Radioiodination of Humic Substances via Azocoupling with 3-[¹²⁵l]lodoaniline

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A new method is described for radiolabeling humic substances (HS) with iodine radioisotopes. The method radiolabels the electron-rich aromatic moieties of HS with the 3-[1251]iodobenzenediazonium ion via azocoupling. The method uses four steps: (i) 3-aminobenzenetrimethylstannane is synthesized and isolated by using a silica gel column, (ii) 3-[1251]iodoaniline is synthesized and isolated by HPLC, with radiochemical yields of up to 60%, (iii) 3-[1251]iodobenzenediazonium chloride is synthesized, and the reaction mixture from this step is used in step iv to radioiodinate HS with radiochemical vields of up to 95% (with reference to 3-[1251]iodoaniline). The advantage of this method is that it is selective radiolabeling, placing the radiolabel in a specific site (the 3-position of the phenyl ring) within HS molecules, which minimizes unwanted secondary chemical interactions. Investigations of the stability of the radiolabel and the effect of photoreductive dehalogenation showed that there was a negligible release of ¹²⁵I. The production of radiolabeled HS using this method allows the sensitive detection of HS in laboratory and field studies. In addition, the method offers the possibility of using different iodine radioisotopes simultaneously in investigations using HS.

Introduction

Humic substances are important because they (i) are part of the global carbon cycle (1), (ii) influence transport processes, which affects plant nutrition (2), (iii) affect pharmacological potency (3), (iv) affect the availability of chemotoxic pollutants (4-6), (v) need to be included in estimates of risk assessments in the disposal of radioactive wastes (7, 8), and (vi) are important in carbon sequestration of atmospheric $CO_2(1, 9)$. Humic substances have therefore been studied for many years, and their properties and behavior have been investigated using a wide range of different analytical methods and techniques, ranging from in situ measurements of HS in the field to the use of advanced spectrometric techniques in the laboratory (10-15). The aims of these investigations were to identify and characterize HS at the molecular level and to investigate the bulk behavior of the rather complicated and heterogeneous HS molecules. The structure(s) of HS have not been experimentally defined, but models describe HS as having a supramolecular-like structure with a sample of HS containing a mixture of molecular weights (16, 17). Because of the ease and sensitivity of the detection of radioactivity, investigations of the behavior of HS are made easier by using radiolabeled HS. Radioactive elements have mostly been used to investigate the reactions of metals with HS (10, 18-21). However, these complexes may be of limited use in some investigations because of their relative instability. Producing radiolabeled HS in which the radiolabel is covalently bound to HS molecules should produce a more stable radiolabel. Whichever method is used, it should not change the inherent properties of the molecules and should produce radiolabeled molecules in high yield and high specific activity. A number of different radiolabeling methods have been reported (23, 24) but, although they are useful, they have limitations in terms of authenticity, specific activity, yields, handling, or stability. In addition to these methods, radioiodination via an electrophilic H/I substitution on aromatic moieties has been reported, which is based on a widely used and reliable mild method of radiolabeling proteins and other biomolecules (22). The advantages of this method are ease of handling, high labeling yield, and the preservation of the properties of the humic material. The covalent bonding of iodine on humic substances has been reported (25) but may be complicated because of secondary interactions with thioland imidazole-like structures and because of the complex aqueous chemistry of iodine (26, 27). These complications may lead to radiolabeling at different sites within the HS molecules (i.e., nonspecific radiolabeling), resulting in radiolabels that have different stabilities with respect to pH, $E_{\rm h}$, etc. These unwanted interactions are considered, and somewhat overcome, by suitable quenching and purification methods reported in ref 22. Secondary reactions may be avoided by using a method that was reported for radiohalogenating amino acids and peptides (28). The method was used to radiolabel the carbon backbone of HS with ¹⁸F via azocoupling using a precursor (4-[18F]fluorobenzenediazonium chloride) (29). The ¹⁸F labeled group (Figure 1) can react with the polyphenolic groups in the HS. The increased effort required to make the precursor is compensated for by the production of a more selective and specific label.

