The 7th International Electronic Conference on Medicinal Chemistry (ECMC 2021)
01–30 NOVEMBER 2021 | ONLINE

Solving a chemical challenge in the synthesis of antiprotozoal agents targeting the DNA minor groove: A high yield synthesis of *trans*-azoxybenzene

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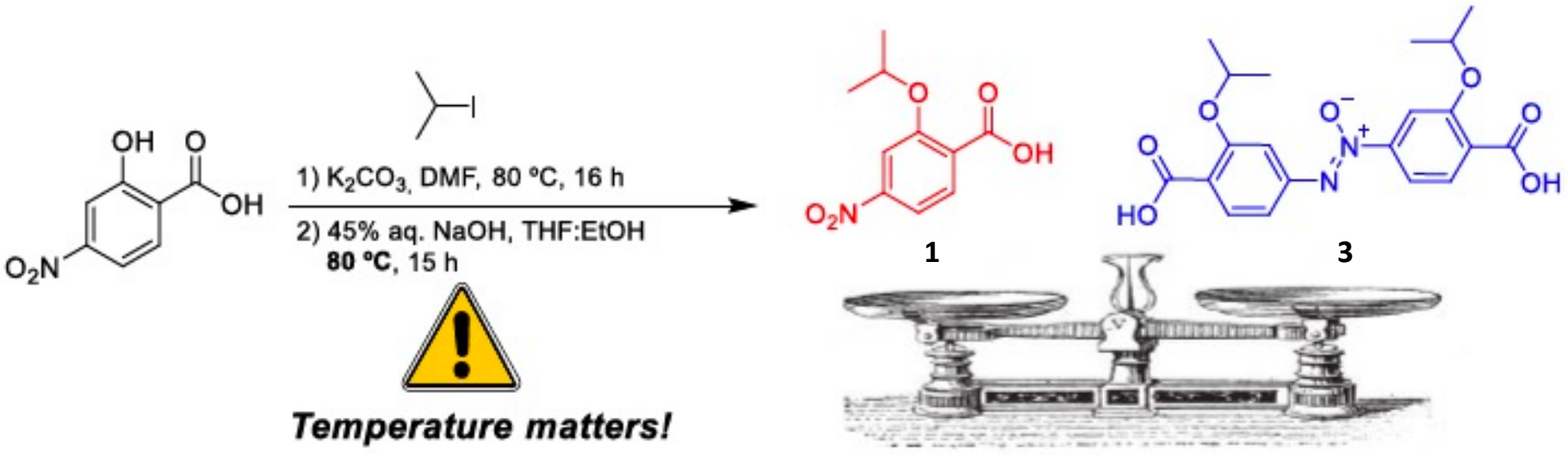


Nué Martinez, J. J.; Alkorta, I.; Dardonville, C. *Arkivoc* **2021**, viii, 265-276.

Abstract:
As part of our research program on the synthesis of new DNA minor groove binders active against kinetoplastid parasites (i.e. *T. brucei*, *T. cruzi*, and *L. donovani*), we needed to prepare 2-isopropoxy-4-nitrobenzoic acid (**1**). A previously reported synthetic approach consisting in the reaction of 2-hydroxy-4-nitrobenzoic acid with an excess of 2-iodopropane had been reported earlier. The di-alkylated isopropyl 2-isopropoxy-4-nitrobenzoate intermediate (**2**), was then saponified using harsh basic conditions (i.e. 45% aqueous NaOH in THF/EtOH at 80 °C) to yield pure **1** (72% overall) by acid workup according to the reported procedure.

However, our efforts to obtain **1** following this protocol were unsuccessful. In its place, (*Z*)-1,2-bis(4-carboxy-3-isopropoxyphenyl)diazene-1-oxide derivative (**3**) was isolated as main product (92%) of the reaction. Full characterization this *trans*-azoxybenzene derivative was carried out by means of IR, ^{1}H, ^{13}C, and ^{15}N NMR spectroscopy.

Adjusting the internal temperature of the saponification step was key to control the outcome of the reaction towards the formation of either products **1** or **3**. We show that working at room temperature and using lithium hydroxide instead of concentrate NaOH is an excellent alternative to achieve the synthesis of the desired 2-isopropoxy-4-nitrobenzoic acid (**1**). On the other hand, working at high temperature (80 °C, as reported previously) is an excellent method for the high-yield synthesis of *trans*-azoxybenzene.

