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Biocatalytic Desulfurization of Arylsulfonates

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Abstract—A microbial strain, *Klebsiella oxytoca* KS3D, has been isolated which is capable of exploiting arylsulfonates as a sole source of sulfur during growth. The desulfurization catalyzed by intact *K. oxytoca* KS3D results in the conversion of arylsulfonates into the corresponding phenols. Even arylsulfonates carrying substituents which significantly alter steric and electronic characteristics are substrates. Only a single regioisomer is produced from substituted arylsulfonates. Based on the products formed from the biocatalytic desulfurizations and incorporation of isotopic oxygen in phenolic product when the desulfurization is run under ^{18}O-enriched oxygen, hydrolysis mechanisms can be eliminated from consideration. Two reaction types which might mimic the chemistry occurring during microbial desulfurization of arylsulfonates were examined. The first reaction involved conversion of appropriately substituted arylsulfonates into phenols by single electron reduction followed by reaction of the radical anions with molecular oxygen. A second reaction using intramolecular reaction of arylsulfonates and arylsulfones with alkoxy radicals failed to achieve desulfurization. In addition to mechanistic evaluation, desulfurization of arylsulfonates catalyzed by *K. oxytoca* KS3D is examined from the perspective of its relevance to desulfurization of the organosulfur components of coal and its possible use for industrial manufacture of phenols.

Introduction

Abiotic and biotic cleavage of carbon–sulfur (C–S) bonds constitutes an enormous ensemble of reactions of significant environmental, industrial and academic importance. These reactions are best categorized according to the hybridization of the carbon atom and the oxidation state of the attached sulfur. In this report, microbial cleavage of aryl carbon atoms bonded to sulfonic acid sulfur is examined. Abiotic, chemical cleavage of arylsulfonate C–S bonds often requires forcing conditions as evidenced by the fusion of benzenesulfonate at 300–320 °C with caustic soda which was once the dominant chemical route for manufacture of phenol.[1] Similar forcing conditions are employed in one strategy for desulfurization of the dibenzothiophene-type organosulfur component of coal where oxidation of the dibenzothiophene sulfur to a sulfone is followed by alkali fusion to form 2-hydroxybiphenyl.[2] A microbe, *Klebsiella oxytoca* KS3D, has now been isolated which biocatalytically desulfurizes arylsulfonates at neutral pH and near-ambient temperatures. Products formed from various substituted and isotopically labeled arylsulfonates provide the basis for mechanistic evaluation of the bioconversion. Chemical model reactions are also examined to assess the reactivity of intermediates possibly involved in microbial desulfurization of arylsulfonates.

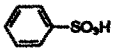
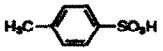
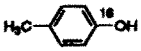
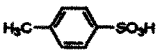
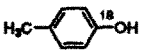
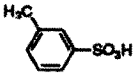
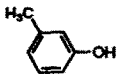
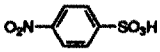
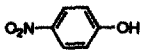
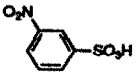
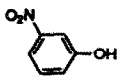
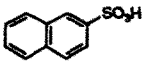
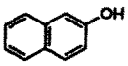
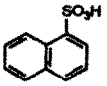
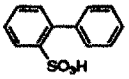
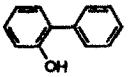
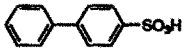
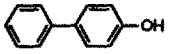
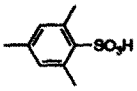
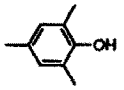
Results and Discussion

Klebsiella oxytoca KS3D was isolated from the activated sludge of a municipal sewage treatment plant. The sludge was used as an inoculant for cultures subsequently spread on solid growth medium where *p*-nitrobenzenesulfonic acid was the only source of sulfur. Single colonies surrounded by a yellowish halo were subcultured leading to the isolation of strain KS3D. The strain was subsequently determined to be *K. oxytoca*.[3] All subsequent mechanistic work was performed using *K. oxytoca* KS3D. A wide range of arylsulfonates including variously substituted benzene-, naphthalene-, and biphenylsulfonates supported growth of *K. oxytoca* KS3D when used as the sole source of sulfur in liquid suspension medium (Table 1). Arylsulfonates were converted into corresponding phenols which accumulated in the culture supernatant where they could be readily extracted into organic solvent and analyzed.

The products of arylsulfonate desulfurization catalyzed by *K. oxytoca* KS3D are significantly different from the products generated by an extensively studied desulfurization system in *Comamonas testosteroni* T2,[4] *Alcaligenes* sp. strain O-1,[5] and other microbial strains.[6] This characterized microbial desulfurization leads to the formation of catechol products as opposed to the phenolics produced by *K. oxytoca* KS3D. The dioxygenase responsible for the desulfurization has been purified to homogeneity.[4d,6d] By contrast, the desulfurization catalyzed by *K. oxytoca* KS3D appears to be a monooxygenase. There is one report and only limited characterization of a *Pseudomonas* sp. which, like *K. oxytoca* KS3D, also catalyzes conversion of arylsulfonates into phenols.[7]

Factors likely responsible for the existence of microbial arylsulfonate desulfurization are worthy of comment. Arylsulfonates, as opposed to alkylsulfonates, are not the products of naturally occurring biosynthesis but are produced by industrial processes. Naphthalenesulfonic acids are used in industrial manufacture of dyes.[1a] Arylsulfonates such as linear alkylbenzenesulfonates (LAS) have found widespread use as synthetic surfactants in cleansing and laundry formulations.[8] It is thus not surprising that microbes capable of arylsulfonate C–S bond cleavage, such

Table 1. Desulfurization products resulting from growth of *Klebsiella oxytoca* KS3D in medium with arylsulfonates as the sole source of sulfur

	Organosulfonate	Growth Conditions	Product
1		a	
2		a,b	
		c	
3		a	
4		a	
5		a	
6		a	
7		a	
8		a	
9		a	
10		a	

