JOC The Journal of Organic Chemistry

Article

pubs.acs.org/joc

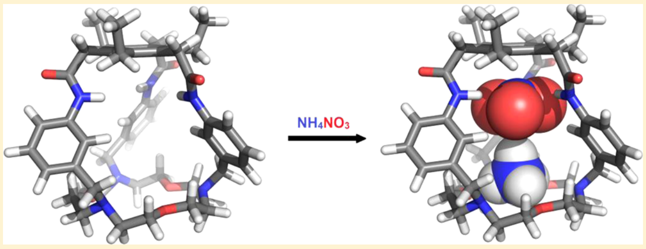
Selective Ammonium Nitrate Recognition by a Heteroditopic Macrotricyclic Ion-Pair Receptor

Jan Romański and Piotr Piątek*

Department of Chemistry, University of Warsaw, Pasteura 1, 02-093 Warsaw, Poland

Supporting Information

ABSTRACT: The heteroditopic macrotricyclic molecular receptor **1**, which bears a tripodal anion binding domain and 4,10,16-triaza-18-crown-6 cation recognition domain, proves to be an effective ion-pair receptor. In the absence of the cobound cation (TBA$^+$ salts) receptor **1** preferably binds nitrate and nitrite over other anions, including basic anions such as acetate or dihydrogenphosphate. Ammonium cation binding by the 4,10,16-triaza-18-crown-6 subunit significantly enhances the strength of the nitrate and nitrite complexation at the triamide recognition site of the receptor. In the presence of ammonium cations, the association constants of nitrate binding reach an impressive value of 1050 M^{-1} in highly polar DMSO-d_6. Interestingly, the binding of other anions such as chloride and bromide is not enhanced in the presence of a cobound NH_4^+ cation. The increased affinity of [**1**·NH_4^+]PF_6^- for anionic species is attributed to a strong cooperative effect that arises from the properly positioned binding sites in the receptor **1** cavity, thus allowing for the formation of the ion pair. Under liquid/liquid conditions, receptor **1** is able to extract NH_4NO_3 from an aqueous to an organic phase, as inferred from ^{1}H NMR spectroscopic and nitrite/nitrate colorimetric analyses.



INTRODUCTION

Nitrate contamination of atmospheric liquids and surface water is a global problem. The main source of nitrate contamination appears to be from agricultural operations, farm runoff, and fertilizer usage.[1] There is also some nitrate formed in the atmosphere through the oxidation of nitrogen oxides that are emitted from power plants and internal combustion engines.[2] An excess of nutrients, particularly nitrates and phosphates, in aquatic systems results in its eutrophication, i.e. excessive plant growth.[3] This phenomenon is one of the most visible examples of human changes to the biosphere, affecting aquatic ecosystems from the Arctic to the Antarctic.

The primary health risks associated with elevated nitrate levels are methemoglobinemia, which causes the "blue baby" syndrome in infants, and the potential formation of carcinogenic nitrosamines.[4]

The trigonal-planar geometry of the nitrate anion, its high hydration energy (ΔG_{hyd} = −300 kJ/mol), large ionic radii (1.79 Å), and low basicity (pK_a = −1.44) result in the low affinity for hydrogen-bonding interactions.[5] Therefore it is particularly challenging to design molecular receptors with appropriately matched complementary recognition motifs for selective and effective nitrate anion recognition, particularly with neutral receptors.

In recent years, some elegant, neutral receptors for nitrate ions have been synthesized. For example, Ansyln and coworkers reported an amide-linked C_3-symmetric macrobicyclic receptor where six converging amide hydrogens were used for selective binding of nitrate anions.[6] In 2001, Hamilton et al. synthesized a rigid macrocyclic triamide of 3′-amino-3-biphenylcarboxylic acid.[7] This molecular receptor strongly binds iodide, *p*-toluenosulfonate, and nitrate anions in weakly polar solvent systems. However, in polar solvents such as DMSO-d_6, the association constants of halide and nitrate anions decreased dramatically. Guided by DFT calculations, Herges, König et al. prepared a macrocyclic tris(thiourea) receptor that binds bromide and nitrate in DMSO-d_6.[8] Very recently Singh and Sun reported a charged, zwitterionic fluorescent chemosensor that displays one of the highest binding affinities for nitrate anions so far reported in polar media (550 M^{-1}, DMSO).[9,10]

The molecular receptors mentioned above bind the nitrate anion in the presence of the soft, noncompeting tetra-*n*-butylammonium countercation (TBA). However, in real-life applications, the anion is accompanied by hard cations such as Na^+, K^+, or NH_4^+, and ion pairing of the target anion with its countercation can lead to diminished receptor-anion affinity.[11] Therefore, to overcome this problem, receptors capable of binding both the anion and cation of an ion pair are needed.[12] Properly designed salt receptors have the potential, due to positive cooperativity, to bind anions more strongly than the monotopic receptors. Moreover, complexation of both the cation and the anion enhances salt lipophilicity, thus facilitating its solubilization, extraction, and membrane transport.[13]

Among the heteroditopic ion-pair receptors the most effective, but unfortunately the most difficult to design, are salt receptors that recognize contact ion pairs. An elegant

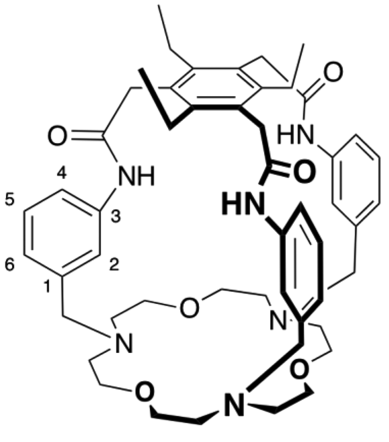
Received: February 15, 2013
Published: April 5, 2013

dx.doi.org/10.1021/jo4003322 | *J. Org. Chem.* 2013, 78, 4341−4347

example of such a heteroditopic receptor consists of 1,10-diaza-18-crown-6 and 1,3-phenylenedicarboxamide subunits, as reported by Smith's group.[14] A single crystal X-ray structural analysis revealed that this receptor is able to bind, as contact ion pairs, Na^+ and K^+ salts of halides, as well as trigonal oxyanions, such as NO_3^- and AcO^-. In solution, in the presence of either Na^+ or K^+ and Cl^-, the association constants for complexation of the corresponding counterion increased significantly. The solution studies of nitrate salts have been limited, althought it has been confirmed that this receptor is able to slowly extract solid $LiNO_3$, $NaNO_3$, and KNO_3 into chloroform.[15,16]

