JOC*Article*

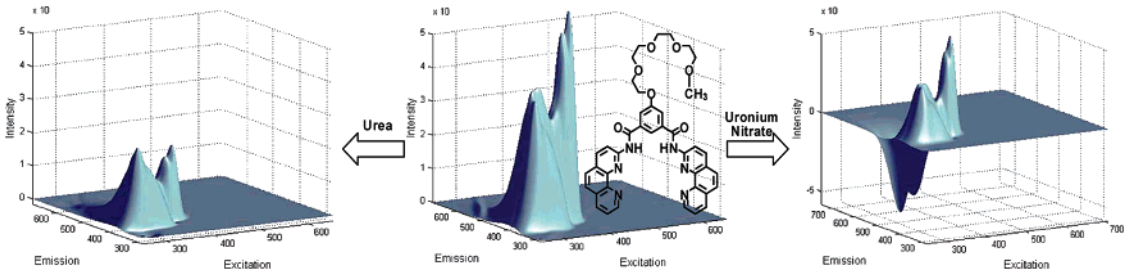
Phenanthroline-Derived Ratiometric Chemosensor for Ureas

Yoni Engel, Adi Dahan, Emily Rozenshine-Kemelmakher, and Michael Gozin*

School of Chemistry, Raymond and Beverly Sackler Faculty of Exact Sciences, Tel Aviv University, Tel Aviv 69978, Israel

cogozin@mgchem.tau.ac.il

Received October 15, 2006



The syntheses of 1,10-phenanthroline fluorophore-based chemosensor **7** and its truncated analog **9** are reported. Interactions of these compounds with urea, thiourea, 1,3-dimethylurea, tetrahydropyrimidin-2(1*H*)-one, imidazolidin-2-one, and selected uronium salts were assessed by three-dimensional excitation−emission spectroscopy, UV−vis absorbance, and fluorescence titrations. Chemosensor **7** was found to be capable of distinguishing between neutral ureas and their salts, by producing a different optical response for each type of compounds. The complexation of urea by **7** was also studied by selective-NOE ^{1}H NMR, ^{13}C NMR (using ^{13}C-labeled guest), and MALDI-TOF mass spectrometry. In addition, we performed DFT calculations (B3LYP 3-21g** level) for structures of complexes of **7** with urea, imidazolidin-2-one, and tetrahydropyrimidin-2(1*H*)-one. Development of chemosensor **7**-type compounds in conjunction with differential excitation−emission spectroscopy represents an important step toward the development of novel tools for ureas and their salts analysis.

Introduction

The development of supramolecular receptors for the binding of urea,[1] its salts,[2] and derivatives[3] including various cyclic ureas, barbiturates, biotin, citrulline, uric acid, uracil, and others, is an active field of research with great potential for environmental and biomedical applications. Remarkably, development of chemosensors for uronium salts, such as uronium nitrate (UN), a powerful improvised explosive frequently used in acts of terror,[4] has received very little attention. In contrast to the substantial number of reports on various chemosensors for urea and its derivatives, only one paper has recently been published, by Almog and co-workers, regarding chemical detection of UN.[5] A chemosensor producing a different optical response to ureas and their salts, thus being capable of distinguishing between these analytes, is unprecedented and could be of considerable importance.

Here we report the synthesis and evaluation of the new ratiometric chemosensor **7**, capable of differential detection of urea and UN. Chemosensor **7** structure is composed of two 1,-10-phenanthroline fluorophores, which also function as binding "arms" (hydrogen-bond acceptors). These "arms" are bridged by a 5-(2,5,8,11-tetraoxatridecan-13-yloxy)isophthalamidyl unit (amide functional group-containing hydrogen bond donor), which carries a methyltetraethyleneglycol moiety for improved solubility in solvents that are better suited to urea/UN analysis. For comparison and mode-of-binding studies, a truncated derivative of chemosensor **7** with a single 1,10-phenanthroline "arm", compound **9**, was prepared and evaluated by the same methods as for **7**.

Results and Discussion

Synthesis. Synthesis of chemosensor **7** included the construction of two key building blocks, namely, 2-amino-1,10-phenanthroline **3**[6] and 5-(2,5,8,11-tetraoxatridecan-13-yloxy)isophthaloyl dichloride **6** and their subsequent coupling (Figure 1). Compound **3** was synthesized by a new route in three steps, starting with the oxidation of 1,10-phenanthroline monohydrate by H_2O_2 in acetic acid, to produce 1,10-phenanthroline-*N*-oxide **1**.[7] This transformation was followed by conversion of com-

* To whom correspondence should be addressed. Tel: +972-3-640-5878. Fax: +972-3-640-5879.

10.1021/jo062130h CCC: $37.00
Published on Web 03/01/2007

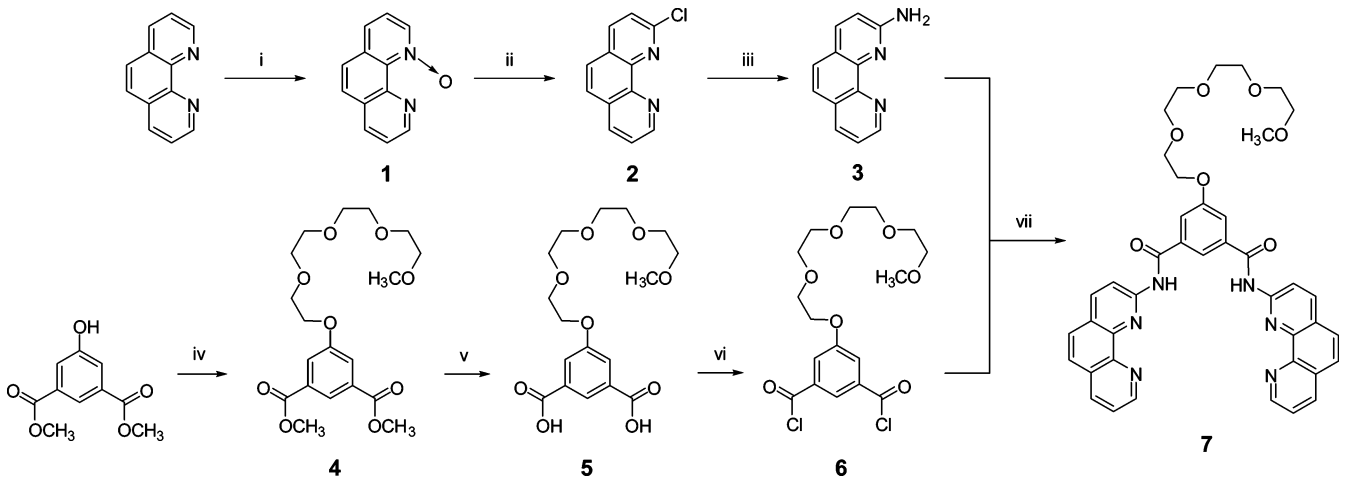


FIGURE 1. Synthesis of chemosensor **7**. Reagents and conditions: (i) H_2O_2, AcOH, 70 °C; (ii) $POCl_3$, NaCl, DMF, 100 °C; (iii) K_2CO_3, acetamide, 200 °C; (iv) K_2CO_3, 2,5,8,11-tetraoxatridecan-13-yl-4-methylbenzenesulfonate, CH_3CN, reflux; (v) 1.0 M $NaOH_{(aq)}$, EtOH, 65 °C; (vi) $SOCl_2$, THF, reflux; (vii) *N*,*N*′-diisopropylethylamine, CH_2Cl_2, 0 °C.

pound **1** to the corresponding 2-chloro-1,10-phenanthroline **2**,[8] using $POCl_3$ as a chlorination agent and DMF as the solvent. Heating of the resultant compound **2** with K_2CO_3 in acetamide to 200 °C led to formation of the first building block **3**.

The second key intermediate dichloride **6** was prepared in three steps, starting with a hydroxyalkylation reaction of dimethyl-5-hydroxyisophthalate with 2,5,8,11-tetraoxa-tridecan-13-yl-4-methylbenzene-sulfonate[9] in refluxing CH_3CN, using K_2CO_3 as a base, to afford dimethyl-5-(2,5,8,11-tetraoxatridecan-13-yloxy)isophthalate **4** in a quantitative yield. Subsequent hydrolysis of diester **4** by 1.0 M NaOH in aqueous EtOH at 65 °C, followed by acidification, resulted in formation of the corresponding 5-(2,5,8,11-tetraoxatridecan-13-yloxy)isophthalic acid **5**. The diacid **5** was quantitatively converted to the diacylchloride **6** by reaction with thionyl chloride in refluxing THF. The target chemosensor **7** was finally obtained in 48% yield, by coupling of **6** with a 2-fold excess of **3** in CH_2Cl_2, using *N*,*N*′-diisopropylethylamine as the base.

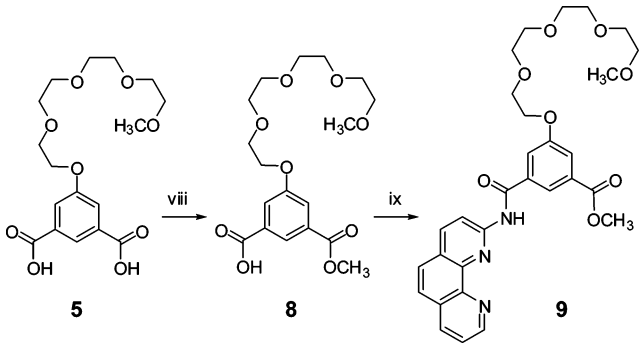


FIGURE 2. Synthesis of **9**. Reagents and conditions: (viii) H_2SO_4 (catalyst), MeOH, THF, 65 °C; (ix) HOBt, DCC, DMF, 0 °C.