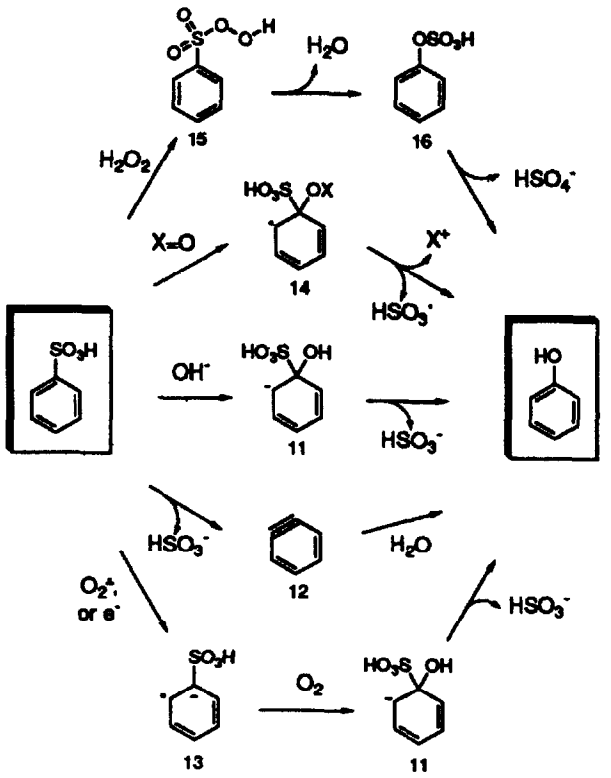
[a]Cultured under $^{16}O_2$ in $H^{16}OH$ containing arylsulfonate along with minimal salts (See General Biology in the Experimental Section); [b]Cultured under $^{16}O_2$ in $H^{18}OH$ containing arylsulfonate along with minimal salts; [c]Cultured under $^{18}O_2$ in $H^{16}OH$ containing arylsulfonate along with minimal salts.

as *K. oxytoca* KS3D were found in activated sludge of a municipal sewage treatment facility. While the focus hashistorically[8] been on the biodegradation of the aliphatic and aromatic portions of these synthetic surfactants, mechanisms for desulfurization which leave the aromatic ring intact have also apparently evolved. Concentrations of arylsulfonates can reach astonishing levels in the environment. Arylsulfonates have been reported to comprise approximately 10% of the pollution load in the Rhine river.[9,7]

Possible microbial arylsulfonate desulfurization mechanisms

Mechanisms for desulfurization of arylsulfonates catalyzed by *K. oxytoca* KS3D can be divided (Scheme I) into hydrolytic and oxidative processes. Enzyme-catalyzed attack of hydroxide on the *ipso* aryl carbon attached to the sulfonic acid sulfur could lead to a hydroxy sulfite adduct **11**. Elimination of sulfite would then lead to phenol formation. Sulfite might also be directly eliminated from the arylsulfonate to generate a benzyne intermediate **12**. Phenol would result from enzyme-mediated hydration of this benzyne intermediate. Alternatively, the arylsulfonate could be first reduced to the radical anion **13** by superoxide or by another reductant. Reaction of the resulting radical anion with molecular oxygen followed by collapse of the hydroxy sulfite adduct **11** would produce phenol and sulfite. Alternatively, *ipso* attack of a metal–oxo species to form intermediate **14** followed by elimination of sulfite radical could lead to phenol products. Finally, formation of a sulfonyl peroxide **15** and subsequent rearrangement might generate an aryl sulfate **16**. Hydrolysis catalyzed by a sulfatase would yield phenol.

Biological precedent relevant to the hydrolytic mechanisms can be found in reported enzyme-catalyzed conversion of *p*-chlorobenzoic acid into *p*-hydroxybenzoic acid.[10] Alkali fusion of arylsulfonates yielding phenolic products provides



Scheme I.

the chemical precedent for the hydrolysis mechanisms of Scheme I.[11] Benzyne intermediacy might be expected to yield regioisomeric phenolic product mixtures due to the two possible products formed from benzyne hydration. No regioisomers of phenols were produced by *K. oxytoca* KS3D. For instance, biocatalytic desulfurization of *m*-, and *p*-nitrobenzenesulfonic acids (**3** and **4**, Table 1) yielded only the corresponding *m*- and *p*-nitrophenol. It is possible that the enzyme might mediate the hydration of the benzyne intermediate such that only one regioisomer was formed. However, the conversion of mesitylenesulfonate (**10**, Table 1) to mesityl alcohol by *K. oxytoca* KS3D proceeds in lieu of any hydrogens *ortho* to the sulfonic acid. This result eliminates benzyne intermediacy from further consideration. Enzyme-catalyzed *ipso* hydroxide attack can be eliminated by the lack of ^{18}O incorporation into *p*-cresol product when the biocatalytic desulfurization of *p*-toluenesulfonic acid proceeded in ^{18}O-enriched water. Incorporation of ^{18}O label into *p*-cresol product when the biocatalytic desulfurization of *p*-toluenesulfonic acid was run under ^{18}O-enriched oxygen establishes the overall biological transformation as an oxidation and not a hydrolysis.

Discriminating between possible oxidative mechanisms (Scheme I) for biocatalytic desulfurization was complicated by the lack of C–S bond cleavage activity in cell-free lysate. Although a variety of different reasons can explain the lack of cell-free activity, the possibility that the desulfurization is catalyzed by an intrinsic membrane enzyme is particularly appealing. Localization of such an enzyme on the periplasmic side of the inner membrane would allow direct access to arylsulfonates in the growth medium. Enzymatic cleavage of arylsulfonate C–S bonds on the periplasmic surface of the inner membrane would then lead to the transport of the sulfate or sulfite ion by standard sulfate/sulfite permeases. This would obviate the need for the microbe to possess (or have evolved) different permease systems for each arylsulfonate or structural class of arylsulfonates as would be the likely requirement if the desulfurizing enzyme was located in the cytosol or on the cytosolic side of the inner membrane.

Chemical models

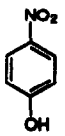
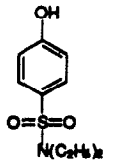
To gain further insights into the mechanism of C–S cleavage in arylsulfonate desulfurization, chemical reactions were designed to model the microbe-catalyzed process. Such chemical modeling helps to bracket what is mechanistically reasonable for the biotic process. These model reactions had to first yield phenolic products. Secondly, the model reactions had to involve a reactive species which could be derived from molecular oxygen. Three classes of reactive oxygen species are involved in molecular oxygen fixation. Oxygen is initially reduced to superoxide.[12] Further single electron reduction of protonated superoxide leads to peroxide formation. Reaction of this hydrogen peroxide with a cofactor-bound metal generates a metal–oxo complex.[13]

One of the requisite model reactions already has been reported in the literature and involves the chemistry of sulfoperoxides.[14] Sulfoperoxides are prepared by reaction of a suitably activated sulfonic acid with hydrogen peroxide. Intramolecular rearrangement of arylsulfoperoxides to phenyl sulfates followed by hydrolysis yields phenols. This intramolecular rearrangement has been reported for benzenesulfonyl peroxide[14b,c] and *m*-trifluoromethylbenzenesulfonyl peroxide.[14d] Rearrangement of benzenesulfonyl peroxide takes place at room temperature.