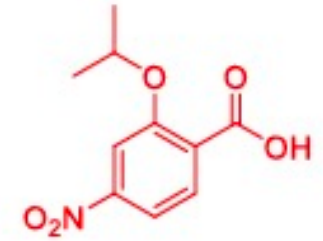
Keywords: Azoxybenzene; DNA-minor groove binder; kinetoplastid parasite; ^{15}N NMR spectroscopy; GIAO

Introduction

Protozoan and helminthic parasitic diseases affect more than 3 billion people worldwide mostly in tropical and subtropical areas. These diseases also have a high prevalence in animals provoking a great health, social and economic loss in the less-developed countries.

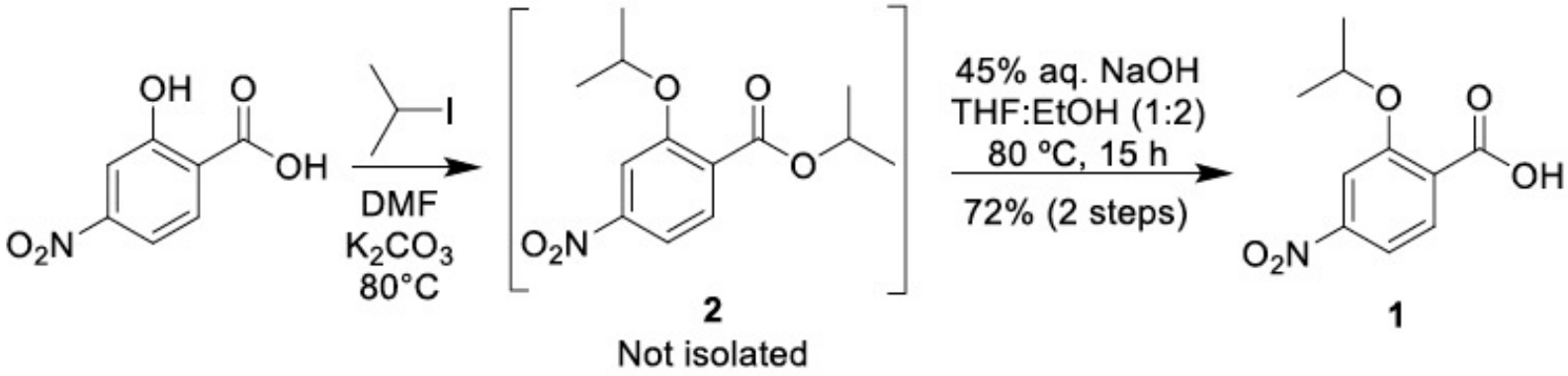
In the last years we have discovered several families of dicationic compounds, bis(2-aminoimidazolinium) and bisguanidinium salts, that act potently *in vitro* and *in vivo* against the protozoan parasites *T. brucei* and *P. falciparum.* This class of dicationic compounds has been shown to interact specifically with the minor groove of DNA.

As part of our project dedicated to the design of new dicationic compounds active against kinetoplastid parasites, we needed to prepare 2-isopropoxy-4-nitrobenzoic acid (**1**).



