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High yield synthesis of *trans*-azoxybenzene versus 2-isopropoxy-4-nitrobenzoic acid: influence of temperature and base concentration

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This article is dedicated to Prof. José Elguero on the occasion of his 86th birthday

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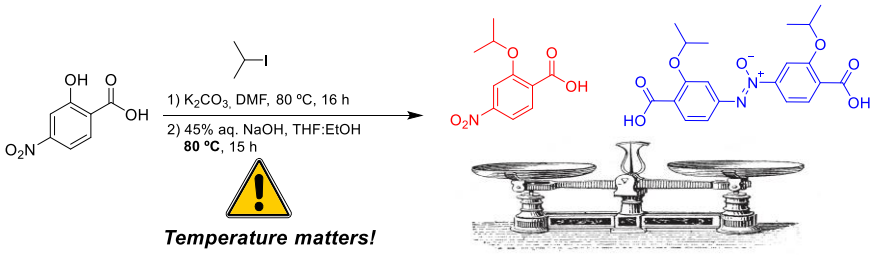
Abstract

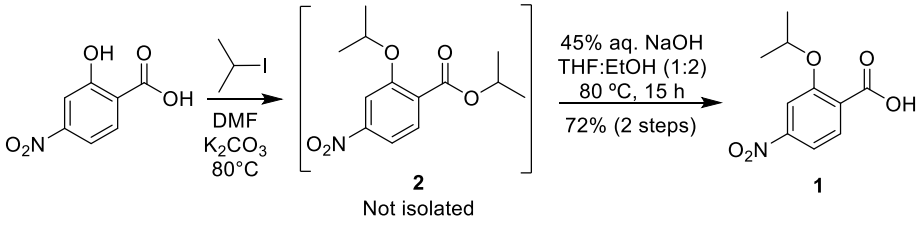
The reported two-step synthesis of 2-isopropoxy-4-nitrobenzoic acid from 2-hydroxy-4-nitrobenzoic acid, using iodopropane/K_2CO_3 and subsequent hydrolysis of the isopropyl 2-isopropoxy-4-nitrobenzoate intermediate with 45% NaOH/THF-EtOH at 80 °C, was reconsidered. (*Z*)-1,2-bis(4-carboxy-3-isopropoxyphenyl)diazene-1-oxide derivative (**3**), which was isolated as main product (92%) of the reaction, was characterized by IR, ^{1}H, ^{13}C, and ^{15}N NMR spectroscopy. The ^{15}N chemical shifts were consistent with the *trans*-configuration for this azoxybenzene derivative. As an alternative, synthesis of 2-isopropoxy-4-nitrobenzoic acid was accomplished in high yield (82%) working at room temperature and using lithium hydroxide instead of concentrate NaOH. Incorrect reaction temperature report or measurement in the published protocol (*J. Org. Chem.* **2011**, *76*, 7040) probably accounts for the discrepancies with our findings.



Keywords: Azoxybenzene; ^{15}N NMR spectroscopy; infrared spectroscopy; 2-isopropoxy-4-nitrobenzoic acid; GIAO

Introduction

During our project dedicated to the design of new dicationic compounds active against kinetoplastid parasites,[1-4] we needed to prepare 2-isopropoxy-4-nitrobenzoic acid (**1**). The two-steps synthesis of this compound had been reported earlier by Adler & Hamilton.[5] Their synthetic approach consisted in the reaction of 2-hydroxy-4-nitrobenzoic acid with an excess of 2-iodopropane to form the di-alkylated intermediate **2**. After an aqueous workup, the crude compound was treated with 45% aqueous NaOH in THF/EtOH at 80 °C to yield pure **1** (72% overall) by acid workup according to the reported procedure (Scheme 1).[5,6]



Scheme 1. Reported two-step synthetic route to 2-isopropoxy-4-nitrobenzoic acid (**1**).[5]

We tried to synthesize **1** following this reported procedure and, to our surprise, compound **1** was not obtained whereas a new compound (**3**), structurally closely related to **1**, was consistently obtained as major product (72–89%) of the synthesis (Scheme 2). This was surprising because the occurrence of this major product was not mentioned by the authors (who reported 72% yield of **1** for this reaction, without any chromatographic purification).[5]

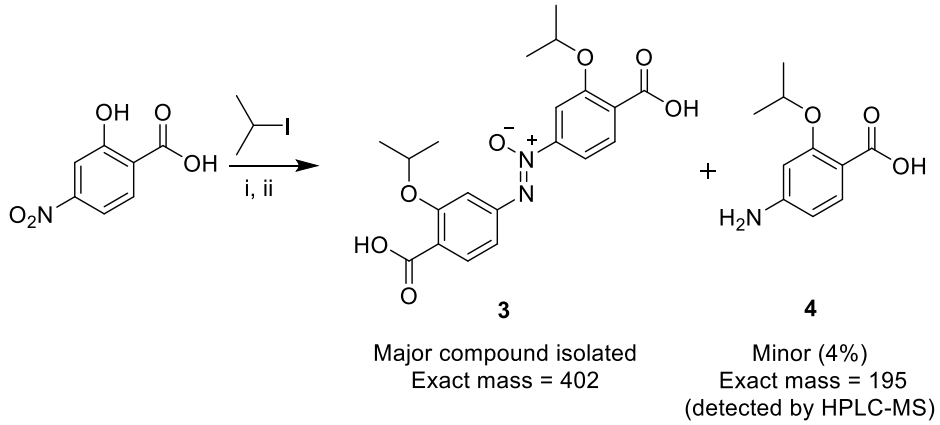
In the present paper, we report the isolation and full spectroscopic characterization by IR, ^{1}H, ^{13}C, and ^{15}N NMR of (*Z*)-1,2-bis(4-carboxy-3-isopropoxyphenyl)diazene-1-oxide (**3**), which is the major product of this synthesis using the reported protocol.[5] We investigated the cause for the different outcome of this reaction with respect to the literature report. Finally, we describe a practical two-step procedure for the synthesis of **1** with 82% overall yield.

