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Fluorescent Chemosensing of Catechol and Catecholamines in Water

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Abstract—It has been known for almost 35 years that catechol complexes reversibly to boronic acids. We observe that 2-anthrylboronic acid complexes catechol in water with K_d 330 μM and concomitant 20-fold reduction in fluorescence intensity. L-DOPA and dopamine behave similarly, suggesting a mechanism for the development of real-time sensing schemes.

Over the past several decades, chemists have devoted their collective attention to the design of molecules that bind other molecules reversibly. A primary goal of such work is the desire to engineer synthetic catalysts from first principles. However, an equally appealing goal, and one with significant practical potential, is the design of chemosensors with selectivity for analytes such as metal ions (e.g. Pb^{2+}), anions (e.g. phosphate), zwitterions (e.g. amino acids), and neutral species (e.g. O_2). Insufficient attention has been paid to this application of ion and molecule recognition, given the potential utility of chemosensors as essential components in the construction of real-time monitoring devices. Because many sensing applications will take place in an aqueous environment, aqueous recognition is a prerequisite. Considerable recent effort has focused on fluorescence as a signal transduction mechanism because of its sensitivity, its potential application to fiber optic-based remote sensing schemes, and because the multiplicity of mechanisms for modulating fluorescence provides for the incorporation of many known binding mechanisms.

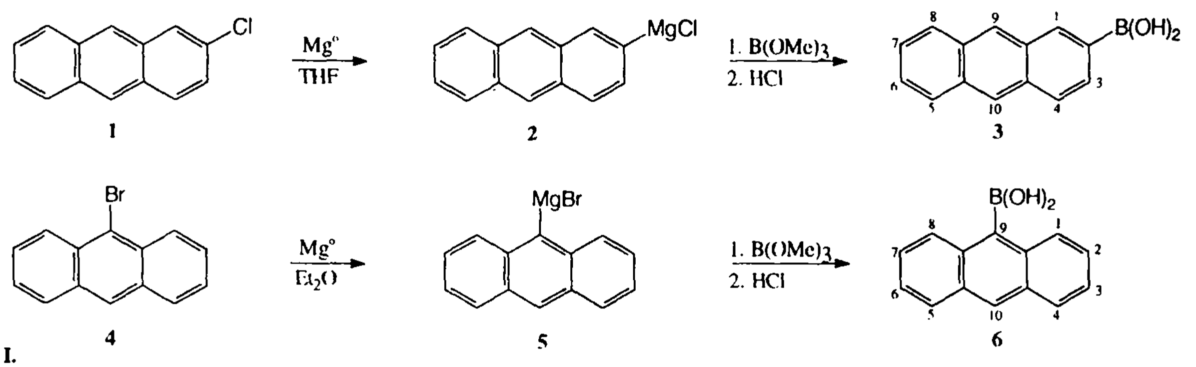
There is a substantial amount of classical literature describing the fluorimetric indication of ions in water,[1] which has been augmented by more recent studies.[2] We have been studying additionally the synthesis of fluorescent chemosensors for *uncharged* organic analytes in water. The design of any fluorescent chemosensor requires knowledge of three topics: (1) how can one bind a species with selectivity from water, (2) how can one generate signals from such binding events that are easy to measure, and (3) what mechanisms for binding and signal transduction intersect. Mechanisms have been elucidated by which ion binding events can be transduced to fluorescent events, but there is little literature available on how to accomplish this with neutral ligands.[3]

The complexation of carbohydrates with phenylboronic acid has been known for at least 40 years,[4] and the reversibility of that interaction serves as a basis for the chromatographic separation of sugars.[5] In 1959, Lorand and Edwards[6] reported association constants (listed here as K_ds) for aqueous associations of phenylboronic acid with many saturated polyols; binding interactions range from very weak (e.g. ethylene glycol, K_d = 360 mM) to moderately strong (e.g. glucose, K_d = 9.1 mM). We have utilized these results to evaluate a mechanism for fluorescent chemosensing based on polyol–boronic acid association.[7] This abiotic sensing scheme yields fluorescence quenching of 40% upon polyol chelation, owing to a modulation of the boronic acid pK_a.

