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Graphical Abstract

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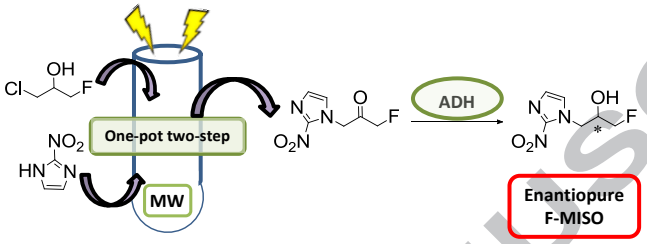

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Biocatalyzed synthesis of both enantiopure fluoromisonidazole antipodes

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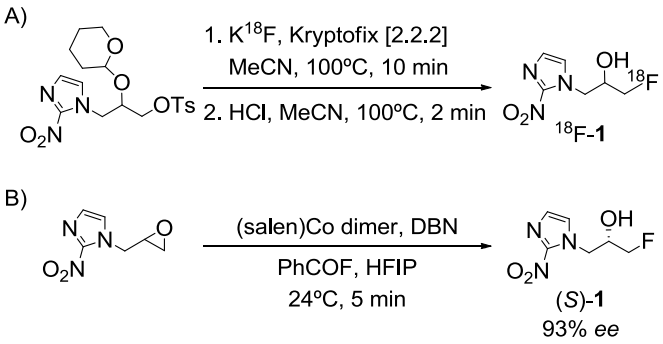
ABSTRACT

Fluoromisonidazole (FMISO or F-MISO) is a radiotracer for positron emission tomography when ^{18}F-labeled and is administrated in its racemic form. Herein, a straightforward synthesis of both enantiopure antipodes is proposed through a one-pot two-step microwave protocol to obtain a fluorinated ketone precursor followed by bioreduction using alcohol dehydrogenases from *Rhodococcus ruber* (ADH-A) or *Lactobacillus brevis* (LBADH)

The relevance of chiral fluorohydrins has been highlighted with the recent development of novel methodologies based on, *e.g.* the use of transition metal catalysis applied to nucleophilic or electrophilic fluorination,[1] through the aperture of epoxides[2] or the reduction of the corresponding ketones.[3] These compounds are very interesting precursors for agrochemicals and pharmaceuticals,[4] and they also present a remarkable role as liquid crystals,[5] and as radiotracers when they are ^{18}F-labeled for positron emission tomography (PET).[6]

In particular, fluoromisonidazole (FMISO or F-MISO), 1-(2-nitroimidazol-1-yl)-3-fluoropropan-2-ol (**1**, Scheme 1), is a derivative of the nitroimidazole group of compounds which have widely been investigated as hypoxic cell sensitizers. ^{18}F-Labeled derivative of F-MISO has extensively been used to image hypoxic tissues in vivo with PET technology and also to predict cancer recurrence in living cells.[7]

Since this compound presents a chiral center at position 2, it is administrated in its racemic form although it is well-known that the biological activity of a racemate can largely differ from each enantiomer.[8] Among the different methodologies described to obtain this derivative, it can be highlighted the synthesis through fluoride displacement of a primary tosylated alcohol, and subsequent deprotection of the tetrahydropyranyl protecting group from the secondary alcohol in acidic conditions (Scheme 1A).[9] Very recently it has been proposed the first asymmetric synthesis of (*S*)-**1** *via* enantioselective aperture of an epoxide precursor with 1,1,1,3,3,3-hexafluoroisopropanol (HFIP), 5-diazabicyclo[4.3.0]non-5-ene (DBN), benzoyl fluoride, and a linked (salen)Co catalyst to achieve the kinetic resolution obtaining it with 93% *ee* and 40% yield (Scheme 1B).[10]



Scheme 1. Previous synthesis of: A) racemic labeled, and B) enantioenriched unlabeled F-MISO.

Due to the short half-life of ^{18}F-labeled F-MISO, it would be highly desirable to find a methodology that could easily afford both stable unlabeled F-MISO antipodes so the different biological properties of each enantiomer could be more easily assessed. To achieve this goal, here we propose the synthesis of a fluorinated ketone precursor (**5**, Scheme 2) that could be selectively reduced employing stereocomplementary alcohol dehydrogenases (ADHs),[11] enzymes able to achieve the selective reduction of carbonyl compounds or the oxidation of alcohol derivatives under mild hydrogen transfer reaction conditions in aqueous medium. Starting from this prochiral ketone, this methodology could afford the final enantiopure fluorohydrin with a theoretical yield of 100%.