■ RESULTS AND DISCUSSION

Design and Synthesis. Inspired by Smith's receptor, we designed a macrotricyclic heteroditopic receptor that displays a three-dimensional molecular architecture (Figure 1). The 4,10,16-triaza-18-crown-6 is the key structural element that allows the construction of receptor **1**.[17] It is well recognized that *N*-alkyl derivatives of this macrocycle are able to selectively bind primary ammonium cations.[18] The structural unit that closes the macrotricyclic architecture of receptor **1** and creates a convergent, trigonal anion binding site is 2,4,6-triethylbenzene-1,3,5-tris(acetic acid).



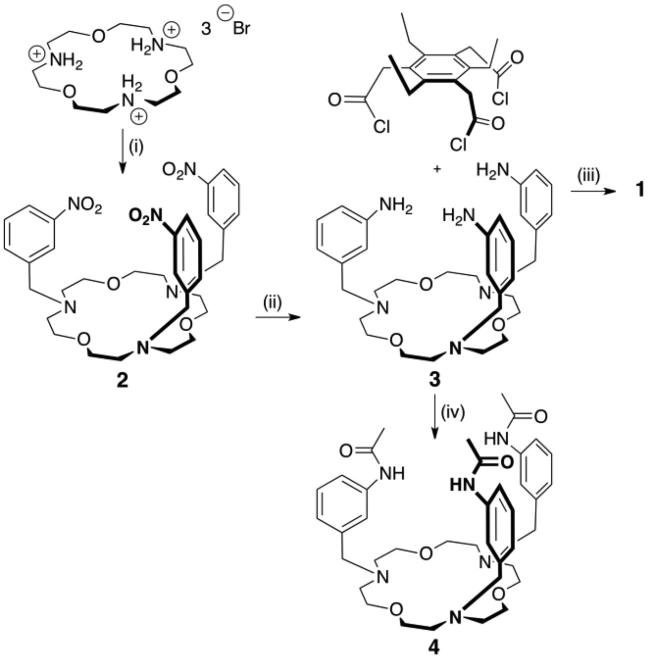
1

Figure 1. Structure of receptor **1**.

The synthesis of the target receptor was accomplished by a three-step synthetic procedure. As shown in Scheme 1, the reaction of the tri-HBr salt of 4,10,16-triaza-18-crown-6 with 3-nitrobenzyl chloride in the presence of excess K_2CO_3 in THF under reflux gave the tris-nitrobenzyl derivative **2** in 72% yield. Subsequent reduction of nitro groups with sodium borohydride in the presence of palladium on carbon then gave the tris-amine **3** in 91% yield. Finally, this compound was condensed with 2,4,6-triethylbenzene-1,3,5-tris(acetyl chloride) under high dilution conditions in CH_2Cl_2, to give macrotricyclic receptor **1** in 42% yield. The relatively high yield of the macrocyclization step could be rationalized in terms of the preorganization of 2,4,6-triethylbenzene-1,3,5-tris(acetyl chloride). The alternating steric interactions in 2,4,6-triethylbenzene-1,3,5-tris(acetic acid) ensure that the carboxylic groups are oriented toward the same face of the aromatic ring.[19] Compound **4**, the open counterpart of **1**, was prepared as a reference compound by acylation of **3** with acetyl anhydride.

Solid/Liquid Extraction. Receptor **1** was initially examined by ^1H NMR for its ability to extract solid Na^+, K^+, and NH_4^+ salts of AcO^-, Cl^-, Br^-, and NO_3^- into $CDCl_3$ solution. However, after 1 h of stirring a solution of **1** in the presence of sodium and potassium salts, the ^1H NMR spectra of the receptor had changed only slightly, which suggests that these salts are extracted rather moderately into the organic phase (see Supporting Information). In contrast, in the presence of ammonium salts, the amide-NH signal shifted downfield considerably by ~1.2 ppm, which indicates hydrogen-bonding interactions of **1** with anions. Additionally, the complex-induced changes in the chemical shifts of the *N*-benzylic and crown ether-CH_2 were consistent with the ammonium cation being encapsulated by the triaza-18-crown-6 moiety.

Scheme 1. Synthesis of 1[a]



[a] Reagents and conditions: (i) 3-nitrobenzyl chloride, K_2CO_3, KI, THF, reflux, overnight, 72%; (ii) $NaBH_4$, Pd/C, THF/MeOH, 1h, 91%; (iii) Et_3N, CH_2Cl_2, slow addition over 6 h, 42%; (iv) Ac_2O, CH_2Cl_2, 25%.

Solution Binding Studies. The association constant between **1** and the nitrate anion accompanied by the bulky, noncoordinating TBA cation was determined, by ^1H NMR titration experiments in $CDCl_3$, to be 115 M^{-1}. The limited solubility of NH_4^+ salts of PF_6^- or ClO_4^- precluded the preparation of a solution of this cation in sufficient concentration. Therefore, an unspecified amount of NH_4PF_6 was introduced into the solution of receptor **1** via solid−liquid extractions. In the presence of the ammonium cation the association constant for nitrate was higher than 5×10^4 and therefore could not be accurately determined by the ^1H NMR titration method. This remarkable positive cooperativity effect enhances the efficacy of nitrate recognition by nearly 3 orders of magnitude in this low polar solvent.

Quantitative information about the binding ability of receptor **1** toward anions, cations, and ion pairs was obtained by ^1H NMR titration experiments in highly polar DMSO-d_6. First, the affinity of the receptor for anions in the presence of the noncoordinating TBA cation was established. The addition of anions to a 2.6 mM solution of **1** caused nonlinear downfield shifts of the amide NHs and aromatic protons (H2) signals directed into the center of the cavity (Figure 1). The former signal was used to determine the association constant for complexes of **1** with a variety of anionic guests. The association constants calculated by the nonlinear regression analysis of the binding isotherms are presented in Table 1.

