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High α/β-Anomer Selectivity in Molecular Recognition of Carbohydrates by Artificial Receptors

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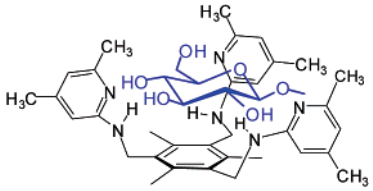
ABSTRACT



New effective, acyclic, pyridine-based receptors 1–3 show remarkable α/β binding selectivity in the recognition of monosaccharides. They are able to participate in cooperative and bidentate hydrogen bonds with sugar hydroxyls as well as in CH–π interactions with CH's of sugar molecules.

A contemporary challenge in supramolecular and biomimetic chemistry is the development of effective artificial receptors for the selective recognition of carbohydrates.[1−3]These studies are of particular importance due to the key roles which sugar molecules play in a wide range of biological processes, including various intercellular recognition processes, such as infection, inflammation, differentiation, and intercellular communication. Artificial receptors provide model systems to study the molecular basis of carbohydrate recognition, which in the future may lead to the development of new chemosensors or therapeutics. The three-dimensional complexity of sugar structures renders the development of selective receptors for these important biomolecules particularly difficult. As discussed in ref 4, the common feature of protein−sugar complexes is the existence of hydrogen bonds between sugar OH groups as well as ring oxygens and polar residues of the protein, supplemented by interactions of sugar CH moieties with aromatic amino acid side chains and numerous van der Waals contacts.

Recently, we reported on an effective receptor system for the recognition of monosaccharides in chloroform solution.[2i,j] These receptors, containing three heterocyclic recognition units covalently attached to a phenyl spacer, simultaneously form both multiple hydrogen bonds and stacking interactions with sugar molecules. Our studies showed that in the case of uncharged hydrogen-bonding interactions only recognition units containing both donors and acceptors for hydrogen bonding are effective for the recognition of monosaccharides, similar to protein−carbohydrate complexes.

(1) For a recent review on carbohydrate recognition through noncovalent interactions, see: Davis, A. P.; Wareham, R. S. *Angew. Chem., Int. Ed.* **1999**, *38*, 2978−2996.

(2) For recent reports on hydrogen-bonding receptors for carbohydrates, see: (a) Ryan, T. J.; Lecollinet, G.; Velasco, T.; Davis, A. P. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 4863−4866 and references therein. (b) Rusin, O.; Lang, K.; Kral, V. *Chem. Eur. J.* **2002**, *8*, 655−663. (c) Droz, A. S.; Neidlein, U.; Anderson, S.; Seiler, P.; Diederich, F. *Helv. Chim. Acta* **2001**, *84*, 2243−2289. (d) Löwik, D. W. P. M.; Lowe, C. R. *Eur. J. Org. Chem.* **2001**, 2825−2839. (e) Benito, J. M.; Gomez-Garcia, M.; Jimenez Blanco, J. L.; Ortiz Mellet, C.; Garcia Fernandez, J. M. *J. Org. Chem.* **2001**, *66*, 1366−1372. (f) Tamaru, S.-i.; Yamamoto, M.; Shinkai, S.; Khasanow, A. B.; Bell, T. W. *Chem. Eur. J.* **2001**, *7*, 5270−5276. (g) Kim, H. J.; Kim, Y. H.; Hong, J. I. *Tetrahedron Lett.* **2001**, *42*, 5049−5052. (h) Bitta, J.; Kubik, S. *Org. Lett.* **2001**, *3*, 2637−2640. (i) Mazik, M.; Sicking, W. *Chem. Eur. J.* **2001**, *7*, 664−670. (j) Mazik, M.; Bandmann, H.; Sicking, W. *Angew. Chem., Int. Ed.* **2000**, *39*, 551−554.

(3) For a recent review on boronic acid based receptors, using covalent interactions for sugar binding, see: James, T. D.; Shinkai, S. *Top. Curr. Chem.* **2002**, *218*, 159−200.

(4) (a) Quiocho, F. A. *Pure. Appl. Chem.* **1989**, *61*, 1293−1306. (b) Weiss, W. I.; Drickamer, K. *Annu. Rev. Biochem.* **1996**, *65*, 441−473. (c) Lis, H.; Sharon, N. *Chem. Rev.* **1998**, *98*, 637−674. (d) Elgavish, S.; Shaanan, B. *Trends Biochem. Sci.* **1998**, *22*, 462−467.

