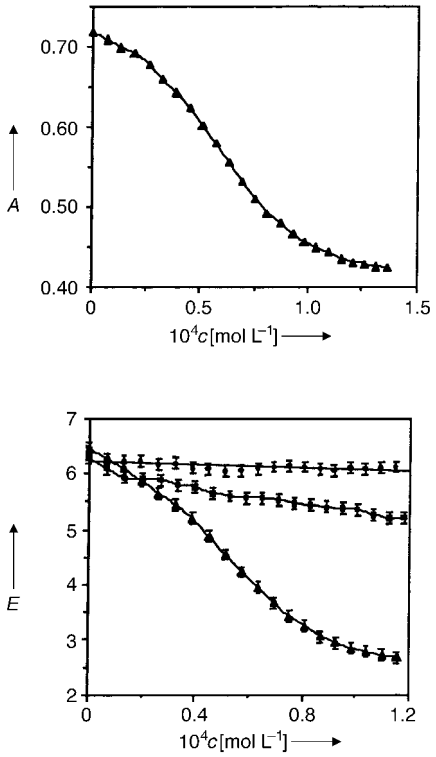


Figure 2. Calibration curves used for the sensor assay (75% methanol, 25% water, 5 mM HEPES buffer, pH 7.4).[19] Top: UV calibration curve (75 mM **1**, 14 mM **2**, $\lambda = 498$ nm). A = absorption, c = concentration of citrate. Bottom: Fluorescence calibration curves (75 mM **1**; 14 mM **2**; excitation at $\lambda = 490$ nm; emission E at $\lambda = 525$ nm). Addition of citrate (▲), succinate (■), and acetate (●) to the sensing assay.

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Finding the concentration of citrate in a large number of beverages was simply a matter of adding between 2 and 50 μL of the beverage to the sensing ensemble, determining the absorbance or fluorescence, and reading the amount of citrate off the calibration curves. The results for several drinks are given in Table 1. To verify the accuracy of the method, known concentrations of citrate dissolved in water were tested, and an NMR technique for measuring citrate was also used as a control. All three methods agree within 10% (except for the beverage "Mountain Dew"), and the agreement between the absorption and emission is typically within 3%. Repetitive measurements indicated a standard deviation not larger than 3%. To demonstrate that competitive media can be analyzed, sport drinks which are very high in electrolyte content (i.e. ionic strength) were tested. "All Sport", for example, contains polyphosphates, benzoate, KH_2PO_4/H_3PO_4, and chloride salts. To show that the method works in the presence of large amounts of sugar, we analyzed "Mountain Dew", a soft drink very high in fructose and sucrose. In fact, the high saccharide concentrations makes the NMR method inaccurate due to a lack of sensitivity in integration of the citrate signal. Finally, to show that the method does not respond to other analytes in the absence of citrate, we analyzed "Coca Cola" and "Diet Coke", soft drinks containing either no citrate or very low concentrations of citrate, respectively. In all cases the sensor ensemble was effective.[20]

Table 1. Concentration of citrate [mM] in beverages determined by different methods.

	Gravimetric	NMR	**1**+**2**, absorption	**1**+**2**, emission
citrate model solution	30.1	–	30.3	29.9
calibration solution	1.33	–	1.205	1.39
orange juice	–	43.1	44.1	44.7
Gatorade	–	15.95	15.05	15.1
Powerade	–	12.4	11.1	11.3
All Sport	–	7.4	7.1	8.1
Mountain Dew	–	7.95	5.5	5.4
tonic water	–	21.0	21.15	20.8
Coca Cola	–	0	0	<0.5
Diet Coke	–	<0.2	<0.4	<0.7

In summary, when the receptor is selective for its guest within a given application, such a receptor can be used in situations typically reserved for antibodies. Further, competition assays can be applied to synthetic receptors, creating very facile assays. Both these concepts can be extended to any synthetic receptor. In fact, we are currently working on using a similar strategy for the sensing of even more complex molecules such as neurotransmitters.

Experimental Section

The detection limit for citrate under the experimental conditions described is approximately 5 mM. By either increasing the amount of methanol and/or manipulating the ratio of **1** and **2**, one can quantitate citrate down to approximately 100 μM as long as the concentrations of competitive components remain below the cross-reactivity concentrations given in ref.[13b].

Conditions for the UV/Vis determination of citrate: 5 mM HEPES buffer, 94 μM **1**, 18 μM 5-carboxyfluorescein in 25 vol% water/75 vol% methanol. The conditions used for fluorescence were the same as for the UV/Vis experiments except that **1** (84 μM) and 5-carboxyfluorescein (5 μM) were more dilute. The average of three scans was used to determine one value. The beverages were adjusted to pH 7.4 using NH_4OH prior to addition to the ensemble.

For the NMR assay the beverage was first filtered through Celite. Aliquots were then adjusted to pH 7.4 with NH_4OH and lyophilized. NMR titration method was used with standard solutions of THF and acetonitrile in D_2O. After integration of the citrate signal, the citrate concentration in the beverage tested was calculated.