Direct literature precedent is lacking for desulfurization resulting from enzyme-catalyzed reaction of the arylsulfonate with superoxide or single electron reduction of the arylsulfonate to a radical anion followed by reaction of the radical anion with oxygen. Alkali fusion of dibenzothiophene sulfone does lead to formation of the radical anion of the sulfone which can be detected by EPR spectroscopy.[24b] The radical anion may or may not be on the reaction pathway, but the observation is suggestive that the observed C–S bond cleavage might proceed via initial electron transfer from the hydroxide to sulfone followed by combination of the two resulting radicals. This type of $S_{RN}1$ mechanism[15] also occurs during room temperature hydrolysis of bromonitrobenzenes[16] with potassium superoxide. The possible relevance of this type of chemistry to microbial desulfurization of arylsulfonates has now been demonstrated (Table 2) with the desulfurization of arylsulfonates and arylsulfonamides having a nitro substituent attached to the aromatic ring.

Potassium superoxide in benzene in the presence of a stoichiometric amount of 18-crown-6 was reacted (Table 2) with the tetrabutylammonium salts of benzene-, *p*-toluene-,

Table 2. Single electron reduction of an arylsulfonate and arylsulfonamide and reaction of the resulting radical anions with molecular oxygen

Organosulfur Starting Material	Reaction Conditions	Isolated Yields (%)	
	———		
17 R = O^- $^+N(C_4H_9)_4$	KO_2 (25 eq.), O_2 18-crown-6 C_6H_6, 60 h, RT	55	
18 R = $N(C_2H_5)_2$	KO_2 (4 eq.), O_2 18-crown-6 C_6H_6, 18 h, RT	35	14
18 R = $N(C_2H_5)_2$	1. SmI_2, -20 °C 2. O_2, THF, -20 °C, 1 h	31	10
18 R = $N(C_2H_5)_2$	1.  , -20°C 2. O_2, THF, -20 °C, 1 h	12	trace

2-naphthalene-, and *p*-nitrobenzenesulfonic acids. No reaction was observed with benzene-, *p*-toluene-, or 2-naphthalenesulfonic acids. However, tetrabutylammonium *p*-nitrobenzenesulfonate (**17**) was cleanly converted into *p*-nitrophenol at room temperature. When a large excess of superoxide (25 equivalents) was used in the reaction, the yield of *p*-nitrophenol reached 55 %. *p*-Nitrophenol was also produced when *N,N*-diethyl-*p*-nitrobenzenesulfonamide (**18**) was treated with superoxide. In this case, formation of *N,N*-diethyl-*p*-hydroxybenzenesulfonamide along with the *p*-nitrophenol indicated that carbon to nitrogen bond cleavage was competitive with carbon to sulfur bond cleavage.

As a reductant, superoxide[12] could donate a single electron to generate a radical anion from tetrabutylammonium *p*-nitrobenzenesulfonate (**17**) or *N,N*-diethyl-*p*-nitrobenzenesulfonamide (**18**). Subsequent reaction of these radical anions with oxygen could lead to the observed desulfurized products. Alternatively, potassium superoxide may merely be functioning as a base catalyzing the hydrolysis of tetrabutylammonium *p*-nitrobenzenesulfonate or *N,N*-diethyl-*p*-nitrobenzenesulfonamide. Reaction of potassium hydroxide with *p*-nitrobenzenesulfonate in benzene with 18-crown-6 did lead to a low yield (5 %) of *p*-nitrophenol. However, formation of *p*-nitrophenol (Table 2) was also observed when *N,N*-diethyl-*p*-nitrobenzenesulfonamide (**18**) was treated with sodium naphthalenide or samarium iodide followed by bubbling of oxygen through the reaction solution. This reactivity is consistent with initial reduction of the *N,N*-diethyl-*p*-nitrobenzenesulfonamide (**18**) to a radical anion. Subsequent reaction with molecular oxygen would then be expected to result in desulfurization and formation of phenol.

Intramolecular attack of alkoxy radicals was chosen as a model for the type of chemistry accessible to metal–oxo complexes which are often electrophilic and have radical character. Pursuit of such a chemical model was based on the C–S bond cleavage observed by Speckamp[17] and more recently by Motherwell[18] in arylsulfone, arylsulfonate, and arylsulfonamide model systems. Carbon-centered radicals attacked the *ipso* carbon attached to the sulfur via five- and six-membered intramolecular cyclic transition states. Related systems (Table 3) were therefore examined where an oxygen-centered radical replaced the carbon-centered radical.

The model chemistry for the metal–oxo species was designed to exploit intramolecular *ipso* attack of an oxygen-centered radical on the aryl carbon atom bonded to the sulfonic acid sulfur. Three alcohols (Table 3), mono(4-methylbenzenesulfonyl)-1,2-ethanediol (**19**), 2-(phenylsulfonyl)ethanol (**20**), and catechol monotosylate (**21**) were chosen as alkoxy radical precursors. 2-(Phenylsulfonyl)ethoxy radical was generated to assess the possible reaction products resulting from intramolecular *ipso* attack via a five-membered cyclic transition state. Intramolecular *ipso* attack via a six-membered cyclic transition state was planned for the mono(4-methylbenzenesulfonyl)-1,2-ethanediol alkoxy radical and catechol monotosylate alkoxy radical.

Photolysis[19] (Table 3) of mono(4-methylbenzenesulfonyl)-1,2-ethanediol (**19**) in the presence of mercuric oxide and iodine in benzene yielded (4-methylbenzenesulfonyl)-2-

phenoxyethanol. Apparently, an alkoxy radical was generated as evidenced by phenyl ether formation although intermolecular reaction of the alkoxy radical was preferred relative to intramolecular *ipso* attack. No products indicative of alkoxy attack on the aromatic ring of the arylsulfonate were formed when carbon tetrachloride replaced benzene as solvent. Mono(4-methoxybenzenesulfonyl)-1,2-ethanediol and mono(mesityl)-1,2-ethanediol were also prepared and photolyzed in the presence of mercuric oxide and iodine. Alkoxy radical attack on the aromatic ring was not observed.

Phenyl ether formation was the primary product of photolysis (Table 3) of 2-(phenylsulfonyl)ethanol (**20**) in the presence of mercuric oxide and iodine in benzene. A small amount of 2,3-dihydro-1,4-benzoxathiin-4,4-dioxide was formed indicating intramolecular attack on aromatic carbon *ortho* to the sulfonic acid. This product became the dominant product when the photolysis was carried out in carbon tetrachloride. A small amount of iodomethyl phenyl sulfone was also formed in this reaction. Generation of this product is consistent with loss of formaldehyde from the initially formed alkoxy radical followed by reaction of the resulting radical with iodine. Nitration instead of either *ipso* or *ortho* attack of catechol alkoxy radical was observed when catechol monotosylate **21** was reacted with ceric ammonium nitrate (Table 3). When the tosylate was reacted with potassium ferricyanide under biphasic conditions,[20] insoluble polymeric material was produced.