However, the disadvantage with using ¹⁸F is its short halflife (109.7 min), which limits its use to experiments of short time scales. A similar method was used for the ¹⁴C labeling of HS (*30*), but the β^- decay may limit the use of the radiolabeled HS.

The limitations can be overcome by the use of radioisotopes of iodine (123 I ($t_{1/2} = 13.2$ h), 124 I ($t_{1/2} = 4.17$ days), 125 I ($t_{1/2} = 60.14$ days), and 131 I ($t_{1/2} = 8.04$ days)), and a promising precursor for azocoupling with HS is 3-[125 I]iodoanaline. This paper describes the synthesis of this precursor, the optimization of the azocoupling reaction to radiolabel HS, and the investigations of the radiolabeled HS. Two different humic acids (HA) were radiolabeled, but the method can be used to radiolabel fulvic acid.

Materials and Methods

Chemicals and solvents were obtained in reagent or analytical quality from VWR. ^{125}I was purchased from Amersham Bioscience as sodium iodide in NaOH solution (pH 8.0–12.0). The certified specific activity was >0.6 TBq/mg iodide with a radionuclidic purity of at least 99.9% ^{125}I .

Thin layer chromatography (TLC) was carried out using plates (Polygram SIL-G/UV254) purchased from Macherey-

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catalyst: tetrakis(triphenylphosphine)palladium

FIGURE 2. Synthesis of 3-aminobenzenetrimethylstannane.

Nagel. TLC plates were contacted with imaging plates (BAS-MS2325, Fujifilm) and afterward scanned with an IP-Reader (BAS-1800II, Fujifilm). The images were recorded and evaluated with the AIDA software (Raytest Isotopenmessgeräte). High performance liquid chromatography (HPLC) was conducted using an Agilent HP1100, which was equipped with a quaternary HPLC pump, a vacuum degasser, an autosampler, a multiwavelength UV/vis absorption detector, and a NaI(TI)-detector. HPLC analysis was controlled by using Chemstation software for LC systems (Agilent). Two different HPLC columns were used: (i) LiChrospher 100CN 5 μ m, 250 mm \times 10 mm and (ii) TOSOH Bioscience G3000 PWxl, 300 mm \times 7.8 mm for high performance size exclusion chromatography (HPSEC).

Radioiodinated HS was purified by using ultrafiltration (Microsep Centrifugal Devices; PALL Corporation; filter media: modified polyethersulfone on polyethylene substrate, MWCO 3000 Da) at the recommended centrifugation parameters (centrifugal force: 7500g, time: 60 min, and maximum sample volume: 3.5 mL) or by precipitation by the addition of acid. The radioactive yield of radiolabeled HS was calculated from measurements of the ¹²⁵I radioactivity in an Aktivimeter ISOMED 1000 (Nuklear-Medizintechnik Dresden GmbH). The following paragraphs describe the preparation of radiolabeled HS.

Step 1: Synthesis of 3-Aminobenzenetrimethylstannane (2). Tetrakis(triphenylphosphin)-palladium(0) (50 mg), 3-bromoaniline (100 μ g), and hexamethyldistannane (250 mg) in toluene (2 mL) were mixed in a 10 mL vial (Figure 2) (31). The mixture was stirred for 12 h at 105 °C under an atmosphere of N₂. After cooling, the black precipitate was filtered off and washed with EtOAc. The remaining EtOAC was evaporated. The residue was redissolved in petroleum ether-ethyl acetate, 80:20 (v/v). The synthesis of 3-aminobenzenetrimethylstannane was monitored by TLC against 3-bromoaniline. The product (2) was isolated from 1 by chromatographic separation (petroleum ether-ethyl acetate, 80:20 (v/v)) using a silica gel filled glass column (180 mm × 35 mm). The progress of elution was monitored by TLC. The fraction containing the product (2) was evaporated to dryness.