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[1] M. A. Morttellaro, D. G. Nocera, *CHEMTECH* **1996**, *26*, 17; D. Diamond, M. A. McKervey, *Chem. Soc. Rev.* **1996**, 16; *Fluorescent Chemosensors for Ion and Molecule Recognition* (Ed.: A. W. Czarnik), Am. Chem. Soc., Washington, D. C., **1992**; *Chemosensors of Ion and Molecule Recognition* (Eds.: J.-P. Desvergne, A. W. Czarnik), Kluwer, New York, **1997**; A. P. de Silva, C. P. McCoy, *Chem. Ind.* **1994**, 992; A. P. de Silva, H. Q. N. Gunaratne, P. L. M. Lynch, *J. Chem. Soc. Perkin Trans. 2* **1995**, 685; R. A. Bissell, A. P. de Silva, H. Q. N. Gunaratne, P. L. M. Lynch, G. E. M. Maguire, K. R. A. S. Sandanayake, *Chem. Soc. Rev.* **1992**, *21*, 187; R. A. Bissell, A. P. de Silva, H. Q. N. Gunaratne, P. L. M. Lynch, G. E. M. Maguire, C. P. McCoy, K. R. A. S. Sandanayake, *Top. Curr. Chem.* **1993**, *168*, 223; D. N. Reinhoudt, *Sensors Actuators B* **1995**, *24–25*, 33; R. J. W. Lugtenberg, Z. Brzozka, A. Casnati, R. Ungarro, J. F. J. Engbersen, D. N. Reinhoudt, *Anal. Chim. Acta* **1995**, *310*, 263.

[2] J. Yoon, A. W. Czarnik, *J. Am. Chem. Soc.* **1992**, *114*, 5874; T. D. James, K. R. A. S. Sandanayake, S. Shinkai, *Angew. Chem.* **1994**, *106*, 2287; *Angew. Chem. Int. Ed. Engl.* **1994**, *33*, 2207; T. D. James, K. R. A. S. Sandanayake, S. Shinkai, *Nature* **1995**, *374*, 345.

[3] S.-Y. Hong, A. W. Czarnik, *J. Am. Chem. Soc.* **1993**, *115*, 3330; A. P. de Silva, H. Q. N. Gunaratne, M. Gunnlaugsson, M. Nieuwenhuizen, *J. Chem. Soc. Chem. Commun.* **1996**, 1967; R. Y. Tsien, *Biochemistry* **1980**, *19*, 2396; A. P. de Silva, H. Q. N. Gunaratne, G. E. M. Maguire, *J. Chem. Soc. Chem. Commun.* **1994**, 1213; H. A. Godwin, J. M. Berg, *J. Am. Chem. Soc.* **1996**, *118*, 6514.

[4] T. W. Bell, Z. Hou, Y. Luo, M. G. B. Drew, E. Chapoteau, B. P. Czech, A. Kumar, *Science* **1995**, *269*, 671.

[5] Z. Pikramenou, K. M. Johnson, D. G. Nocera, *Tetrahedron Lett.* **1993**, *34*, 3531.

[6] D. H. Vance, A. W. Czarnik, *J. Am. Chem. Soc.* **1994**, *116*, 9397.

[7] S. R. Adams, A. T. Harootunian, Y. J. Buechler, S. S. Taylor, R. Y. Tsien, *Nature* **1991**, *349*, 694.

[8] A chemosensor is a device that requires molecular recognition not relying on a biotic recognition element. A. W. Czarnik, *Acc. Chem. Res.* **1994**, *27*, 302.

[9] *Monoclonal Antibodies: Principles and Applications* (Eds.: J. R. Birch, E. S. Lennox), Wiley, New York, **1995**.

[10] A. W. Czarnik, *Chem. Biol.* **1995**, *2*, 423.

[11] L. Fabbrizzi, A. Poggi, *Chem. Soc. Rev.* **1995**, *24*, 197.

[12] P. N. Kale, P. G. Adsule, *Handbook of Fruit Science and Technology*, Chapter 3 ("Citrus"), **1995**, pp. 39–65.

[13] a) A. Metzger, V. M. Lynch, E. V. Anslyn, *Angew. Chem.* **1997**, *109*, 911–914; *Angew. Chem. Int. Ed. Eng.* **1997**, *36*, 862–865. b) Dissociation constants for citrate, succinate, and acetate are 147 μM, 4.5 mM, and >0.1 M, respectively. This yields cross-reactivities of simple dicarboxylic acids and monocarboxylic acids of approx-