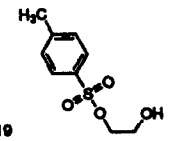
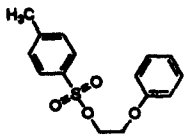
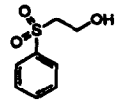
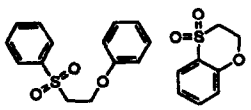
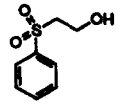
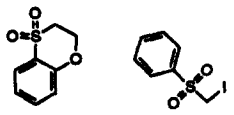
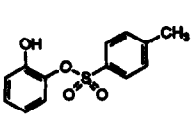
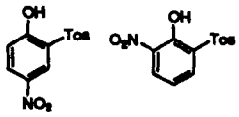
Microbial desulfurization of arylsulfonates may have followed a mechanistic pathway dictated by the electronic characteristics of arylsulfonates. As an electron withdrawing substituent, a sulfonate would be expected to deactivate the aromatic ring towards attack of electrophiles. This is illustrated in the model reactions with mono(4-methylbenzenesulfonyl)-1,2-ethanediol alkoxy radical and 2-(phenylsulfonyl)ethoxy radical run in benzene where intermolecular reaction of the alkoxy radicals with benzene solvent predominates over intramolecular reaction of the alkoxy radicals. Some *ortho* attack was observed which suggests that other factors may also play a role in the absence of *ipso* attack.

Single electron reduction of the arylsulfonate by superoxide or another reductant followed by reaction with molecular oxygen appears to be more compatible with the intrinsic electronic characteristics of an aromatic sulfonate. Nonetheless, an additional nitro substituent was necessary to achieve reduction of the arylsulfonate in the chemical model reaction. It is possible that the enzyme which catalyzes desulfurization of arylsulfonates can polarize the aromatic ring upon binding of substrate to expedite subsequent reduction. Polarization of the aromatic ring by the nitro substituent in the chemical model might mimic polarization of the aromatic ring that occurs at the enzyme active site.

Opportunities for biocatalysis

Biocatalytic desulfurization of arylsulfonates indicates that it is possible to effect the cleavage of bonds consisting of aryl carbon and sulfonic acid sulfur under mild reaction conditions. This reactivity sharply contrasts with the high temperatures and extreme alkaline conditions which have previously been used for chemically converting benzenesulfonate (**1**), *m*-benzenedisulfonate, naphthalene-1-sulfonate (**7**) and naphthalene-2-sulfonate (**6**) into phenol,

Table 3. Reaction of arylsulfonates and arylsulfones with alkoxy radicals

Organosulfur Starting Material	Reaction Conditions	Isolated Products
 19	HgO/I_2 C_6H_6 hv 12 h, RT	 15%
 20	HgO/I_2 C_6H_6 hv 12 h, RT	 14% 3%
 20	HgO/I_2 CCl_4 hv 12 h, RT	 10% 2%
 21	$Ce(NH_4)_2(NO_2)_6$ CH_3CN RT, 30 min	 11% 18%

resorcinol, α-naphthol and β-naphthol. Hock processes for conversion for cumene into phenol and *m*-diisopropylbenzene into resorcinol employ comparatively mild reaction conditions and have largely displaced the older route for fusion of benzenesulfonate and *m*-benzenedisulfonate with caustic soda to form phenol and resorcinol.[1a] As evidenced by Table 1, *K. oxytoca* KS3D can catalyze desulfurization of benzenesulfonate, naphthalene-1-sulfonate and naphthalene-2-sulfonate with formation, respectively, of phenol, α-naphthol, and β-naphthol. This suggests that a biocatalytic process could be an option for the manufacture of these phenols. Developing an understanding of the biocatalytic desulfurization might also lead to new reaction conditions for more environmentally compatible, chemical conversions of arylsulfonates into phenols.

Biocatalytic desulfurization of arylsulfonates is also relevant to the ongoing search for methods that can remove sulfur from coal without significant reduction of the oxidizable carbon content of the fossil fuel. Coal desulfurization techniques often use dibenzothiophene as a model compound since this molecule is structurally representative of the sulfur-containing organic component of coal. Chemical methodology is now available for reductive cleavage of carbon–sulfur bonds in dibenzothiophene giving rise to biphenyl and sulfide.[21] Biocatalytic desulfurization of dibenzothiophene can also lead to biphenyl formation.[22] In another chemical approach, dibenzothiophene is first oxidized to the sulfone which is then converted into 2-hydroxybiphenyl and sulfite by alkali fusion.[2] Biocatalytic desulfurization of dibenzothiophene by *Rhodococcus rhodochrous* IGTS8[23] and other microbes[23a,24] has also been observed to lead to 2-hydroxybiphenyl although the mechanism[23c] appears to differ from the abiotic, chemical route.

In both the chemical and biocatalytic desulfurization of dibenzothiophene, conversion of arylsulfonates such as biphenyl-2-sulfonate to phenols is an essential step. Notably, *K. oxytoca* KS3D has been discovered to catalyze the conversion of biphenyl-2-sulfonate (**8**) (Table 1) into 2-hydroxybiphenyl. Elaboration of the mechanism of arylsulfonate desulfurization catalyzed by *K. oxytoca* KS3D might therefore provide inspiration for new chemical methods for coal desulfurization. Alternatively, a heterologous, coal-desulfurizing microbe might be constructed by introduction of genes isolated from a variety of microbes such as *K. oxytoca* KS3D into a single host. Each introduced gene would encode an enzyme which catalyzed an individual step required for desulfurization. The resulting heterologous biocatalytic desulfurization may be mechanistically related but more efficient than that found in *R. rhodochrous* IGTS8 or may follow a different route modeled after chemical desulfurization involving biphenyl-2-sulfonate intermediacy. For either route, the gene(s) encoding the enzyme(s) responsible for desulfurization of arylsulfonates in *K. oxytoca* KS3D could play a key role.