Lorand and Edwards also described the binding of phenylboronic acid to catechol, with significantly stronger association (K_d = 57 μM) than that exhibited by carbohydrates.[6] As efficient intramolecular quenching has been observed previously by other electron-rich organic compounds,[8] it seemed reasonable that the catechol complexes of anthrylboronic acids might similarly demonstrate chelation-enhanced quenching (CHEQ). Accordingly, we now report that anthrylboronic acids bind catechol and catecholamines from water, K_d = 330 μM (for the 2-isomer), with concomitant 20-fold changes in fluorescence intensity.[9]

Chemosensors **3** and **6** were synthesized as shown in Scheme I. In each case, the anthryl halide was converted to the corresponding Grignard reagent, followed by quenching with trimethylborate and acid hydrolysis. Both **3**[10] and **6**[11] have appeared in the literature previously, but without experimental (or characterization) detail. The fluorimetrically-determined pK_a of **3** has been reported to be 8.8,[7] which compares favorably to the known phenylboronic acid pK_a of 8.83. In contrast, we determine the pK_a of **6** to be 11.0 (fluorimetry), making **6** a weaker acid than **3** by a factor of 160-fold. The basic ionization of boronic acids entails an sp^2 to sp^3 rehybridization at boron, requiring a trigonal to tetrahedral geometry change. A tetrahedral boronate would appear to be accomodated readily at the anthracene 2-position (i.e. **3**) as in phenylboronic acid; however, that at the 9-position (i.e. **6**) predictably experiences significant steric interaction with H-1 and H-8. Such sterically repulsive *peri*-interactions at the anthracene 9-position are well precedented.[12]

Fluorescence titrations of **3** and **6** with catechol (1,2-dihydroxybenzene; **7**) were carried out using an 0.75 μM solution of chemosensor at room temperature and p*H* 7.4 (20 mM phosphate buffer containing 1% v/v DMSO), and the results are shown in Figure 1. Excitation was at 348 nm, and emission was observed at 416 nm; because catechol itself displays weak fluorescence (centered at 396 nm), the data shown include subtraction of anthracene-free reference solutions. Several observations are notable. First, the reversible complexation of catechol to each anthrylboronic acid results in an approximately 20-fold CHEQ on saturation. This is as predicted by the rationale outlined in Schemes II and III. Association of **3** or **6** with catechol (**7**) yields boronic esters **8** and **9**, respectively (Scheme II). The 57-fold weaker interaction with **6** may be attributable to *peri*-interactions, although this is only speculation. As a working hypothesis, we speculate that boronic esters **8** and **9** are of lower fluorescence due to photoinduced electron transfer (**PET**) from catechol into the excited S_1 state of anthracene (Scheme III). This pathway for fluorescence quenching via electron transfer is well precedented,[13] although the details of radiationless (thermal) energy relaxation are not well established.



Scheme I.