Table 1. Association Constants (K_a) Values for Interactions of 1 with Various Anions (TBA Salts)[a]

	AcO^-	$H_2PO_4^-$	Cl^-	Br^-	NO_2^-	NO_3^-
K_a (M^{-1})	120	110	30	155	290	280

[a] ^{1}H NMR, solvent: DMSO-d_6, temperature 293 K, (**1**) = 2.6 mM, anions added as TBA salts (TBAX) ~20 mM, Errors < 10%. No appreciable change in the ^{1}H NMR spectrum of **1** was seen upon treatment with excess $TBAPF_6$.

As can be clearly seen in Table 1, receptor **1** selectively associates with nitrate and nitrite anions with fairly high binding constants in polar solvents such as DMSO-d_6. Because the ionic volumes of bromide and nitrate anions are comparable,[20] bromide could interact strongly with receptors that bind nitrate.[8] However, this is not the case with receptor **1**, which binds bromide more weakly than nitrate. The chloride ion is apparently too small to be effectively recognized by **1**. Interestingly, a moderate affinity to basic anions, such as dihydrogenphosphate and even acetate, which has the same trigonal geometry as nitrate, is observed. Such a high selectivity toward nitrate and nitrite ions is, to our knowledge, unprecedented in abiotic chemical systems.

The cation binding properties of receptor **1** were probed by monitoring the aromatic protons H2 using ^{1}H NMR spectroscopy in DMSO-d_6. These studies revealed that receptor **1** binds Na^+, K^+, and NH_4^+ cations (as hexafluorophosphate salts) with similar strengths (K_a ~35 M^{-1}). Analogous titrations conducted in the presence of 1 equiv of NO_3^- anions showed significant enhancement of association constants for K^+ (K_a = 80 M^{-1}) and NH_4^+ (K_a = 50 M^{-1}) cations.

To gain more insight into the cooperative enhancement of anion recognition by the presence of the cation, ^{1}H NMR titrations of receptor **1** were conducted with anions in the presence of 1 equiv of hard cations such as Na^+, K^+, and NH_4^+. The calculated association constants are listed in Table 2.

Table 2. Association Constant (K_a) Values for Interactions of 1 with Various Anions in the Presence of 1 equiv of Cation[a]

	TBA^+	Na^+	K^+	NH_4^+
Cl^-	30	35	50	30
Br^-	155	280	290	180
NO_2^-	290	220	250	980
NO_3^-	275	280	765	1050

[a] ^{1}H NMR, solvent DMSO-d_6, temperature 293 K, (**1**) = 2.6 mM, cations added as PF_6^- salts (MPF_6) = 2.6 mM, anions added as TBA salts (TBAX) ~20 mM; M^{-1}, Errors < 10%.

Examination of these data reveals a number of trends. First, from the perspective of halogen anions binding, only the presence of K^+ cations results in a notable increase of anion association constants. Other cations generally have little influence on the halogen anion binding abilities of **1**, with the exception of NaBr. In contrast, nitrite anion recognition is actually slightly reduced in the presence of both K^+ and Na^+ cations. However, in the presence of NH_4^+ cations the value of the association constants increases more than 3-fold. The recognition of nitrate is significantly enhanced by the presence of K^+, yet the largest positive cooperativity factor (K_{NH_4}/K_{TBA} = 3.8) is observed for simultaneous binding of nitrate and NH_4^+ cations. In this case the association constants of NO_3^- binding reach an impressive value of 1050 M^{-1} in DMSO-d_6. Interestingly, the presence of NH_4^+ cations not only enhances the affinity for nitrate but also increases the selectivity of receptor **1** toward this anion. This is due to the fact that the binding of halogen anions is not enhanced by ammonium cations and the association of AcO^- and $H_2PO_4^-$ with **1** is actually suppressed in the presence of NH_4^+ (see Supporting Information). The ability of **1** to strongly associate with NH_4NO_2 is also worthy of note because it is a highly toxic anion that is an intermediate product of both the nitrification and denitrification processes, and therefore it is present in biological systems along with the NO_3^- anion.[21]

Interestingly, when the reference compound **4** was titrated with the nitrate anion in the presence and absence of the ammonium cation, no binding was detected. This supports the notation that the observed binding affinity for receptor **1** and nitrates mainly originates from its tricyclic structure and the consequent proper orientation of the binding domains.

Taking advantage of the sufficient solubility of ammonium nitrate in DMSO-d_6, ion-pair NMR titrations were conducted. Specifically, a 2.5 mM solution of receptor **1** was titrated with a 50 mM solution of NH_4NO_3 in DMSO-d_6. The association constant value was determined to be 75 M^{-1}. That surprisingly low value, in comparison to titration with $TBANO_3$, can be explained in terms of the ion pairing of NH_4NO_3 in organic solvent, which must be much greater than that for $TBANO_3$.[22] In the case of anion titration, in the presence of 1 equiv of cation, the $TBANO_3$ salt was added to the solution of **1** pretreated with NH_4PF_6 (Figure 2). The ion-pair titration was therefore conducted in an analogous manner, and the binding constant was calculated to be 340 M^{-1}. That binding constant enhancement can be rationalized in terms of the formation of the $[\mathbf{1}\cdot NH_4^+]PF_6^-$ complex. The binding of the ammonium cation by receptor **1** creates a positively charged complex, which holds an unoccupied anion binding domain (no binding of PF_6^- was evidenced by ^{1}H NMR analysis). Moreover this "positively charged anion receptor" possesses an additional hydrogen bonding donor which can interact with anions (see Figure 3). Therefore the ability of the $[\mathbf{1}\cdot NH_4^+]PF_6^-$ complex for anion binding is stronger than that of free receptor **1**. These data suggest that receptor **1** binds salts in a sequential manner.[23]