Step 2: Synthesis of 3-[¹²⁵**I**]**iodoaniline (3).** 3-Aminobenzenetrimethylstannane (1 mg) and IODOGEN (50 μ g) were dissolved in dichloromethane (550 μ L) and then added to an aqueous solution (500 μ L) containing [¹²⁵I]NaI (0.75 MBq) (Figure 3). The mixture was stirred for 30 min. The organic phase was then separated and evaporated. The residue was dissolved in the HPLC mobile phase and injected into an HPLC column (LiChrospher 100CN, eluent: petroleum etherethyl acetate, 95:5, v/v and mobile phase flow rate: 5 mL min⁻¹). 3-Iodoaniline was used to determine the retention



2: 3-Aminobenzenetrimethylstannane

3: 3-[¹²⁵]]lodoaniline

FIGURE 3. Synthesis of nca 3-[¹²⁵I]iodoaniline.



3: 3-[¹²⁵]lodoaniline

4: 3-[¹²⁵I]lodobenzenediazonium ion

FIGURE 4. Synthesis of nca 3-[1251]iodobenzenediazonium ion.

TABLE 1. Buffer Solutions

	Dutter	description
	tris	0.2 M tris(hydroxymethyl)-aminomethane
		(250 mL)
		0.1 M HCI (315 mL)
		H ₂ O (435 mL)
	phosphate	0.667 M KH ₂ PO ₄ (31 mL)
		0.667 M Na ₂ HPO ₄ × 2H ₂ O (969 mL)
	borate	0.05 M Na₂B₄Oァ (559 mL)
		0.1 M HCI (441 mL)

time (13.05 min) of **3**. The fraction containing the product was acidified (1 M HCl, 100 μ L) and then evaporated.

Step 3: Synthesis of 3-[¹²⁵]]Iodobenzenediazonium Chloride (4). The 1 M sodium nitrite (20 μ L) and 1 M HCl (830 μ L) were added to 3-[¹²⁵]]iodoaniline in 1 M HCl (100 μ L) (Figure 4). After 10 min at 0 °C, 1 M urea (50 μ L) was added to the mixture at 0 °C for 20 min.

Step 4: Preparation of ¹²⁵**I Radiolabeled HA (6).** Two different HAs were radiolabeled—HA purchased from Aldrich (Aldrich HA) and peat bog humic acid (peat bog HA), which was extracted from the ombrotrophic raised bog Kleiner Kranichsee, situated in the upper Ore Mountains (Erzgebirge), Saxony, Germany (32). The peat bog HA was extracted and isolated using the procedure reported by Aiken (33). Different buffers (Table 1) were used for the preparation of a 200 mg/L HA stock solution (0.02 M KCl).

A total of 100 μ L of the reaction mixture from step 3, containing 3-[¹²⁵I]iodobenzenediazonium chloride (**4**) in 1 M HCl, 1 M sodium hydroxide (100 μ L), and tris buffer (300 μ l), was added to 500 μ L of HA stock solution (**5**) (Figure 5). After an incubation time of 20 min at 0 °C, [¹²⁵I]I-[¹²⁵I]I-HA (**6**) was separated by ultrafiltration or precipitation (addition of 50 μ L of 1 M HCl).

Results and Discussion

Optimization and Yields. Step 2. A total of 70–80% of the ¹²⁵I radioactivity from Na¹²⁵I was found in the organic phase after extracting the radioactive product from the aqueous phase. After evaporation of the organic phase and redissolving it in the HPLC mobile phase, 3-[¹²⁵I]iodoaniline was further purified by HPLC (Figure 6) to remove organic impurities that might (i) interfere with the diazotization process and/or (ii) be incorporated in the HA structure, thereby changing the properties of the HA. The radiochemical yield after HPLC



4: 3-[125]lodobenzenediazonium ion

5: HA-constituent

6: ¹²⁵I labeled HA

FIGURE 5. Reaction scheme for the preparation ¹²⁵I radiolabeled HAs.



FIGURE 6. Chromatographic separation of 3-[¹²⁵I]iodoaniline (radioactivity: ¹²⁵I, gray line and UV absorption (254 nm): black line). Retention time of the product: 13.05 min.

was up to 75%, and step 2 was in total 60%. The diazotization reaction is reported extensively in the literature (*29, 30, 34, 35*). A yield can not be given for the product (**4**) because of the high instability of this intermediate. However, a high excess of nitrite ensured a quantitative chemical reaction (Figure 6).