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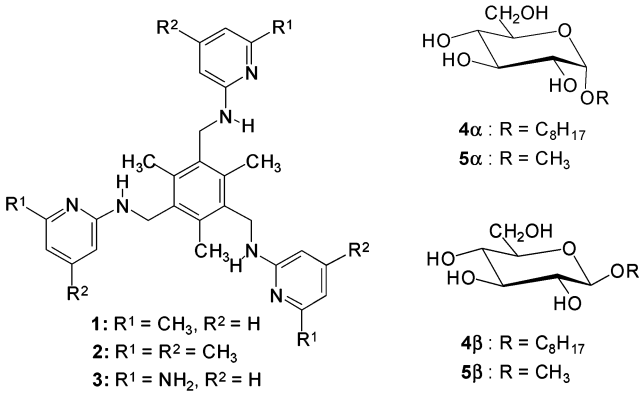
With the aim of increasing both the binding affinity and the selectivity we now designed pyridine-based receptors with enhanced basicity of the heterocyclic recognition units, incorporating multiple, adjacent hydrogen bonding sites and π-bonds for facilitating CH$-\pi$ interactions. In this paper we report the complexation properties of three representatives (**1**−**3**) of this novel series of hydrogen-bonding receptors for monosaccharides, which show a high binding affinity and diastereoselectivity. Compounds **1**−**3** were synthesized from 1,3,5-tris(bromomethyl)-2,4,6-trimethylbenzene[5] and 2-amino-6-methylpyridine, 2-amino-4,6-dimethylpyridine, or 2,6-diaminopyridine, respectively (THF or CH_3CN, K_2CO_3, room temperature).

The aminomethyl groups attached to the pyridine units as well as a second methyl group at the 4-position of the pyridine ring in host **2** or an additional α-amino group in **3** favorably increased the basicity of the pyridine moieties.[6] The increase in the electron density on the pyridine nitrogen should cause an enhancement of the receptor affinity mainly due to enthalpic factors.[7] In addition, the incorporation of a second amino group in **3** provides an additional binding site for carbohydrates. The substituted central phenyl ring in **1**−**3** should be able to participate in effective CH$-\pi$ interactions with CH's of sugar molecules. Moreover, the methyl groups increase the solubility of the hosts in chloroform. As shown by Davis et al., receptors which are designed for solubility in nonpolar media can be studied in a two-phase system, demonstrating the ability to extract or transport some carbohydrates.[2a]

As a starting point, we examined the adaptability of receptors **1**−**3** for the recognition of glucopyranosides. The octyl derivatives **4α** and **4*β*** were selected to evaluate the recognition capabilities of the receptors for glucopyranoside in aprotic solvents such as chloroform and compare their binding properties with the properties of previously studied receptors. In addition, the affinity of the receptors for methyl glucopyranosides **5α** and **5*β*** was tested. Since the methyl glucopyranosides are insoluble in $CDCl_3$, the binding properties were determinated by the extraction method.



The interactions of hosts **1**−**3** and glucopyranosides **4α**−**4*β*** were investigated by ^{1}H NMR spectroscopy. The binding constants were determined in chloroform at 25 °C by titration experiments and the titration data were analyzed by nonlinear regression analysis.[8] The stoichiometry of receptor−sugar complexes was determined by the curve-fitting analysis of the titration data and by mole ratio plots.[9] Self-aggregation of the receptors in the applied concentration range was excluded on the basis of dilution experiments.

The complexation between the receptors **1**−**3**[10] and glucopyranosides **4α** and **4*β*** was evidenced by a significant downfield shift of the receptor amine protons and moderate upfield shift of the CH_2 resonances. The curve fitting of the titration data for receptor **1** and octyl-β-D-glucopyranoside (**4*β***) suggested the existence of both 1:1 and 1:2 receptor−sugar complexes in the chloroform solution, with a strong 1:1 association constant (K_{a1} = 10 500 M^{-1}) and a rather weak association constant for a 1:2 receptor−sugar complex (K_{a2} = 250 M^{-1}). A ^{1}H NMR titration of **2** with glucopyranoside **4*β*** produced similar spectral changes. In particular, the signal due to the amine NH moved downfield by about 1.3 ppm ($\Delta\delta_{max}$) and the methylene CH_2 moved upfield by 0.15 ppm (Figure 1).