(1) (a) Harkema, S.; Van, Hummel, G. J.; Daasvatn, K.; Reinhoudt, D. N. *J. Chem. Soc., Chem. Commun.* **1981**, *8*, 368−369. (b) Crego, M.; Marugan, J. J.; Raposo, C.; Sanz, M. J.; Alcazer, V.; Cruz C., M.; Moran, J. R. *Tetrahedron Lett.* **1991**, *32* (33), 4185−4188. (c) Aarts, V. M. L. J.; Van Staveren, C. J.; Grootenhuis, P. D. J.; Van Eerden, J.; Kruise, L.; Harkema, S.; Reinhoudt, D. N. *J. Am. Chem. Soc.* **1986**, *108* (16), 5035−5036. (d) Van Staveren, C. J.; Fenton, D. E.; Reinhoudt, D. N.; Van Eerden, J.; Harkema, S. *J. Am. Chem. Soc.* **1987**, *109* (11), 3456−3458. (e) Chou, H.-C.; Hsu, C.-H.; Cheng, Y.-M.; Cheng, C.-C.; Liu, H.-W.; Pu, S.-C.; Chou, P.-T. *J. Am. Chem. Soc.* **2004**, *126* (6), 1650−1651. (f) Van Staveren, C. J.; Van Eerden, J.; Van Veggel, F. C. J. M.; Harkema, S.; Reinhoudt, D. N. *J. Am. Chem. Soc.* **1988**, *110* (15), 4994−5008. (g) Van Staveren, C. J.; Aarts, V. M. L. J.; Grootenhuis, P. D. J.; Droppers, W. J. H.; Van Eerden, J.; Harkema, S.; Reinhoudt, D. N. *J. Am. Chem. Soc.* **1988**, *110* (24), 8134−8144. (h) Aarts, Veronika M. L. J.; Grootenhuis, Peter D. J.; Reinhoudt, David N.; Czech, A.; Czech, B. P.; Bartsch, R. A. *Recl. Trav. Chim. Pays-Bas* **1988**, *107* (2), 94−103. (i) Hung, C. Y.; Hopfner, T.; Thummel, R. P. *J. Am. Chem. Soc.* **1993**, *115* (26), 12601−12602. (j) Reinhoudt, D. N. *J. Coord. Chem.* **1988**, *18* (1−3), 21−43. (k) Nijenhuis, W. F.; Van Doorn, A. R.; Reichwein, A. M.; De Jong, F.; Reinhoudt, D. N. *J. Am. Chem. Soc.* **1991**, *113* (9), 3607−3608. (l) Van Doorn, A. R.; Schaafstra, R.; Bos, M.; Harkema, S.; Van Eerden, J.; Verboom, W.; Reinhoudt, D. N. *J. Org. Chem.* **1991**, *56* (21), 6083−6094. (m) Reichwein, A. M.; Verboom, W.; Harkema, S.; Spek, A. L.; Reinhoudt, D. N. *J. Chem. Soc., Perkin Trans. 2* **1994**, *6*, 1167−1172. (n) Goswami, S.; Mukherjee, R. *Tetrahedron Lett.* **1997**, *38* (9), 1619−1622. (o) Goswami, S.; Mukherjee, R.; Ray, J. *Org. Lett.* **2005**, *7* (7), 1283−1285. (p) Bell, T. W.; Hou, Z. *Angew. Chem., Int. Ed.* **1997**, *36* (13/14), 1536−1538. (q) Ray, J. K.; Haldar, M. K.; Gupta, S.; Kar, G. K. *Tetrahedron* **2000**, *56* (6), 909−912. (r) Bell, T. W.; Liu, J. *J. Am. Chem. Soc.* **1988**, *110* (11), 3673−3674. (s) Bell, T. W.; Hext, Nicholas M. *Chem. Soc. Rev.* **2004**, *33* (9), 589−598. (t) Bell, T. W.; Hext, N. M.; Khasanov, A. B. *Pure Appl. Chem.* **1998**, *70* (12), 2371−2377.

(2) (a) Uiterwijk, J. W. H. M.; Harkema, S.; Reinhoudt, D. N.; Daasvatn, K.; den Hertog, H. J., Jr.; Geevers, J. *Angew. Chem.* **1982**, *94* (6), 462−463. (b) Aarts, V. M. L. J.; Geevers, J.; Reinhoudt, D. N.; Lengton, W.; Bos, M.; Uiterwijk, J. W. H. M.; Harkema, S. *Tetrahedron* **1987**, *43* (3), 617−624. (c) Uiterwijk, J. W. H. M.; Van Hummel, G. J.; Harkema, S.; Aarts, V. M. L. J.; Daasvatn, K.; Geevers, J.; Den Hertog, H. J., Jr.; Reinhoudt, D. N. *J. Inclusion Phenom.* **1988**, *6* (1), 79−100.

(3) (a) Van Loom, J. D.; Janssen, R. G.; Verboom, W.; Reinhoudt, D. N. *Tetrahedron Lett.* **1992**, *33* (35), 5125−5128. (b) Naher, S.; Hiratani, K.; Karikomi, M.; Haga, K. *J. Heterocycl. Chem.* **2005**, *42* (4), 575−582. (c) Kim, Y.; Chung, C. *Synth. Met.* **2001**, *117* (1−3), 301−303. (d) Claramunt, R. M.; Herranz, F.; Santa Maria, M. D.; Jaime, C.; de Federico, M.; Elguero, J. *Biosens. Bioelectron.* **2004**, *20* (6), 1242−1249. (e) Claramunt, R. M.; Herranz, F.; Santa Maria, M. D.; Pinilla, E.; Torres, M. R.; Elguero, J. *Tetrahedron* **2005**, *61* (21), 5089−5100. (f) Herranz, F.; Santa Maria, M. D.; Claramunt, R. M. *J. Org. Chem.* **2006**, *71* (8), 2944−2951. (g) Goswami, S.; Dey, S. *J. Org. Chem.* **2006**, *71* (19), 7280−7287. (h) Ge, Y.; Miller, L.; Ouimet, T.; Smith, D. K. *J. Org. Chem.* **2000**, *65* (26), 8831−8838. (i) Hegde, V.; Madhukar, P.; Madura, J. D.; Thummel, R. P. *J. Am. Chem. Soc.* **1990**, *112* (11), 4549−4550. (j) McClenaghan, N. D.; Grote, Z.; Darriet, K.; Zimine, M.; Williams, R. M.; De Cola, L.; Bassani, D. M. *Org. Lett.* **2005**, *7* (5), 807−810. (k) Chang, S.-K.; Hamilton, A. D. *J. Am. Chem. Soc.* **1988**, *110*, 1318−1319. (l) Li, S.; Sun, L.; Chung, Y.; Weber, S. G. *Anal. Chem.* **1999**, *71* (11), 2146−2151. (m) Westwood, J.; Coles, S. J.; Collinson, S. R.; Gasser, G.; Green, S. J.; Hursthouse, M. B.; Light, M. E.; Tucker, J. H. R. *Organometallics* **2004**, *23* (5), 946−951. (n) Dirksen, A.; Hahn, U.; Schwanke, F.; Nieger, M.; Reek, J. N. H.; Voegtle, F.; De Cola, L. *Chem. Eur. J.* **2004**, *10* (8), 2036−2047.

(4) Kapur, G. B.; Hutson, H R.; Davis, M. A.; Rice, P. L. *J. Trauma: Inj., Infect., Crit. Care* **2005**, *59* (6), 1436−1444.

(5) Almog, J.; Klein, A.; Tamiri, T.; Shloosh, Y.; Abramovich-Bar S. *J. Forensic Sci.* **2005**, *50* (3), 582−586.

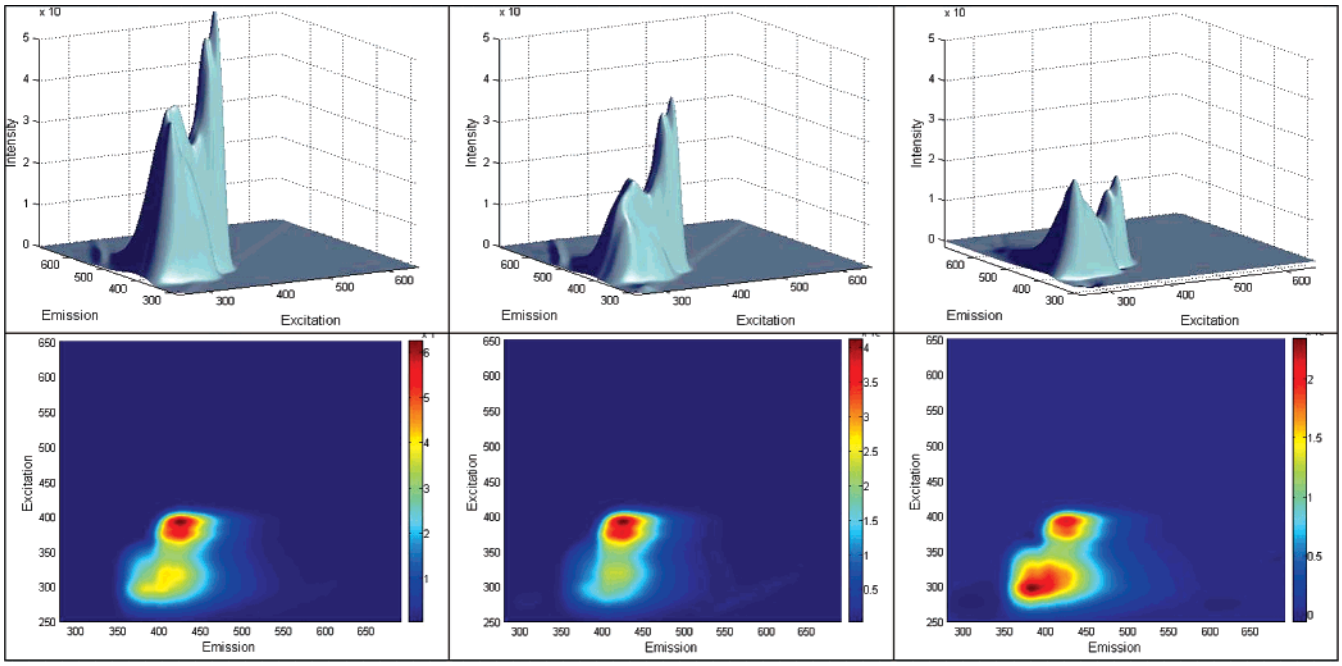


FIGURE 3. Side and top view of (left column) EEM spectrum of free chemosensor **7**, (middle column) EEM spectrum obtained after addition of 100 equiv excess of urea to **7** (complex **10**), and (right column) differential EEM spectrum obtained for free chemosensor **7** and its interaction with 100 equiv excess of urea.

Compound **9** was prepared in two synthetic steps, using the diacid **5** as the starting material (Figure 2). Sulfuric acid catalyzed monoesterification of **5** in MeOH/THF solution afforded the 3-(2,5,8,11-tetraoxatridecan-13-yloxy)-5-(methoxycarbonyl)-benzoic acid **8**. Subsequent DCC-mediated coupling of **8** with the 2-amino-1,10-phenanthroline **3** in DMF led to the formation of the target compound **9** in 70% yield.

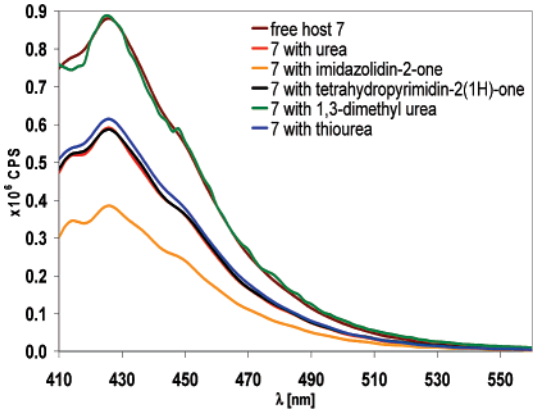


FIGURE 4. Fluorescence spectra (λ_{Ex} = 396 nm) measured for **7** and its interactions with excess of various urea derivatives.

