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Complexation of Metals with Piperazine-Containing Azamacrocyclic Fluorophores

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Received April 1, 2004

Azamacrocyclic fluorophores containing piperazine units were synthesized using sequential rhodium-catalyzed regioselective hydroformylation−reductive amination. A piperazine unit is introduced into the macrocycles to act simultaneously as electron donor and binding site. The macrocycles chelate divalent cations, either Zn^{2+} or Co^{2+}, which considerably enhanced fluorescence. Complexation with Zn^{2+} was additionally confirmed by NMR.

Introduction

The design and construction of molecular scale sensors is a challenging aspect of modern nanotechnology.[1] Molecular sensors are devices that are used for the sensitive and rapid detection of various organic compounds and metals. The detection of toxic metals is crucial not only for environmental protection but also in medicine or pharmacology.[2] Fluorescence spectroscopy, because of its sensitivity, is often used as a tool for detection. It is governed by three principal parameters: lifetime of excited states, intensity, and quantum yields.[3] Fluorescence techniques are used in combination with imaging techniques in various biological and material research areas.[3]

Typical fluorescent sensors contain signaling and recognition parts.[4] The recognition moiety within molecules is responsible for the selectivity and efficiency of binding with guests.[5] To date, various sensors based on signaling and recognition units have been synthesized and investigated.[6] In this manuscript, a new approach to the design of sensors is presented. Piperazine was introduced into the macrocycle to act as an electron donor and simultaneously as a recognition moiety (Figure 1). Motivation for chosing piperazine as a building block of the macrocycle was to investigate the effect on the fluorescence sensitivity during the metal complexation of piperazine, since detectable changes should occur with the same group acting as electron donor of the fluorophore and also as the host for the complexation−recognition moiety of the potential sensor.

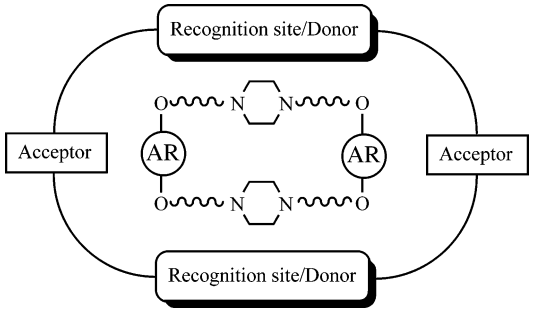

FIGURE 1. Scheme of the fluorescent sensor in which the same group acts as a receptor and electron donor.

The synthesis of various azamacrocycles containing piperazine has been reported, and their complexation characteristics have been investigated.[7] In this paper we report a new and convenient method for incorporating piperazine into a macrocycle, as well as fluorescence investigations that, to our knowledge, have not previosly been carried out with this type of compounds. The

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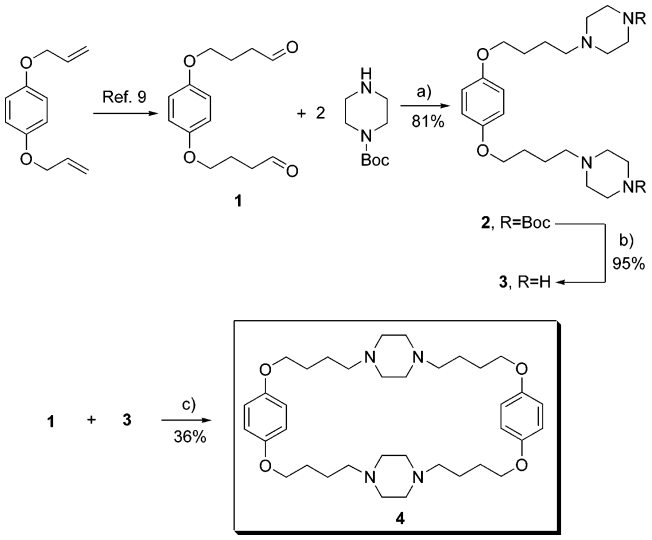
10.1021/jo049465o CCC: $27.50
Published on Web 07/08/2004

SCHEME 1. Synthesis of Azamacrocycle 4[a]