Therefore, in order to obtain ketone **5**, the straightforward synthesis shown in Scheme 2 was envisaged. Starting from commercially available 2-nitroimidazole **2** and 1,3-

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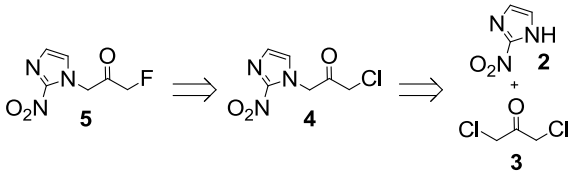
dichloroacetone **3**, the chlorinated ketone **4** could be synthesized through nucleophilic substitution, which would afford desired ketone **5** using a fluoride source.

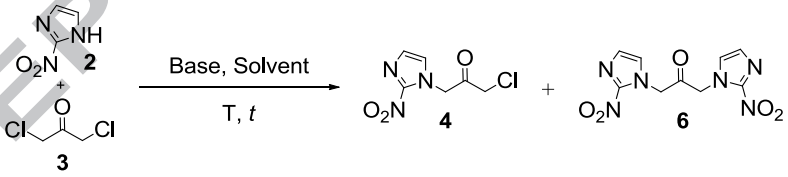


Scheme 2. Proposed retrosynthesis to obtain fluorinated ketone **5**.

In a first set of experiments, different ratios of compounds **2** and **3** were treated with several solvents and bases under various temperatures to synthesize ketone **4**, although it was noticed that in many cases disubstituted derivative **6** was also obtained as by-product (Table 1). Thus, using an equimolar ratio between compounds **2** and **3** to minimize the formation of **6**, dichloromethane (entry 1), acetone (entries 2 and 3), and acetonitrile (entries 4 and 5) were employed at different temperatures in the presence of sodium carbonate as base, observing that while for the first solvent no reaction occurred, probably due to the low solubility of the base, better results were achieved with the other two solvents, although undesired ketone **6** (14-30%) was obtained as by-product. Since the use of acetonitrile allows higher reaction temperatures, we further studied more conditions with this organic solvent. When the process was performed under reflux (entries 6 and 7), the employment of 3 equivalents of the base helped to achieve a higher conversion of ketone **4**, obtaining 57% of conversion after 2 h. Potassium carbonate was also used (entry 8) under these conditions, affording 64% of conversion of ketone **4** after 2 h of reaction. In another set of experiments, triethylamine was studied as base to get access to the desired derivative (entries 9 to 14). While at room temperature a maximum of 57% of conversion at 24 h was achieved using a 1:1 ratio between **2** and **3** (entry 11), the employment of lower temperatures and a higher amount of 1,3-dichloroacetone afforded very low yields of undesired ketone **6**, although conversions of chlorinated derivative **4** were also low (up to 31%, entries 13 and 14).

To achieve a faster and more efficient synthesis, the reaction was carried out under microwave conditions,[12] studying again different parameters in order to optimize this transformation (Table 2). Thus, when this process was performed in the absence of a base (entries 1 to 3), even at temperatures of 175ºC the conversion of ketone **4** was extremely low. Therefore, several basic compounds were added into the reaction medium to obtain higher conversions with good selectivities. While the proportion between **2** and **3** was kept 1:3 to minimize the formation of **6**, a substoichiometric amount of the base (0.5 equivalents) was selected to compare their efficiency as catalysts, and among them, triethylamine (entry 7), 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU, entry 11), and potassium carbonate (entry 14) afforded the best results (43-75%) after 10 min at 80ºC, forming disubstituted ketone **6** in very low quantities (<10%). A lower amount of the base (entry 6) or the addition of 1,3-dichloroacetone **3** in portions (entry 13) did not improve the previous results, although leaving the reaction for a longer time (entry 12), slightly enhanced the formation of derivative **4**. Then, the transformations were carried out with these three bases in stoichiometric quantity during 30 min (entries 15 to 17), and it was clearly observed that K_2CO_3 appeared as the best one affording a high conversion of the desired compound (86%) avoiding the formation of ketone **6**. Gladly, under these conditions and just after 2 min of reaction, the same results were observed (entry 19), and the employment of higher temperatures (entry 20), did not improve the conversion obtained, so conditions shown in entry 19 were selected as the most appropriate to synthesize derivative **4**.