Results and Discussion

Synthesis of compound 1

In all our attempts (from 180 mg to 5 g scale) to prepare 2-isopropoxy-4-nitrobenzoic acid (**1**) following the reported[5] two-step protocol shown in Scheme 1, **1** was not detected in the crude reaction mixture whereas azoxybenzene **3** was isolated by crystallization from EtOAc and fully characterized (see below).

Since azoxybenzene **3** was formed in the second step of the reaction, different conditions of hydrolysis of benzoate **2** (i.e. base, concentration, temperature) were tested (Table 1). When the reaction was performed with 45% aq. NaOH at 60 °C and 40 °C (entries 1-2), the expected acid **1** was obtained as major product (70 and 81% detected by HPLC-MS, respectively).



Scheme 2. Main compound isolated (**3**) using the Adler & Hamilton two-step strategy to synthesize **1**.[5] Reagents and conditions: i) K_2CO_3, DMF, 80 °C, 16 h; ii) 45% aq. NaOH, THF:EtOH (1:2), 80 °C, 15 h.

However, **1** was not detected with temperature ≥80 °C (entries 3-4) whereas **3** and a new by-product (*m/z* 195), possibly corresponding to 4-amino-2-isopropoxybenzoic acid (**4**), were obtained predominantly (Scheme 2). When the reaction was performed with 10% aq. NaOH solution (entries 5-7), acid **3** was obtained as major product (approximately 77%) regardless of the temperature used in the reaction. Altogether, azoxybenzene **3** was obtained as major by-product (≥ 19%) in all cases. Of note, the use of 45% aq. KOH instead of 45% NaOH (entry 8) was less efficient in producing azoxybenzene **3**.

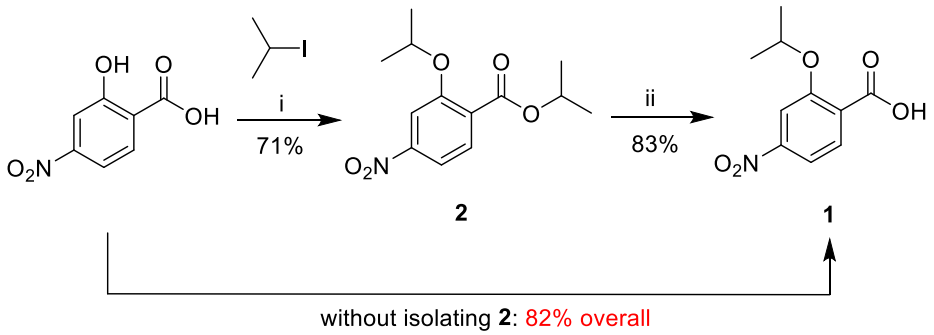
Table 1. Conditions tested and products formed during the hydrolysis of benzoate **2** with concentrated aqueous sodium hydroxide solution

Entry	Conditions[a]		Detected product[b] (%)		
	Base	T (°C)[c]	**1**	**3**	**4**
1	45% aq. NaOH	40	81	19	0
2		60	70	23	7
3		80	0	96	4
4		100	0	49	51
5	10% aq. NaOH	40	76	24	0
6		60	76	24	0
7		100	79	21	0
8	45% aq. KOH	80	39	61	0

[a] Reactions were performed at 1 mmol scale following the same protocol as reported[5] with the conditions indicated in the Table. [b] The products were detected by HPLC–MS. [c] The internal temperature of the reaction mixture was controlled with a thermometer.

When the synthesis was repeated working at room temperature, compound **2** was isolated in 71% yield after silica chromatography (Scheme 3). Treatment of pure benzoate **2** with lithium hydroxide in THF/water at room temperature yielded benzoic acid **1** (83%) after column chromatography. When both steps of the reaction were performed at room temperature, an overall yield of 82% was achieved without the necessity of

isolating intermediate **2**. It should be noted that in the first step of this synthesis, the dialkylated intermediate **2** was detected (HPLC–MS) as a mixture with approximately 15–33% of the final product **1**.



Scheme 3. Synthesis of **3** in two steps. Reagents and conditions: i) K_2CO_3, DMF, rt, 20 h; ii) LiOH, THF:H_2O (1:1), rt, 12 h, then 1M HCl.

Elucidation of compound 3 structure

^{1}H nuclear magnetic resonance spectroscopy. The ^{1}H NMR data of compounds **1**, **2**, and **3** are gathered in Table 2. Compound **1** spectrum shows one septuplet for the $CH(Me)_2$ (H-12) whereas compound **2** shows two septuplets: one accounting for the phenolic H-12 of the O^{i}Pr group at 4.87 ppm (J = 6.0 Hz, 1H) and the other one corresponding to the benzoate O^{i}Pr group that appear downfield at 5.14 ppm (J = 6.0 Hz, 1H, H-15). The ^{1}H NMR spectrum of **3** in DMSO-d_6 is consistent with an unsymmetrical molecule, with two septuplets corresponding to two C-H groups from the isopropoxy substituents at 4.81 and 4.69 ppm, respectively (Table 2). The septuplet at 4.8 ppm, with similar chemical shifts in compounds **1**, **2**, and **3**, was attributed to the phenolic isopropyl H-12. In contrast, the septuplet corresponding to H-12' appears upfield at 4.69 ppm (Table 2). Six aromatic H are observed in compound **3** with multiplicities corresponding to two ABX patterns for H-1,3,4 and H-1',3',4', respectively. Peak assignation was done with the help of Heteronuclear Multiple-bond Correlation (HMBC) and Heteronuclear Simple Quantum Correlation (HSQC) experiments.