[a] (a) Ti(O-*i*Pr)$_4$, NaBH$_3$CN, EtOH; (b) TFA, CH_2Cl_2; (c) 40 bar H_2, [Rh(acac)(CO)$_2$], toluene.

piperazine unit proved to be a very good host for many heavy metals. We chose to investigate two of them, cobalt and zinc, since both form dications, with the former strongly complexing many similar multidentate ligands, while the latter is an important cation present in biological systems.[6d,8]

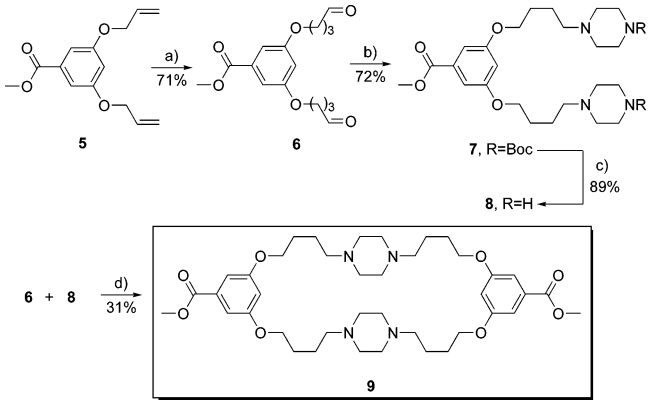
Results and Discussion

Synthesis. The macrocycles were synthesized by improving our general method for the synthesis of such compounds.[9] Starting from aromatic diolefins, dialdehydes were synthesized via regioselective hydroformylation. The piperazine unit was incorporated within the macrocycles by a convergent strategy with dialdehydes, employing a convenient reductive amination/deprotection/reductive amination sequence. Ring closure in the rhodium-catalyzed last reductive amination step occurred in relatively high yields (31−36%) for these types of macrocycles.

The first step was the reductive amination of dialdehyde **1**[9] with monoprotected Boc-piperazine[10] using Ti(O-*i*Pr)$_4$ and NaBH$_3$CN. The reduction was performed with 81% isolated yield of **2**, which underwent TFA cleavage of the Boc protecting groups to give **3** in 95% yield. Finally, rhodium-catalyzed reductive amination of **1** with **3** gave macrocycle **4** in 36% yield. (Scheme 1).

Additionally, using the same methodology, azamacrocycle **9** was synthesized from diolefin **5**.[11,12] First, the synthesis of dialdehyde **6** via regioselective Rh-catalyzed *n*-hydroformylation using XANTPHOS ligand was accomplished in 71% yield. Compounds **7** and **8** were then prepared analogously to **2** and **3** in 72% and 89% yields, respectively. Finally, azamacrocycle **9** was synthesized again by rhodium-catalyzed reductive amination from aldehyde **6** and amine **8** in 31% yield (Scheme 2).

SCHEME 2. Synthesis of Azamacrocycle 9[a]



[a] (a) 20 bar CO/H_2 (1:1), [Rh(acac)(CO)$_2$], XANTPHOS, 70 °C, toluene; (b) Ti(O-*i*Pr)$_4$, NaBH$_3$CN, EtOH; (c) TFA, CH_2Cl_2; (d) 40 bar H_2, [Rh(acac)(CO)$_2$], toluene.

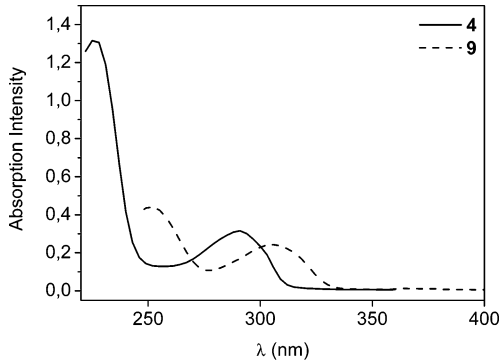


FIGURE 2. UV absorption spectra of **4** and **9** in CH_3CN, c = 0.1 mM.