The radiolabeling of HA (6) was optimized with respect to (i) the buffer used, (ii) the concentration of HA, and (iii) the method used to purify the radiolabeled HA. According to ref 29, radiolabeling should be carried out at pH 8. Nevertheless, different buffer systems (borate buffer, phosphate buffer, tris buffer, pH adjusted by means of NaOH/ HCl) were tested to determine the optimum radiolabeling conditions. Two purification procedures (ultrafiltration and precipitation) were used to purify the radiolabeled HA. Depending on the purification step used, radiolabeling yields of between 50 and 95% were obtained (Figure 7). Purification by precipitation resulted in similar high yields for Aldrich HA and peat bog HA using tris buffer. However, using tris buffer and ultrafiltration, higher yields were observed from peat bog HA than from Aldrich HA. In addition to producing a high vield, a buffer must be noncomplexing (i.e., should not complex with metals if the aim of the experimental work is to investigate metal-HS interactions as well as HS



FIGURE 8. Influence of HA (peat bog HA) concentration on radiolabeling yield after precipitation.

interactions). With these considerations in mind, the use of phosphate and borate buffers was excluded (*36*). The following studies were therefore conducted using tris buffer, which would enable the use of HPSEC and investigations of metal interactions.

Both purification methods (ultrafiltration and precipitation), which were used in step 4 to separate [¹²⁵I]I-HA from the reaction mixture, showed comparable radiolabeling yields. Ultrafiltration is gentler than precipitation, but precipitation is a fast alternative (but cannot be used with fulvic acid). A partial loss of small size fractions of humic material is possible due to filtration, but suitable filters with small molecular weight cutoffs (500 to 100 Da) and a mild centrifugation would minimize the loss at the expense of time. Alteration of HA caused by precipitation is unlikely since precipitation is widely used for its isolation. The radiochemical yields differ with the origin of HA and the purification method (Figure 7). As expected, a higher radiolabeling yield was obtained with increasing concentrations of peat bog HA because of the increasing number of binding sites (Figure 8). Almost a 100% yield was achieved with concentrations of 20 mg/L and above.

Stability of the Radiolabel. The stability of the radiolabel with respect to changes in pH and to physical stress was tested by repeated precipitation and ultrafiltration. When



FIGURE 7. Dependence of radioabeling yield on HA origin (black: peat HA and white: Aldrich HA), used buffer system (1: borate buffer, 2: phosphate buffer, 3: NaOH/HCI, and 4: tris buffer), and purification method.



FIGURE 9. Radiochemical yield dependence on the number of precipitation steps of [¹²⁵1]I-HA (peat bog HA).



FIGURE 10. Longer term stability of the radiolabeled HA (\diamond peat bog HA and \Box Aldrich HA) tested by ultrafiltration.

investigating precipitation (Figure 9), there was a loss of approximately 5% of ¹²⁵I after the fifth precipitation. The loss was probably because of incomplete precipitation of HA. When investigating ultrafiltration (Figure 9), nearly 45% of the total amount of ¹²⁵I was detected in the <3000 Da fraction after five filtrations. The loss was probably because of a partial temporary break of the interaction forces within the molecular humic assembly, which resulted in HA passing through the filter membrane.

Longer Term Stability of the Label. Radiolabeled samples of peat bog HA and Aldrich HA were prepared and purified by precipitation. The longer term stabilities of the radiolabels were tested over a period of 45 days (arbitrarily chosen period of time) (Figure 10) by storing the samples in the dark at 4 °C and taking samples at regular intervals for ultrafiltration to determine the release of radioiodine. Results showed that, although there was a constant loss of approximately 5%, there was no significant time dependent release of radioiodine. Since the release of ¹²⁵I was not time dependent, the loss was probably caused by ¹²⁵I remaining on the filter and surfaces or to HA passing through the filter. It can be concluded that the [¹²⁵I]I-HA species were stable during these investigations.