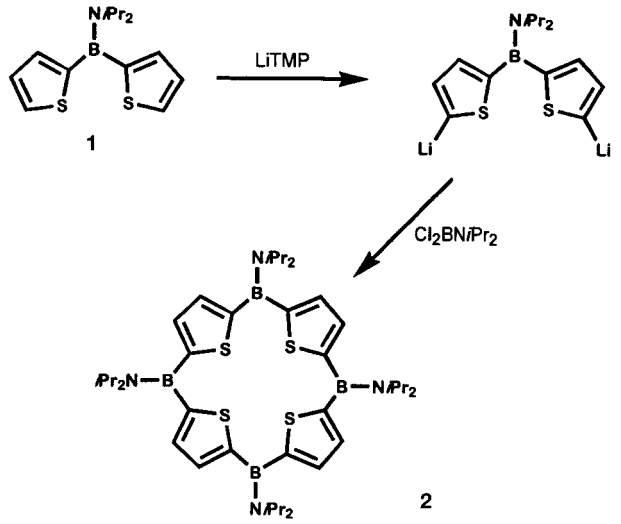
imately 28% and 0.14%, respectively. c) The unsymmetrical binding shown was revealed by X-ray crystal structure analysis.
[14] C. L. Hannon, E. V. Anslyn, *Bioorg. Frontiers* **1993**, *3*, 143.
[15] K. V. Kilway, J. S. Siegel, *J. Am. Chem. Soc.* **1995**, *114*, 255.
[16] J. Bramhall, J. Hofmann, R. DeGuzman, S. Montestruque, S. Schell, *Biochemistry* **1987**, *26*, 6330; M. L. Graber, D. DiLillo, B. L. Friedman, E. Pastoriza-Munoz, *Anal. Biochem.* **1986**, *156*, 202.
[17] H. A. Benesi, J. H. Hildebrandt, *J. Am. Chem. Soc.* **1949**, *71*, 2703;
F. Diederich, K. Dick, D. Griebel, *J. Am. Chem. Soc.* **1986**, *108*, 2273.
[18] K. A. Connors, *Binding Constants, The Measurement of Molecular Complex Stability*, Wiley, New York, **1987**.
[19] Typical immunoassay calibration curves show hyperbolic behavior while sigmoidal behavior is common with semilogarithmic plots (J. Wyman, S. J. Gill, *Binding and Linkage*, University Science Books, Mill Valley, CA, USA, **1990**, pp. 55–57). We have sigmoidal behavior in linear plots because **1** is in large excess over **2**. As we add citrate it first binds to the free **1** before it competes significantly with **2**.
[20] We have further validated the method by HPLC analyses. A series of citric acid samples obtained from an industrial source were analyzed by our fluorescence method. The results were compared to those determined independently by the industrial donor using their in-house HPLC protocol. In all cases the results differed by at most 5%.

A Boron-Bridged Tetrathiaporphyrinogen**

Francis H. Carré, Robert J.-P. Corriu,* Thomas Deforth, William E. Douglas,* Walter S. Siebert,* and Wolfgang Weinmann

Few porphyrinoid macrocycles based on thiophene are known,[1] and to our knowledge none containing boron have been previously described, although various mono- and diborylthiophenes were isolated many years ago.[2] The first thiophene-containing porphyrinoids to be reported were a colorless carbon-bridged tetrathiaporphyrinogen and the violet tetrathiaporphyrin dication generated from it on oxidation.[3] Their more soluble ethyl-substituted analogues have also been described,[4] as has a carbon-bridged neutral aromatic porphyrinoid based on thiophene.[5] Recently silicon-bridged silatetrathiaporphyrinogens and a phosphorus-bridged phosphatetrathiaporphyrinogen have been reported.[6] Several such silicon-bridged macrocycles based on thiophene have also been prepared.[7]

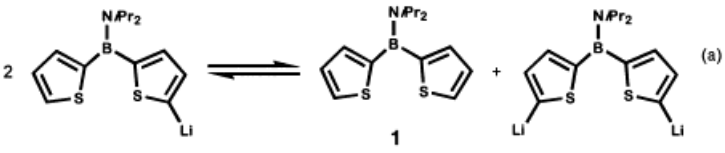
The novel boron-bridged tetrathiaporphyrinogen **2** reported here (see Scheme 1), which contains both Lewis base (sulfur) and Lewis acid (boron) sites, was isolated during an investigation into the synthesis of polymers containing boron and thiophene (or other aromatic) units in the backbone. Initial evidence for the formation of **2** was found in the mass spectrum of the product obtained from the reaction of Cl_2BNiPr_2 with 2,5-dilithiothiophene. The yield can be improved by starting from the *N*,*N*-diisopropyl derivative **1** of the previously reported *N*,*N*-dimethyl-1,1-di-2-thienylboranamine[2b] in which part of the porphyrinoid ring structure has been preformed. Thus, dimetalation of **1** followed by treatment with the stoichiometric quantity of Cl_2BNiPr_2 gave **2** in 62% yield (Scheme 1); no polymer formation was observed. To avoid any attack at boron, the sterically hindered



Scheme 1.

strong base lithium 2,2,6,6-tetramethylpiperidide (LiTMP) was used.

Remarkably, even monolithiation of **1** and treatment with the aminodichloroborane gives rise to **2**. As with the corresponding silicon system,[8] this can be explained in terms of the equilibrium between **1** and the mono- and dilithiated species [Eq. (a)].



Crystals of **2** suitable for an X-ray structure determination[9] were obtained from a solution of the crude reaction product in

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