Experimental Section

General chemistry

^{1}H NMR Spectra were recorded at 300 MHz on a General Electric QE-300 spectrometer. Chemical shifts for ^{1}H NMR are reported (in parts per million) relative to tetramethylsilane [$(CH_3)_4Si$, $\delta = 0.0$ ppm]. ^{13}C NMR Spectra were recorded at 75 MHz on a General Electric QE-300 spectrometer. Chemical shifts for ^{13}C NMR are reported (in parts per million) relative to $CDCl_3$ ($\delta = 77.2$ ppm). Low resolution electron impact (EI, 70 eV) and chemical ionization (CI, 70 eV) mass spectra (MS) were recorded on a Finnigan 4000 mass spectrometer, and high resolution mass spectra (HRMS) were recorded on Kratos MS50 or MS25 mass spectrometers. Silica gel 60 (230–400 mesh, 40–63 μm, E. Merck) was used for flash chromatography. Radial chromatography was carried out with a Harrison Associates Chromatotron, using a 1 mm layer of silica gel 60 PF_{254} containing gypsum (E. Merck). Biphenyl 2-sulfonate was prepared by literature procedure.[2e] All other sulfonates, phenols and reagents were purchased from Aldrich or Lancaster and used as received. [^{18}O]-O_2 (95 %) and [^{18}O]-H_2O (95 %, normalized) were obtained from Icon Services. Benzene, ethyl acetate, hexanes and methylene chloride were distilled from calcium hydride. Acetonitrile was distilled from phosphorus pentoxide and stored over 4 Å sieves.

General biology

The bacterial strain used was *Klebsiella oxytoca* KS3D. Liquid arylsulfonate medium contained dibasic sodium phosphate (42 mM), monobasic potassium phosphate (22 mM), ammonium chloride (19 mM), sodium chloride (10 mM), magnesium chloride (1 mM), glucose (23 mM) and an arylsulfonate (1 mM). Serial dilution cultures were incubated at 30 °C in a G76 water bath shaker (New Brunswick Scientific) and large scale cultures were incubated at 30 °C in a G25 incubator shaker. Extracts were analyzed on a Hewlett Packard HP5890A gas chromatograph. Extract components were separated by gas chromatography on a 15 m, 0.25 mm (i.d.) SE-52 capillary column. Products were characterized by coinjection with authentic samples and by GC–MS analysis on a Hewlett Packard 5970 mass spectrometer.

Microbial desulfurization of arylsulfonates

Arylsulfonate medium was transferred in aliquots (5 mL) to five sterile test tubes. Serial dilutions (100-fold) of *K. oxytoca* KS3D were then prepared in these tubes. Inoculated solutions were cultured in an incubated shaker at 30 °C. Bacterial growth in the tube having the highest dilution of the initial inoculant was diluted by 10^6 and used as the inoculant (50 μL) for 50 mL of arylsulfonate medium in a 250 mL Erlenmeyer flask. Incubation of this culture at 30 °C until the growth solution was cloudy was followed by centrifugation of the growth at 10,000 *g* for 5 min. The culture supernatant was acidified with 5 mL of 1

M HCl and then extracted with ethyl acetate (50 mL). Washing the organic solution with brine and drying over sodium sulfate was followed by removal of the ethyl acetate *in vacuo*. The residue, after dissolving in ethyl acetate (250 μL), was analyzed by GC and GC–MS.

Microbial desulfurization in the presence of [^{18}O]-labeled oxygen[7,13b,25]

A 250 mL Erlenmeyer flask containing 50 mL of sterile *p*-toluenesulfonate medium was sealed with a septum. The flask was subsequently evacuated followed by introduction of nitrogen (99.999 %). This cycle was repeated three times. After evacuating a fourth time, [^{18}O]-labeled oxygen was added to the flask using a gas-tight syringe. An inoculant (100 μL) prepared as described previously was then added via sterile syringe. After growth at 30 °C for five days, the cloudy suspension culture was analyzed for *p*-cresol formation as previously described. The isotopic composition of the *p*-cresol produced by the microbial desulfurization of *p*-toluenesulfonate was determined by GC–MS analysis: m/z (rel. int.) EI 107 (100), 108 (87), 109 (35), 110 (21). Incorporation of ^{18}O was indicated by comparison with the GC–MS of unlabeled *p*-cresol: m/z (rel. int.) EI 107 (100), 108 (88), 109 (5.44), 110 (0.08).

Microbial desulfurization in the presence of [^{18}O]-labeled water

p-Toluenesulfonate medium was transferred in aliquots (5 mL) to five sterile test tubes. Serial dilutions (100-fold) of *K. oxytoca* KS3D were then prepared in these tubes. After growth at 30 °C, an aliquot (50 μL) from the 10^8 dilution culture was used to inoculate a second, 100-fold serial dilution. The test tube corresponding to the 10^6 dilution of this series contained 2 mL of *p*-toluenesulfonate medium and 0.5 mL of 95 % enriched [^{18}O]-water. After culturing in the isotopically enriched *p*-toluenesulfonate medium, *p*-cresol was isolated by standard procedure and analyzed by GC–MS: m/z 107 (100), 108 (84), 109 (2), 110 (0.10). No ^{18}O incorporation was observed based on comparison with the GC–MS of a sample of unlabeled *p*-cresol (see above).

Reaction of tetrabutylammonium p-nitrobenzenesulfonate (17) (Table 2) with potassium superoxide[16]

Tetrabutylammonium hydroxide (40 %, in water) was added to a solution of *p*-nitrobenzenesulfonyl chloride in methylene chloride. The biphasic mixture was stirred for 2 h. After extraction of the aqueous phase with methylene chloride, the combined organic solutions were washed with brine and then dried over Na_2SO_4. Removal of solvent *in vacuo* yielded the tetrabutylammonium salt: ^{1}H NMR ($CDCl_3$) δ 1.00 (t, 12H, J = 7.3 Hz), 1.46 (m, 8H), 1.67 (m, 8H), 3.29 (m, 8H), 8.08 (d, 2H, J = 8.8 Hz), 8.19 (d, 2H, J = 8.8 Hz); ^{13}C NMR ($CDCl_3$) δ 13.5, 19.5, 23.8, 58.6, 123.1, 127.3, 147.8, 153.3; MS, m/z (rel. int.) CI 444 (M^-, 12), 259 (52), 202 (100), 186 (17), 127 (46); Anal. calcd for $C_{22}H_{40}N_2O_5S$: C, 59.43; H, 9.07; N, 6.30, found: C, 59.47; H, 9.15; N, 6.28.