Photophysical Studies. All photophysical studies of chemosensor **7** binding of urea and urea derivatives (which included thiourea, 1,3-dimethylurea, tetrahydropyrimidin-2(1*H*)-one, and imidazolidin-2-one) were conducted in CH_3CN, due to sufficient solubility of both chemosensor **7** and the urea analytes in this solvent. To establish optimal parameters for fluorescence-based

(6) (a) Claus, K. G.; Rund, J. V. *Inorg. Chem.* **1969**, *8* (1), 59−63. (b) De Zwart, M. A. H.; Bastiaans, H. M. M.; Van der Goot, H.; Timmerman, H. *J. Med. Chem.* **1991**, *34* (3), 1193−1201. (c) Ogawa, S.; Gotoh, N. *Kogyo Kagaku Zasshi* **1971**, *74* (1), 83. (d) Pijper, P. J.; Van der Goot, H.; Timmerman, H.; Nauta, W. T. *Eur. J. Med. Chem.* **1984**, *19* (5), 399−404.

(7) (a) Maerker, G.; Case, F. H. *J. Am. Chem. Soc.* **1958**, *80*, 2745−2748. (b) Corey, E. J.; Borror, A. L.; Foglia, T. *J. Org. Chem.* **1965**, *30* (1), 288−290. (c) Ogawa, S.; Gotoh, N. *Kogyo Kagaku Zasshi* **1971**, *74* (10), 2127−2129. (d) Dega-Szafran, Z. *Rocz. Chem.* **1972**, *46* (5), 827−36. (e) Mlochowski, J.; Kloc, K. *Rocz. Chem.* **1973**, *47* (4), 727−734. (f) Engbersen, J. F. J.; Koudijs, A.; Joosten, M. H. A.; Van der Plas, H. C. *J. Heterocycl. Chem.* **1986**, *23* (4), 989−990. (g) Rozen, S.; Dayan, S. *Angew. Chem., Int. Ed.* **1999**, *38* (23), 3472−3473. (h) Balicki, R.; Golinski, J. *Synth. Commun.* **2000**, *30* (8), 1529−1534.

(8) (a) Halcrow, B. E.; Kermack, W. O. *J. Chem. Soc.* **1946**, 155−157. (b) Pitie, M.; Donnadieu, B.; Meunier, B. *Inorg. Chem.* **1998**, *37* (14), 3486−3489. (c) Fife, T. H.; Pujari, M. P. *J. Am. Chem. Soc.* **1988**, *110* (23), 7790−7797. (d) Ogawa, S.; Nakajima, T.; Gotoh, N. *Seisan Kenkyu* **1969**, *21* (7), 474−475. (e) Lewis, J.; O'Donoghue, T. D. *J. Chem. Soc., Dalton Trans.* **1980**, *5*, 736−742. (f) Rice, C. R.; Anderson, K. M. *Polyhedron* **2000**, *19* (4), 495−498.

(9) (a) Hyatt, J. A. *J. Org. Chem.* **1978**, *43* (9), 1808−1811. (b) Schultz, R. A.; White, B. D.; Dishong, D. M.; Arnold, K. A.; Gokel, G. W. *J. Am. Chem. Soc.* **1985**, *107* (23), 6659−6668. (c) Andrews, M. P.; Ozin, G. A. *Inorg. Chem.* **1986**, *25* (15), 2587−2595. (d) Ouchi, M.; Inoue, Y.; Wada, K.; Iketani, S.; Hakushi, T.; Weber, E. *J. Org. Chem.* **1987**, *52* (12), 2420−2427. (e) Ouchi, M.; Inoue, Y.; Liu, Y.; Nagamune, S.; Nakamura, S.; Wada, K.; Hakushi, T. *Bull. Chem. Soc. Jpn.* **1990**, *63* (4), 1260−1262. (f) Achilefu, S.; Selve, C.; Stebe, M.-J.; Ravey, J.-C.; Delpuech, J.-J. *Langmuir* **1994**, *10* (7), 2131−2138. (g) Allcock, H. R.; O'Connor, S. J. M.; Olmeijer, D. L.; Napierala, M. E.; Cameron, C. G. *Macromolecules* **1996**, *29* (23), 7544−7552. (h) Lauter, U.; Meyer, W. H.; Wegner, G. *Macromolecules* **1997**, *30* (7), 2092−2101. (i) Brunsveld, L.; Zhang, H.; Glasbeek, M.; Vekemans, J. A. J. M.; Meijer, E. W. *J. Am. Chem. Soc.* **2000**, *122* (26), 6175−6182. (j) Imbert, D.; Baret, P.; Gaude, D.; Gautier-Luneau, I.; Gellon, G.; Thomas, F.; Serratrice, G.; Pierre, J.-L. *Chem. Eur. J.* **2002**, *8* (5), 1091−1100. (k) Snow, A. W.; Foos, E. E. *Synthesis* **2003**, *4*, 509−512. (l) Brunner, H.; Gruber, N. *Inorg. Chim. Acta* **2004**, *357* (15), 4423−4451. (m) Kishimoto, K.; Suzawa, T.; Yokota, T.; Mukai, T.; Ohno, H.; Kato, T. *J. Am. Chem. Soc.* **2005**, *127* (44), 15618−15623. (n) Kung, C.; Kenski, D. M.; Krukenberg, K.; Madhani, H. D.; Shokat, K. M. *Chem. Biol.* **2006**, *13* (4), 399−407.

(10) (a) Yang, Y.; Lowry, M.; Schowalter, C. M.; Fakayode, S. O.; Escobedo, J. O.; Xu, X.; Zhang, H.; Jensen, T. J.; Fronczek, F. R.; Warner, I. M.; Strongin, R. M. *J. Am. Chem. Soc.* **2006**, *128*, 14081−14092. (b) Cory, R. M.; McKnight, D. M. *Environ. Sci. Technol.* **2005**, *39* (21), 8142−8149. (c) Munoz de la Pena, A.; Mora Diez, N.; Bohoyo Gil, D.; Olivieri, A. C.; Escandar, G. M. *Anal. Chim. Acta* **2006**, *569* (1−2.), 250−259. (d) Wu, F. C.; Mills, R. B.; Evans, R. D.; Dillon, P. J. *Anal. Chem.* **2004**, *76* (1), 110−113. (e) Patel-Sorrentino, N.; Mounier, S.; Benaim, J. Y. *Water Res.* **2002**, *36* (10), 2571−2581.

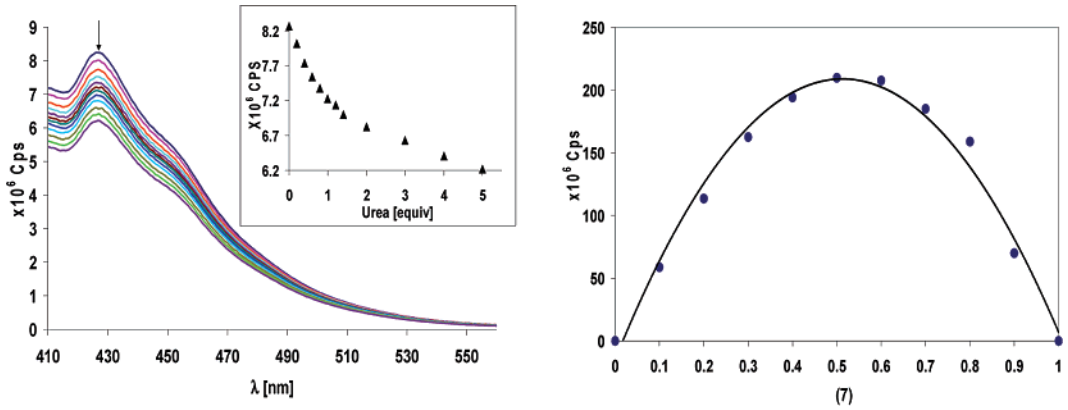


FIGURE 5. (Left) Results of fluorescence titration of **7** with urea. (Left, insert) titration profile obtained for **7** and urea, monitored at $\lambda_{maxEm} = 426$ nm. (Right) Job's plot obtained for **7** and urea.

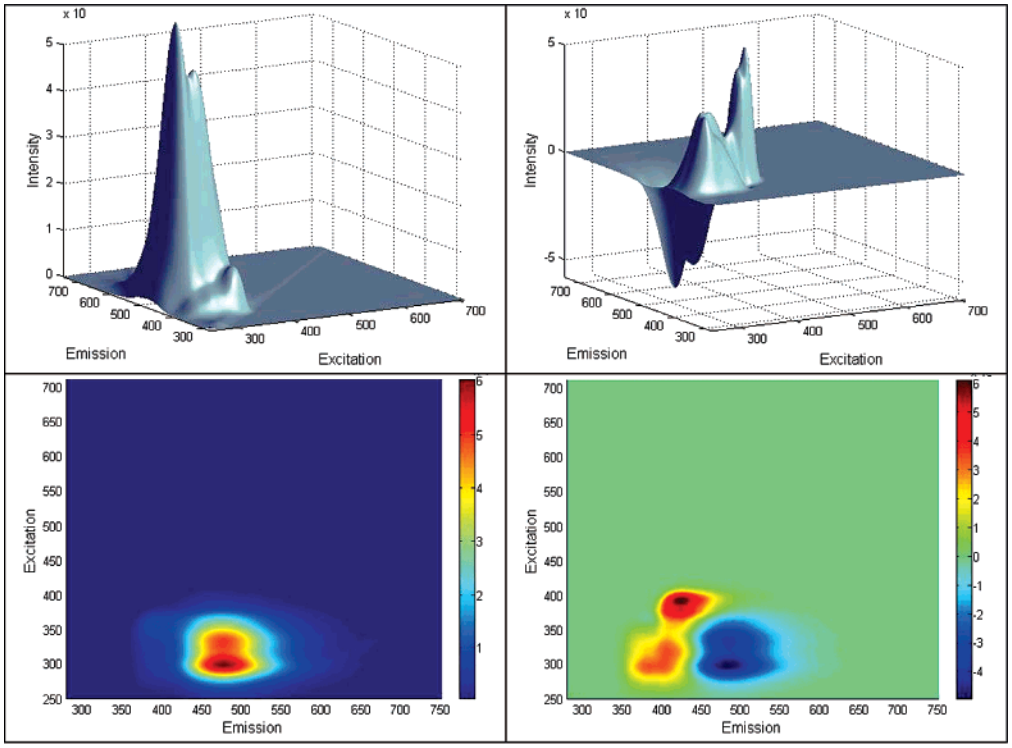


FIGURE 6. Side and top view of (left column) EEM spectrum obtained after addition of 100 equiv excess of UN to **7** and (right column) differential EEM spectrum obtained for free chemosensor **7** and its interaction with 100 equiv excess of UN.