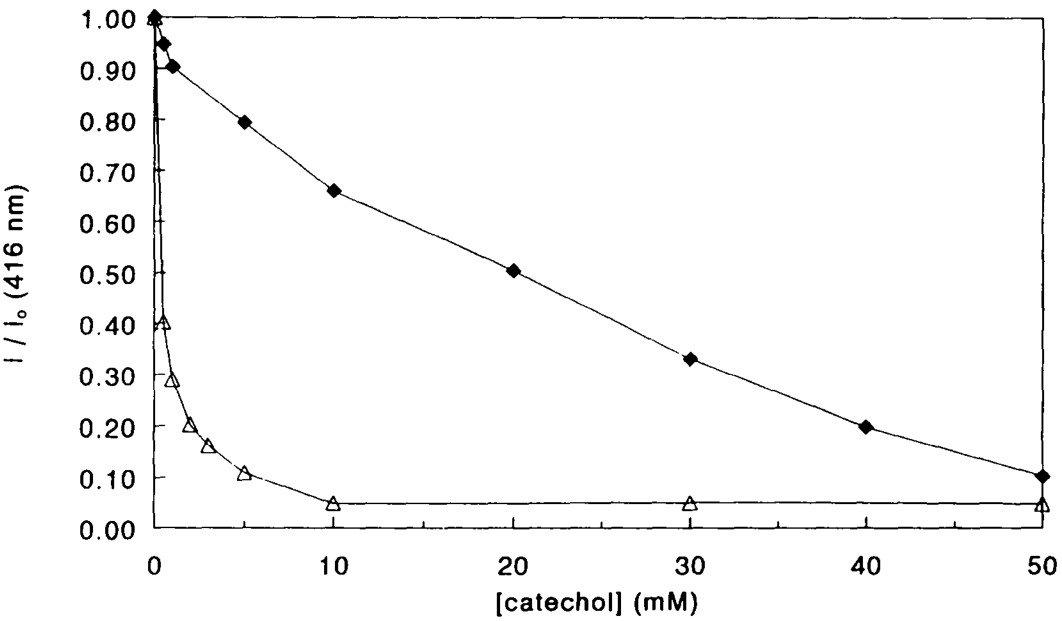
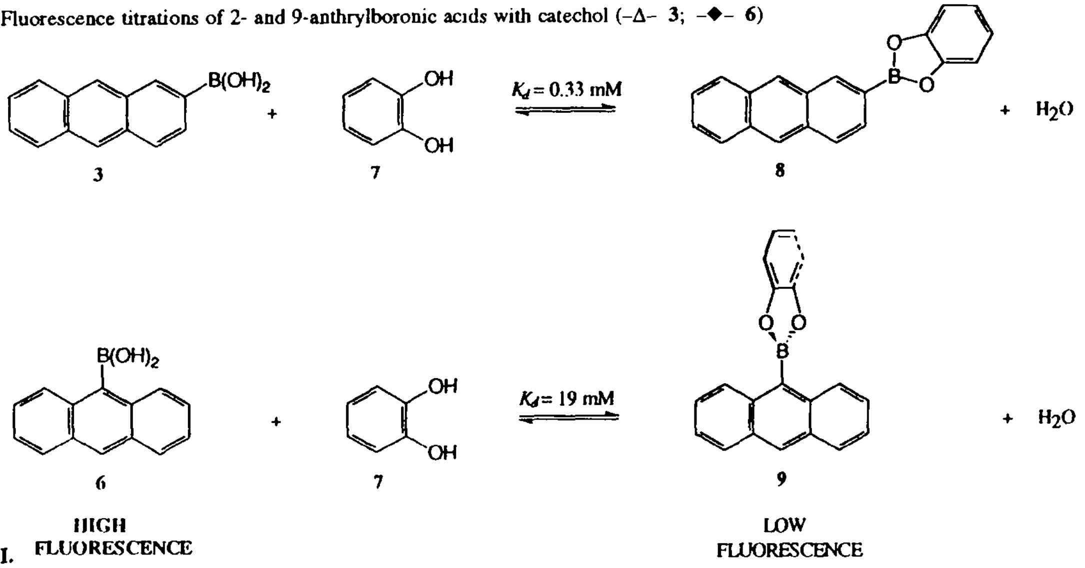
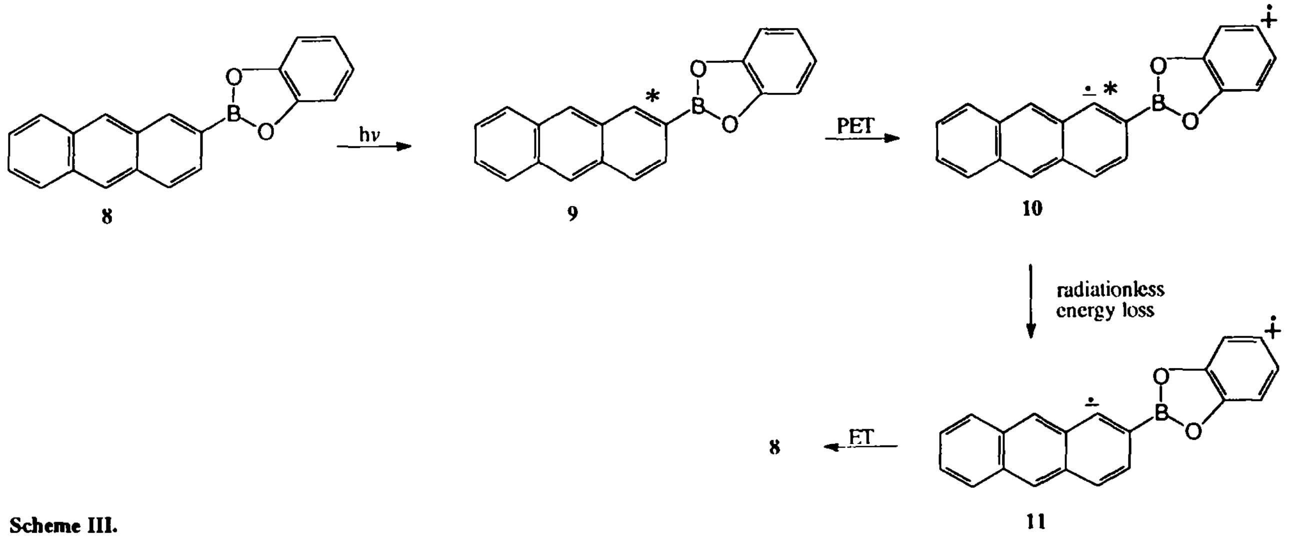