Molecular Modeling. The remarkable affinity of receptor **1** toward nitrate in the presence of ammonium was also investigated by means of Density Functional Theory (DFT) calculations. The structures of the **1** + NH_4NO_3 complex were optimized by DFT using the accurate M06-2X functional with the 6-31+G* basis set in DMSO solution described by a polarizable continuum solvent model (see Experimental Section for full details). These calculations lead to two low energy structures that have almost the same energy (ΔE = 0.6 kcal/mol, Figure 3). Both structures have C_3 symmetry, with the principal axis passing through the nitrogen atom of the nitrate anion, the nitrogen of the ammonium cation, and the center of the hexasubstituted aromatic ring of the receptor. The nitrate anion is bound inside the cavity by H-bonds between O-atoms of the nitrate anion and three amide N–H protons

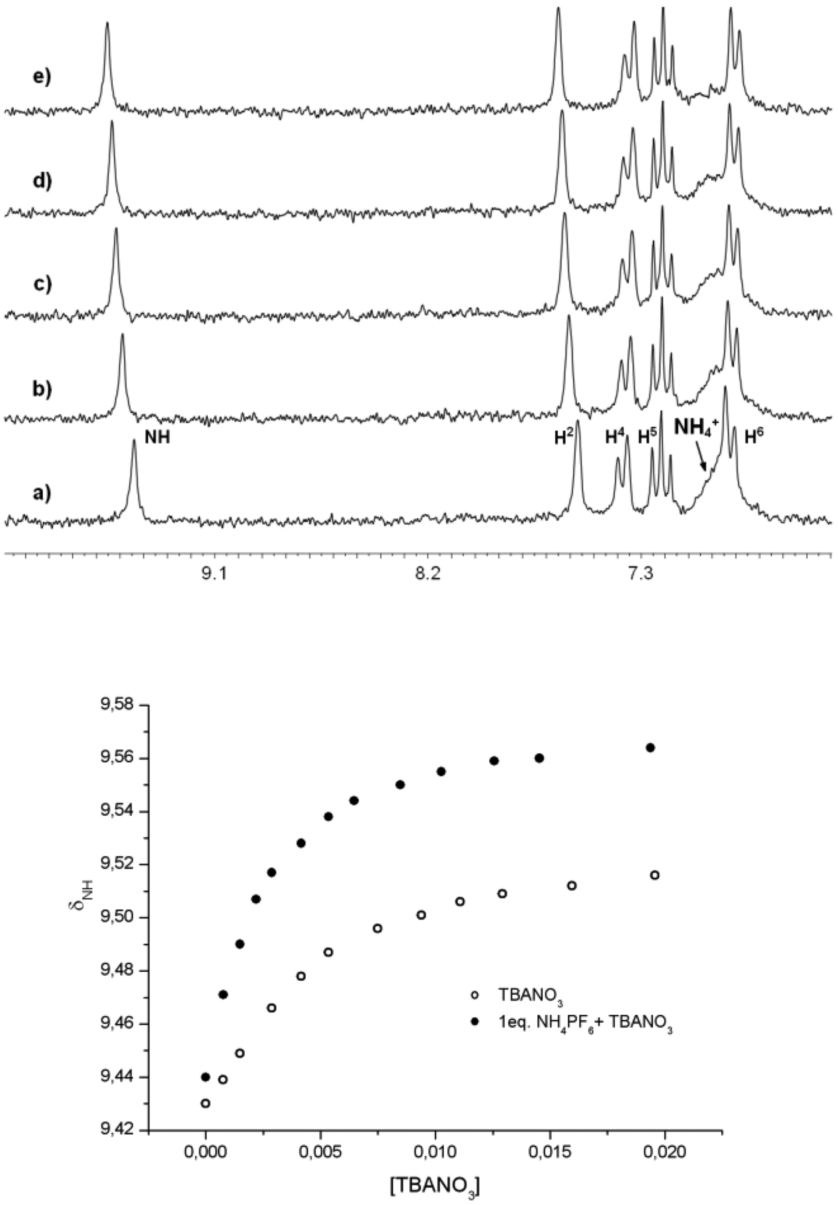


Figure 2. Top: ^{1}H NMR (200 MHz) partial spectra of receptor **1** in the presence of NH_4PF_6 upon progressive addition of $TBANO_3$ (0, 0.6, 1.1, 2.2, and 7.5 equiv from bottom to top). Bottom: Chemical shift of NH signals of **1** as a function of increasing amounts of $TBANO_3$ in the absence and presence of NH_4PF_6.

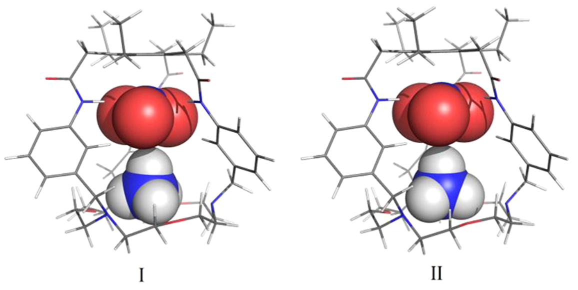


Figure 3. DFT optimized structures of **1**·NH_4NO_3.

(dN−H···O from 3.01 to 3.07 Å, θN−H···O from 158.1° to 159.2°). The N−H···O interactions observed here are directed primarily toward the lone pairs on the NO_3^- oxygens. The distances and angles between amide N−H and nitrate anion O-atoms are almost identical in the second structure. Moreover, in the calculated structures, the nitrate anion is located parallel to the plane defined by three amide N-atoms. This observation is in agreement with NMR titration experiments. According to the calculations of the nitrate shielding surface, a deshielding effect is observed in the range of 0° to 60° above/below the plane of the NO_3^- anion, whereas a shielding effect is observed directly above/below the N-atom.[13] Therefore the downfield shift of the NH and H2 protons of receptor **1** upon addition of the nitrate anion indicates that the NO_3^- anion is located in the deshielding range. This observation rules out a perpendicular location of the NO_3^- anion inside the receptor cavity.