^{13}C and ^{15}N NMR spectroscopy and GIAO calculations. The geometry of compound **3** has been optimized at the B3LYP/6-311++G(d,p) computational level[7-9] with the Gaussian program (Gaussian-16, A03).[10] Frequency calculations have been carried out to confirm that the structure obtained corresponds to an energetic minimum. The ^{1}H, ^{13}C, and ^{15}N chemical shifts of compound **3** reported in Table 3 were obtained with the gauge invariant atomic orbitals (GIAO) method[11] (GIAO/B3LYP/6-311++G(d,p) calculations)[7-9,12] and a set of linear correlations[13,14] established earlier to transform absolute shielding values (σ, ppm) into chemical shifts (δ, ppm). The solvent has been simulated using the polarizable continuum model (PCM)[15] with the DMSO parameter in the geometry optimization, frequency and NMR calculations. The Cartesian coordinates of the minimum are gathered in the Supporting Information. These calculated values were in good agreement with the experimental ones (Table 3).

Table 2 .[1]H NMR experimental data of compounds **1**, **2** and **3** in DMSO-d_6

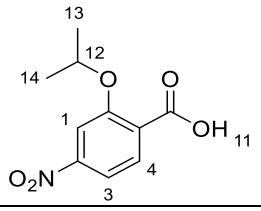
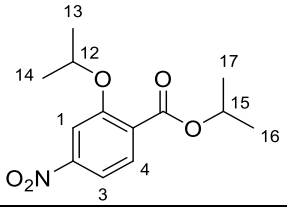
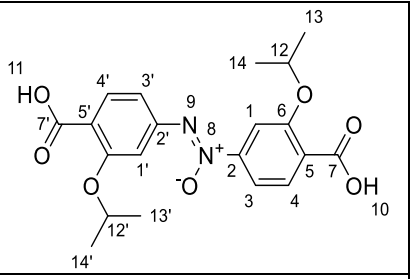
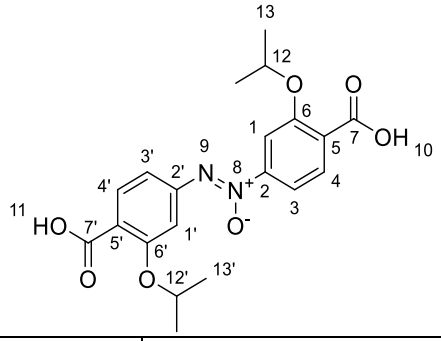
			
Atom	**1** (300 MHz, DMSO-d_6)	**2** (400 MHz, DMSO-d_6)	**3** (500 MHz, DMSO-d_6)
H-1	7.85 (d, *J* = 2.0 Hz, 1H)	7.87 (d, *J* = 2.0 Hz, 1H)	7.91 (d, *J* = 2.0 Hz, 1H)
H-1’			7.79 (d, *J* = 1.8 Hz, 1H)
H-3	7.81 (dd, *J* = 8.4, 2.0 Hz, 1H)	7.82 (dd, *J* = 8.4, 2.0 Hz, 1H)	7.86 (dd, *J* = 8.4, 2.0 Hz, 1H)
H-3’			7.62 (dd, *J* = 8.3, 1.8 Hz, 1H)
H-4	7.75 (d, *J* = 8.4 Hz, 1H)	7.75 (d, *J* = 8.4 Hz, 1H)	7.77 (d, *J* = 8.4 Hz, 1H)
H-4’			7.74 (d, *J* = 8.3 Hz, 1H)
H-11			12.82 (br)
H-12	4.84 (sept, *J* = 6.0 Hz, 1H)	4.87 (sept, *J* = 6.0 Hz, 1H),	4.81 (sept, *J* = 6.1 Hz, 1H)
H-12’			4.69 (sept, *J* = 6.1 Hz, 1H)
H-13,14	1.30 (d, *J* = 6.0 Hz, 6H).	1.31 (d, *J*= 6.0 Hz, 6H)	1.32 (d, *J* = 6.1 Hz, 6H)
H-13’,14’			1.31 (d, *J* = 6.0 Hz, 6H)
H-15		5.14 (sept, *J* = 6.0 Hz, 1H)	
H-16,17		1.30 (d, *J*= 6.0 Hz, 6H)	

Table 3. NMR chemical shifts (ppm) of compound **3**



Atom	**Experimental** (DMSO-d_6)			**Calcd** (GIAO/PCM)			$\lvert\Delta\delta\rvert$ [a]		
	δ_H	δ_C	δ_N	δ_H	δ_C	δ_N	δ_H	δ_C	δ_N
1	7.91	108.9		8.10	106.7		0.19	2.2	
2		149.9			153.5			3.6	
3	7.86	114.0		8.21	114.1		0.35	0.1	
4	7.77	130.8		8.30	134.9		0.53	4.1	
5		127.4			120.4			7.0	
6		156.3			158.4			2.1	