Figure 1. Fluorescence titrations of 2- and 9-anthrylboronic acids with catechol (-Δ- 3; -◆- 6)



Scheme II.



Scheme III.

Deboronation is a possible side reaction, and would be predicted to occur more rapidly under strongly acidic or basic conditions.[14] However, we do not observe deboronation of **3** or **6** under our sensing conditions. Deboronation of either anthrylboronic acid would yield anthracene itself, which is soluble at these very low concentrations and would have led to fluorescence enhancement rather than diminution. We further evaluated the **3**+**7**$\rightleftharpoons$**8** reaction using HPLC,[15] which demonstrated no loss of **3** and no observable formation of anthracene under the time frame of our experiments. Finally, addition of $B(OH)_3$ to a solution of **3** (0.75 μM) fully quenched by catechol (10 mM) leads to a titrated increase of fluorescence as the $B(OH)_3$ concentration is raised from 0 to 0.5 M (in the absence of catechol, $B(OH)_3$ has no influence on the fluorescence of **3**). $B(OH)_3$ competes for catechol binding, as reported previously.[6]

To evaluate further both the selectivity and intensity range for this mechanism of signal transduction, fluorescence titrations with a variety of potential ligands were compiled (Figure 2). Guaicol (**12**) and phenol (**13**) (Scheme IV), each structurally related to catechol, also complex with CHEQ, although the association is predictably weaker. Glucose (**14**), whose association with **3** has been described by us previously, also yields CHEQ; however, because the mechanism for fluorescence quenching is different, the magnitude is smaller. Additionally, the binding of catechol is considerably stronger than that of glucose.

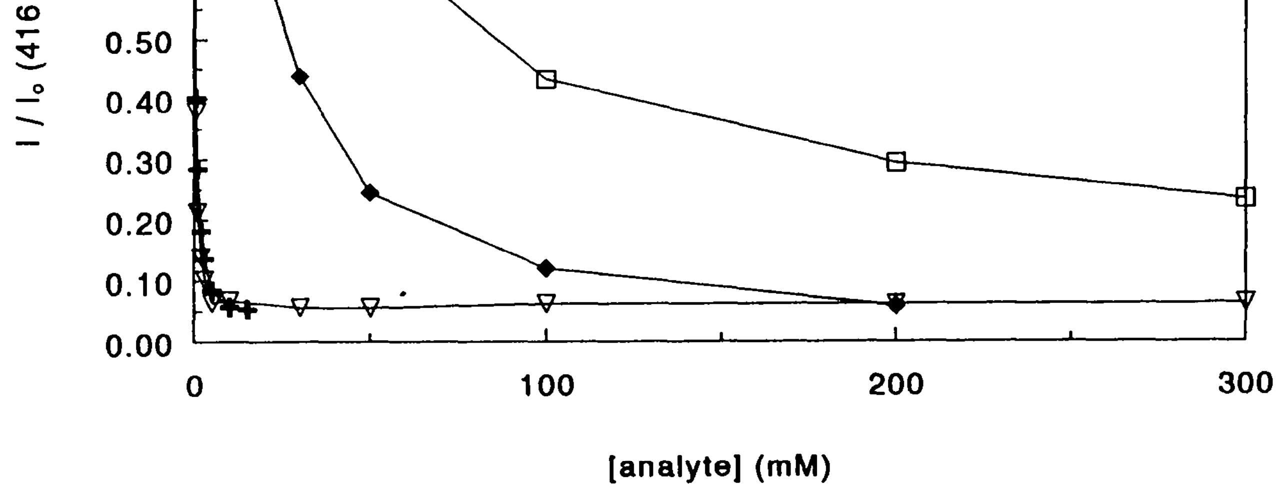
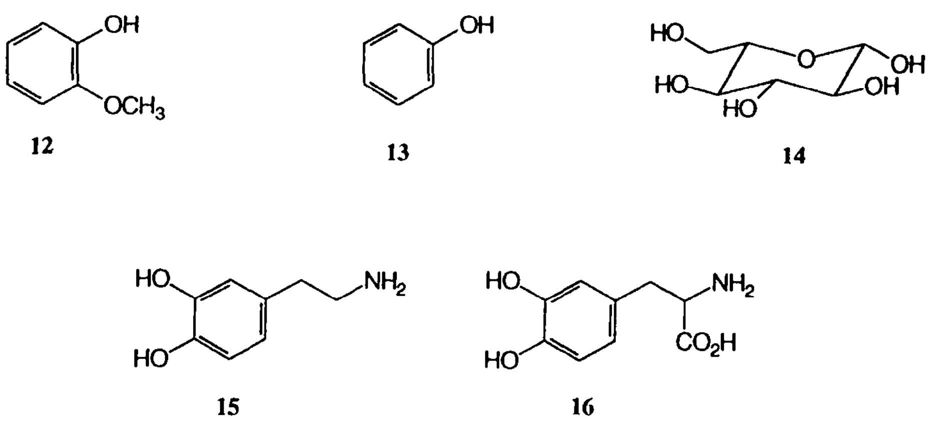