TABLE 1. Binding Constants of Chemosensor 7 with Various Urea Derivatives and Their Binding Stoichiometry

	guest	binding constant	binding stoichiometry
1	urea	$3.0 \pm 0.5 \times 10^4$	1:1
2	thiourea	$6.5 \pm 1.0 \times 10^4$	1:1
3	imidazolidin−2-one	$3.0 \pm 0.2 \times 10^3$	1:1
4	tetrahydropyrimidin-2(1*H*)-one	$5.0 \pm 1.0 \times 10^3$	1:1
5	1,3-dimethylurea	0.0	n/a

titration experiments of **7** with the listed ureas, three-dimensional excitation−emission (EEM) spectra[10] of free chemosensor **7** and its complexes with the urea derivatives were measured. These complexes were prepared by addition of 100-fold excess analyte to a 4.6×10^{-6} M solution of **7**. Addition of analyte in larger excess did not result in any further changes. Figure 3 shows representative EEM spectra of **7**, its complex with urea (**10**), and a differential EEM spectrum (the overall fluorescence response of **7** to binding of urea), which was calculated by subtracting the EEM spectrum of **10** from that of free chemosensor **7**. The fluorescence quantum efficiency values for the chemosensor **7** and its complex with urea were found to be 0.18 and 0.11, respectively (using quinine sulfate as a reference). A commonly accepted mechanism for the phenanthroline quenching phenomena involves an inversion between the strongly emissive $\pi\pi^*$ and the poorly emissive $n\pi^*$ states of this fluorophore. Such an inversion could result from a hydrogen bond interaction of phenanthroline nitrogens with urea, which leads to stabilization of the $n\pi^*$ state with respect to the $\pi\pi^*$ state and decrease in the fluorescence emission intensity.[11]

The results of the EEM experiments allowed us to unambiguously establish that urea, thiourea, tetrahydropyrimidin-2(1*H*)-one, and imidazolidin-2-one can form complexes with **7**, as

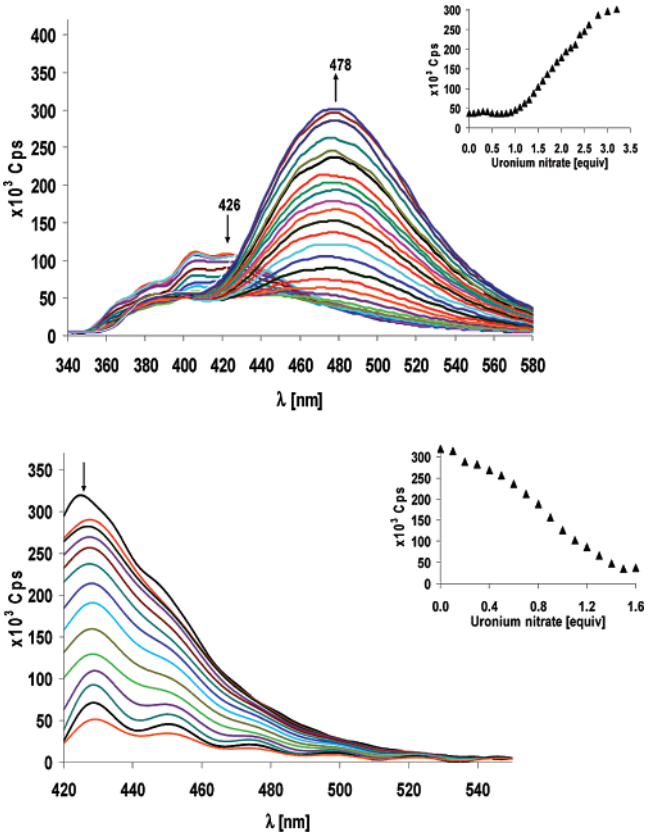


FIGURE 7. (Top) Results of fluorescence titration of **7** with UN (at $\lambda_{Ex} = 297$ nm). (Top, insert) Titration profile obtained for **7** and UN, monitored at $\lambda_{maxEm} = 478$ nm (at $\lambda_{Ex} = 297$ nm). (Bottom) Results of fluorescence titration of **7** with UN (at $\lambda_{Ex} = 396$ nm). (Bottom, insert) Titration profile obtained for **7** and UN, monitored at $\lambda_{maxEm} = 426$ nm (at $\lambda_{Ex} = 396$ nm).

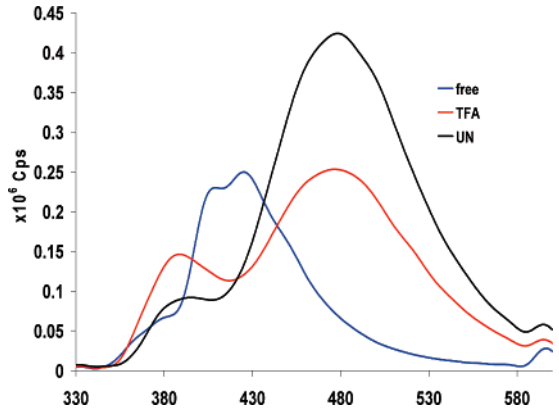


FIGURE 8. Fluorescence spectra of **7** before (blue spectrum), after the addition of 100 equiv excess of trifluoroacetic acid (red spectrum), and after the addition of 100 equiv excess of UN (black spectrum), monitored at $\lambda_{Ex} = 297$ nm.

reflected by substantial changes (quenching) in the fluorescence spectrum of **7** (Figure 4).

In contrast, addition of 1,3-dimethylurea did not produce any measurable response, most probably as a result of its inability

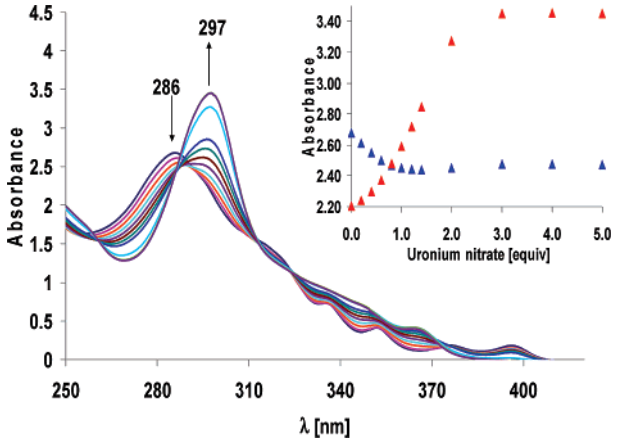


FIGURE 9. Results of UV−vis absorbance titration of **7** with UN. Insert: titration profile obtained for **7** with UN, monitored at $\lambda_{maxAb} =$ 286 nm (blue triangles) and 297 nm (red triangles).

to form a complex with chemosensor **7** in CH_3CN. From analysis of the EEM experiments, we also established that for all urea analytes the optimum wavelength of excitation (at which the biggest absolute changes could be detected) is 396 nm with a corresponding $\lambda_{maxEm} = 426$ nm.

On the basis of these findings, the next step of our evaluation involved a series of fluorescence titration experiments with chemosensor **7** (at 5.2×10^{-5} M) and all aforementioned ureas. Representative results of titration of **7** with $(NH_2)_2CO$ are shown in Figure 5.

Least-square analysis of the spectral titration data, using Hyperquad software,[12] allowed us to determine the binding constants for the evaluated ureas and the binding stoichiometries of their complexes with **7**. The binding stoichiometry for each urea was also validated by continuous variation technique (Job's plot, Figure 5). Table 1 summarizes the results obtained for **7** and all evaluated ureas.

The presented results clearly exhibit preferential binding of certain ureas by chemosensor **7** in CH_3CN. Specifically, thiourea was found to have a higher affinity to **7** than urea, by a factor of 2. These observations can be rationalized by the higher basicity of urea's oxygen relative to thiourea's sulfur (as reflected by pK_a values of 0.1 and −1.0 for uronium and thiouronium cations, respectively),[13] allowing the urea to form stronger hydrogen bonds with protons of the amide functional groups of **7** than thiourea. On the other hand, thiourea hydrogens are more acidic (compared with hydrogens of urea)[14] and thus create stronger hydrogen bonds with nitrogens of phenanthroline binding moieties of **7**,[15] which seems to be the more dominant factor in ureas' binding to **7**. Although somewhat higher binding

(11) (a) Bazzicalupi, C.; Bencini, A.; Bianchi, A.; Borsari, L.; Danesi, A.; Giorgi, C.; Lodeiro, C.; Mariani, P.; Pina, F.; Santarelli, S.; Tamayo, A.; Valtancoli, B. *Dalton Trans.* **2006**, 4000. (b) Pina, F.; M. Bernardo, A.; García-España, E. *Eur. J. Inorg. Chem.* **2000**, 2143−2157.

(12) (a) http://www.hyperquad.co.uk/hq2000.htm. (b) Gans, P.; Sabatini, A.; Vacca A., *Talanta* **1996**, *43*, 1739−1753.

(13) (a) Perrin, D.D. *Dissociation Constants of Organic Bases in Aqueous Solution*; Butterworths: London, 1965; Supplement, 1972. (b) Albert, A. Ionization Constants of Heterocyclic Substances. In *Physical Methods in Heterocyclic Chemistry*; Katritzky, A. R., Ed.; Academic Press: New York, 1963. (c) *CRC Handbook of Biochemistry*; Sober, H. A., Ed.; CRC Press: Boca Raton, FL, 1968. (d) Albert, A.; Serjeant, E. P. *The Determination of Ionization Constants*, 3rd ed.; Chapman and Hall: London, 1984. (e) *The Merck Index*, 12th ed.; Budavari, S., Ed.; Merck & Co.: Whitehouse Station, NJ, 1996.

(14) Gomez, D. E.; Fabbrizzi, L.; Licchelli, M.; Monzani, E. *Org. Biomol. Chem.* **2005**, *3* (8), 1495−1500.

(15) (a) Oepen, G.; Voegtle, F. *Lieb. Ann. Chem.* **1979**, *12*, 2114−2117. (b) Donnelly, P. S.; Skelton, B. W.; White, A. H. *Aust. J. Chem.* **2003**, *56* (12), 1249−1253.