7		166.9			165.3			1.6	
8			-55.1			-60			4.9
9			-49.6			-54.4			4.8
10	12.82			11.17			1.65		
11	12.82			11.25			1.57		
12	4.81	71.7			76.9		0.06	5.2	
13	1.32	21.7		1.38	19.7		0.06	0.2	
1’	7.79	111.4		9.16	106.2		1.37	5.2	
2’		146.3			148.4			2.1	
3’	7.62	116.9		7.41	124.0		0.21	7.1	
4’	7.74	130.77		8.34	134.0		0.6	3.2	
5’		124.0			118.5			5.5	
6’		156.2			158.4			2.2	
7’		167.0			165.5			1.5	
12’	4.69	71.3		5.09	75.9		0.40	4.6	
13’	1.31	21.8		1.56	19.9		0.25	1.5	

[a] Absolute value of the difference between the calculated and experimental chemical shifts

When recording the ^{15}N NMR spectrum of **3** in DMSO-d_6 by g-HMBC using the standard J value of 8 Hz, no clear signals were observed due to high signal to noise ratio. Since the intensity of cross peaks depends on the heteronuclear (^{15}N-^{1}H) long-range coupling constants, we surmised that the J value used in the g-HMBC experiment was not a convenient one.[16] Hence, we calculated the $^{3}J_{NH}$ and $^{4}J_{NH}$ coupling constants of **3** at the B3LYP/6-311++G(d,p) level of theory. As shown in Figure 1, the calculated $^{3}J_{NH}$ and $^{4}J_{NH}$ values are in the range 0.41 to 2.38 Hz, which explains the failure of the first experiment using J = 8 Hz. In the repeated g-HMBC experiment optimized for J = 2.0 Hz, two cross-peaks at -55.1 and -49.6 ppm were observed that are consistent with the chemical shifts of N-8 (N^{+}–O^{-}) and N-9, respectively. These chemical shifts were in good agreement with the calculated values (Table 3) and the reported values for *trans*-azoxybenzene (-54.1 and -46.7 ppm, respectively)[17] and 4’-substituted *trans*-azoxybenzenes.[18,19]

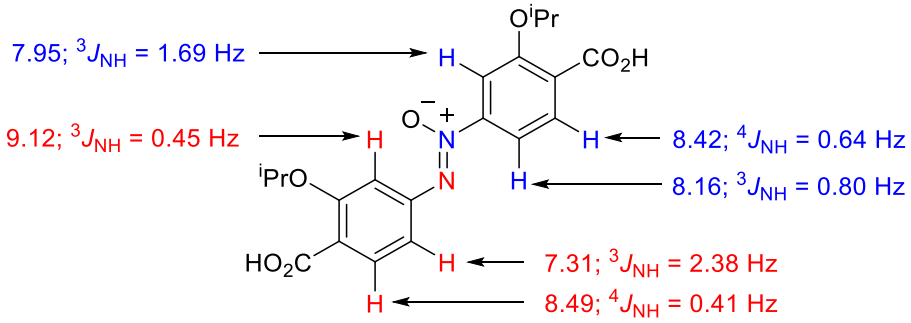


Figure 1. Calculated $^{3}J_{NH}$ and $^{4}J_{NH}$ coupling constants and chemical shifts of **3**

Infrared spectroscopy. The IR spectrum of **3** was recorded from 4000 to 400 cm^{-1} on a FT-IR spectrometer fitted with a diamond single-bounce ATR. A broad band at 2971 cm^{-1} (COO-H) and a strong band at 1682 cm^{-1} (C=O) are indicative of a dicarboxylic acid derivative. The antisymmetric stretching (ν_{as}) of -N=N-O is observed

at 1485 cm^{-1}. The N=N stretch vibration is observed at 1408-1359 cm^{-1} which fits within the region of trans-azoxybenzenes.[20] The strong bands at 1290 – 1237 cm^{-1} are assigned to the CN(O) stretch with the oxygen-coordinated nitrogen. The assignment of the N→O bond stretch is difficult due to overlapping with other vibrations. In the literature, it has been assigned to modes in the 1330-1295 cm^{-1} region for trans-azoxybenzene.[21] In our experiment, this could be related to the strong signal at 1290 cm^{-1}. The N→O stretch contributes to the peak observed at 1136-1077 cm^{-1}. The peak at 1178 cm^{-1} (non-resolved doublet) is indicative of the isopropyl group bonded to an oxygen. A medium band at 1112 cm^{-1} is assigned to phenyl CC stretch/CH in-plane bends in azoxybenzenes. A medium band at 855 cm^{-1} is assigned to azoxybenzenes.

Discussion

Our repeated attempts to synthesize 2-isopropoxy-4-nitrobenzoic acid **1** using the two-step protocol reported previously led to the formation of the unknown (*Z*)-1,2-bis(4-carboxy-3-isopropoxyphenyl)diazene-1-oxide (**3**) almost exclusively. Even though **1** was obtained as minor product of the synthesis in some cases, we were unable to reproduce the reported results. This was disconcerting because this major product was not even mentioned by the authors who reported an intriguing 72% yield of **1**, without any chromatographic purification, for this reaction.[5]