2-isopropoxi-4-nitrobenzoic acid **1**

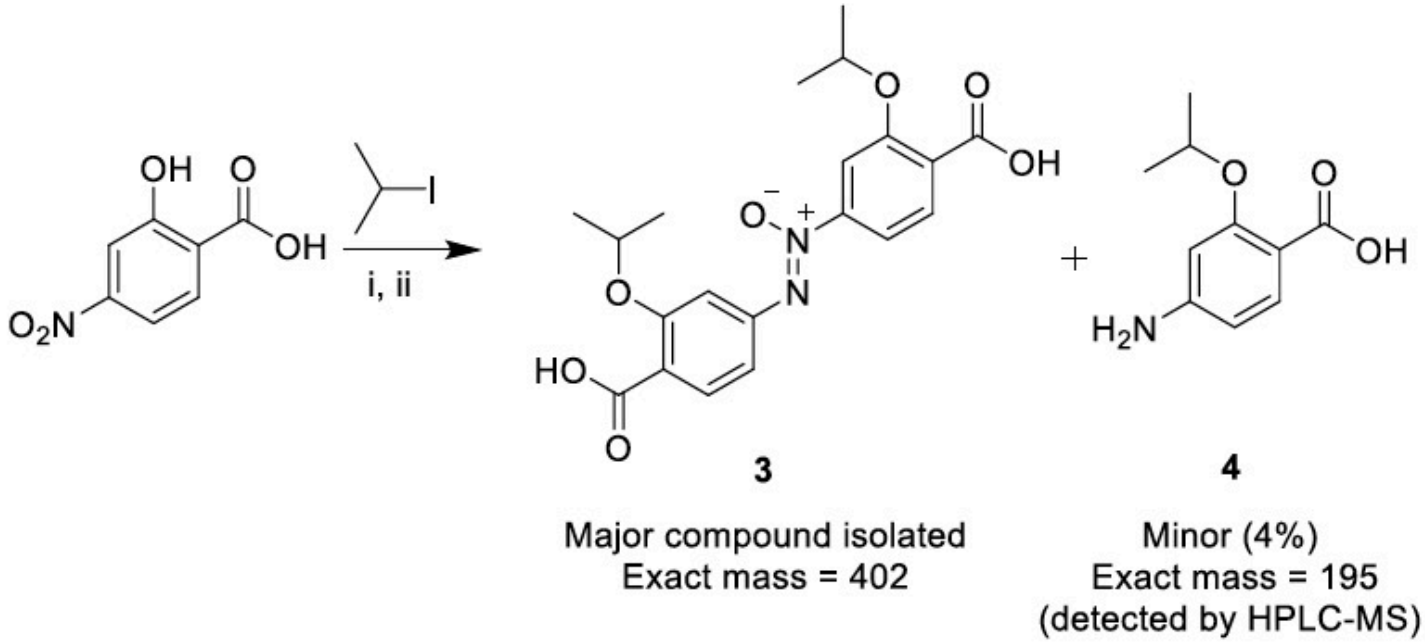
The two-steps synthesis of this compound had been reported earlier by Adler & Hamilton [1]. According to their protocol (Scheme 1), the reaction of 2-hydroxy-4-nitrobenzoic acid with an excess of 2-iodopropane forms the di-alkylated intermediate **2**, which is subsequently treated with 45% aqueous NaOH in THF/EtOH at 80 °C to yield pure **1** without chromatographic purification (72% overall).



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

[1] Adler, M. J.; Hamilton, A. D. *J. Org. Chem.* **2011**, *76*, 7040.

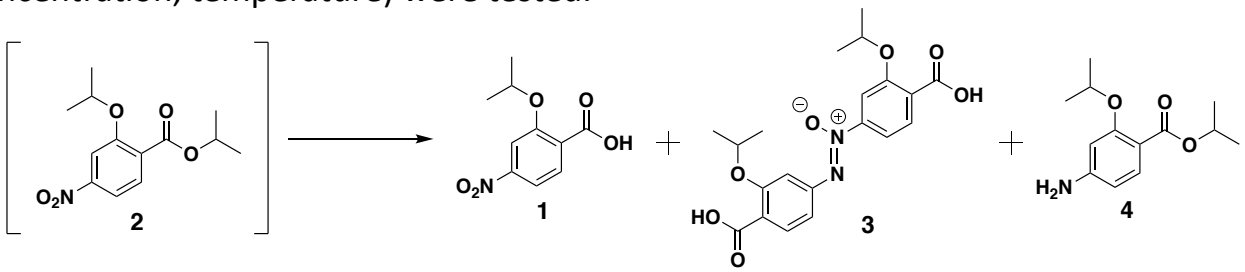
We tried to synthesize **1** following this reported procedure [1] and, to our surprise, compound **1** was not obtained. A new compound (**3**), structurally closely related to **1**, was consistently obtained as major product (72–89%) of the synthesis (Scheme 2). Even though **1** was obtained as minor product of the synthesis in some cases, we were unable to reproduce the reported results.



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

Results and discussion

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.



Scheme 3: Hydrolisis of benzoate **2**.

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 Adler's protocol with the conditions indicated in the Table. [b] The products were detected by HPLC–MS. [c] Thermometer reported temperature.

- With 45% aq. NaOH at 60 °C and 40 °C (entries 1-2), the expected acid **1** was obtained as major product.
- With 10% aq. NaOH solution (entries 5-7), acid **1** was obtained as major product regardless of the temperature.
- Azoxybenzene **3** was obtained as major by-product (≥ 19%) in all cases.
- Using 45% aq. KOH instead of 45% NaOH (entry 8) was less efficient in producing azoxybenzene **3**.