The motions of the NH of **2** were consistent with 1:1 and 1:2 binding (Figure 2a), providing association constants of 20 950 M^{-1} (K_{a1}) and 790 M^{-1} (K_{a2}), respectively, indicating stronger binding than with **1**.

These data reveal that the effect of the increased basicity of the pyridine moieties causes a marked increase in the value of association constants in comparison with previously studied receptors.[2i,j] Possible structures for the complexes

(5) van der Made, A. W.; van der Made, R. H. *J. Org. Chem.* **1993**, *58*, 1262−1263.

(6) The increase in pK_a of substituted pyridines is generally greater for α- and γ- than for β-amino or -methyl groups: Katritzky, A. R.; Pozharski, A. F. *Handbook of Heterocyclic Chemistry*; Pergamon: Amsterdam, The Netherlands, 2000; p 178.

(7) For examples on the use of the effect of increased basicity of pyridine moieties in other hydrogen-bonded systems, see: (a) Inouye, M.; Miyake, T.; Furusyo, M.; Nakazumi, H. *J. Org. Chem.* **1999**, *64*, 8170−8176. (b) Tecilla, P.; Dixon, R. P.; Slobodkin, G.; Alavi, D. S.; Waldeck, D. H.; Hamilton, A. D. *J. Am. Chem. Soc.* **1990**, *112*, 9408−9410.

(8) Wilcox, C. S.; Glagovich, N. M. Program HOSTEST 5.6, University of Pittsburgh. This program is designed to fit data to different binding models, which include both "pure" binding models, taking into consideration the formation of only one type of complex in solution, and "mixed" binding models containing more than one type of complex in solution.

(9) For examples on the use of the mole ratio method, see: (a) Tsukube, H.; Furuta, H.; Odani, A.; Takeda, Y.; Kudo, Y.; Inoue, Y.; Liu, Y.; Sakamoto, H.; Kimura, K. In *Comprehensive Supramolecular Chemistry*; Atwood, J. L., Davis, J. E. D., MacNicol, D. D, Vögtle, F., Eds.; Pergamon: Oxford, UK, 1996; Vol. 8, pp 425−482. (b) Liu, M. T. H.; Bonneau, R. *J. Am. Chem. Soc.* **1990**, *112*, 3915−3919.

(10) Selected physical and spectroscopic data for compounds **1**−**3**. *1*,3,5-Tris[(6-methylpyridin-2-yl)aminomethyl]-2,4,6-trimethylbenzene (**1**): Yield 53%. Mp 160−162 °C. ^{1}H NMR ($CDCl_3$) δ 2.36 (s, 9H, 3 × CH_3), 2.39 (s, 9H, 3 × CH_3), 4.17 (t, 3H, 3 × NH, J = 4.3 Hz), 4.39 (d, 6H, 3 × CH_2, J = 4.3 Hz), 6.25 (d, 3H$_{pyr}$, J = 8.2 Hz), 6.46 (d, 3H$_{pyr}$, J = 7.3 Hz), 7.34 (t, 3H$_{pyr}$, J = 7.3 Hz). ^{13}C NMR ($CDCl_3$) δ 15.89, 24.40, 41.77, 103.08, 112.33, 133.75, 136.82, 137.80, 157.11, 158.12. HR-MS calcd for $C_{30}H_{36}N_6$: 480.3001. Found: 480.2999. 1,3,5-Tris[(4,6-dimethylpyridin-2-yl)aminomethyl]-2,4,6-trimethylbenzene (**2**): Yield 60%. Mp. 195 °C. ^{1}H NMR ($CDCl_3$) δ 2.21 (s, 9H, 3 × CH_3), 2.33 (s, 9H, 3 × CH_3), 2.38 (s, 9H, 3 × CH_3), 4.11 (t, 3H, 3 × NH, J = 4.2 Hz), 4.37 (d, 6H, 3 × CH_2, J = 4.2 Hz), 6.08 (s, 3H$_{pyr}$), 6.32 (s, 3H$_{pyr}$). ^{13}C NMR ($CDCl_3$) δ 15.85, 21.09, 24.18, 41.85, 103.42, 113.94, 133.74, 136.77, 148.76, 156.74, 158.41. HR-MS calcd for $C_{33}H_{42}N_6$: 522.3471. Found: 522.3477. 1,3,5-Tris[(6-aminopyridin-2-yl)aminomethyl]-2,4,6-trimethylbenzene (**3**): Yield 30%. Mp 130−132 °C. ^{1}H NMR ($CDCl_3$) δ 2.38 (s, 9H, 3 × CH_3), 4.01 (t, 3H, 3 × NH, J = 4.4 Hz), 4.15 (s, 6H, 3 × NH_2), 4.35 (d, 6H, 3 × CH_2, J = 4.4 Hz), 5.81−5.84 (m, 6H$_{pyr}$), 7.24 (d, 3H$_{pyr}$). ^{13}C NMR ($CDCl_3$) δ 15.83, 41.65, 95.70, 96.81, 133.78, 136.84, 139.25, 157.68, 157.99. HR-MS calcd for $C_{27}H_{33}N_9$: 483.2859. Found: 483.2867.