The ammonium cation resides in proximity to the triazacrown ether binding domain. However, in one structure (Figure 3, structure I) the ammonium NHs are directed toward the central N-atom of the nitrate anion (dN−H···N 3.97 Å, θN−H···N 177.1°) and the oxygen atoms of crown ether (dN−H···O from 2.871 Å to 2.90 Å, θN−H···O from 176.6° to

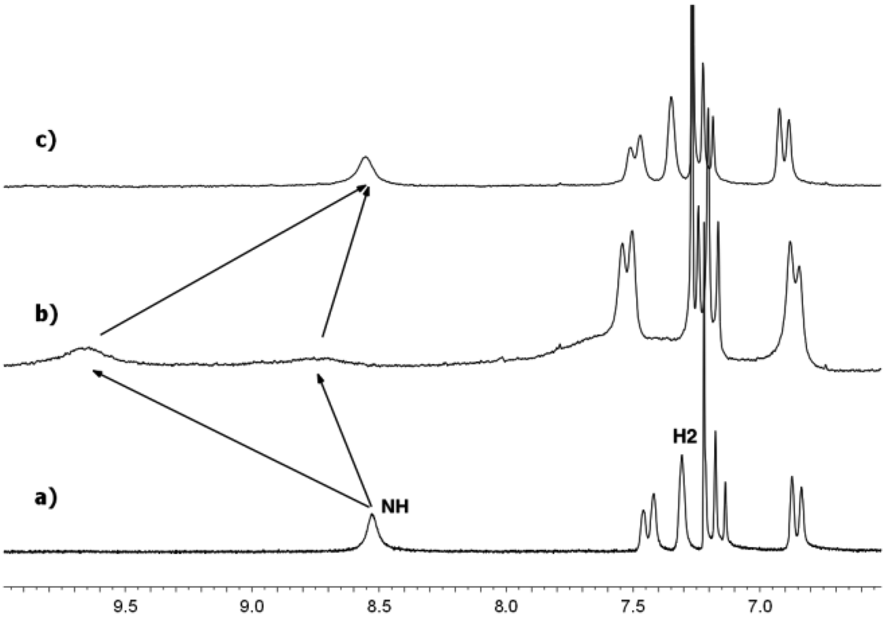


Figure 4. Partial ^1H NMR (200 MHz) spectra of receptor **1**: (a) 11.8 mM solution in wet $CDCl_3$; (b) after NH_4NO_3 extraction from water phase; (c) after back-extraction to distilled water.

179.0°). In contrast, in the second structure (Figure 3, structure II) ammonium NHs form three H-bonds to nitrogen rather than the oxygen atoms of the triazacrown moiety (dN−H···N from 3.23 Å to 3.26 Å, θN−H···O from 170.4° to 172.0°). These modeling results indicate that NH_4^+ flips between two equally occupied positions.

Liquid/Liquid Extractions. As receptor **1** has a high affinity and selectivity for NH_4NO_3 salt in polar solvents, the liquid/liquid extraction behavior of this receptor was examined by means of ^1H NMR spectroscopy and nitrate anion colorimetric analysis. A 1.7 M solution of NH_4NO_3 in distilled water was layered onto a 11.8 mM solution of **1** in $CDCl_3$. The two layers were thoroughly mixed and then separated, and the ^1H NMR spectrum was recorded (Figure 4b). Inspection of this spectrum revealed that signals corresponding to the amide NHs and aromatic protons (H2) are broadened. Furthermore, a new broad signal at 9.60 ppm appeared. These results indicate that in wet $CDCl_3$ the complexation/decomplexation process is slow on the NMR time scale, and the new signal can be attributed to the NH_4NO_3 complex of **1**. The extraction efficiency, i.e. the fraction of receptor molecules occupied by the complex in the organic phase, as determined by NMR integration, is ~65%. The organic phase was then back-extracted into H_2O. The ^1H NNR spectrum of the $CDCl_3$ phase is essentially identical to the spectrum of the free receptor (Figure 4a and 4c). The nitrate content in the aqueous layer was determined by means of a nitrite/nitrate colorimetric method to be 20.4 mg/L, which corresponds to a 71% extraction efficiency. The high preference of **1** toward both ammonium cations and nitrate anions was confirmed in extraction experiments, since no extraction of $NaNO_3$ or NH_4Cl was observed.

■ CONCLUSIONS

In summary, a heteroditopic macrotricyclic receptor for the NH_4NO_3 ion pair has been developed. The receptor is capable of effectively and selectively associating with nitrate anions in the presence of a noncoordinating TBA cation in highly polar DMSO-d_6. However, remarkable enhancement of both nitrate association strength and selectivity in the presence of a cobound ammonium cation has been observed. The increased affinity of $[\mathbf{1}\cdot NH_4^+]PF_6^-$ for anionic species is attributed to a strong cooperative effect that arises from the properly positioned binding sites in the receptor **1** cavity, thus allowing the formation of the ion pair. With receptor **1** as a liquid−liquid extractant, extraction of the NH_4NO_3 ion pair from an aqueous to an organic phase in a recyclable manner has been achieved. As the most effective strategy for reducing the content of "nitrogen" in aquatic systems is removal of both ammonium cations and nitrate anions, the receptor ability to extract NH_4NO_3 is of great value. Ongoing efforts are focused on the incorporation of receptor **1** derivatives into a polymeric matrix in order to create materials that could effectively separate nitrate salts from an aqueous solution.[24]

■ EXPERIMENTAL SECTION

The tri-HBr salt of 4,10,16-triaza-18-crown-6 and 2,4,6-triethylbenzene-1,3,5-tris(acetyl chloride) were synthesized according to the literature procedures.[25,26] Other reagents and chemicals were of reagent grade quality and purchased commercially. The anion TBA and cation PF_6 salts were dried under high vacuum at 30−45 °C prior to use. ^1H and ^{13}C NMR spectra as well as titrations experiments were recorded on a 200 MHz spectrometer. ^1H NMR chemical shifts δ are reported in ppm referenced to the tetramethylsilane ($CDCl_3$) or protonated residual solvent signal (DMSO-d_6).