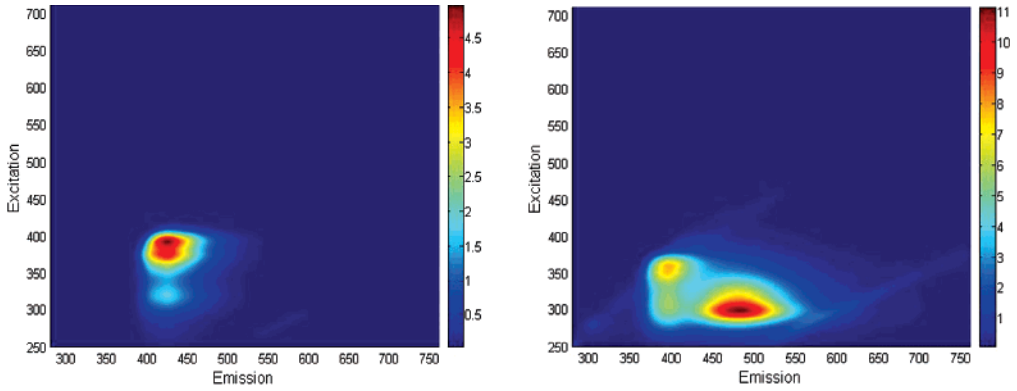


FIGURE 10. Top view of (left) EEM spectrum of free compound **9** and (right) EEM spectrum obtained after addition of 100 equiv excess of UN to **9**.

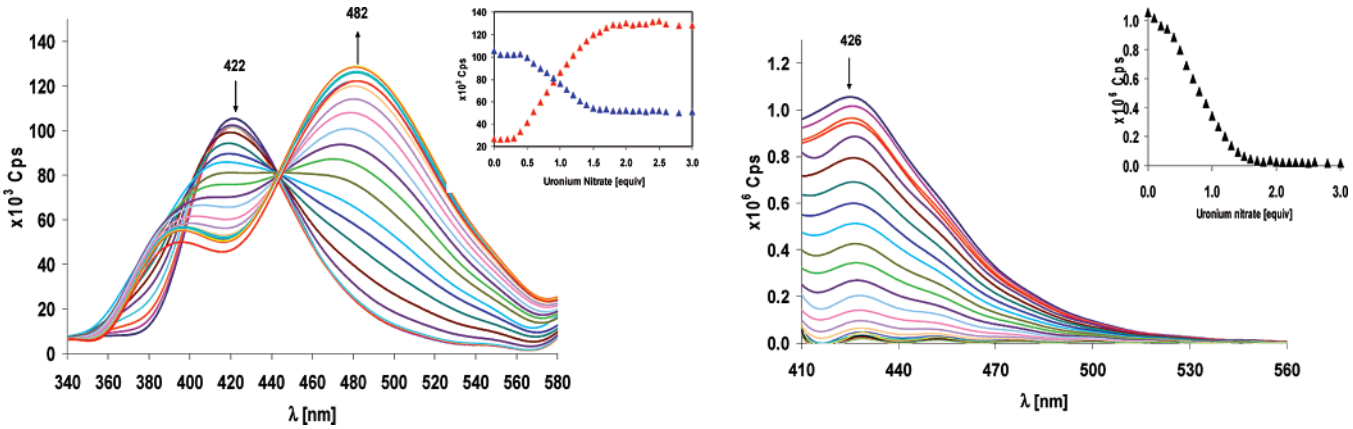


FIGURE 11. (Left) Results of fluorescence titration of **9** with UN (at λ_{Ex} = 297 nm). (Left insert) Titration profile obtained for **9** and UN, monitored at λ_{maxEm} = 422 nm (blue) and 482 nm (red) (at λ_{Ex} = 297 nm). (Right) Results of fluorescence titration of **9** with UN (at λ_{Ex} = 396 nm). (Right insert) Titration profile obtained for **9** and UN, monitored at λ_{maxEm} = 426 nm (at λ_{Ex} = 396 nm).

constants for urea complexes with other neutral organic hosts have been reported,[1] in most cases those results were obtained in solvents less polar and competitive than CH_3CN.

For the cyclic ureas, imidazolidin-2-one and tetrahydropyrimidin-2(1*H*)-one, 1 order of magnitude lower binding constants (relative to those of urea and thiourea) with host **7** were determined. These results could be partially explained by the lower acidity of the cyclic ureas' nitrogen protons, in comparison to those of urea. Additional factors could be related to the size of these cyclic ureas and their fitting into the binding cavity of chemosensor **7** (see computational results further on, in the Supporting Information section). It is important to mention that despite their bearing only two NH groups, the evaluated cyclic ureas were still able to exhibit binding constants in a range of several thousands, strongly suggesting that properly oriented NH groups are still sufficient for complex formation with the chemosensor **7**, on the one hand, and implying that mostly "upper" aromatic nitrogens (*ortho* to the amide group) of 1,10-phenanthroline moieties are actually engaged in binding, on the other. The lack of interaction between chemosensor **7** and 1,3-dimethylurea could be explained by NH groups' preferred orientation,[1i] as the latter urea derivative is incapable of forming proper "in-plane" interactions with both 1,10-phenanthroline moieties of this chemosensor.

In addition to neutral urea ligands, we evaluated response of chemosensor **7** to the uronium cation, using uronium trifluoroacetate, and uronium hydrogensulfate and UN as representative analytes. As already described, in order to find optimized

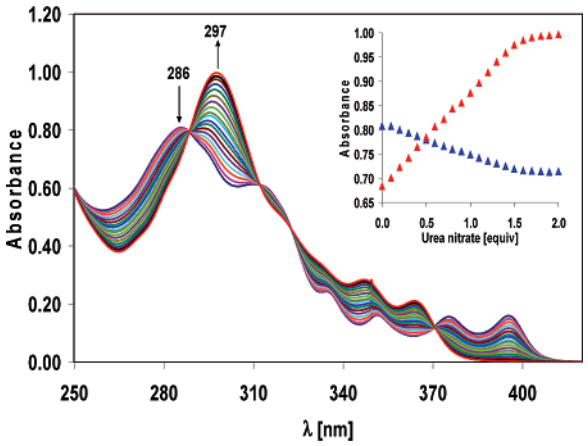


FIGURE 12. Results of UV−vis absorbance titration of **9** (at 3.2 × 10^{-5} M) with UN. Insert: titration profile obtained for **9** and UN, monitored at λ_{maxAb} = 286 nm (blue) and 297 nm (red).

parameters for titration experiments with UN, the EEM spectrum of **7** (at concentration of 4.6 × 10^{-6} M) with the addition of about 100 equiv excess UN was measured (Figure 6). We discovered that exposure of **7** to the uronium salts produces a fluorescent response completely different than that observed when this chemosensor is exposed to neutral urea derivatives. The overall shape of the resulting fluorescence signal changed and its three maximum peaks at $\lambda_{Ex}/\lambda_{maxEm}$ = 315/410, 376/

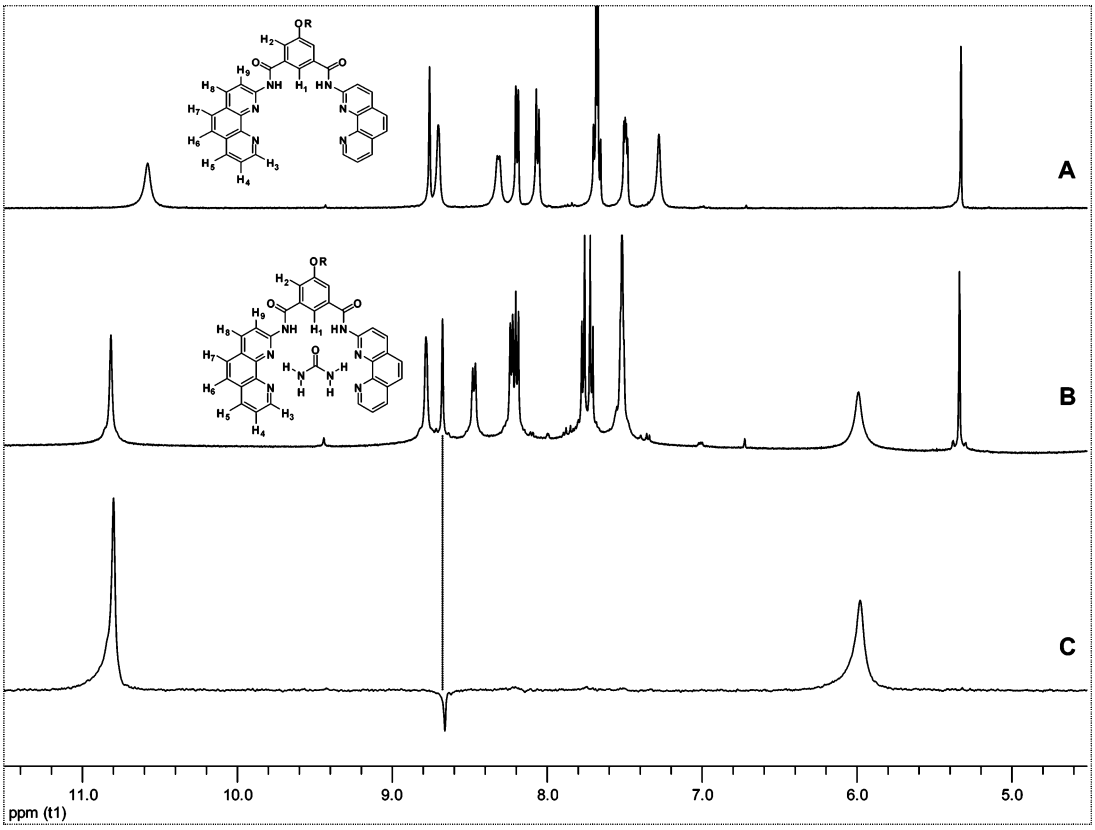


FIGURE 13. (A) ^{1}H NMR spectra of **7** in CD_2Cl_2. (B) ^{1}H NMR spectra of the complex **10** in CD_2Cl_2. (C) Results of ^{1}H NMR selective-NOE experiment with **10** in CD_2Cl_2 (when the amide protons at 10.80 ppm were irradiated).

426, and 396/426 nm were shifted to $\lambda_{Ex}/\lambda_{maxEm} = 297/478$ and 336/478 nm (Figure 6). These changes could be clearly seen in the differential EEM spectrum, obtained from subtraction of the EEM spectrum of **7** with excess UN from the EEM spectrum of free chemosensor **7** (Figure 6).

Based on these EEM results, a series of fluorescence titration experiments with chemosensor **7** (at 4.6×10^{-6} M) and UN were performed at $\lambda_{Ex} = 297$ and 396 nm (Figure 7).

Very similar results were obtained upon titrating chemosensor **7** with trifluoroacetic acid ($pK_a = 0.52$), clearly indicating that UN is acting as an acid, protonating rather than binding to the 1,10-phenanthroline moieties of **7** (Figure 8). Note that other investigators have also reported that upon acidification of 1,-10-phenanthroline and its derivatives, fluorescent peaks of these compounds shift by at least 50 nm to longer wavelengths.[16]

These conclusions were strongly supported by UV−vis absorption spectroscopy experiments. In contrast to all evaluated neutral urea derivatives, the addition of which did not result in any detectable changes in the UV−vis absorption spectrum of **7**, substantial changes in this spectrum were observed upon addition of the UN salt. The ratiometric response of **7** (at 5.4×10^{-4} M) to the presence of UN in solution was evaluated by a titration experiment conducted in CH_3CN, which showed a gradual shift in λ_{max} absorbance from 286 to 297 nm (Figure 9).