The conversion of nitrobenzenes to azoxybenzenes by heating nitro derivatives with alkaline solution such as alcoholic sodium or potassium alkoxide has been known for more than one hundred years ago.[22,23] The formation of azoxybenzene is thought to occur through the condensation of an aryl nitroso with an aryl hydroxylamine formed *in situ* upon reduction of nitroarenes (Figure 2). Recently, Wei and Shi showed that nitrobenzenes could be selectively reduced to azoxybenzenes in 74% yield with alcohols (1-propanol> ethanol) as the hydrogen source and KOH as the promoter (e.g. octane/1-propanol/50 °C/24 h). With these conditions, sodium hydroxide appeared to be less effective.[24] Accordingly, the harsh conditions used in the hydrolysis step by Adler & Hamilton (45% aq. NaOH/ THF–EtOH/ 80 °C/ 15 h) were highly compatible with the formation of azoxybenzene as reported in the literature.[22-25] Our experiments confirmed that azoxybenzene **3** is a major by-product of this synthesis when concentrated aqueous solution (10% or 45%) of sodium hydroxide in THF-EtOH is used for the hydrolysis step. However, compound **1** was obtained in reasonable yield (approximately 75% detected by HPLC–MS of the crude reaction mixture) when lower temperatures were used (i.e. 40 and 60 °C). Interestingly, and in contrast to the findings of Wei and Shi,[24] the protocol reported here using 45% NaOH/THF-EtOH/80 ºC was more efficient than KOH to produce the azoxybenzene derivative.

Two more synthetic protocols to prepare **1** have been reported in the literature posterior to Adler's work.[6,26,27] These three-steps syntheses consist in the protection of 2-hydroxy-4-nitrobenzoic acid as methyl ester followed by alkylation of the 2-OH group with 2-propanol under Mitsunobu conditions[26] or with 2-bromopropane in the presence of base (e.g. K_2CO_3).[27] Then, the methyl ester is hydrolysed with aqueous NaOH working at room temperature or 65 °C to give the desired product **1** with 63% and 82% overall yields, respectively. These synthetic protocols, which use lower temperature for the hydrolysis step with aqueous NaOH, are consistent with our own observations. Hence, the most probable explanation for the discrepancy between our results and the reported ones is an inadequate measurement (or report) of the reaction temperature in Adler & Hamilton's work. These results underscore the importance of accurate internal temperature control during the hydrolysis step when concentrated aqueous NaOH is used.

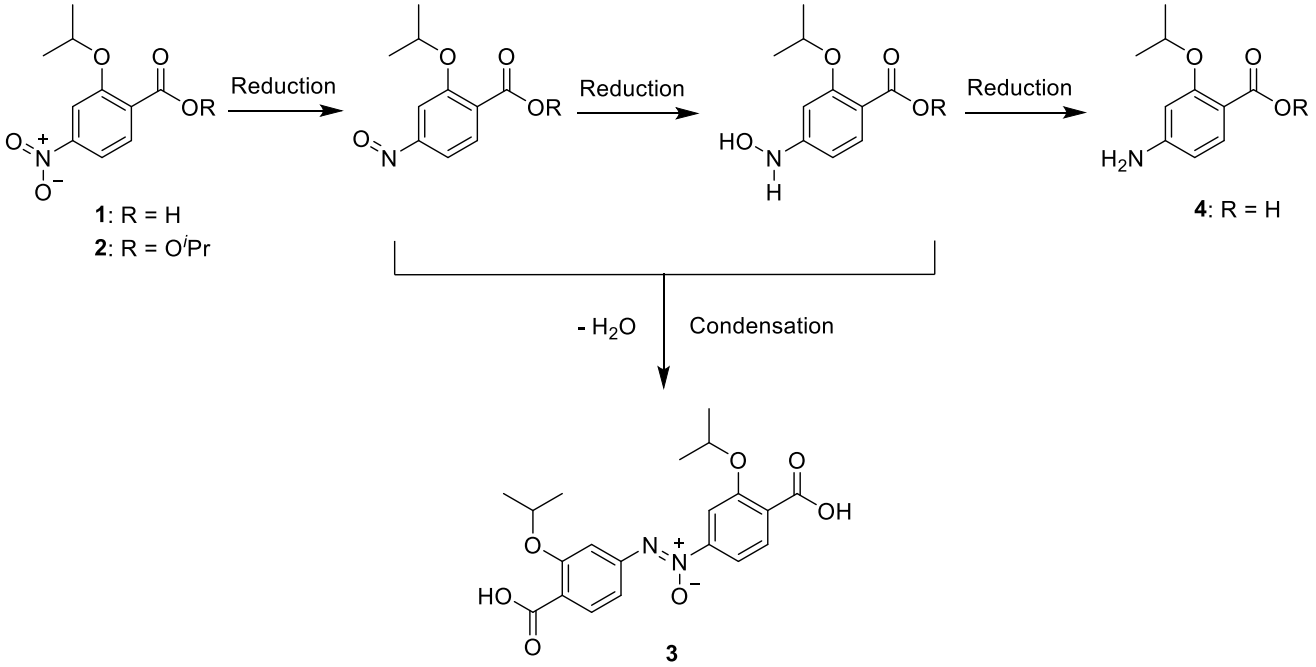


Figure 2. Putative mechanism for the formation of azoxybenzene **3**.

Conclusions

To conclude, we have identified and fully characterized azoxybenzene **3** as main product (92%) of the synthesis of 2-isopropoxy-4-benzoic acid (**1**) using the harsh hydrolysis conditions (45% aq. NaOH/ THF–EtOH/ 80 °C/ 15 h) reported earlier.[5] This synthetic protocol may be useful for the gram scale synthesis of 2-alkoxy trans-azoxybenzene derivatives. The use of GIAO/density-functional calculations to predict chemical shifts and coupling constants was useful in the elucidation of the NMR spectra of **3**, as was shown previously.[16]
Alternatively, the synthesis of **1** was achieved successfully in high yield (82% overall) working at room temperature with a two-step procedure using lithium hydroxide as base instead of concentrated NaOH/EtOH-THF.