Photoreductive Dehalogination. Photoreductive dehalogenation of halophenols is well-known (*37, 38*), and this was investigated using white light (40 W light bulb held 50 cm from the sample) to simulate the working conditions in a laboratory. Radiated and nonradiated [¹²⁵I]I-HA stock solutions were kept at 0 °C over a period of 110 h. At regular intervals, aliquots were taken from these solutions, and the possible release of iodine was measured by using ultrafiltration. The release of ¹²⁵I caused by photoreductive dehalogination is shown in Figure 11. After 110 h of radiation, 3.8% of total ¹²⁵I was released from [¹²⁵I]I-HA. No significant difference between radiolabeled peat bog HA or Aldrich HA



FIGURE 11. Release of ¹²⁵I caused by photoreductive dehalogination after radiation with white light as a function of time (three parallel experiments with \diamond peat bog HA and \Box Aldrich HA).



FIGURE 12. Comparison of radioactivity (¹²⁵I, gray line) and UV absorption (254 nm) (black line) chromatograms of radioiodinated peat bog HA.

was observed in repeated investigations. If necessary, samples should be kept in the dark, which is common laboratory practice to avoid alterations in humic material.

HPSEC Investigation. HPSEC was used to compare the radiochromatogram of [¹²⁵I]I-HA with the UV absorption (254 nm) chromatogram to investigate as to whether the complete range of molecular weights in the peat bog HA was radio-labeled (Figure 12). Similar results were obtained for Aldrich HA. Figure 12 clearly shows that ¹²⁵I coelutes with the UV absorbing HA constituents, demonstrating that radiolabeling was not selective to a particular fraction of molecular weights but that all molecular weights and sizes of HA molecules were radiolabeled by this method. Nonselectivity is essential if radiolabeled HA is to be used in investigations where the bulk behavior of HA is to be investigated.

The combination of radiolabeling at a specific site in a molecule, nonselectivity toward molecules or fractions of molecules, and the possibility of using a number of different radioisotopes of iodine in the same experiment offers an excellent and reliable tool for geochemical investigations. The simultaneous use of different radioisotopes allows the study of complex exchange processes of HA in coagulation, flocculation, transport, and sorption processes.

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Literature Cited

 Swift, R. S. Sequestration of carbon soil. Soil Sci. 2001, 166 (11), 858–871.