***N,N′,N″*-Tris(3-nitrobenzyl)-4,10,16-triaza-18-crown-6 (2).** To a stirred solution of the tri-HBr salt of 4,10,16-triaza-18-crown-6 (1.5 g, 3 mmol), potassium carbonate (2.48 g, 18 mmol, 6 equiv) and a catalytic amount of potassium iodide (70 mg) in 150 mL of dry THF 3-nitrobenzyl chloride (0.268 g, 1.56 mmol, 3 equiv) were added. The solution was refluxed overnight under argon. THF was removed under vacuum, and the solid residue taken up in CH_2Cl_2 and washed with distilled water. The organic phase was dried over Na_2SO_4, and the organic solvent was removed under reduced pressure. The residue was dissolved in a minimal amount of CH_2Cl_2 and loaded on silica gel. The silica gel was eluted first with 80/20 AcOEt/hexanes, and then AcOEt afforded **2** in the form of a thick light yellow oil (1.5 g, 72%). R_f (5% $MeOH/CH_2Cl_2$) = 0.47; ^1H NMR (200 MHz, $CDCl_3$) δ = 8.26 (3H, s), 8.07 (3H, d, J = 8.2 Hz), 7.68 (3H, d, J = 7.8 Hz), 7.44 (3H, t, J = 7.8 Hz), 3.78 (6H, s), 3.59 (12H, t, J = 5.6 Hz), 2.82 (12H, t, J = 5.6 Hz); ^{13}C NMR (50 MHz, $CDCl_3$) δ = 148.5, 142.0, 134.8, 129.2, 123.5, 122.1, 69.9, 59.1, 54.3; IR (thin-film) 3078, 2956, 1620, 1582 cm^{-1}; ESI HR calcd for $C_{33}H_{42}N_6O_9Na$ 689.2911, found 689.2894.

***N,N′,N″*-Tris(3-aminobenzyl)-4,10,16-triaza-18-crown-6 (3).** Sodium borohydride (210 mg, 5.1 mmol, 4.5 equiv) was added to a vigorously stirred suspension of **2** (755 mg, 1.13 mmol) and palladium on carbon (150 mg) in 50 mL of a 4:1 mixture of THF/methanol. After 1 h, the mixture was filtered through a pad of Celite and solvents were evaporated. The residual solid was taken up in 50 mL of $CHCl_3$ and washed twice with distilled water. The organic layer was dried over Na_2SO_4. Evaporation of the solvent gave the triamine **3**, as a colorless thick oil (594 mg, 98%). R_f (10% MeOH/CH_2Cl_2) = 0; ^{1}H NMR (200 MHz, $CDCl_3$) δ = 7.02 (3H, t, J = 7.8 Hz), 6.71 (3H, bs), 6.64 (3H, d, J = 7.4 Hz), 6.51 (3H, dd, J = 7.4 Hz, J = 2.4 Hz), 3.56−3.51 (18H, m), 2,74 (12H, t, J = 5.6 Hz); ^{13}C NMR (50 MHz, $CDCl_3$) δ = 146.6, 141.0, 129.0, 119.1, 115.5, 113.7, 69.9, 60.0, 54.1; IR (thin-film) 3321, 3041, 2982, 1659, 1552 cm^{-1}; ESI HR calcd for $C_{33}H_{48}N_6O_3Na$ 599.3686, found 599.3676.

Receptor 1. The solutions of 2,4,6-triethylbenzene-1,3,5-tris(acetyl chloride) (400 mg, 1.03 mmol) and the triamine **3** (590 mg, 1.02 mmol) in CH_2Cl_2 (20 mL each) were simultaneously added via a syringe pump to a vigorously stirred solution of triethylamine (0.6 mL, 4.4 mmol, 4.4 equiv) in CH_2Cl_2 (150 mL) over 6 h at room temperature. After 12 h of additional stirring, solvent was removed under vacuum and the solid residue was redissolved in dichloromethane (60 mL) and washed with distilled water (40 mL). The organic layer was dried over 4A molecular sieves, and solvent was removed under reduced pressure. The residue was dissolved in a minimal amount of CH_2Cl_2 and loaded on silica gel. The silica gel was eluted first with 50% acetone/CH_2Cl_2 and then 70% acetone/CH_2Cl_2, affording **1** in the form of a white powder (419 mg, 48%). R_f (10% MeOH, acetone) = 0.12; ^{1}H NMR (200 MHz, $CDCl_3$) δ = 8.35 (3H, bs), 7.46 (3H, d, J = 8.2 Hz), 7.36 (3H, s), 7.23 (3H, t, J = 8.2 Hz), 7.92 (3H, d, J = 7.4 Hz), 3.91 (6H, s), 3.53−3.45 (18H, m), 2.74−2.68 (18H, m), 1,22 (H9, t, J = 7.2 Hz); ^{13}C NMR (50 MHz, $CDCl_3$) δ = 170.6, 143.6, 140.9, 138.0, 129.6, 128.6, 125.7, 122.1, 121.0, 70.3, 60.3, 38.2, 24.2, 14.6; IR (thin-film) 3479, 3271, 2965, 2873, 1656, 1598, 1543 cm^{-1}; ESI HR calcd for $C_{51}H_{66}N_6O_6Na$ 881.4936, found 881.4941.

***N,N′,N″*-Tris(3-acetamidobenzyl)-4,10,16-triaza-18-crown-6 (Reference Compound 4).** To a stirred solution of the triamine **3** (300 mg, 0.52 mmol) and triethylamine (1.4 mL, 10 mmol) in 20 mL of dry dichloromethane acetic anhydride (0.8 mL, 7.8 mmol) was added. The solution was stirred overnight under argon. The organic phase was then extracted with sat. $NaHCO_3$ and dried over Na_2SO_4, and the organic solvent was removed under reduced pressure. The residue was dissolved in a minimal amount of acetone and loaded on silica gel. The silica gel was eluted with acetone, affording **4** in the form of colorless oil (91 mg, 25%). R_f (40% MeOH, chloroform) = 0.15; ^{1}H NMR (200 MHz, $CDCl_3$) δ = 8.97 (3H, s), 7.72 (3H, d, J = 8.2 Hz), 7.46 (3H, bs), 7.20 (3H, t, J = 9.6 Hz), 6.95 (3H, d, J = 7.6 Hz), 3.65−3.46 (18H, m), 2.75 (12H, t, J = 4.6 Hz), 2.05 (9H, s). ^{13}C NMR (50 MHz, $CDCl_3$) δ = 169.6, 138.9, 138.4, 128.9, 125.2, 121.1, 119.6, 68.7, 59.5, 54.5, 24.4. IR (thin-film) 3253, 3055, 2955, 1673, 1553 cm^{-1}; ESI HR calcd. for $C_{39}H_{54}N_6O_6Na$ 725.4003, found 725.0412.