Experimental Section

General. ^{1}H NMR and ^{13}C NMR were recorded on a Varian Inova-300 (^{1}H: 300 MHz, ^{13}C: 75.5 MHz), Varian System-500 (^{1}H: 500 MHz, ^{13}C: 125.8 MHz), and Bruker Avance III HD-400 (^{1}H: 400.13 MHz, ^{13}C: 100.62 MHz, ^{15}N: 40.54 MHz) spectrometers using solvent peak as internal reference (DMSO-d_6: 2.49 ppm for ^{1}H and 39.5 ppm for ^{13}C). For ^{15}N NMR, nitromethane (0.00 ppm) was used as external standard. Inverse proton detected heteronuclear shift correlation spectra, (^{1}H-^{13}C) gs-HMQC, (^{1}H-^{13}C) gs-HMBC, and (^{1}H-^{15}N) gs-HMQC, were carried out with the standard pulse sequences to assign the ^{1}H, ^{13}C, and ^{15}N signals. The chemical shifts (δ) and coupling constants (J) are expressed in ppm and hertz respectively. Carbon attribution C, CH, CH_2 and CH_3 were determined by ^{13}C, HMBC and HMQC experiments. InfraRed (IR) spectra were recorded on a PerkinElmer Spectrum Two FT-IR spectrometer fitted with a diamond single-bounce ATR. The spectrum was collected at 4 cm^{-1} spectral resolution. The compound was pressed on the diamond crystal and measured directly without any further sample preparation. Melting points were determined by using a Mettler Toledo MP70 apparatus.

Merck silica gel (0.043-0.063 mm) was used for flash chromatography. Anhydrous solvents and starting materials were directly used as obtained commercially. Aqueous solutions of sodium hydroxide (Panreac® NaOH pellets, pure, pharma grade, 98.8% CoA purity) and potassium hydroxide (Panreac® KOH 85%, (86.2% CoA purity) pellets, USP-NF, Bp, Ph. Eur.) were prepared with distilled water. The solutions were allowed to cool to room temperature and filtered through filter paper before use.

Procedures. Synthesis of **3** following the two-step protocol reported earlier[5] for the preparation of **1** (similar results were obtained in different attempts working at 1 and 27 mmol scale).

To a round-bottom screw cap pressure flask was added sequentially 2-hydroxy-4-nitrobenzoic acid (1099 mg, 6 mmol), anhydrous *N*,*N*-dimethylformamide (12.5 mL), K_2CO_3 (2070 mg, 15 mmol), and 2-iodopropane (1.5 mL, 15 mmol). The flask was capped and the reaction was stirred at 80 °C for 20 h. After being allowed to cool to room temperature, the mixture was diluted with ethyl acetate and washed successively with water (2×) and brine. The organic layer was dried over magnesium sulfate, filtered, and then the solution was evaporated to dryness to obtain a brownish oil. This material was taken up in tetrahydrofuran (5 mL), to which was added ethanol (12 mL) and 45% aqueous NaOH solution (12 mL). This mixture was allowed to stir at 80 °C (i.e. internal temperature) for 15 h. The resulting solution was allowed to cool to ambient temperature and then diluted with distilled water and washed twice with ethyl acetate. The aqueous solution was then acidified using 1 M aqueous HCl and extracted twice with diethyl ether. The combined organic layers were washed with an aqueous saturated sodium chloride solution, dried over magnesium sulfate, filtered, and solvent was removed under vacuum, Recrystallization from ethyl acetate yielded (*Z*)-1,2-bis(4-carboxy-3-isopropoxyphenyl)diazene-1-oxide (**3**) as yellowish solid (1237 mg, 92%). mp 225.7 °C. IR (solid, ν_{max}, cm^{-1}): 2971, 2925, 2869, 2641, 2563, 1682, 1580, 1551, 1485, 1446, 1408, 1387, 1290, 1237, 1178, 1137, 1112, 920. ^1H NMR data appear in Tables 2 and 3. $^{13}C\{^1H\}$ NMR (DMSO-d_6, 101 MHz) δ_C 167.0 (C-7'), 166.9 (C-7), 156.3 (C-6), 156.2 (C-6'), 149.9 (C-2), 146.3 (C-2'), 130.82 (C-4), 130.77 (C-4'), 127.4 (C-5), 124.0 (C-5'), 116.9 (C-3'), 114.0 (C-3), 111.4 (C-1'), 108.9 (C-1), 71.7 (C-12), 71.3 (C-12'), 21.8 (C-13'), 21.7 (C-13). Anal. calcd. for $C_{20}H_{22}N_2O_7$ (403.14): C, 59.70; H, 5.51; N, 6.96. Found: C, 59.83; H, 5.79; N, 6.76.