Fluorescence Experiments. To determine the best emission wavelengths for **4** and **9**, UV absorbance experiments were performed (Figure 2). UV spectra show the absorbance between 260 and 320 nm, which relates to electronic transitions of the aromatic ring.[13] A small peak at higher wavelengths (around 370 nm) was not observed for macrocycles that do not contain piperazine units, so it can be assumed that this absorbance is caused by a charge-transfer transition within the azamacrocycles.[14] Different charge-transfer mechanisms via space or conjugate units or by intraanular interactions have been addressed previously.[15] In the present example the possible mechanism of this charge transfer is still under investigation.

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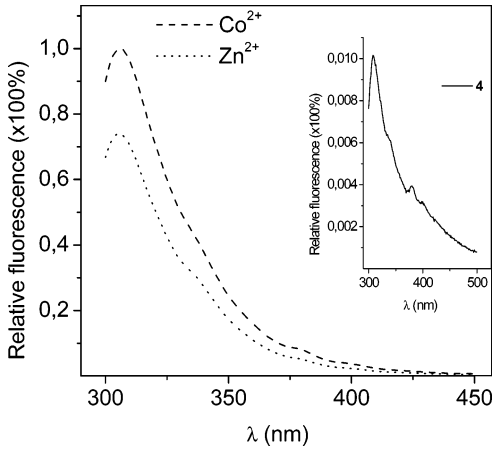

FIGURE 3. Fluorescence spectra of **4**, before and upon addition of equimolar amounts of Co^{2+} and Zn^{2+} in CH_3CN, $c = 20\ \mu M$, $\lambda_{ex} = 280$ nm.

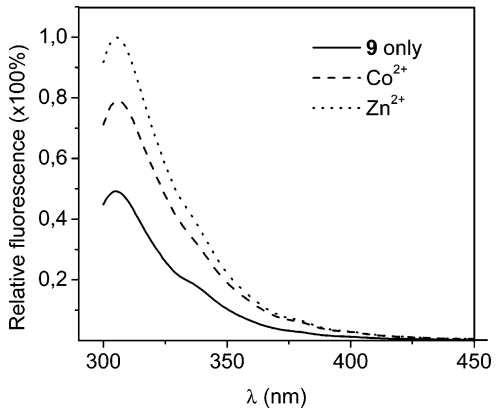

FIGURE 4. Fluorescence spectra of **9**, before and after the addition of equimolar amounts of Co^{2+} and Zn^{2+} in CH_3CN, $c = 20\ \mu M$, $\lambda_{ex} = 280$ nm.

For the fluorescence experiments, the macrocycles were excited at 280 nm, and the corresponding emissions were recorded. Using the same conditions, fluorescence of macrocycles **4** and **9** was also recorded in the presence of equimolar amounts of $CoCl_2$ and $ZnCl_2$. Figures 3 and 4 show the fluorescence spectra of **4** and **9** without the cations and with equimolar amounts of Co^{2+} and Zn^{2+} at the chosen excitation wavelength. The addition of metals does not change the position of the maximum in the emission spectrum, which is expected when taking into account that only weak physical interactions take place between the corresponding macrocycles **4** and **9** and metal ions.[6c] However, strong changes of the fluorescence intensity were observed, as in the case of previously described chemosensors.[6b,c]

The fluorescence was enhanced approximately 100 times (10,000%) for macrocycle **4** and 1.6 times (60%) for the macrocycle **9** as a result of the complexation of macrocycles with Co^{2+} at an excitation wavelength of 280 nm. At the same wavelength upon addition of Zn^{2+}, the enhancement is 70 times (7,000%) and 2 times (100%) higher for the macrocycles **4** and **9** respectively.

TABLE 1. Fluorescence Enhancement Due to Complexation of 4 and 9 with Metals[a]

	$\lambda_{ex} = 280$ nm	
	4	**9**
Co^{2+}	100×	1.6×
Zn^{2+}	70×	2×

[a] $c = 20\ \mu M$, [macrocycle]/[cation] 1:1, CH_3CN.

The fluorescence intensities at 280 nm and their changes due to complexation with metals are summarized in the Table 1.

From the presented data, it is possible to conclude that both macrocycles **4** and **9** interact with the metals, and these interactions are easily monitored by observing the changes in fluorescence intensity. Taking into account the concentrations used in the fluorescence experiments, one estimates that the dissociation constants for complexation of metals with macrocycles should be in the low micromolar range.