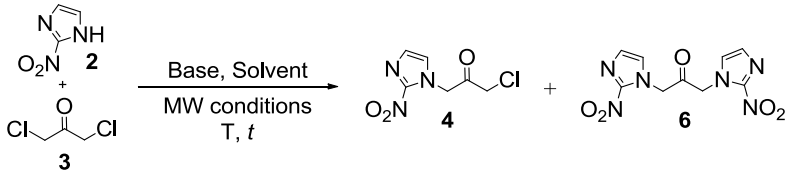
Table 1. Synthesis of α-chloro ketone **4** by reaction of nitroimidazole **2** and 1,3-dichloroacetone **3** in the presence of different bases.



Entry	Ratio **2**:**3**	Conditions	*t* (h)	**2** (%)[a]	**4** (%)[a]	**6** (%)[a]
1	1:1	CH_2Cl_2, 41ºC, inert atmosphere, Na_2CO_3 (1 equiv.)	24	>97	<3	<3
2	1:1	acetone, rt, Na_2CO_3 (0.5 equiv.)	48	28	51	21
3	1:1	acetone, 56ºC, Na_2CO_3 (0.5 equiv.)	24	15	57	28
4	1:1	CH_3CN, rt, Na_2CO_3 (0.5 equiv.)	48	40	46	14
5	1:1	CH_3CN, 56˚C, Na_2CO_3 (0.5 equiv.)	24	12	58	30
6	1:1	CH_3CN, 82ºC, Na_2CO_3 (0.5 equiv.)	2	<3	43	57
7	1:1	CH_3CN, 82ºC, Na_2CO_3 (3 equiv.)	2	<3	57	40
8	1:1	CH_3CN, 82ºC, K_2CO_3 (3 equiv.)	2	26	64	10
9	1:1	CH_3CN, rt, Et_3N (1 equiv.)	1	67	28	5
10	1:1	CH_3CN, rt, Et_3N (1 equiv.)	2	57	36	7
11	1:1	CH_3CN, rt, Et_3N (1 equiv.)	24	22	57	21
12	1:1	CH_3CN, rt, Et_3N (1 equiv.)	48	22	56	22
13	1:2	CH_3CN, 0˚C, Et_3N (1 equiv.)	1	80	20	<3
14	1:2	CH_3CN, 0˚C, Et_3N (1 equiv.)	2	65	31	4

[a] Measured by ^{1}H-NMR.

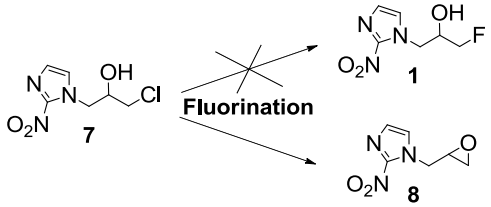
Table 2. Synthesis of α-chloro ketone **4** by reaction of nitroimidazole **2** and 1,3-dichloroacetone **3** (molar proportion 1:3) under microwave conditions.