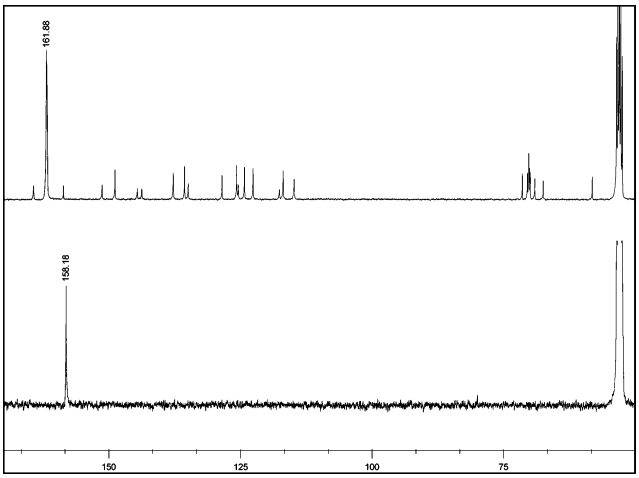


FIGURE 14. (Top) ^{13}C NMR spectra of complex **10** with $(NH_2)_2{}^{13}CO$ in CD_2Cl_2. (Bottom) ^{13}C NMR spectra of $(NH_2)_2{}^{13}CO$ in CD_2Cl_2.

Analysis of these results revealed that the interaction between **7** and UN occurs via a multistep process, as evidenced by the lack of an exact isosbestic point in the absorbance and fluorescence titrations of **7** with UN (Figure 9). This could be attributed to the presence of two 1,10-phenanthroline groups in **7**, which most probably do not undergo simultaneous protonation during UN addition. Similar changes in the absorption and fluorescence spectra of 1,10-phenanthroline as a function of pH have been described by de Melo and others.[17]

For comparison and to determine which functional group in chemosensor **7** is more important for ureas binding, we repeated some of the above-described experiments with compound **9**. In

(16) (a) Sugiyasu, K.; Fujita, N.; Takeuchi, M.; Yamada, S.; Shinkai, S. *Org. Biomol. Chem.* **2003**, *1* (5), 895−899. (b) Yamada, M.; Kimura, M.; Nishizawa, M.; Kuroda, S.; Shimao, I. *Bull. Chem. Soc. Jpn.* **1991**, *64* (6), 1821−1827. (c) Armaroli, N.; Ceroni, P.; Balzani, V.; Kern, J.-M.; Sauvage, J.-P.; Weidmann, J.-L. *J. Chem. Soc., Faraday Trans.* **1997**, *93* (23), 4145−4150.

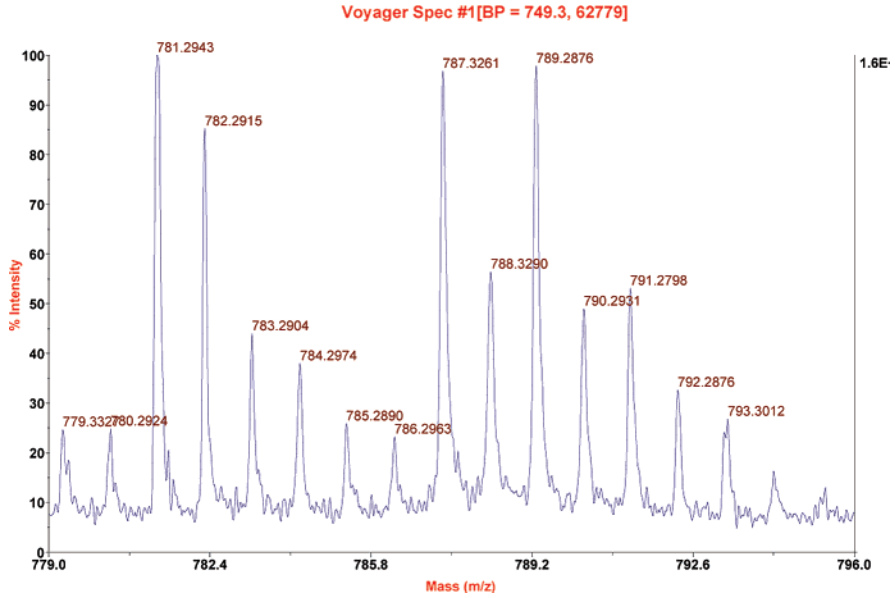


FIGURE 15. MALDI-TOF MS results obtained for complex **10**.

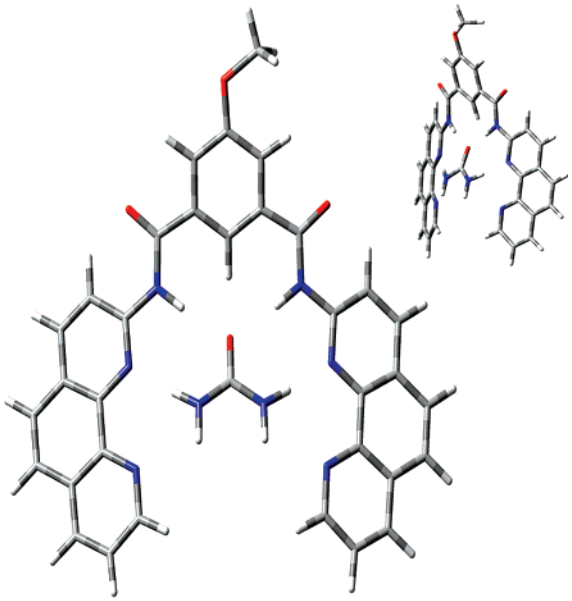


FIGURE 16. Top and side view of the Energy-minimized (DFT B3LYP 3-21G** level) structure of complex **10**.

CH_3CN, we were not able to detect any changes in the fluorescence or absorbance spectra of **9** upon addition of the afore-listed neutral ureas. These results confirmed our previous conclusion regarding the dominant and essential role of phenanthroline moieties in the binding of various ureas.

The response of compound **9** to the presence of UN was also evaluated in the manner described for **7**. Thus, exposure of **9** (at concentration of 4.9×10^{-6} M) to 100 equiv excess of UN produced a substantial fluorescence response, as shown in Figure 10. As in the case of chemosensor **7** (which shares the same fluorophore unit with **9**), the overall shape of the resulting fluorescence signal of **9** changed, and its three maximum peaks at $\lambda_{Ex}/\lambda_{maxEm}$ = 315/410, 376/422, and 396/426 nm shifted to $\lambda_{Ex}/\lambda_{maxEm}$ = 297/478 nm.

Based on the EEM results, a series of titration experiments with **9** (at 4.9×10^{-6} M) and UN was performed at λ_{Ex} = 297 and 396 nm (Figure 11).

Considerable similarity was found between the results of the fluorescence and absorbance titrations (Figure 12) of **9** with UN and those of chemosensor **7** with the same analyte in CH_3CN. The major differences were related to the single-step transition from a neutral free **9** to its corresponding monoprotonated cation, as manifested by clear isosbestic points at 444 nm (in fluorescence titration) and at 288, 312, 322, and 370 nm (in absorbance titration), confirming our previous conclusions.

NMR Studies. In addition to photophysical studies, we evaluated the formation of complex **10** in a series of NMR experiments conducted in CD_2Cl_2. It should be mentioned that because of the very limited solubility of urea in dichloromethane, titration of chemosensor **7** could not be performed and therefore its exact binding constants with this analyte to could not be determined.

To measure the ^{1}H NMR of complex **10**, the compound was prepared directly in deuterated solvent by sonicating a solution of **7** in CD_2Cl_2 with 10-fold excess of solid urea. When the transformation of **7** to **10** was complete (as monitored by ^{1}H NMR), the remaining excess of solid urea was filtered out. Comparison of the ^{1}H NMR spectrum of the free chemosensor **7** to a spectrum of the resultant product revealed substantial changes, undoubtedly indicating formation of a stable complex between **7** and urea (Figure 13). Specifically, a new peak, assigned to the urea protons, appeared at 5.98 ppm. With respect to the response of the host **7**, a chemical shift from 10.57 to 10.80 ppm ($\Delta\delta$ = 0.23 ppm) was observed in its amide functional groups. In contrast to reports by other investigators for related complexes,[1n,o] in our case the *peri* proton of the 5-(2,5,8,11-tetraoxatridecan-13-yloxy)isophthalamidyl fragment

(17) (a) Pina, J.; Seixas de Melo, J.; Pina, F.; Lodeiro, C.; Lima, J. C.; Parola, A. J.; Soriano, C.; Clares, M. P.; Albelda, M. T.; Aucejo, R.; Garcia-Espana, E. *Inorg. Chem.* **2005**, *44* (21), 7449−7458. (b) Kanzaki, R.; Umebayashi, Y.; Maki, T.; Ishiguro, S.-I. *J. Solution Chem.* **2004**, *33* (6/7), 699−709. (c) Armaroli, N.; De Cola, L.; Balzani, V.; Sauvage, J.-P.; Dietrich-Buchecker, C. O.; Kern, J.-M. *J. Chem. Soc., Faraday Trans.* **1992**, *88* (4), 553−556. (d) Henry, M. S.; Hoffman, M. Z. *J. Phys. Chem.* **1979**, *83* (5), 618−625. (e) Winefordner, J. D.; Schulman, S. G.; Tidwell, P. T.; Cetorelli, J. J. *J. Am. Chem. Soc.* **1971**, *93* (13), 3179−3183. (f) Linnell, R. H.; Kaczmarczyk, A. *J. Phys. Chem.* **1961**, *65*, 1196−1200.