It is also possible to conclude that macrocycle **4** is a much better sensor for metals than **9** because of the observed high changes in the intensity for **4**. This might be due to differences in the distance of the donor and acceptor groups and/or due to different conformational flexibilities of the macrocycles.

NMR Studies. To confirm the complexation between the macrocyclic ligand and the metal, NMR spectra of **9** were recorded with and without Zn^{2+} ions in solution. The strongest changes in the NMR signals of macrocycles were expected in the area of piperazine subunit, where complexation with the metals should actually take place. As the signals of all 16 protons within the piperazines are not resolvable, ^1H NMR could not be used for proving complexation. Therefore, ^{13}C NMR investigations were performed. Several NMR techniques such as 1D-NOE, g-COSY, and g-HSQC were used to assign the signals to the macrocycle **9**. The results indicate that the signal at 53.8 ppm belongs to the piperazine methylenes (position *a*) and the signal at 58.4 ppm belongs to the methylenes adjacent to the piperazine ring system (position *b*) (Figure 5, bottom).

For the complexation studies, ^{13}C NMR experiments were performed on **9** alone and upon addition of 1 and 2 equiv of $ZnCl_2$ (Figure 5, top). The shift differences are summarized in Table 2.

The strongest shifting and broadening of signals occurs for carbons inside the piperazine ring (position *a*, downfield shift more than 2.0 ppm). Contrary to what was expected, shifting does not decrease from position *a* to *d*, since the resonance of position *c* (β to the nitrogen, downfield shift around 1.2 ppm) shifts more than that of position *b* (adjacent to the piperazine ring system, downfield shift around 0.8 ppm). Finally, carbons at position *d* (γ to the nitrogen) have a downfield shift of around 0.6 ppm, while the ether methylenes (δ to the nitrogen) have the smallest shift (downfield shift of 0.3 ppm). For all other carbon atoms no shift of signals was observed.

From these data, it can be concluded that the center of the complexation of Zn^{2+} with **9** is in the area of

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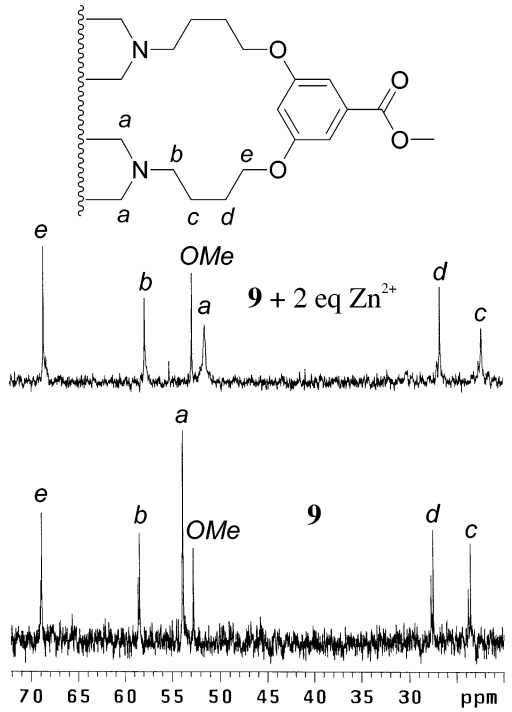

FIGURE 5. ^{13}C NMR spectra before (bottom) and after (top) addition of 2 equiv of Zn^{2+} to **9** and assignment of resonances in compound **9.** Solvent: $CD_3CN/CDCl_3$ (9:1).

TABLE 2. Shifts, Shift Differences ($\Delta\delta$), and Signal Broadening in ^{13}C NMR of 9[a]

	position and shift (ppm)				
	a	b	c	d	e
9	53.8	58.4	23.5	27.5	68.9
9 + Zn^{2+}	51.8 b	57.6	22.4 b	26.9	68.6
$\Delta\delta_1$	2.0	0.8	0.9	0.6	0.3
9 + $2Zn^{2+}$	51.5 vb	57.8	22.3 b	26.7	68.6
$\Delta\delta_2$	2.3	0.6	1.2	0.8	0.3

[a] b = broad, vb = very broad; $\Delta\delta_1$ = (**9** + 1 equiv of Zn^{2+}) − **9**; $\Delta\delta_2$ = (**9** + 2 equiv of Zn^{2+}) − **9**.

piperazine units. Upon complexation, part of the molecule probably assumes a boat conformation where carbons in position *b* and *d* are almost at the same distance from Zn^{2+} while carbons between them are slightly closer.