Solid/Liquid Extraction. Inorganic salts were used as received; therefore, water content may vary. The solid Na^+, K^+, and NH_4^+ salts of AcO^-, Cl^-, and NO_3^- were added to 0.6 mL of the 2.5 mM solution of **1** in $CDCl_3$. After 1 h of stirring all solids were filtered off, and ^{1}H NMR spectra of the clear solution were recorded.

^{1}H NMR Titration Experiments. The ^{1}H NMR titrations were performed on a 200 MHz spectrometer, at 298 K in DMSO-d_6. In each case, 500 μL of a freshly prepared 2.6 mM solution of receptor **1** was added to a 5 mm NMR tube. Where applicable the solution also contained 1 mol equiv of hexafluorophosphate cation salt (or tetrabutylammonium anion salt). Small aliquots of an ~20 mM solution of tetrabutylammonium anion salts (or hexafluorophosphate cation salts), containing **1** at a 2.6 mM concentration, were added, and a spectrum was acquired after each addition. Titration isotherms for NH protons were fitted to a 1:1 binding model using the HypNMR 2000 program. All measurements were carried out in at least duplicate using independent samples. The 1:1 binding stoichiometries were verified by a Job plot analysis.

Liquid/Liquid Extractions. A commercially available NO_2^-/NO_3^- colorimetric test was used for quantitative determination of the nitrate content in the water phase, after the back-extraction of the organic phase containing a complex of NH_4NO_3 and receptor **1**. First, nitrate standard solutions were prepared by diluting the 500 mg/L stock solution of KNO_3 with distilled water in the range from 25 to 0.05 mg/L of nitrate. To these solutions, according to the user manual, appropriate reagents were added. For each solution UV−vis spectra were acquired, and a calibration curve was generated by plotting an absorbance at 540 nm as a function of nitrate concentration. As described in the manuscript a 1.7 M solution of NH_4NO_3 in distilled water was layered onto an 11.8 mM solution of **1** in $CDCl_3$. The two layers were thoroughly mixed and then separated. The organic phase was then back-extracted into H_2O. A 1 mL aliquot of the aqueous phase was diluted in a volumetric flask to 25 mL, and then, after treatment with appropriate reagents, UV−vis spectra of that solution were acquired. Using the calibration curve the nitrate content in the aqueous layer was determined to be 20.4 mg/L, which corresponds to a 71% extraction efficiency.

Molecular Modeling. The model of the $\mathbf{1}\cdot NH_4NO_3$ complex was built using the Maestro suite (Schrodinger LLC, 2012) maintaining the C_3 symmetry and minimized initially using Macromodel. NH_4^+ and NO_3^- ions were place inside the structure in positions according to similar structures reported earlier.[27,28] The whole structure was optimized without any constraints using the hybrid M06-2X density functional method in DMSO (simulated by means of polarizable continuum model PCM), in a standard 6-31+G* basis set suitable for treating ionic species, as implemented in the Gaussian 09 software suite.[29,30]

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

^{1}H and ^{13}C NMR spectra, details of solid/liquid experiments, selected titration isotherms, molecular modeling, and Cartesian coordinates for the optimized structures. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: ppiatek@chem.uw.edu.pl.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was supported by the Faculty of Chemistry University of Warsaw (501-64-BST-163220). We thank Dr. Bartosz Trzaskowski for his invaluable assistance with the DFT calculations.

■ REFERENCES

(1) Keeneya, D. R.; Hatfieldb, J. L. In *Nitrogen in the Environment: Sources, Problems, and Management*; Hatfield, J. L., Follett, R .F., Eds.; Elsevier: Oxford, U.K., 2008; pp 7−12.

(2) Keeneya, D. R.; Hatfieldb, J. L. In *Nitrogen in the Environment: Sources, Problems, and Management*; Hatfield, J. L., Follett, R. F., Eds.; Elsevier: Oxford, U.K., 2008; pp 453−458.

(3) Smith, V. H.; Schindler, D. W. *Trends Ecol. Evol.* **2009**, *24*, 201−207.

(4) Benjamin, N. In *Nitrate, Agriculture and the Environment*; Addiscott, T. M., Ed.; CABI Publishing: Wallingford, U.K., 2005; pp 145−152.

(5) Steed, J. W.; Atwood, J. L. *Supramolecular Chemistry*; Wiley: Chichester, U.K., 2009; p 226.

(6) Bisson, A. P.; Lynch, V. M.; Monahan, M. K. C.; Anslyn, E. V. *Angew. Chem., Int. Ed.* **1997**, *36*, 2340−2342.

(7) Choi, K.; Hamilton, A. D. *J. Am. Chem. Soc.* **2001**, *123*, 2456−2457.

(8) Herges, R.; Dikmans, A.; Jana, U.; Köhler, F.; Jones, P. G.; Dix, I.; Fricke, T.; König, B. *Eur. J. Org. Chem.* **2002**, *2002*, 3004−3014.

(9) Singh, A. S.; Sun, S. S. *J. Org. Chem.* **2012**, *77*, 1880−1890.

(10) For examples of other charged receptors that bind NO_3^-, see: (a) Mason, S.; Clifford, T.; Seib, L.; Kuczera, K.; Bowman-James, K. *J. Am. Chem. Soc.* **1998**, *120*, 8899−8900. (b) Cronin, L.; McGregor, P. A.; Parsons, S.; Teat, S.; Gould, R. O.; White, V. A.; Long, N. J.; Robertson, N. *Inorg. Chem.* **2004**, *43*, 8023−8029. (c) Isiklan, M.; Saeed, M. A.; Pramanik, A.; Wong, B. M.; Fronczek, F. R.; Hossain, M. A. *Cryst. Growth Des.* **2011**, *11*, 959−963.

(11) Shukla, R.; Kida, T.; Smith, B. D. *Org. Lett.* **2000**, *2*, 3099−3102.

(12) (a) McConnell, A. J.; Beer, P. D. *Angew. Chem., Int. Ed.* **2012**, *51*, 5052−5061. (b) Kim, S. K.; Sessler, J. L. *Chem. Soc. Rev.* **2010**, *39*, 3784−3809.