TABLE 2. Comparison of Calculated and Measured[19] Urea Bond Lengths in Bound Urea (Complex 10) and Unbound Urea

bond	calculated bond length in bound urea [Å]	calculated bond length in unbound urea [Å]	measured bond length in unbound urea [Å]
C═O	1.286	1.239	1.221
C−N	1.352	1.386	1.378
$N-H_1$	1.020	1.001	1.021
$N-H_2$	1.001	1.001	0.998

TABLE 3. Calculated Distances and Angles for Complex 10

bond	distance [Å]	angle [deg]
$H_{amide}\cdots O_{urea}$	1.950 ± 0.002	173.16 ± 0.33 ($H_{amide}\cdots O{=}C_{urea}$)
$N_{1/phen}\cdots H_{1/urea}$	1.950 ± 0.002	174.43 ± 1.58 ($N_{1/phen}\cdots H-N_{urea}$)
$N_{2/phen}\cdots H_{1/urea}$	2.462 ± 0.003	104.71 ± 0.46 ($N_{2/phen}\cdots H-N_{urea}$)
$N_{2/phen}\cdots H_{2/urea}$	2.555 ± 0.013	99.56 ± 0.60 ($N_{2/phen}\cdots H-N_{urea}$)

shifted upfield from 8.75 to 8.66 ppm ($\Delta\delta = 0.09$ ppm). This relatively small shift of the *peri* proton might result from conformational changes in the structure of host **7** upon complexation of urea, resulting in turning the 5-(2,5,8,11-tetraoxatridecan-13-yloxy)isophthalamidyl aromatic ring away from urea's oxygen (see computational results further on, in Figure 16). Additional support for this assumption could be found in the significant downfield shift of two other aromatic ring protons (H_2) from 7.27 to 7.50 ppm ($\Delta\delta = 0.23$ ppm), even though these protons are clearly not participating in the urea guest binding. We estimate that this shift resulted not only from changes in electron density on carbonyl oxygens of host **7** after complexation but also from changes in the distance and dihedral angle between the protons H_2 and these oxygens, as a consequence of the 5-(2,5,8,11-tetraoxatridecan-13-yloxy)-isophthalamidyl aromatic ring tilting. Measurable differences in chemical shifts, upon complex **10** formation, were found between the "upper" and "lower" parts of the 1,10-phenanthroline moieties. While the protons of the "lower" part, H_3 and H_5, were shifted downfield by just 0.08 and 0.03 ppm, respectively (H_5 protons did not show any response at all), protons H_8 and H_9 of the "upper" part displayed a significant downfield shift with $\Delta\delta$ of 0.16 and 0.13 ppm, respectively. These results strongly suggest that mostly the "upper" part of the phenanthroline moieties is engaged in urea binding.

An ^{1}H NMR selective-NOE experiment with complex **10** further supported the proposed mode of urea binding by chemosensor **7** by displaying a clear correlation between the amide protons at 10.80 ppm and the urea protons at 5.98 ppm; no direct interactions between urea protons and other protons was detected (Figure 13). An additional correlation was observed inside the host between the amide protons and the *peri* proton of the 5-(2,5,8,11-tetraoxatridecan-13-yloxy)isophthalamidyl aromatic ring.

An additional technique for studying the interaction between urea and its hosts is ^{13}C NMR, although until now ^{13}C NMR has never been considered as a useful tool for the evaluation of such complexes. For this purpose, preparation of complex **10** was repeated, using ^{13}C-labeled urea. The reference experiment included measurement of noncomplexed $(NH_2)_2{}^{13}CO$ in CD_2Cl_2, which exhibited a peak at 158.18 ppm (Figure 14). Due to the aforementioned low solubility of urea in this solvent, this signal could only be observed after overnight acquisition of the ^{13}C NMR spectrum. In contrast, complex **10** had good solubility in dichloromethane and quickly showed a peak at 161.88 ppm (downfield shift of $\Delta\delta = 3.70$ ppm) with an intensity that could be assigned only to the carbonyl group of bound $(NH_2)_2{}^{13}CO$, indicating the increased solubility of urea due to complexation and providing unambiguous evidence for the formation of urea complex with chemosensor **7**.

Mass Spectrometry Studies. MALDI-TOF MS analysis of sample of complex **10** afforded a high-resolution spectrum in which the peak corresponding to the correct molecular mass of this compound was present (M + 1, *m/z* 787.3261, Figure 15). To the best of our knowledge, prior to this work, the mass spectrum of a noncovalent complex of urea with any of its receptors has never been reported.

Molecular Modeling and Calculations. We studied the structures of the chemosensor **7** complexes with various ureas by DFT calculations using Gaussian 03.[18] After initial geometry optimization, energy minimization at the B3LYP 3-21g** level was performed for each calculated structure. All of the structures were optimized without symmetry constrains. Figure 16 presents the resultant structure of the complexe **10**, (structures of complexes of imidazolidin-2-one and tetrahydropyrimidin-2(1*H*)-one with chemosensor **7**, see in Supporting Information).

A comparison of calculated bond lengths in the bound urea (complex **10**) with the unbound urea (calculated and, for reference, measured by microwave spectroscopy in the gas phase[19]) is presented in Table 2. The subsequent trend could be observed: following complexation, the urea's carbonyl and $N-H_1$ bonds increased slightly in their lengths by 0.047 and 0.019 Å, respectively. In contrast, the urea's $N-H_2$ bond length remained unchanged, as these hydrogens are most probably not involved in binding with the host. The urea's C−N bond length decreased by 0.034 Å, probably due to a stronger interaction between these atoms (more double-bond character) caused by carbonyl bond weakening (Table 2). Similar results were also obtained for cyclic urea ligands and they are consistent with reports from other investigators.[1]

Examining distances and angles between the guest and host in complex **10** (Table 3), we found that in the "upper" part of the complex their values are very close to the optimal lengths and angles known for hydrogen bonds, whereas values for the "lower" part of the complex are substantially different (too distant and too bent), clearly indicating that no hydrogen bonds are present in this part of the complex.

These calculated data were consistent with our spectroscopic results, strongly supporting the proposed structure of **10** and related complexes with other ureas.

(18) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Montgomery, J. A., Jr.; Vreven, T.; Kudin, K. N.; Burant, J. C.; Millam, J. M.; Iyengar, S. S.; Tomasi, J.; Barone, V.; Mennucci, B.; Cossi, M.; Scalmani, G.; Rega, N.; Petersson, G. A.; Nakatsuji, H.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Klene, M.; Li, X.; Knox, J. E.; Hratchian, H. P.; Cross, J. B.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Ayala, P. Y.; Morokuma, K.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Zakrzewski, V. G.; Dapprich, S.; Daniels, A. D.; Strain, M. C.; Farkas, O.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Ortiz, J. V.; Cui, Q.; Baboul, A. G.; Clifford, S.; Cioslowski, J.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M. W.; Gonzalez, C.; Pople, J. A. *Gaussian 03*, Revision B.05; Gaussian, Inc.: Wallingford, CT, 2004.

(19) Godfrey, P. D.; Brown, R. D.; Hunter, A. N. *J. Mol. Struct.* **1997**, *413−414*, 405−414.

Conclusions

We prepared a multidentate receptor **7**, incorporating two 1,-10-phenanthroline fluorophores as chelating/reporting subunits attached to a central 5-(2,5,8,11-tetraoxatridecan-13-yloxy)-isophthalamidyl moiety. This compound was found to be an effective ratiometric and differential chemosensor for urea (and some of its derivatives) and uronium nitrate, capable of distinguishing between these analytes by producing a corresponding optical response for each of them. Three-dimensional excitation−emission spectroscopy was used for preliminary functional evaluation of chemosensor **7** and its truncated analog **9**, enabling us to conveniently pinpoint all the spectral changes taking place in the fluorescence of these compounds upon addition of each analyte. Stability and binding mode of chemosensor **7** complex with urea and several urea derivatives were evaluated by titrations and a series of spectroscopic measurements, as well as by comparison to **9**. In the course of these studies, we determined that the "upper" aromatic nitrogens of the 1,10-phenanthroline subunits are mainly responsible for the binding of urea ligands. In addition, the chemosensor **7** complex with urea (complex **10**) was found to be stable enough to sustain MALDI-TOF mass spectrometric analysis and showed NOE enhancement between the bound host and guest and changes in chemical shift in ^{13}C NMR of the bound ^{13}C-labeled urea. We believe that this new chemosensor may represent an important step toward the development of new chemistry for ureas and their salts analysis.

Experimental Section

All operations with air- and moisture-sensitive compounds were performed by Schlenk techniques under an argon atmosphere. All solvents were of analytical grade or better; dry solvents were purchased as anhydrous. ^1H and ^{13}C NMR signals are reported in ppm. ^{13}C NMR spectra interpretations were supported by DEPT experiments. Mass spectra were obtained on a spectrometer equipped with CI, EI, and FAB probes, on s spectrometer equipped with an ESI probe, or on a MALDI-TOF spectrometer. Progress of reactions was monitored by TLC (SiO_2) and visualized by UV light. Flash chromatography was carried out on SiO_2 (0.04−0.063 mm).

Dimethyl-5-(2,5,8,11-tetraoxatridecan-13-yloxy)isophthalate (4). To a solution of dimethyl-5-hydroxyisophthalate (2.44 g, 11.61 mmol) and 2,5,8,11-tetraoxatridecan-13-yl-4-methylbenzenesulfonate (4.42 g, 12.20 mmol) in an CH_3CN (85 mL) was added K_2CO_3 (1.77 g, 12.81 mmol) powder, and the mixture was refluxed for 16 h. After cooling to room temperature, solids were filtered, and the solvent was evaporated to afford pure **4** as an oil (4.54 g, 98% yield). ^1H NMR (500 MHz, CD_3CN): δ 8.17 (t, $J = 1.4$ Hz, 1H), 7.76 (d, $J = 1.4$ Hz, 2H), 4.24 (m, 2H), 3.93 (s, 6H), 3.84 (m, 2H), 3.66 (m, 2H), 3.61 (m, 2H), 3.57 (m, 6H), 3.48 (m, 2H), 3.31 (s, 3H). ^{13}C NMR (125 MHz, CD_3CN): δ 167.0, 160.5, 133.5, 123.5, 120.9, 72.9, 71.7, 71.5, 71.3, 70.4, 69.7, 59.1, 53.2. IR ($CHCl_3$): 3012, 2886, 2358, 1722, 1598, 1444, 1337, 1246, 1114 cm^{-1}. HRMS (CI+): calcd for $C_{19}H_{29}O_9$ 401.1812, found 401.1816. λ_{max} ($CHCl_3$): 247, 305 nm.

5-(2,5,8,11-Tetraoxatridecan-13-yloxy)isophthalic Acid (5). To a solution of **4** (4.54 gr, 11.34 mmol) in ethanol (115 mL) was added 1.0 M aqueous NaOH (46 mL), and the mixture was heated to 65 °C for 8 h. After cooling to room temperature, the ethanol was evaporated, and the resulting aqueous solution was cooled to 4 °C and acidified to pH 3.0 with 3.0 M HCl (about 15 mL), affording a white precipitate, which was separated from liquids, dried under vacuum, and redissolved in EtOAc (50 mL). The resulting solution was filtered again and evaporated to afford pure **5** as a white wax (3.26 g, 77%). ^1H NMR (400 MHz, $CDCl_3$): δ 9.93 (s, 2H), 8.04 (t, $J = 1.2$ Hz, 1H), 7.57 (d, $J = 1.1$ Hz, 2H), 4.15 (m, 2H), 3.90 (m, 2H), 3.80 (br s, 4H), 3.74 (m, 2H), 3.71 (m, 2H), 3.66 (m, 2H), 3.56 (m, 2H), 3.37 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.4, 158.5, 130.9, 123.8, 120.2, 71.8, 70.6, 70.5, 70.4, 69.6, 67.8, 58.9. IR (KBr): 3459, 2894, 2587, 2055, 1706, 1598, 1453, 1315, 1255, 1112, 942 cm^{-1}. HRMS (MALDI-TOF+): calcd for $C_{17}H_{25}O_9$ 373.1499, found 373.1512. λ_{max} ($CHCl_3$): 248, 309 nm.