Furthermore, upon addition of the second equivalent of Zn^{2+} only slight additional downfield shifts are observed, with an additional broadening of signals, which indicates the formation of a 1:1 complex between the macrocycle and Zn^{2+}, with the cation inside the macrocyclic cavity.

Conclusion

Azamacrocycles containing piperazine units are designed as potential chemosensors for metal cations. Complexation of cations was detected by fluorescence spectroscopy and confirmed by NMR. Different sensitivities of macrocycles for different metals offer a variety of applications of macrocycles in biological research. The observed intramolecular charge transfer within nonconjugated macrocycles is an additional interesting property of these compounds and is currently under further investigation.

Experimental Section

4-(4-{4-[4-(4-*tert*-Butylcarboxy-piperazin-1-yl)-butoxy]-phenoxy}-butyl)-piperazine-1-carboxylic Acid *tert*-Butyl Ester (2). A mixture of **1** (1.26 g, 5.03 mmol), mono-Boc-piperazine (1.90 g, 10.20 mmol), and $Ti(O\text{-}iPr)_4$ (3.59 g, 12.63 mmol) was stirred at room temperature. After 1 h, the viscous solution was diluted with 10 mL of absolute EtOH. $NaBH_3CN$ (423 mg, 6.73 mmol) was added, and the solution was stirred for 20 h. Water (10 mL) was added with stirring, and the resulting inorganic precipitate was filtered and washed with EtOH. The solvent was removed with a rotary evaporator, and the residue was purified by column chromatography (silica gel, CH_2Cl_2/MeOH 95:5) to give 2.40 g (81%) of **2** as a colorless, viscous oil. ^1H NMR (400 MHz, $CDCl_3$): δ 6.73 (s, 4H), 3.85 (t, J = 6.3 Hz, 4H), 3.38 (t, J = 4.8 Hz, 8H), 2.38−2.35 (m, 12H), 1.75−1.68 (m, 4H), 1.65−1.59 (m, 4H), 1.39 (s, 18H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 154.6, 153.0, 115.3, 79.6, 68.1, 58.1, 52.9, 28.3, 27.2, 23.1. HRMS−FAB for $C_{32}H_{54}N_4O_6$: calc 591.4122 $[M + H]^+$, found 591.4125.

1-{4-[4-(4-Piperazine-1-yl-butoxy)-phenoxy]-butyl}-piperazine (3). TFA (5 mL) was added to a solution of **2** (1.95 g, 3.30 mmol) in CH_2Cl_2 (15 mL). The mixture was stirred at room temperature for 30 min. After 30 min, the solvent was removed with a rotary evaporator, and 30 mL of 1 N NaOH was added to the residue. The aqueous layer was extracted with CH_2Cl_2 (3 × 50 mL), and the organic phase was washed with brine and dried over $MgSO_4$. The solvent was removed in vacuo to give 1.23 g (95%) of **3** as a colorless viscous oil. ^1H NMR (400 MHz, $CDCl_3$): δ 6.73 (s, 4H), 3.84 (t, J = 6.3 Hz, 4H), 2.82 (t, J = 4.8 Hz, 8H), 2.37−2.28 (m, 12H), 1.92−1.82 (m, 2H), 1.73−1.66 (m, 4H), 1.61−1.54 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 152.8, 115.1, 68.0, 58.6, 54.1, 45.7, 27.1, 22.9. HRMS−FAB for $C_{22}H_{38}N_4O_2$: calc 391.3073 $[M + H]^+$, found 391.3085.