Figure 2. Fluorescence titrations of 2-anthrylboronic acid with several analytes (-◆- **12**; -□- **13**; -▲- **14**; -▽- **15**; -✚- **16**)



Scheme IV.

Figure 2 also documents the chemosensing of the catecholamine dopamine (**15**) and its metabolic pregenitor, L-Dopa (**16**). We observe that each compound complexes to anthrylboronic acid **3** with roughly equal binding affinities and fluorescence quenching as does catechol (K_d 330 µM); this is again predictable, as both compounds possess the catechol group in their structures. Because dopamine is an important neurotransmitter that regulates functions in the brain, heart, kidney, vasculature, and gut, advances in the ability to visualize it in real-time may prove of interest.

In summary, we have demonstrated for the first time that catechol sensing in aqueous solution can be achieved using a photoinduced electron transfer mechanism for fluorescence quenching. Of course, these results do not in-and-of themselves permit for the immediate formulation of useful sensing devices. This work does, however, point to a heretofore unreported mechanism upon which progress towards this end can be founded.

References and Notes

1. (a) Schwarzenbach, G.; Flaschka, H. *Complexometric Titrations* (translation by H. Irving), Methuen; London, 1969; (b) West, T. S. *Complexometry with EDTA and Related Reagents*, BDH; London, 1969; (c) Bishop, E. (Ed.) *Indicators*, Pergamon; NY, 1972; (d) Guilbault, G. G. *Practical Fluorescence*, Chapter 6, Marcel Dekker; NY, 1973.

2. For lead references to recent work by several authors, see: Czarnik, A. W. Fluorescent chemosensors for metal and non-metal ions in aqueous solution based on the 'CHEF' paradigm, In *Advances in Supramolecular Chemistry*, Vol. 3, Gokel, G. W., Ed.; JAI Press; Greenwich, 1993.

3. A notable exception is found in recent work by Ueno: Ueno, A., Kuwabara, T., Nakamura, A.; Toda, F. *Nature* **1992** *356*, 136.

4. Kuivila, H. G.; Keough, A. H.; Soboczenki, E. J. *J. Org. Chem.* **1954**, *19*, 780.

5. (a) Weith, H. L.; Wiebers, J. L.; Gilham, P. T. *Biochemistry* **1970**, *9*, 4396; (b) Schott, H. *Angew. Chem., Int. Ed. Eng.* **1972**, *11*, 824; (c) Barker, S. A.; Hatt, B. W.; Somers, P. J.; Woodbury, R. R. *Carbohydr. Res.* **1973**, *26*, 55; (d) Yurkevich, A. M.; Kolodkina, I. I.; Ivanova, E. A.; Pichuzhkina, E. I. *Carbohydr. Res.* **1975**, *43*, 215.