Synthesis of 2-isopropoxy-4-nitrobenzoic acid (1) using LiOH. Step 1. To a round-bottom flask containing a magnetic stir bar was added sequentially 2-hydroxy-4-nitrobenzoic acid (2.8 g, 15.2 mmol), anhydrous *N*,*N*-dimethylformamide (20 mL), K_2CO_3 (5.24 g, 38 mmol), and 2-iodopropane (3.8 mL, 38 mmol). The reaction was stirred at room temperature for 16 h. The mixture was diluted with dichloromethane and washed with distilled water twice followed by an aqueous saturated sodium chloride solution (brine). The organic layer was dried over magnesium sulfate, filtered, and the solvent was evaporated under vacuum. Column chromatography was performed using hexane/ethyl acetate: 100/0 → 97/3 as elution system, yielding isopropyl 2-isopropoxy-4-nitrobenzoate (**2**) as yellowish oil (2.9 g, 71.4 %). ^1H NMR: see Table 2. $^{13}C\{^1H\}$ NMR (DMSO-d_6, 101 MHz) δ_C 164.8 (C-7), 156.0 (C-6), 149.9 (C-2), 130.7 (C-4), 128.8 (C-5), 115.0 (C-3), 108.8 (C-1), 71.8 (C-12), 68.9 (C-15), 21.52 (C-13, C-14), 21.49 (C-16, C-17). MS (ESI^+) *m/z* 268.37 (M+H). Anal. calcd for $C_{13}H_{17}NO_5$: C, 58.42; H, 6.41; N, 5.24. Found: C, 58.00; H, 6.33; N, 5.36.

Step 2. A Kimax tube was loaded with **2** (300.5 mg, 1.1 mmol), LiOH (135.3 mg, 5.7 mmol) and 16 mL THF/H_2O (1:1). The reaction mixture was stirred at room temperature for 12 h. THF was then evaporated under vacuum and the aqueous solution acidified with 1 M aqueous HCl. The product was extracted with dichloromethane, washed with brine, and dried over magnesium sulfate. Finally, the solvent was removed under vacuum. Recrystallization from ethyl acetate yielded **1** as a light yellow solid (212 mg, 83.4%). mp 142-143 °C (lit.[6] 146-147 °C). HPLC-UV: 100%. ^1H NMR: see Table 2. $^{13}C\{^1H\}$ NMR (DMSO-d_6, 126 MHz) δ 166.7 (C-7), 156.0 (C-6),

149.7 (C-2), 130.8 (C-4), 129.8 (C-5), 115.1 (C-3), 109.4 (C-1), 71.9 (C-12), 21.6 (C-13, C-14). MS (ESI^+) *m/z* 226.1 (M+H). Anal. calcd for $C_{10}H_{11}NO_5 \cdot 0.5H_2O$: C, 51.28; H, 5.16; N, 5.98. Found: 50.89; H, 4.95; N, 5.95.

Alternative protocol for the synthesis of **1** (without isolating the benzoate intermediate): to a stirred solution of 2-hydroxy-4-nitrobenzoic acid (500 mg, 2.7 mmol) in dry DMF (5 mL) was added K_2CO_3 (943 mg, 6.8 mmol) at once. After 5 minutes stirring, 2-iodopropane (0.7 mL, 6.8 mmol) was added dropwise to the mixture. After 16 hours stirring at room temperature, the crude reaction mixture was diluted with EtOAc and extracted with 1M aq. HCl. The organic phase was washed with saturated NaCl solution, dried over Na_2SO_4, filtered and the solvent was evaporated under vacuum to yield a yellowish oil (HPLC > 95%). The crude oil was diluted in a mixture of THF/H_2O (1:1, 10 mL), followed by the addition of LiOH (240 mg, 10 mmol). After 6 hours stirring at room temperature, the crude reaction mixture was diluted with CH_2Cl_2 and extracted with 1M aq. HCl. The organic phase was washed with brine, dried over Na_2SO_4 and evaporated under vacuum. Compound **1** was obtained as yellowish solid (369 mg, 82 %) by recrystallization from EtOAc.

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Supplementary Material

NMR spectra of compounds **1**-**3**. Electronic energy (hartree), number of imaginary frequencies and cartesian coordinates (Å) of compound **3** calculated at B3LYP/6-311++G(d,p)/PCM(DMSO) computational level.

References

1. Dardonville, C.; Nué Martínez, J. J. *Curr. Med. Chem.* **2017**, *24*, 1. http://dx.doi.org/10.2174/0929867324666170623091522

2. Ríos Martínez, C. H.; Nué Martínez, J. J.; Ebiloma, G. U.; de Koning, H. P.; Alkorta, I.; Dardonville, C. *Eur. J. Med. Chem.* **2015**, *101*, 806. http://dx.doi.org/10.1016/j.ejmech.2015.07.013

3. Ríos Martínez, C.; Miller, F.; Ganeshamoorthy, K.; Glacial, F.; Kaiser, M.; de Koning, H.; Eze, A.; Lagartera, L.; Herraiz, T.; Dardonville, C. *Antimicrob. Agents Chemother.* **2015**, *59*, 890. http://dx.doi.org/10.1128/AAC.03958-14

4. Montalvo-Quirós, S.; Taladriz-Sender, A.; Kaiser, M.; Dardonville, C. *J. Med. Chem.* **2015**, *58*, 1940. http://dx.doi.org/10.1021/jm5018303

5. Adler, M. J.; Hamilton, A. D. *J. Org. Chem.* **2011**, *76*, 7040.
http://dx.doi.org/10.1021/jo200917d

6. Azzarito, V.; Prabhakaran, P.; Bartlett, A. I.; Murphy, N. S.; Hardie, M. J.; Kilner, C. A.; Edwards, T. A.; Warriner, S. L.; Wilson, A. J. *Org. Biomol. Chem.* **2012**, *10*, 6469.
http://dx.doi.org/10.1039/C2OB26262B