Macrocycle 4. Compounds **1** (48 mg, 0.19 mmol) and **3** (75 mg, 0.19 mmol) and $[Rh(acac)(CO)_2]$ (4 mg, 16 μmol) were mixed in 40 mL of toluene and stirred for 1 h. The solution was placed in an autoclave, pressurized with 40 bar H_2, and heated at 50 °C for 18 h. The solvent was removed with a rotary evaporator, and the crude product was purified by column chromatography (silica gel, CH_2Cl_2/MeOH 95:5) to give 42 mg (36%) of **4** as a yellow viscous oil that solidifies upon standing. ^1H NMR (400 MHz, $CDCl_3$): δ 6.74 (s, 8H), 3.92 (t, J = 6.0 Hz, 8H), 2.50−2.35 (m, 16H), 2.34 (t, J = 7.3 Hz, 8H), 1.73−1.67 (m, 8H), 1.62−1.57 (m, 8H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 153.0, 115.6, 68.1, 57.8, 52.8, 27.1, 22.6. HRMS−FAB for $C_{36}H_{56}N_4O_4$: calc 609.4380 $[M + H]^+$, found 609.4402.

3,5-Bis(4-oxo-butoxy)-benzoic Acid Methyl Ester (6). Compound **5** (226 mg, 0.91 mmol), $[Rh(acac)(CO)_2]$ (3 mg, 12 μmol), XANTPHOS (30 mg, 52 μmol), and toluene (15 mL) were dissolved in the autoclave. The solution was pressurized with 20 bar CO/H_2 (1:1) and heated at 70 °C for 18 h. The solvent was removed with a rotary evaporator, and the crude mixture was purified by column chromatography (silica gel, CH_2Cl_2) to give 200 mg (71%) of **6** as a colorless, viscous oil. ^1H NMR (400 MHz, $CDCl_3$): δ 9.76 (s, 2H), 7.07 (d, J = 2.3 Hz, 2H), 6.52 (t, J = 2.3 Hz, 1H), 3.94 (t, J = 6.0 Hz, 4H), 3.62 (s, 3H), 2.59 (dt, J = 5.8, 1.2 Hz, 4H), 2.08−2.01 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 201.6, 166.6, 159.6, 131.9, 107.7, 106.8, 66.9, 52.2, 40.4, 21.8. EA for $C_{16}H_{20}O_6$ (308.33 g/mol): calc C, 62.3; H, 6.5. Found: C, 62.4; H, 6.3. HRMS−FAB: calc 309.1338 $[M + H]^+$, found 309.1346.

3,5-Bis[4-(4-*tert*-butylcarboxy-piperazin-1-yl)-butoxy]-benzoic Acid Methyl Ester (7). Following the procedure for the synthesis of **2**, starting from **6** and Boc-piperazine, compound **7** was prepared in 72% yield as a colorless, viscous oil.

3,5-Bis(4-piperazin-1-yl-butoxy)-benzoic Acid Methyl Ester (8). Following the procedure for the synthesis of **3**, starting from **7**, compound **8** was prepared in 89% yield as a colorless, viscous oil.

Macrocycle 9. Following the procedure for the synthesis of **4**, starting from **6** and **8**, azamacrocycle **9** was prepared in 31% yield as a yellow, viscous oil that solidifies upon standing.

NMR Experiments for Complexation Investigations. All experiments were run on a 600 MHz spectrometer. 2D experiments (g-COSY and g-HSQC) and 1D-NOE were performed at 40 °C in $CDCl_3$, and ^{13}C NMR experiments were performed in $CD_3CN/CDCl_3$ (9:1) solution at 27 °C. Experiments were performed on compound **9** before and after addition on 1 and 2 equiv of $ZnCl_2$. The chemical shift changes on all carbons were monitored.

Acknowledgment. Authors would like to acknowledge Deutscher Akademischer Austauschdienst (DAAD, G.A.) and DFG (B.K.) for financial support. We also thank Degussa AG Düsseldorf for donations of chemicals and MPG for technical support.

Supporting Information Available: ^1H NMR, ^{13}C NMR and HRMS−FAB data of **7**, **8** and **9**; UV spectra of **4** and **9** after addition of Co^{2+} and Zn^{2+}; ^1H and ^{13}C NMR (APT) of **4** and **9**; 1D-NOE, g-COSY, g-HQSC of **9** and ^{13}C NMR complexation experiments of **9** with Zn^{2+}. This material is available free of charge via the Internet at http://pubs.acs.org.

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