5-(2,5,8,11-Tetraoxatridecan-13-yloxy)isophthaloyl Dichloride (6). To a solution of **5** (641 mg, 1.72 mmol) in THF (6 mL) was added thionyl chloride (1.35 mL, 18.59 mmol) dropwise, and the reaction mixture was heated to reflux for 3 h. Then, the liquids were evaporated under vacuum to afford **6** as yellow oil. ^1H NMR (500 MHz, $CDCl_3$): δ 8.33 (t, $J = 1.2$ Hz, 1H), 7.85 (d, $J = 1.5$ Hz, 2H), 4.20 (m, 2H), 3.85 (m, 2H), 3.58 (m, 12H), 3.29 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 166.7, 159.5, 135.1, 125.7, 122.8, 71.7, 70.7, 70.4, 69.7, 69.2, 68.6, 58.7. This compound was used without purification.

5-(2,5,8,11-Tetraoxatridecan-13-yloxy)-N^1,N^3-di(1,10-phenanthrolin-2-yl)isophthalamide (7). To a solution of **3** (706 mg, 3.62 mmol) in dry *N*,*N*-diisopropylethylamine (3.0 mL) and CH_2Cl_2 (15 mL) was added a solution of **6** (705 mg, 1.72 mmol) in dry CH_2Cl_2 (5 mL) dropwise at 0 °C, under inert atmosphere. After addition was completed the solution was allowed to warm up to room temperature and stirred for 10 h. Then, the liquids were evaporated, and the residue was redissolved in CH_2Cl_2 (20 mL) and washed with water (3 × 15 mL). Solvent was evaporated, and the solid residue was purified by flash chromatography (SiO_2; 6:94, CH_2Cl_2/MeOH; $R_f = 0.15$), which was followed by precipitation from CH_2Cl_2/hexane solution to afford **7** as a light yellow solid (600 mg, 48%). ^1H NMR (500 MHz, CD_2Cl_2): δ 10.57 (s, 1H), 8.75 (s, 1H), 8.69 (s, 2H), 8.31 (d, $J = 7.8$ Hz, 2H), 8.19 (d, $J = 7.7$ Hz, 2H), 8.05 (d, $J = 8.6$ Hz, 2H), 7.68 (d, $J = 8.7$ Hz, 2H), 7.65 (d, $J = 8.7$ Hz, 2H), 7.48 (dd, $J = 4.2$ Hz, $J = 7.7$ Hz, 2H), 7.27 (s, 2H), 4.14 (m, 2H), 3.91 (m, 2H), 3.79 (m, 2H), 3.74 (m, 2H), 3.67 (m, 2H), 3.63 (m, 2H), 3.58 (m, 2H), 3.49 (m, 2H), 3.32 (s, 3H). ^{13}C NMR (100 MHz, CD_2Cl_2): δ 164.2, 164.1, 164.0, 158.6, 151.3, 149.1, 144.5, 143.7, 138.0, 135.7, 135.1, 128.6, 125.8, 125.4, 124.3, 122.8, 117.7, 116.7, 114.5, 71.8, 70.8, 70.5, 70.4, 70.3, 69.4, 67.8, 58.5. IR (KBr): 3376, 2876, 1685, 1577, 1536, 1506, 1484, 1387, 1317, 1257, 1099 cm^{-1}. HRMS (MALDI-TOF+): calcd for $C_{41}H_{39}N_6O_7$ 727.2880, found 727.2883. λ_{max} (CH_3CN): 227 (ϵ = 69,150), 286 (ϵ = 49,800), 336 (ϵ = 13,350), 353 (ϵ = 7,350), 376, 396 nm.

3-(2,5,8,11-Tetraoxatridecan-13-yloxy)-5-(methoxycarbonyl)-benzoic Acid (8). To a solution of **5** (1.30 g, 3.50 mmol) in MeOH (4.0 mL) and THF (13.0 mL) was added concentrated H_2SO_4 (150 μL), and the reaction mixture was heated to 65 °C for 4.5 h. After cooling to room temperature, the liquids were evaporated, and the crude residue was purified by flash chromatography (SiO_2; 3:97, MeOH/CH_2Cl_2; $R_f = 0.38$) to afford **8** as a yellow oil (471 mg, 35%). ^1H NMR (400 MHz, CD_3CN): δ 8.16 (t, 1H, $J = 1.4$ Hz), 7.75 (d, 2H, $J = 1.4$ Hz), 4.23 (m, 2H), 3.91 (s, 3H), 3.82 (m, 2H), 3.65 (m, 2H), 3.59 (m, 2H), 3.56 (m, 6H), 3.47 (m, 2H), 3.30 (m, 3H). ^{13}C NMR (100 MHz, CD_3CN): δ 167.0, 166.5, 159.9, 132.8, 123.5, 120.5, 120.4, 72.5, 71.3, 71.1, 70.9, 70.0, 69.1, 58.8, 53.0. IR (CH_2Cl_2): 3009, 2884, 1721, 1698, 1597, 1440, 1226 cm^{-1}. HRMS (MALDI-TOF+): calcd for $C_{18}H_{26}O_9$ 387.1577, found 387.1607. λ_{max} (CH_3CN): 245, 313 nm.

Methyl-3-(1,10-phenanthrolin-2-ylcarbamoyl)-5-(2,5,8,11-tetraoxatridecan-13-yloxy)benzoate (9). To a solution of **3** (240 mg, 1.23 mmol), **8** (450 mg, 1.16 mmol), and HOBt (188 mg, 1.23 mmol) in dry DMF (11.0 mL) was added a solution of DCC (266 mg, 1.29 mmol) in dry DMF (8.0 mL) dropwise at 0 °C under inert atmosphere. The reaction mixture was stirred for 1.5 h at 0 °C and then was allowed to warm up to room temperature and stirred for an additional 20 h. Formed precipitate was filtered out, and the solution was evaporated under vacuum. The crude residue was purified by flash chromatography (SiO_2; 1:5:94, NH_4OH (25%)/MeOH/EtOAc; $R_f = 0.35$) to afford **9** as a yellow wax (460 mg,

70%). ^{1}H NMR (500 MHz, $CDCl_3$): 9.47 (br s, 1H), 9.12 (d, J = 4.3, 1.5 Hz, 1H), 8.75 (d, J = 8.7 Hz, 1H), 8.30 (d, J = 8.8 Hz, 1H), 8.23 (d, J = 7.9 Hz, 2H), 7.78 (s, 1H), 7.76 (d, J = 9.1 Hz, 2H), 7.69 (d, J = 8.7 Hz, 1H), 7.60 (dd, J = 4.3, 8.0 Hz, 1H), 4.23 (m, 2H), 3.93 (br s, 3H), 3.87 (m, 2H), 3.73 (m, 2H), 3.64 (m, 9H), 3.50 (m, 2H), 3.32 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 167.8, 165.2, 159.2, 151.1, 149.9, 144.8, 144.1, 138.8, 136.3, 135.6, 131.9, 129.1, 126.3, 126.2, 125.0, 123.0, 120.3, 119.6, 118.3, 115.7, 71.8, 70.8, 70.5, 70.4, 69.4, 68.1, 58.9, 52.3. IR (CH_2Cl_2): 2886, 1724, 1682, 1597, 1483, 1318, 1265, 1107 cm^{-1}. HRMS (MALDI-TOF+): calcd for $C_{30}H_{34}N_3O_8$ 564.2346, found 564.2300. λ_{max} (CH_3CN): 228, 286, 313, 336, 352, 375, 395 nm.

5-(2,5,8,11-Tetraoxatridecan-13-yloxy)-*N*^{1},*N*^{3}-di(1,10-phenanthrolin-2-yl)isophthalamid Complex with Urea (10). To a solution of **7** (50 mg, 6.88 $\times$ 10^{-5} mole) in CD_2Cl_2 (2.0 mL) was added solid $(NH_2)_2CO$ (42 mg, 0.70 mmole) was added, and the mixture was sonicated (with ^{1}H NMR monitoring) for about 20 min at room temperature. Then, the excess of urea was filtered out, and the solvent was evaporated to afford complex **10** as a yellow solid. ^{1}H NMR (500 MHz, CD_2Cl_2): δ 10.80 (s, 2H), 8.77 (s, 2H), 8.66 (s, 1H), 8.45 (d, J = 8.4 Hz, 2H), 8.21 (d, J = 7.9 Hz, 2H), 8.18 (d, J = 8.7 Hz, 2H), 7.75 (d, J = 8.7 Hz, 2H), 7.70 (d, J = 8.7 Hz, 2H), 7.50 (m, 4H), 5.97 (br s, 4H), 4.21 (m, 2H), 3.90 (m, 2H), 3.77 (m, 2H), 3.71 (m, 2H), 3.65 (m, 2H), 3.62 (m, 2H), 3.59 (m, 2H), 3.50 (m, 2H), 3.32 (s, 3H). ^{13}C NMR (100 MHz, CD_2Cl_2): δ 165.0, 162.5, 159.0, 151.7, 148.9, 145.0, 144.2, 138.0, 135.8, 135.3, 128.8, 126.0, 125.9, 124.5, 122.7, 118.3, 117.5, 115.8, 71.8, 70.8, 70.5, 70.4, 70.3, 69.4, 67.9, 58.5. IR (KBr): 3392, 2962, 1686, 1592, 1534, 1507, 1485, 1386, 1317, 1261, 1097 cm^{-1}. HRMS (MALDI-TOF+): calcd for $C_{42}H_{43}N_8O_8$ 787.3204, found 787.3261. λ_{max} ($CHCl_3$): 227, 286, 337, 353, 376, 396 nm.

Acknowledgment. The authors thank Dr. Shlomit Gali, Dr. Dan Grinstein, and Mrs. Sharon Gil-Chaimov for their contribution and the Israeli Science Foundation and Tel Aviv University for their generous financial support.

Supporting Information Available: Experimental details for synthesis of compounds **1**, **2**, and **3** and 2,5,8,11-tetraoxatridecan-13-yl-4-methylbenzenesulfonate, characterization data, ^{1}H NMR, ^{13}C NMR, MS, FT-IR spectra, and computational data. This material is available free of charge via the Internet at http://pubs.acs.org.

JO062130H