Tetrabutylammonium *p*-nitrobenzenesulfonate (0.110 g, 0.25 mmol) and 18-crown-6 (0.132 g, 0.50 mmol) were dissolved in benzene (25 mL) in a 50 mL flask fitted with a drying tube. Potassium superoxide (0.440 g, 6.25 mmol) was added to the mixture which was then stirred for 60 h at room temperature in the absence of light. The reaction mixture was poured into ethyl acetate, then extracted sequentially with 1 M hydrochloric acid solution and brine. The organic solution was dried (Na_2SO_4) and the solvent was removed *in vacuo* to afford a white solid that was redissolved in ethyl acetate (5 mL). This organic solution was extracted three times with 1 M NaOH solution (10 mL). The combined base extractions were neutralized with concentrated hydrochloric acid and extracted twice with ethyl acetate (10 mL). The combined organic extracts were washed with brine and dried (Na_2SO_4). The solvent was removed to give 0.019 g of *p*-nitrophenol (0.14 mmol, 55 %), which was found to be spectroscopically identical to a sample of authentic material.

Reaction of N,N-*diethyl*-p-*nitrobenzenesulfonamide (18) (Table 2) with potassium superoxide*

The sulfonamide[26] (0.52 g, 2.0 mmol) and 18-crown-6 (1.0 g, 4.0 mmol) were dissolved in benzene (200 mL) in a 500 mL flask with a drying tube attached. Potassium superoxide (0.57 g, 8.0 mmol) was added to the mixture, which was then stirred for 18 h at room temperature in the absence of light. The reaction mixture was poured into ethyl acetate, then extracted with 1 M hydrochloric acid solution followed by brine. The organic solution was dried (Na_2SO_4) and the solvent was removed *in vacuo* to afford a yellow oil that was purified by flash chromatography (1:9 ethyl acetate:toluene, v/v), to give 0.098 g of *p*-nitrophenol (0.70 mmol, 35 %), 0.066 g of *N,N*-diethyl-*p*-hydroxybenzenesulfonamide[27] (0.029 mmol, 14 %) and 0.041 g of recovered starting material (0.016 mmol, 8 %). For *N,N*-diethyl-*p*-hydroxybenzenesulfonamide: ^{1}H NMR ($CDCl_3$) δ 1.12 (t, 6H, J = 7.1 Hz), 3.19 (q, 4H, J = 7.1 Hz), 6.68 (d, 2H, J = 8.7 Hz), 7.58 (d, 2H, J = 8.7 Hz); ^{13}C NMR ($CDCl_3$) δ 14.2, 42.1, 116.0, 129.3, 130.8, 160.3; MS m/z (rel. int.) EI 229 (M^+, 11), 214 (72), 157 (68), 93 (33), 58 (100); HRMS (EI) calcd for $C_{10}H_{15}NO_3S$ (M^+) 229.0773, found 229.0768.

Stepwise reaction of N,N-*diethyl*-p-*nitrobenzenesulfonamide (18) (Table 2) with samarium iodide followed by oxygen*

The sulfonamide (0.065 g, 0.25 mmol) was dissolved in THF (10 mL) in a dried, 25 mL, three-necked flask equipped with a nitrogen line and a bubbler. The bubbler was initially closed off from the reaction. The solution was freeze–thaw degassed three times, then cooled to –20 °C for 10 min. Samarium iodide (1.25 mmol, as a 0.1 M solution in THF) was added slowly via syringe. After the reaction was stirred for 10 min at –20 °C, the nitrogen line was replaced with a thermometer adapter fitted with a glass tube connected to an oxygen line. The tip of the glass tube was placed in the THF solution, the valve to the bubbler was

opened and oxygen was bubbled through the reaction mixture for 1 h at –20 °C. The reaction mixture was poured into ethyl acetate, then extracted with 1 M hydrochloric acid solution followed by brine. The organic solution was dried (Na_2SO_4) and the solvent was removed *in vacuo*. The residue was purified by flash chromatography (1:9 ethyl acetate:toluene, v/v), affording a *p*-nitrophenol-containing fraction (0.031 g) and 0.006 g of *N,N*-diethyl-*p*-hydroxybenzenesulfonamide (0.026 mmol, 10 %). The *p*-nitrophenol containing fraction was subsequently base extracted to give 0.011 g of *p*-nitrophenol (0.079 mmol, 31 %).

Stepwise reaction of N,N-diethyl-p-nitrobenzenesulfonamide (18) (Table 2) with sodium naphthalenide followed by oxygen

A 0.5 M solution of sodium naphthalenide was prepared by stirring naphthalene (1.54 g, 12.0 mmol) and sodium (0.28 g, 12.0 mmol) in THF (24 mL) for 90 min. The sulfonamide (0.53 g, 2.0 mmol) was dissolved in THF (20 mL) in a dried, 100 mL, three-necked flask equipped with a nitrogen line and a bubbler. The bubbler was initially closed off from the reaction. The solution was freeze–thaw degassed three times, then cooled to –20 °C for 10 min. Sodium naphthalenide (8.0 mmol) was added slowly via syringe. After the reaction was stirred for 10 min at –20 °C, the nitrogen line was replaced with a thermometer adapter fitted with a glass tube connected to an oxygen line. The tip of the glass tube was placed in the THF solution, the valve to the bubbler was opened and oxygen was bubbled through the reaction mixture for 1 h at –20 °C. The reaction mixture was poured into ethyl acetate, then extracted with 1 M hydrochloric acid solution followed by brine. The organic solution was dried (Na_2SO_4), the solvent was removed *in vacuo* and the residue was redissolved in ethyl acetate (25 mL). The solution was base extracted three times with 1 M sodium hydroxide solution. The combined extracts were neutralized with concentrated hydrochloric acid and extracted with ethyl acetate (50 mL). The organic solution was washed with brine, dried (Na_2SO_4) and the solvent was removed *in vacuo* to give a mixture of *p*-nitrophenol and naphthalene. The extract was purified by chromatography (gravity column) using a step gradient (hexanes; 1:49 ethyl acetate:hexanes, v/v; 1:24 ethyl acetate:hexanes, v/v; 1:9 ethyl acetate/hexanes, v/v; 1:4 ethyl acetate:hexanes, v/v) to give 0.035 g of *p*-nitrophenol (0.25 mmol, 12 %). The non-extractable material was purified by flash chromatography using a step gradient (1:9 ethyl acetate:hexanes, v/v; 1:4 ethyl acetate:hexanes, v/v; 1:1 ethyl acetate:hexanes) to afford 0.14 g of starting material (0.53 mmol, 26 %) and 0.005 g of *N,N*-diethyl-*p*-hydroxybenzenesulfonamide (0.021 mmol, 1 %).