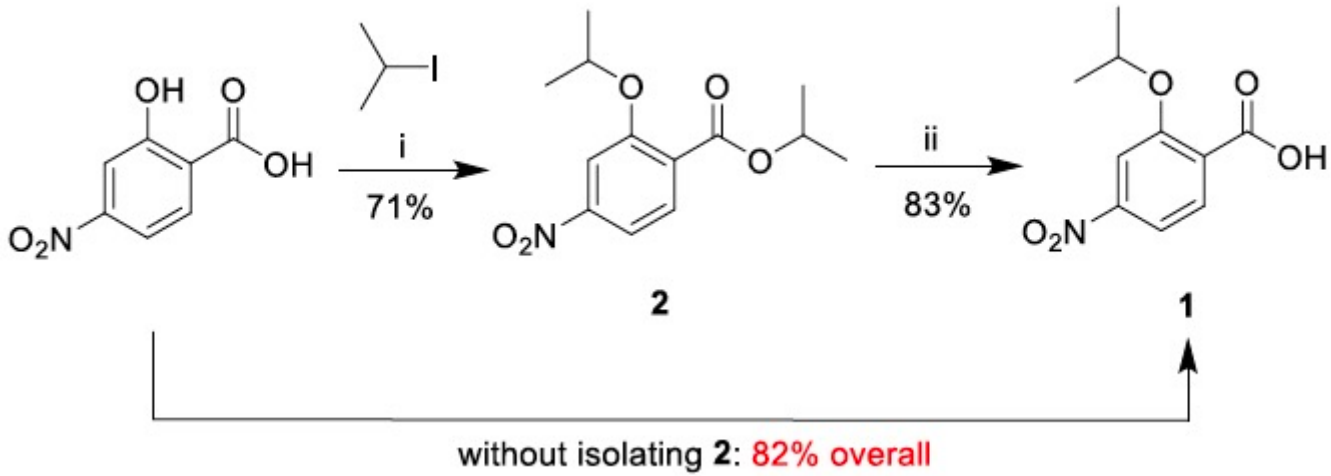
Two more synthetic protocols to prepare **1** have been reported in the literature posterior to Adler's work [2,3]. These synthetic protocols, which use lower temperature for the hydrolysis step with aqueous NaOH, are consistent with our own observations.

Hence, the most probable explanation for the discrepancy between our results (i.e. azoxybenzene **3** as major product when working at 80 °C) and the reported ones is an inadequate measurement (or report) of the reaction temperature in Adler & Hamilton's work [1].

These results underscore the importance of accurate internal temperature control during the hydrolysis step when concentrated aqueous NaOH is used.

[1] Adler, M. J.; Hamilton, A. D. *J. Org. Chem.* **2011**, *76*, 7040.
[2] 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.
[3] 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.

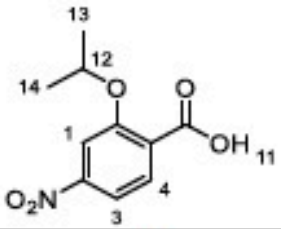
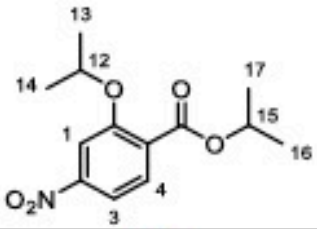
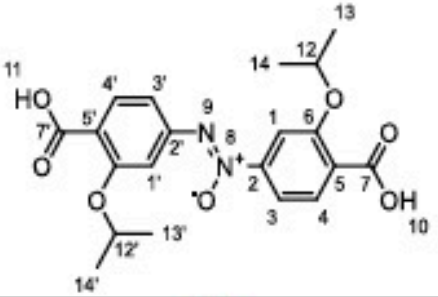
When the synthesis was repeated working at room temperature and using lithium hydroxide in THF/water at room temperature for the hydrolysis step, benzoic acid **1** was isolated in 83% yield after silica chromatography. When both steps of the reaction were performed at room temperature, an overall yield of 82% was achieved without the necessity of isolating intermediate **2** [4].



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.

[4] Nué Martinez, J. J.; Alkorta, I.; Dardonville, C. *Arkivoc* **2021**, viii, 265-276.