(13) Mahoney, J. M.; Nawaratna, G. U.; Beatty, A. M.; Duggan, P. J.; Smith, B. D. *Inorg. Chem.* **2004**, *43*, 5902−5907.

(14) Mahoney, J. M.; Beatty, A. M.; Smith, B. D. *J. Am. Chem. Soc.* **2001**, *123*, 5847−5848.

(15) Mahoney, J. M.; Stucker, K. A.; Jiang, H.; Carmichael, I.; Brinkmann, N. R.; Beatty, A. M.; Noll, B. C.; Smith, B. D. *J. Am. Chem. Soc.* **2005**, *127*, 2922−2928.

(16) For the solid state study of Cs^+ salts of F^-, Cl^-, and NO_3^- complexes with a heteroditopic receptor, see: Kim, S. K.; Sessler, J. L.; Gross, D. E.; Lee, C. H.; Kim, J. S.; Lynch, V. M.; Delmau, L. H.; Hay, B. P. *J. Am. Chem. Soc.* **2010**, *132*, 5827−5836.

(17) Griffin, J. L. W.; Coveney, P. V.; Whiting, A.; Davey, R. *J. Chem. Soc., Perkin Trans. 2* **1999**, 1973−1981.

(18) Lehn, J. M.; Vierling, P. *Tetrahedron Lett.* **1980**, *21*, 1323−1326.

(19) (a) Hennrich, G.; Anslyn, E. V. *Chem.—Eur. J.* **2002**, *8*, 2218−2224. (b) Steed, J. W. *Chem. Commun.* **2006**, 2637−2649.

(20) Marcus, Y.; Jenkins, B. H. D.; Glasser, L. *J. Chem. Soc., Dalton Trans.* **2002**, 3795−3798.

(21) Keeneya, D. R.; Hatfieldb, J. L. In *Nitrogen in the Environment: Sources, Problems, and Management*; Hatfield, J. L., Follett, R .F., Eds.; Elsevier: Oxford, U.K., 2008; p 178.

(22) Unfortunately there is no data in the literature concerning ion-pairing of NH_4NO_3 in polar aprotic solvent, although the ion-pairing constant of even $TBAClO_4$ salt in acetonitrile is 53: Anslyn, E. V.; Dougherty, D. A. In *Modern Physical Organic Chemistry*; University Science Books: Sausalito, CA, 2006; pp 228−232. Limited data are available on ion pairing in DMSO. For example, data for sodium benzoate (K_{IP} = 200 M^{-1}) and potassium benzoate (K_{IP} = 48 M^{-1}) in DMSO have been reported: Olmstead, W.; Bordwell, F. G. *J. Am. Chem. Soc.* **1980**, *45*, 3299−3305. Also data cocerning NaSCO (K_{IP} = 46 M^{-1}) and KSCO (K_{IP} = 16 M^{-1}) ion pairing: Chingakule, D. D. K.; Gans, P.; Gill, J. B.; Longdon, P. J. *Monatsh. Chem.* **1992**, *123*, 521.

(23) Kim, S. K.; Vargas-Zuniga, G. I.; Hay, B. P.; Young, N. J.; Delmau, L. H.; Masselin, C.; Lee, C. H.; Kim, J. S.; Lynch, V. M.; Moyer, B. A.; Sessler, J. L. *J. Am. Chem. Soc.* **2012**, *134*, 1782−1792.

(24) (a) Aydogan, A.; Coady, D. J.; Kim, S. K.; Akar, A.; Bielawski, C. W.; Marquez, M.; Sessler, J. L. *Angew. Chem., Int. Ed.* **2008**, *47*, 9648−9652. (b) Romanski, J.; Piatek, P. *Chem. Commun.* **2012**, *48*, 11346−11348. (c) Rambo, B. M.; Kim, S. K.; Kim, J. S.; Bielawski, C. W.; Sessler, J. L. *Chem. Sci.* **2010**, *1*, 716−722.

(25) Griffin, J. L. W.; Coveney, P. V.; Whiting, A.; Davey, R. *J. Chem. Soc., Perkin Trans. 2* **1999**, 1973−1981.

(26) (a) Sather, A. C.; Berryman, O. B.; Rebek, J., Jr. *J. Am. Chem. Soc.* **2010**, *132*, 13572−13574. (b) Kim, J.; Raman, B.; Ahn, K. H. *J. Org. Chem.* **2006**, *71*, 38−45.

(27) Mahoney, J. M.; Stucker, K. A.; Jiang, H.; Carmichael, I.; Brinkmann, N. R.; Beatty, A. M.; Noll, B. C.; Smith, B. D. *J. Am. Chem. Soc.* **2005**, *127*, 2922−2928.

(28) Işıklan, M.; Saeed, M. A.; Pramanik, A.; Wong, B. M.; Fronczek, F. R.; Hossain, Md. A. *Cryst. Growth Des.* **2011**, *11*, 959−963.

(29) Zhao, Y.; Truhlar, D. G. *Theor. Chem. Acc.* **2008**, *120*, 215−241.

(30) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Scalmani, G.; Barone, V.; Mennucci, B.; Petersson, G. A.; Nakatsuji, H.; Caricato, M.; Li, X.; Hratchian, H. P.; Izmaylov, A. F.; Bloino, J.; Zheng, G.; Sonnenberg, J. L.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Vreven, T.; Montgomery, Jr., J. A.; Peralta, J. E.; Ogliaro, F.; Bearpark, M.; Heyd, J. J.; Brothers, E.; Kudin, K. N.; Staroverov, V. N.; Kobayashi, R.; Normand, J.; Raghavachari, K.; Rendell, A.; Burant, J. C.; Iyengar, S. S.; Tomasi, J.; Cossi, M.; Rega, N.; Millam, J. M.; Klene, M.; Knox, J. E.; 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.; Martin, R. L.; Morokuma, K.; Zakrzewski, V. G.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Dapprich, S.; Daniels, A. D.; Farkas, Ö.; Foresman, J. B.; Ortiz, J. V.; Cioslowski, J.; Fox, D. J. *Gaussian 09*, revision A.1; Gaussian, Inc.: Wallingford, CT, 2009.