Preparation of mono(4-methylbenzenesulfonyl)-1,2-ethanediol (19) (Table 3)[28]

To a solution of ethylene glycol (45 g, 750 mmol), triethylamine (7.5g, 75 mmol) and 4-dimethylaminopyridine (0.075 g, 0.6 mmol) in methylene chloride (75 mL) was added dropwise *p*-toluenesulfonyl chloride (14.3 g, 75 mmol) in methylene chloride (75 mL). The mixture was stirred overnight at room temperature, then extracted with saturated ammonium chloride solution, followed by water and saturated brine. The solution was dried (Na_2SO_4), the solvent was removed *in vacuo* and the remaining mixture was purified by flash chromatography, eluting with a step gradient (toluene; 1:9 ethyl acetate:toluene, v/v; 1:2 ethyl acetate:toluene, v/v) to give 3.10 g of the tosylate as a colorless oil (14.3 mmol, 19 %). ^{1}H NMR ($CDCl_3$) δ 2.44 (s, 3H), 3.79 (m, 2H), 4.13 (m, 2H), 7.35 (d, 2H, J = 8.1 Hz), 7.79 (d, 2H, J = 8.1 Hz); ^{13}C NMR ($CDCl_3$) δ 21.7, 60.8, 71.8, 128.1, 130.1, 132.8, 145.2; MS m/z (rel. int.) CI 217 ($M + H^+$, 100), 107 (11); HRMS (EI) calcd for $C_9H_{12}O_4S$ (M^+) 216.0456, found 216.0447.

Mono(4-methylbenzenesulfonyl)-1,2-ethanediol hypoiodide formation and photolysis in benzene[19]

Mono(4-methylbenzenesulfonyl)-1,2-ethanediol, (0.22 g, 1.0 mmol) was dissolved in benzene (50 mL) in a 100 mL, three-necked flask equipped with a nitrogen line, a vacuum line and a magnetic stir bar. The solution was freeze–thaw degassed three times. Red mercuric oxide (0.22 g, 1.0 mmol) and iodine (0.25 g, 1.0 mmol) were added to the flask. The rapidly stirred, heterogeneous mixture was irradiated with two 100 W light bulbs for 12 h. Insoluble material was filtered away, then washed with ethyl acetate. The combined organic solutions were washed with sodium thiosulfate solution followed by brine and then dried (Na_2SO_4). The solvent was removed *in vacuo* and the remaining material was purified by flash chromatography using a step gradient (1:9 ethyl acetate:hexanes, v/v; 1:2 ethyl acetate:hexanes, v/v), affording 0.045 g of 1-(4-methylbenzenesulfonyl)-2-phenoxyethanol[29] (0.15 mmol, 15 %) and 0.16 g unreacted material (0.71 mmol, 71 %). ^{1}H NMR ($CDCl_3$) δ 2.45 (s, 3H), 4.15 (m, 2H), 4.37 (m, 2H), 6.79 (d, 2H, J = 8.5 Hz), 6.96 (t, 1H, J = 7.4 Hz), 7.26 (dd, 2H, J = 7.4, 8.5 Hz), 7.34 (d, 2H, J = 8.3 Hz), 7.82 (d, 2H, J = 8.3 Hz); ^{13}C NMR δ 21.8, 65.4, 68.3, 114.7, 121.5, 128.1, 129.6, 130.0, 133.0, 145.0, 158.1; MS m/z (rel. int.) EI 292 (M^+, 12), 199 (63), 155 (25), 91 (100); HRMS (EI) calcd for $C_{15}H_{16}O_4S$ (M^+) 292.0769, found 292.0763 .

2-Phenylsulfonylethanol hypoiodide formation and photolysis in benzene

2-Phenylsulfonylethanol (0.19 g, 1.0 mmol) was dissolved in benzene (50 mL) in a 100 mL, three-necked flask equipped with a nitrogen line, a vacuum line and a magnetic stir bar. The solution was freeze–thaw degassed three times. Red mercuric oxide (0.22 g, 1.0 mmol) and iodine (0.25 g, 1.0 mmol) were added to the flask. The rapidly stirred, heterogeneous mixture was irradiated with two 100 W light bulbs for 12 h. Insoluble material was filtered away, then washed with ethyl acetate. The combined organic solutions were washed with sodium thiosulfate solution followed by brine and then dried (Na_2SO_4). The solvent was removed *in vacuo* and the remaining material was purified by flash chromatography using a step gradient (1:9 ethyl acetate:hexanes, v/v; 1:4

ethyl acetate:hexanes, v/v; 1:2 ethyl acetate:hexanes, v/v; 1:1 ethyl acetate:hexanes, v/v) to give 0.036 g of 2-phenoxyethyl phenyl sulfone[30] (0.14 mmol, 14 %), 0.006 g of 2,3-dihydro-1,4-benzoxathiin-4,4-dioxide[31] (0.032 mmol, 3 %) and 0.029 g of unreacted material (0.016 mmol, 16 %), for 2-phenoxyethyl phenyl sulfone: ^{1}H NMR ($CDCl_3$) δ 3.61 (t, 2H, J = 6.0 Hz), 4.35 (t, 2H, J = 6.0 Hz), 6.64 (t, 2H, J = 8.7 Hz), 6.95 (t, 1H, J = 7.3 Hz), 7.23 (dd, 2H, J = 7.3, 8.7 Hz), 7.56 (dd, 2H, J = 6.4, 8.2 Hz), 7.68 (t, 1H, J = 6.4 Hz), 7.95 (d, 2H, J = 8.2 Hz); ^{13}C NMR ($CDCl_3$) δ 51.9, 61.4, 114.4, 121.6, 128.3, 129.3, 129.6, 133.9, 139.9, 157.5; MS m/z (rel. int.) CI 263 (M + H$^+$, 100); EI 262 (M$^+$, 6), 169 (3), 141 (13), 77 (100); HRMS (EI) calcd for $C_{14}H_{14}O_3S$ (M$^+$) 262.0664, found 262.0661.