- (2) Capasso, R.; De Martino, A.; Cristinzio, G. Production, characterization, and effects on tomato of humic acid-like polymerin metal derivatives from olive oil mill waste waters. *Environ. Sci. Technol.* 2002, 50 (14), 4018–4024.
- (3) Paciolla, M. D.; Davies, G.; Jansen, S. A. Generation of hydroxyl radicals from metal-loaded humic acids. *Environ. Sci. Technol.* 1999, 33 (11), 1814–1818.
- (4) Chen, Z.; Valentine, R. L. Modeling the formation of *N*nitrosodimethylamine (NDMA) from the reaction of natural organic matter (NOM) with monochloramine. *Environ. Sci. Technol.* **2006**, 40 (23), 7290–7297.
- (5) Piol, M. N.; Lopez, A. G.; Mino, L. A.; dos Santos Afonso, M.; Verrengia Guerrero, N. R. The impact of particle-bound cadmium on bioavailability and bioaccumulation: A pragmatic approach. *Environ. Sci. Technol.* **2006**, *40* (20), 6341–6347.
- (6) Buschmann, J.; Kappeler, A.; Lindauer, U.; Kistler, D.; Berg, M.; Sigg, L. Arsenite and arsenate binding to dissolved humic acids: Influence of pH, type of humic acid, and aluminum. *Environ. Sci. Technol.* **2006**, *40* (19), 6015–6020.
- (7) Maes, A.; Bruggeman, C.; Geraedts, K.; Vancluysen, J. Quantification of the interaction of Tc with dissolved boom clay humic substances. *Environ. Sci. Technol.* **2003**, *37* (4), 747–753.
- (8) Monsallier, J. M.; Schussler, W.; Buckau, G.; Rabung, T.; Kim, J. I.; Jones, D.; Keepax, R.; Bryan, N. Kinetic investigation of Eu(III)–humate interactions by ion exchange resins. *Anal. Chem.* 2003, 75 (13), 3168–3174.
- (9) Hayes, M. H. B.; Clapp, C. E. Humic substances: Considerations of compositions, aspects of structure, and environmental influences. *Soil Sci.* 2001, *166* (11), 723–737.
- (10) Kupsch, H.; Franke, K.; Degering, D.; Tröger, W.; Butz, T. Speciation of aquatic heavy metals in humic acids by Cd-111m/ Hg-199m TDPAC. *Radiochim. Acta* **1996**, *73* (3), 145–147.
- (11) Siripinyanond, A.; Worapanyanond, S.; Shiowatana, J. Field-flow fractionation-inductively coupled plasma mass spectrometry: An alternative approach to investigate metal-humic substances interaction. *Environ. Sci. Technol.* 2005, 39 (9), 3295– 3301.
- (12) Gustafsson, J. P.; Persson, I.; Kleja, D. B.; vanSchaik, J. W. J. Binding of iron(III) to organic soils: EXAFS spectroscopy and chemical equilibrium modeling. *Environ. Sci. Technol.* 2007, 41 (4), 1232–1237.
- (13) Peuravuori, J.; Pihlaja, K. Preliminary study of lake dissolved organic matter in light of nanoscale supramolecular assembly. *Environ. Sci. Technol.* 2005, 38 (22), 5958–5967.
- (14) Mao, J. D.; Schmidt-Rohr, K. Absence of mobile carbohydrate domains in dry humic substances proven by NMR and implications for organic-contaminant sorption models. *Environ. Sci. Technol.* 2006, 40 (6), 1751–1756.
- (15) Simpson, A. J.; Song, G.; Smith, E.; Lam, B.; Novotny, E. H.; Hayes, M. H. B. Unraveling the structural components of soil humin by use of solution-state nuclear magnetic resonance spectroscopy. *Environ. Sci. Technol.* **2007**, *41* (3), 876–883.
- (16) Sutton, R.; Sposito, G. Molecular structure in soil humic substances: The new view. *Environ. Sci. Technol.* 2005, 39 (23), 9009–9015.
- (17) Plancque, G.; Amekraz, B.; Moulin, V.; Toulhoat, P.; Moulin, C. Molecular structure of fulvic acids by electrospray with quadrupole time-of-flight mass spectrometry. *Rapid Commun. Mass Spectrom.* 2001, *15* (10), 827–835.
- (18) Torres, R. A.; Choppin, G. R. Europium (II) and americium (III) stability constants with humic acid. *Radiochim. Acta* 1984, 35 (3), 143–148.
- (19) Röβler, D.; Franke, K.; Süβ, R.; Becker, E.; Kupsch, H. Synthesis and chromatographic characterization of [Tc-99m]technetium– humic acid species. *Radiochim. Acta* 2000, 88 (2), 95–100.
- (20) Schafer, T.; Artinger, R.; Dardenne, K.; Bauer, A.; Schuessler, W.; Kim, J. I. Colloid-borne americium migration in Gorleben

groundwater: Significance of iron secondary phase transformation. *Environ. Sci. Technol.* **2003**, *37* (8), 1528–1534.