6. Lorand, J. P.; Edwards, J. O. *J. Org. Chem.* **1959**, *24*, 769.

7. Yoon, J.-Y.; Czarnik, A. W. *J. Am. Chem. Soc.* **1992**, *114*, 5874.

8. Stahley, S. MS Thesis, 1989, The Ohio State University, Columbus, OH.

9. The current method is to be distinguished clearly from the well-known fluorimetric trihydroxyindol assay for catecholamines, which consumes the analyte (i.e. it is a batch analysis) and is not intended to afford real-time response: von Euler, U. S.; Lishajko, F. *Acta Physiol. Scand.* **1961**, *51*, 348.

10. 2-Anthrylboronic acid (**3**) has been reported (Ref. 7). The synthesis method we employed follows: A mixture of 2-chloroanthracene (2.0 g, 9.4 mmol), magnesium powder (460 mg, 19 mmol), and anhydrous tetrahydrofuran (60 mL) was heated to reflux under argon. Five drops of ethylene dibromide was added, and reflux was continued for 2 h. After being cooled, the reaction mixture was added to a pre-cooled solution of trimethylborate (970 mg, 9.4 mmol) in dry ether (20 mL) dropwise over 1 h, the addition being carried out under argon in an acetone–dry ice slush bath. The reaction was allowed to warm to room temperature overnight. The Grignard complex was decomposed with 10% hydrochloric acid (30 mL), the organic layer collected, extracted with aqueous sodium hydroxide and the aqueous phase acidified with hydrochloric acid. The white precipitate that formed after acidification was precipitated from benzene to give 2-anthrylboronic acid (530 mg, 25%); m.p. 320 °C dec.; ^{1}H NMR [$(CD_3)_2SO$] δ 7.47–7.52 (m, 2H), 7.80–7.84 (d, 1H), 7.97–8.02 (d, 1H), 8.02–8.11 (m, 2H), 8.23 (s, 2H, OH), 8.52 (s, 1H), 8.56 (d, 2H); ^{13}C NMR [$(CD_3)_2SO$] δ 125.43, 125.58, 125.84, 126.56, 126.86, 128.01, 128.31, 129.76, 130.85, 131.21, 131.73, 131.99, 135.78; EI mass spectrum, m/e 222 (M^+), 204 (M^+ - H_2O). High-resolution EI mass spectrum, calcd for $C_{14}H_{11}BO_2$, 222.0852; measured, 222.0851.

11. 9-Anthrylboronic acid (**6**) has been reported: Dodson, V. H.; Fisher, W. E. *Ohio J. Sci.* **1958**, *58*, 141. The synthesis method we employed follows: A mixture of 9-bromoanthrancence (5.1 g, 20 mmol), magnesium powder (1.0 g, 40 mmol), and anhydrous ether (100 mL) was heated to reflux under argon. Five drops of ethylene dibromide were added, and reflux was continued for 7 h. After being cooled, the reaction mixture was added to a pre-cooled solution of trimethylborate (4.16 g, 40 mmol) in anhydrous ether (20 mL) dropwise over 1 h, the addition being carried out under argon in an acetone–dry ice slush bath. Continued reaction and workup as for the 2-isomer yielded, after acidification, a white

precipitate that was analytically pure 9-anthrylboronic acid (1.89 g, 43%): m.p. 187–190 °C (lit.[11] 180–184 °C); ^{1}H NMR [$(CD_3)_2SO$] δ 7.45–7.53 (m, 4H), 7.98–8.08 (m, 4H), 8.5 (s, 1H), 8.8 (s, 2H, OH); ^{13}C NMR [$(CD_3)_2SO$] δ 125.108, 125.151, 125.981, 125.451, 129.022, 130.082, 132.802. High-resolution EI mass spectrum, calcd for $C_{14}H_{11}BO_2$, 222.0852; measured, 222.0848.

12. For example, 9,10-diisopropylanthracene isomerizes with acid with loss of the central aromatic ring: Cho, H.; Harvey, R. G.; Rabideau, P. W. *J. Am. Chem. Soc.* **1975**, *97*, 1140.

13. Knibbe, H.; Röllig, K.; Schäfer, F. P.; Weller, A. *J. Chem. Phys.* **1967**, *47*, 1184.

14. (a) Ainley, A. D.; Challenger, F. *J. Chem. Soc.* **1930**, 2171; (b) Nahabedian, K. V.; Kuivila, H. G. *J. Am. Chem. Soc.* **1961**, *83*, 2167.

15. HPLC was accomplished on a C_{18} column and elution with methanol/water (2:1).