2-Phenylsulfonylethanol hypoiodide formation and photolysis in carbon tetrachloride

The alcohol (0.19 g, 1.0 mmol) was dissolved in carbon tetrachloride (50 mL) in a 100 mL, three-necked flask equipped with a nitrogen line, a vacuum line and a magnetic stir bar. The solution was freeze–thaw degassed three times. Red mercuric oxide (0.22 g, 1.0 mol) and iodine (0.25 g, 1.0 mmol) were added to the flask. The rapidly stirred, heterogeneous mixture was irradiated with two 100 W light bulbs for 12 h. Insoluble material was filtered away, the solution was washed with sodium thiosulfate solution to remove iodine and the solvent was removed *in vacuo*. The residue was taken up in ethyl acetate, and the insoluble material from the reaction mixture was washed with ethyl acetate. The combined organic solutions were washed with sodium thiosulfate solution followed by brine and then dried (Na_2SO_4). The solvent was removed *in vacuo* and the mixture of products was purified by chromatography (gravity column), eluting with a step gradient (1:9 ethyl acetate:hexanes, v/v; 1:4 ethyl acetate:hexanes, v/v; 1:2 ethyl acetate:hexanes, v/v; 1:1 ethyl acetate:hexanes, v/v; ethyl acetate) to afford 0.005 g of iodomethyl phenyl sulfone[32] (0.018 mmol, 2 %), 0.019 g of 2,3-dihydro-1,4-benzoxathiin-4,4-dioxide (0.010 mmol, 10 %) and 0.029 g of unreacted material (0.016 mmol, 16 %), for iodomethyl phenyl sulfone: ^{1}H NMR ($CDCl_3$) δ 4.46 (s, 2H), 7.60 (m, 2H), 7.70 (t, 1H, J = 7.5 Hz), 7.96 (d, 2H, J = 8.2 Hz); ^{13}C NMR ($CDCl_3$) δ 16.8, 129.1, 129.5, 134.7, 136.1; MS m/z (rel. int.) EI 282 (M$^+$, 13), 141 (26), 125 (50), 77 (100); HRMS (EI) calcd for $C_7H_7IO_2S$ (M$^+$) 281.9213, found 281.9213, for 2,3-dihydro-1,4-benzoxathiin-4,4-dioxide: ^{1}H NMR ($CDCl_3$) δ 3.55 (m, 2H), 4.82 (m, 2H), 6.99 (d, 1H, J = 8.4 Hz), 7.15 (t, 1H, J = 8.2 Hz), 7.45 (m, 1H), 7.82 (dd, 1H, J = 1.8, 8.2 Hz); ^{13}C NMR ($CDCl_3$) δ 50.1, 65.2, 118.8, 122.3, 124.1, 126.4, 134.5, 148.8, 153.9; MS m/z (rel. int.) CI 185 (M + H$^+$, 100); EI 184 (M$^+$, 100), 156 (21), 140 (78); HRMS (EI) calcd for $C_8H_8O_3S$ (M$^+$) 184.0194, found 184.0201.

Preparation of catechol monotosylate (19) (Table 3)[33]

To a solution of catechol (0.55 g, 5.0 mmol) and triethylamine (0.50 g, 5.0 mmol) in methylene chloride, a solution of *p*-toluenesulfonyl chloride (0.95 g, 5.0 mmol) was added in a dropwise fashion over 1 h. The solution was stirred for 12 h, extracted with saturated ammonium chloride solution, washed with water followed by saturated brine and dried (Na_2SO_4). The solvent was removed *in vacuo*, the product mixture was purified by flash chromatography (1:4 ethyl acetate:hexanes, v/v) and the product-containing fraction was purified by flash chromatography, eluting with a step gradient (1:1 toluene:hexanes, v/v; toluene) to afford 0.16 g of catechol monotosylate (0.61 mmol, 12 %). ^{1}H NMR ($CDCl_3$) δ 2.47 (s, 3 H), 5.95 (s, 1H), 6.76 (m, 2H), 7.01 (d, 1H, J = 7.8 Hz), 7.14 (m, 1H), 7.35 (d, 2H, J = 8.3 Hz), 7.76 (d, 2H, J = 8.3 Hz); ^{13}C NMR ($CDCl_3$) δ 21.9, 118.5, 121.0, 123.3, 128.6, 128.7, 130.1, 131.3, 137.3, 146.3, 148.5; MS m/z (rel. int.) CI 265 (M + H$^+$, 100); EI 264 (M$^+$, 49), 155 (85), 109 (37), 91 (100).

Oxidation of catechol monotosylate with CAN (21) (Table 3)[34]

Ceric ammonium nitrate (0.22 g, 0.40 mmol) was added as a solid to a solution of catechol monotosylate (0.011g, 0.40 mmol) in acetonitrile (10 mL), the mixture was stirred for 1 h, then partitioned between equal volumes of ethyl acetate and water. The aqueous solution was extracted with ethyl acetate, the combined organic solutions were washed with brine, dried (Na_2SO_4) and the solvent was removed *in vacuo*. The product mixture was separated by radial chromatography (1 mm plate) (1:9 ethyl acetate/hexanes, v/v; 1:1 ethyl acetate:hexanes, v/v) to afford 0.022 g of 6-nitro-2-(4-methylbenzenesulfonyloxy)phenol (0.071 mmol, 18 %), and 0.014 g of 4-nitro-2-(4-methylbenzenesulfonyloxy)phenol (0.045 mmol, 11 %). For 6-nitro-2-(4-methylbenzenesulfonyloxy)phenol: ^{1}H NMR ($CDCl_3$) δ 2.47 (s, 3H), 6.97 (dd, 1H, J = 7.8, 8.1 Hz), 7.35 (d, 2H, J = 8.3 Hz), 7.56 (dd, 1H, J = 1.2, 8.1 Hz), 7.83 (d, 2H, J = 8.3 Hz), 8.04 (dd, 1H, J = 1.2, 7.8 Hz); ^{13}C NMR δ 21.9, 118.9, 123.6, 128.7, 129.9, 131.8, 132.6, 134.8, 139.2, 146.0, 149.0; MS m/z (rel. int.) CI 310 (M + H$^+$, 100), 293 (4), 280 (6), 157 (15); EI 309 (M$^+$, 7), 155 (65), 91 (100); HRMS (EI) calcd for $C_{13}H_{11}NO_6S$ (M$^+$) 309.0307, found 309.0301. For 4-nitro-2-(4-methylbenzenesulfonyloxy)phenol: ^{1}H NMR ($CDCl_3$) δ 2.49 (s, 3H), 7.12 (d, 1H, J = 9.1 Hz), 7.41 (d, 2H, J = 8.2 Hz), 7.70 (d, 1H, J = 2.6 Hz), 7.81 (d, 2H, J = 8.2 Hz), 8.08 (d, 1H, J = 2.6, 9.1 Hz); ^{13}C NMR ($CDCl_3$) δ 22.0, 118.3, 119.7, 124.4, 128.8, 130.5, 136.3, 140.2, 141.0, 147.3, 154.7; MS m/z (rel. int.) CI 310 (M + H$^+$, 100), 280 (2), 157 (6); EI 309 (M$^+$, 2), 155 (60), 91 (100); HRMS (EI) calcd for $C_{13}H_{11}NO_6S$ (M$^+$) 309.0307, found 309.0301. Anal. calcd for $C_{13}H_{11}NO_6S$: C, 50.48, H, 3.58, found: C, 50.78; H, 3.59.

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