- (21) Franke, K.; Röβler, D.; Kupsch, H. Use of radioactive tracers for the characterization of humic and fulvic acids in HPSEC. In *Humic Substances—Nature's Most Versatile Materials*, Ghabbour, E., Davis, G., Eds.; Taylor & Francis: New York, 2004.
- (22) Warwick, P.; Carlsen, L.; Randall, A.; Zhao, R.; Lassen, P. C-14 and I-125 labeling of humic material for use in environmental studies. *Chem. Ecol.* **1993**, *8*, 65–80.
- (23) Pompe, S.; Schmeide, K.; Bubner, M.; Geipel, G.; Heise, K. H.; Bernhard, G.; Nitsche, H. Investigation of humic acid complexation behavior with uranyl ions using modified synthetic and natural humic acids. *Radiochim. Acta* **2000**, *88* (9–11), 553– 558.
- (24) Badun, G. A.; Pozdnyakova, V. Y.; Kudryavtsev, A. V.; Perminova, I. V. Preparation of tritium-labeled humic substances using thermal activation methods. *Ecol. Future Bulg. J. Ecol. Sci.* 2003, 2 (3–4), 26–27.
- (25) Moulin, V.; Reiller, P.; Amekraz, B.; Moulinet, C. Direct characterization of iodine covalently bound to fulvic acids by electrospray mass spectrometry. *Rapid Commun. Mass Spectrom.* 2001, *15* (24), 2488–2496.
- (26) Li, C. H. Kinetics and mechanism of 2,6-di-iodotyrosine formation. J. Am. Chem. Soc. 1942, 64 (5), 1147–1152.
- (27) Schlegel, M. L.; Reiller, P.; Mercier-Bion, F.; Barre, N.; Moulin, V. Molecular environment of iodine in naturally iodinated humic substances: Insight from X-ray absorption spectroscopy. *Geochim. Cosmochim. Acta* 2006, *70* (22), 5536–5551.
- (28) Patt, J. T.; Patt, M. Action of [F-18]4-fluorobenzenediazonium cations with cysteine or the cysteinyl group: Preparation of F-18labeled S-aryl-cysteine and a radiolabeled peptide. *J. Label. Comp. Radiopharm.* **2002**, 45 (14), 1229–1238.
- (29) Franke, K.; Patt, J. T.; Patt, M.; Kupsch, H.; Steinbach, J. A new technique for radiolabeling of humic substances. *Radiochim. Acta* 2004, 92 (4–6), 359–362.
- (30) Mansel, A.; Kupsch, H. Radiolabelling of humic substances with ¹⁴C by azo coupling [¹⁴C]phenyldiazonium ions. *Appl. Radiat. Isotop.* **2007**, 65 (7), 793–797.
- (31) Hiller, A.; Patt, J. T.; Steinbach, J. On the reaction of 4-substituted trimethyltin aromatics with perchlorylfluoride. *J. Organomet. Chem.* **2006**, 691 (18), 3737–3742.
- (32) Gottschalch, U. Concentration, Characterization, and Identification of Humic Substances by Means of Isoelectric Focusing Electrophoresis. Ph.D. Thesis, Department of Chemistry, University of Leipzig, Leipzig, 1998.
- (33) Aiken, G. R. Isolation and concentration techniques for aquatic humic substances. In *Humic Substances in Soil, Sediment, and Water: Geochemistry and Isolation*; Aiken, G. R., McKnight, D. M., Wershaw, R. L., MacCarthy, P., Eds.; Wiley-Interscience: New York, 1985.
- (34) Houben, H.; Weyl, T. *Methods of Organic Chemistry*; Thieme: Stuttgart, 1965.
- (35) Patai, S. *The Chemistry of Diazonium and Diazo Groups*; Wiley: Chichester, U.K., 1978.
- (36) Schmitt-Kopplin, P.; Hertkorn, N.; Garrison, A. W.; Freitag, D.; Kettrup, A. Influence of borate buffers on the electrophoretic behavior of humic substances in capillary zone electrophoresis. *Anal. Chem.* **1998**, *70* (18), 3798–3808.
- (37) Kuchmii, S. Y.; Korzhak, A. V.; Kulik, S. V.; Belous, A. I.; Kryukov, A. I. Photoreduction dehalogenation of polychlorobenzenes by ethanol. *Theor. Exp. Chem.* **1994**, *30* (1), 30–33.
- (38) Wada, Y.; Kitamura, T.; Yanagida, S.; Yin, H. Photoreductive dechlorination of chlorinated benzene derivatives catalyzed by ZnS nanocrystallites. *Chem. Commun. (Cambridge, U.K.)* 1998, 24, 2683–2684.

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