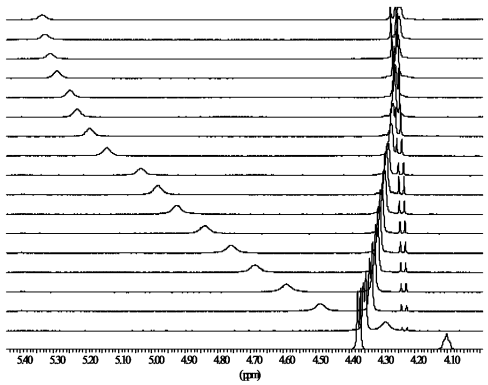


Figure 1. Titration of **2** with octyl β-D-glucopyranoside (**4β**). ^{1}H NMR spectra ($CDCl_3$, 25 °C) of receptor **2** (NH and CH_2 resonances are shown) after addition of (from bottom to top) 0.00, 0.18, 0.36, 0.45, 0.54, 0.63, 0.72, 0.81, 0.90, 1.08, 1.26, 1.44, 1.62, 1.81, 2.26, 2.71, 3.16, and 3.62 equiv of **4β** ([**2**] = 1.35×10^{-3} mol/L).

of **1**, **2**, and the glucopyranosides were obtained by molecular modeling and are shown exemplary for the complex **2·4β** in Figure 3. Interestingly, after addition of small amounts of water (0.07%) to the chloroform solutions the binding affinity of the receptors increases by a factor of 2−3, probably due to the formation of water-mediated hydrogen bonds,[11] in line with the observations in protein−carbohydrate complexes, where the hydrogen bonds are both direct and water mediated.[4a,b]

In contrast to the strong binding of β-glucopyranoside **4β** by **1** and **2**, binding of the α-anomer **4α** is relatively weak. The chemical shifts of the host signals observed during the titration with the derivative **4α** varied in a more linear way with the sugar concentration, indicating a much weaker complex (Figure 2b).

The fit of NMR shift changes of the NH as well as the CH_2 group agreed with a 1:1 association model for **4α** with both receptors **1** and **2**, yielding association constants of 690 and 800 M^{-1}, respectively. Thus, **1** and **2** show a minimum β/α selectivity ratio of 15 and 26, respectively, which is

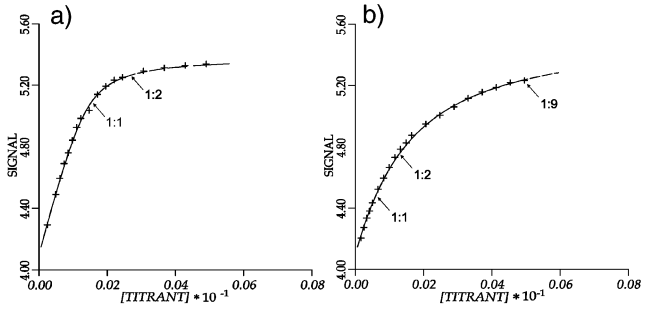


Figure 2. Plot of the observed (×) and calculated (—) downfield chemical shifts of the NH resonances of **2** as a function of added β-glucopyranoside **4β** (a) or α-glucopyranoside **4α** (b). The [receptor]/[glucopyranoside] ratio is marked.