Table 2 .^{1}H NMR experimental data of compounds **1**, **2** and **3** in DMSO-d_6

			
	1	**2**	**3**
Atom	(300 MHz, DMSO-d_6)	(400 MHz, DMSO-d_6)	(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-10, H-11			12.82 (br, 2H)
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)	

[4] Nué Martinez, J. J.; Alkorta, I.; Dardonville, C. *Arkivoc* **2021**, viii, 265-276.

The conversion of nitrobenzenes to azoxybenzenes by heating nitro derivatives with alkaline solution has been known for more than one hundred years ago [5,6].

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.

Accordingly, the harsh conditions used in the hydrolysis step by Adler & Hamilton [1] (45% aq. NaOH/ THF–EtOH/ 80 °C/ 15 h) were highly compatible with the formation of azoxybenzene as reported in the literature [5-8].

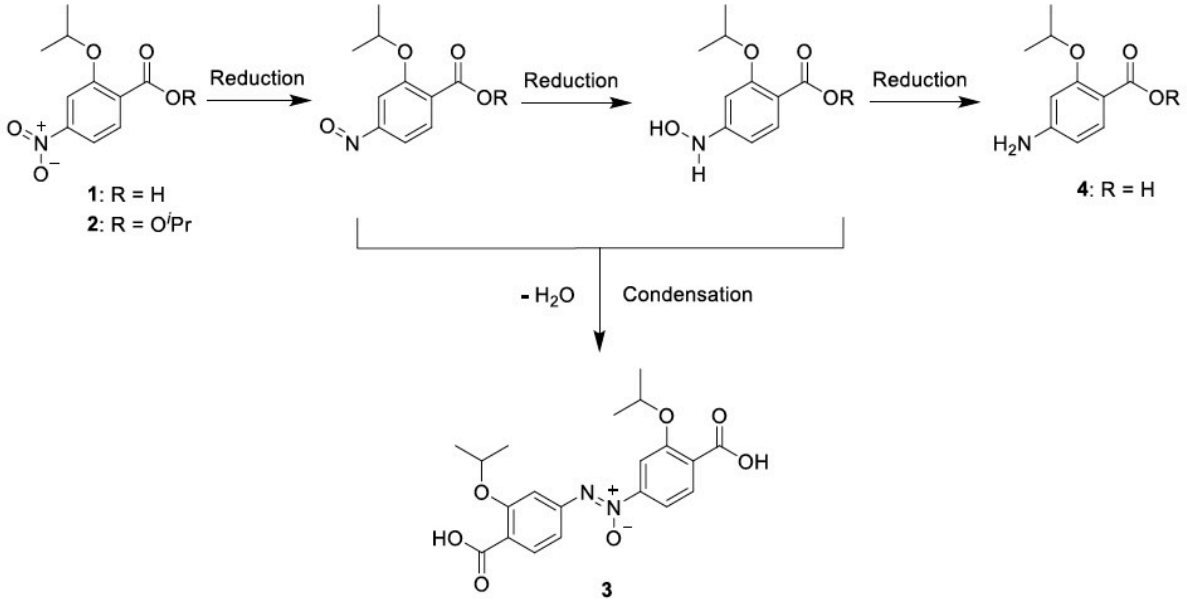


Figure 2. Putative mechanism for the formation of azoxybenzene **3**.

[5] Vančik, H. In Aromatic C-nitroso Compounds; Springer: Dordrecht, **2013**. [6] Suter, C. M.; Dains, F. B. *J. Am. Chem. Soc.* **1928**, 50, 2733. [7] Wei, R. P.; Shi, F. *Synth. Commun.* **2019**, 49, 688. [8] Reid, E. B.; Pritchett, E. G. *J. Org. Chem.* **1953**, 18, 715.

Conclusions

The described synthetic protocol may be useful for the gram scale synthesis of 2-alkoxy trans-azoxybenzene derivatives.

Azoxybenzene **3** was identified and fully characterized 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.

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.

Acknowledgments

This research was funded by Ministerio de Ciencia, Innovación y Universidades (MCIU/AEI/FEDER, UE), grant RTI2018-093940-B-I00.

J.J.N.-M. was recipient of a PhD fellowship from the Programa Nacional de Becas y Crédito Educativo (PRONABEC) of the Peruvian Government.

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