7. Becke, A. D. *J. Chem. Phys.* **1993**, *98*, 5648.
http://dx.doi.org/10.1063/1.464913

8. Lee, C.; Yang, W.; Parr, R. G. *Phys. Rev. B* **1988**, *37*, 785.
http://dx.doi.org/ 10.1103/PhysRevB.37.785

9. Frisch, M. J.; Pople, J. A.; Binkley, J. S. *J. Chem. Phys.* **1984**, *80*, 3265.
http://dx.doi.org/10.1063/1.447079

10. Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Scalmani, G.; Barone, V.; Petersson, G. A.; Nakatsuji, H.; Li, X.; Caricato, M.; Marenich, A. V.; Bloino, J.; Janesko, B. G.; Gomperts, R.; Mennucci, B.; Hratchian, H. P.; Ortiz, J. V.; Izmaylov, A. F.; Sonnenberg, J. L.; Williams; Ding, F.; Lipparini, F.; Egidi, F.; Goings, J.; Peng, B.; Petrone, A.; Henderson, T.; Ranasinghe, D.; Zakrzewski, V. G.; Gao, J.; Rega, N.; Zheng, G.; Liang, W.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Vreven, T.; Throssell, K.; Montgomery Jr., J. A.; Peralta, J. E.; Ogliaro, F.; Bearpark, M. J.; Heyd, J. J.; Brothers, E. N.; Kudin, K. N.; Staroverov, V. N.; Keith, T. A.; Kobayashi, R.; Normand, J.; Raghavachari, K.; Rendell, A. P.; Burant, J. C.; Iyengar, S. S.; Tomasi, J.; Cossi, M.; Millam, J. M.; Klene, M.; Adamo, C.; Cammi, R.; Ochterski, J. W.; Martin, R. L.; Morokuma, K.; Farkas, O.; Foresman, J. B.; Fox, D. J. Wallingford, CT, 2016.

11. Ruud, K.; Helgaker, T.; Bak, K. L.; Jo/rgensen, P.; Jensen, H. J. r. A. *J. Chem. Phys.* **1993**, *99*, 3847.
http://dx.doi.org/10.1063/1.466131

12. Ditchfield, R.; Hehre, W. J.; Pople, J. A. *J. Chem. Phys.* **1971**, *54*, 724.
http://dx.doi.org/10.1063/1.1674902

13. Silva, A. M. S.; Sousa, R. M. S.; Jimeno, M. L.; Blanco, F.; Alkorta, I.; Elguero, J. *Magn. Reson. Chem.* **2008**, *46*, 859.
http://dx.doi.org/10.1002/mrc.2272

14. Blanco, F.; Alkorta, I.; Elguero, J. *Magn. Reson. Chem.* **2007**, *45*, 797.
http://dx.doi.org/10.1002/mrc.2053

15. Tomasi, J.; Mennucci, B.; Cammi, R. *Chem. Rev.* **2005**, *105*, 2999.
https://doi.org/10.1021/cr9904009

16. Alkorta, I.; Dardonville, C.; Elguero, J. *Angew. Chem. Int. Ed.* **2015**, *54*, 3997.
http://dx.doi.org/10.1002/anie.201412144

17. Schwotzer, W.; Leuenberger, C.; Hoesch, L.; Dreiding, A. S.; von Philipsborn, W. *Org. Magn. Reson.* **1977**, *9*, 382.
https://doi.org/10.1002/mrc.1270090620

18. Masami, S.; Yoshio, T.; Takanori, T.; Terukiyo, H.; Masao, O.; Yuho, T. *Bull. Chem. Soc. Jpn* **1990**, *63*, 702.
https://doi.org/10.1246/bcsj.63.702

19. Mason, J. In *PATAI'S Chemistry of Functional Groups*; Rappoport, Z., Ed.; John Wiley & Sons, Ltd.: 2009.
https://doi.org/10.1002/9780470682531.pat0148

20. Tecklenburg, M. M. J.; Kosnak, D. J.; Bhatnagar, A.; Mohanty, D. K. *J. Raman Spectrosc.* **1997**, *28*, 755.
https://doi.org/10.1002/(SICI)1097-4555(199710)28:10%3C755::AID-JRS143%3E3.0.CO;2-V

21. Gruger, A.; Le Calvé, N. *Spectrochim. Acta Part A* **1972**, *28*, 1253.
https://doi.org/10.1016/0584-8539(72)80095-3

22. Vančik, H. In *Aromatic C-nitroso Compounds*; Springer: Dordrecht, 2013.

23. Suter, C. M.; Dains, F. B. *J. Am. Chem. Soc.* **1928**, *50*, 2733.
https://doi.org/10.1021/ja01397a024

24. Wei, R. P.; Shi, F. *Synth. Commun.* **2019**, *49*, 688.
https://doi.org/10.1080/00397911.2019.1566472

25. Reid, E. B.; Pritchett, E. G. *J. Org. Chem.* **1953**, *18*, 715.
https://doi.org/10.1021/jo01134a017

26. Durcik, M.; Lovison, D.; Skok, Ž.; Durante Cruz, C.; Tammela, P.; Tomašič, T.; Benedetto Tiz, D.; Draskovits, G.; Nyerges, Á.; Pál, C.; Ilaš, J.; Peterlin Mašič, L.; Kikelj, D.; Zidar, N. *Eur. J. Med. Chem.* **2018**, *154*, 117.
https://doi.org/10.1016/j.ejmech.2018.05.011

27. Prabhakaran, P.; Azzarito, V.; Jacobs, T.; Hardie, M. J.; Kilner, C. A.; Edwards, T. A.; Warriner, S. L.; Wilson, A. J. *Tetrahedron* **2012**, *68*, 4485.
https://doi.org/10.1039/c6ob00078a