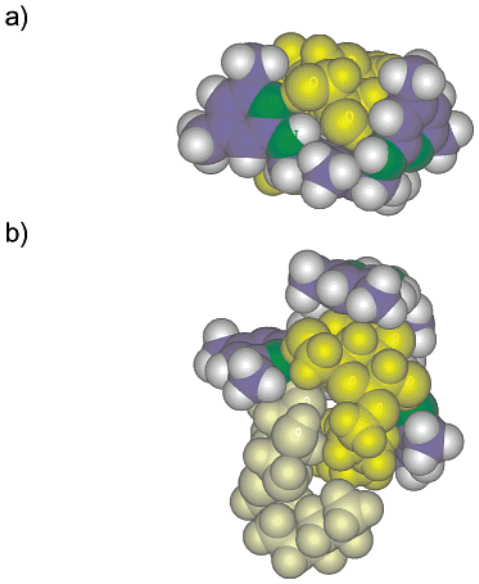


Figure 3. Energy-minimized structure of the complex formed between receptor **2** and octyl β-D-glucopyranoside (**4β**) (MacroModel V.6.5, Amber* force field, Monte Carlo conformational searches, 50 000 steps): (a) 1:1 and (b) 1:2 receptor−glucopyranoside complex.

significantly higher than observed previously.[2i,j] The stoichiometries observed by fitting of the binding isotherms agree with the determination by the ratio method.

Additional evidence for the preferred complexation of the β-anomer was obtained from extraction experiments, where α- and β-methyl-glucopyranoside (**5α**, **5β**) were extracted from the solid state into a $CDCl_3$ solution of receptor **1** or **2**. The ^{1}H NMR signals of the anomeric CH and the OCH_3 protons of α- and β-methyl-glucopyranoside were integrated with respect to the host's proton signals to provide the [guest]/[host] ratio. In case of the extraction experiments with receptor **2** and β-anomer **5β** about 0.7 equiv is extracted into the $CDCl_3$ solution of **2**. The NH signal at 4.20 ppm of **2** is shifted downfield by ca. 0.9 ppm and is broadened. The aromatic CH pyridine signal at 6.18 ppm also broadenes significantly. These effects indicate strong interactions between the receptor and the sugar. In contrast, the extraction experiments with α-anomer **5α** have provided spectra similar to those obtained from the control experiments.

Receptor **3**, carrying a second amino group at the pyridine rings, also exhibits a similar level of affinity toward glucopyranosides. According to the titration curve analysis and mole ratio plots, in this case 1:1 and 2:1 receptor−sugar complexes with **4β** are formed. The 2:1 complex displays hydrogen bonding between **3** and **4β**, as well as between the two receptor molecules (see molecular modeling, Figure 4).

For the complex with α-anomer **4α** again a 1:1 stoichiometry was found. The NMR signals of the NH and NH_2

(11) A similar effect was previously observed for other systems. See: (a) Bonar-Law, R. P.; Sanders, J. K. M. *J. Am. Chem. Soc.* **1995**, *117*, 259−271. (b) Mizutani, T.; Kurahashi, T.; Murakami, T.; Matsumi, N.; Ogoshi, H. *J. Am. Chem. Soc.* **1997**, *119*, 8991−9001.

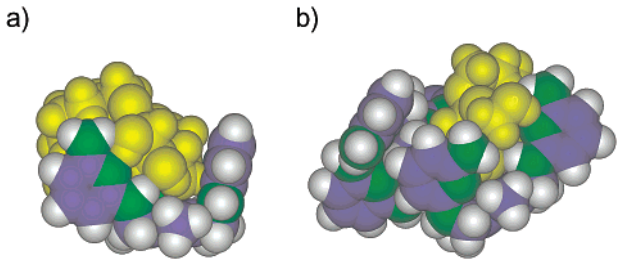


Figure 4. Energy-minimized structure of the complex formed between receptor **3** and octyl β-D-glucopyranoside (**4*β***) (MacroModel V.6.5, Amber* force field, Monte Carlo conformational searches, 50 000 steps): (a) 1:1 and (b) 2:1 receptor−glucopyranoside complex.

groups of **3** were substantially shifted downfield, suggesting the formation of hydrogen bonds between these groups and the sugar OH's. The binding constants of octyl-β-glucopyranoside (**4*β***) and receptor **3** were found to be 9500 (K_{a1}) and 4800 M^{-1} (K_{a2}), while the binding constant for octyl-α-glucopyranoside (**4α**) and **3** amounts to 620 M^{-1} (Table 1). Thus, again significantly weaker binding for the α-anomer was determinated.

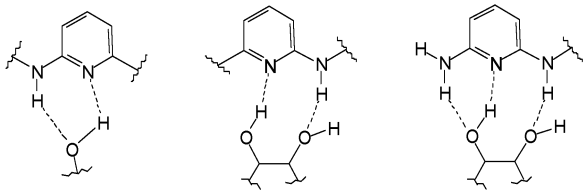
Table 1. Association Constants K_a[a] and Corresponding Free Energy Changes $\Delta G°$ for Receptors **1**−**3** and Glucopyranosides **4α**−**4*β***

host−guest complex	K_{a1} [M^{-1}] ($\Delta G°$ [kJ mol^{-1}])	K_{a2} [M^{-1}] ($\Delta G°$ [kJ mol^{-1}])	$\Delta\delta_{max}$ [ppm][d]
1·4*β*	10500 (−22.5)	250 (−13.7)[b]	1.30
1·4α	690 (−16.2)		1.42
2·4*β*	20950 (−24.7)	790 (−16.5)[b]	1.30
2·4α	800 (−16.6)		1.45
3·4*β*	9500 (−22.7)	4800 (−21.0)[c]	1.20
3·4α	620 (−15.9)		1.50

[a] Average K_a values from multiple titrations ($CDCl_3$, stored over activated molecular sieves and deacidified with Al_2O_3). The reproducibility of the K_a values was ±10−20%. Uncertainty in a single K_a estimation was ±2−10%. Dilution experiments show that receptors do not self-aggregate in the used concentration range. [b] 1:2 receptor−glucopyranoside complex.[c] 2:1 receptor−glucopyranoside complex. [d] Complexation-induced shifts observed for the NH of receptor; values provided by HOSTEST.

The results obtained with receptors **1**−**3** apparently indicate that the interactions involving the amino-pyridine units significantly affect the binding affinity and selectivity of the receptors, which show high affinity for β-D-glucopyranoside and marked β vs α selectivity. An important design criterion is that heterocyclic recognition units of **1**−**3** are able to participate in cooperative and bidentate hydrogen bonds with the sugar hydroxyls, similar to biological systems, where the hydrogen bonding displays the property of cooperativity.[12] Typical hydrogen-bonding motifs found by molecular modeling studies are shown in Scheme 1.

Scheme 1. Cooperative and Bidentate Hydrogen Bond Pattern in Carbohydrate Complexes with Receptors **1**−**3**



The hydrogen bonds between amine-NH/pyridine-N and the sugar OH groups seem to provide the major driving force for complexation. Further improvement of the complexation properties is expected with receptors based on the 2,4,6-triethylbenzene frame, which should be capable of favorable preorganization.[13] Synthesis of new receptors of this type and further complexation studies with numerous sugar molecules are in progress.

Acknowledgment. This work was supported by the Deutsche Forschungsgemeinschaft (SFB 452), Fonds der Chemischen Industrie, and Dr. Otto Röhm Stiftung. We thank Prof. R. Sustmann for helpful discussions and Prof. C. S. Wilcox for access to his HOSTEST program.

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(12) Jeffrey, G. A.; Saenger, W. A. *Hydrogen Bonding in Biological Structures*;, Springer-Verlag: Berlin, Germany, 1994.

(13) For examples on the use of other systems based on the triethylbenzene frame, see: (a) Stack, T. D. P.; Hou, Z.; Raymond, K. N. *J. Am. Chem. Soc.* **1993**, *115*, 6466. (b) Niikura, K.; Metzger, A.; Anslyn, E. V. *J. Am. Chem. Soc.* **1998**, *120*, 8533−8534. (c) Kim, S.-G.; Ahn, K. H. *Chem. Eur. J.* **2000**, *6*, 3399−3403.