Entry	Conditions	MW setup	*t* (min)	**2** (%)[a]	**4** (%)[a]	**6** (%)[a]
1	CH_3CN	150˚C, 300 W, 250 psi	10	>97	<3	<3
2	CH_3CN	175˚C, 300 W, 250 psi	30	96	4	<3
3	CH_3CN/DMSO (4:1)	150˚C, 200 W, 250 psi	10	>97	<3	<3
4	CH_3CN, NH_3 (0.5 equiv.)	80˚C, 200 W, 250 psi	10	92	8	<3
5	CH_3CN, Py (0.5 equiv.)	80˚C, 200 W, 250 psi	10	>97	<3	<3
6	CH_3CN, Et_3N (0.2 equiv.)	80˚C, 200 W, 250 psi	10	79	20	<3
7	CH_3CN, Et_3N (0.5 equiv.)	80˚C, 200 W, 250 psi	10	54	43	3
8	CH_3CN, DMAP (0.5 equiv.)	80˚C, 200 W, 250 psi	10	82	16	<3
9	CH_3CN, DABCO (0.5 equiv.)	80˚C, 200 W, 250 psi	10	82	13	5
10	CH_3CN, piperidine (0.5 equiv.)	80˚C, 200 W, 250 psi	10	79	20	<3
11	CH_3CN, DBU (0.5 equiv.)	80˚C, 200 W, 250 psi	10	48	48	4
12	CH_3CN, DBU (0.5 equiv.)	80˚C, 200 W, 250 psi	30	43	53	4
13[b]	CH_3CN, DBU (0.5 equiv.)	80˚C, 200 W, 250 psi	10+10+10	43	43	14
14	CH_3CN, K_2CO_3 (0.5 equiv.)	80˚C, 200 W, 250 psi	10	15	75	10
15	CH_3CN, Et_3N (1 equiv.)	80˚C, 200 W, 250 psi	30	46	48	6
16	CH_3CN, DBU (1 equiv.)	80˚C, 200 W, 250 psi	30	34	62	4
17	CH_3CN, K_2CO_3 (1 equiv.)	80˚C, 200 W, 250 psi	30	14	86	<3
18	CH_3CN, K_2CO_3 (1 equiv.)	80˚C, 200 W, 250 psi	10	17	83	<3
19	**CH_3CN, K_2CO_3 (1 equiv.)**	**80˚C, 200 W, 250 psi**	**2**	**14**	**86**	**<3**
20	CH_3CN, K_2CO_3 (1 equiv.)	120˚C, 200 W, 250 psi	2	16	84	<3

[a] Measured by ^{1}H-NMR. [b] 1,3-Dichloroacetone was added in three portions every 10 min of microwave reaction.

This transformation was achieved at 2 mmol scale obtaining, after 2 min of MW reaction and purification, compound **4** with 78% isolated yield. As the next step, fluorination of this ketone to obtain **5** (Scheme 2), was tried under several conditions. Hence, the use of KF and ZnF_2 in CH_3CN at 120˚C for 24 h;[13] PhCOF, HFIP and DBU in TBME at 50˚C for 24 h;[10] or CsF in CH_3CN under reflux for 24 h in the presence or in the absence of 18-crown-6, did not afford any conversion recovering the starting chlorinated ketone. Due to this, the corresponding chlorohydrin **7** was synthesized by reduction of **4** using $NaBH_4$ in MeOH at 0ºC in 97% yield, and then the nucleophilic fluorination over this compound was also tried. In this case it was observed the formation of a new derivative when employing KF and ZnF_2 or CsF with 18-crown-6 in CH_3CN, although unfortunately it was not the desired racemic F-MISO but the epoxide **8** (Scheme 3).

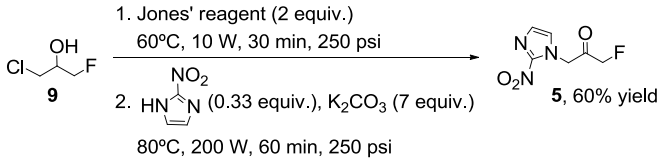


Scheme 3. Fluorination conditions over chlorohydrin **7** afforded epoxide **8**.

Due to the fact that this strategy seemed to be not appropriate to obtain the fluorinated ketone **5**, precursor of enantioenriched F-MISO antipodes, the synthetic route was modified. Since 1-chloro-3-fluoropropan-2-ol (**9**) is commercially available, it was envisaged the oxidation of this derivative into 1-chloro-3-fluoroacetone (**10**), which under the previous optimized MW reaction conditions could directly afford the final derivative **5**.

Due to the high difficulty to oxidize halohydrins,[14] it was envisioned the employment of a potent oxidant such as Jones' reagent. Thus, following a typical procedure,[15] using a mixture of acetone/water (40:1 v v^{-1}) at 0ºC afforded, by TLC, the desired compound, but when the solvent was evaporated under vacuum to obtain **10**, the high volatility of this ketone avoided its adequate isolation. In order to circumvent this fact, the use of microwave conditions in the absence of an organic solvent employing just alcohol **9** with the Jones' reagent under different setups was done (see Supplementary Data). Hence, a temperature of 80ºC was too high since several by-products were observed in the reaction crude, therefore 60ºC was fixed as the most adequate. Maintaining a pressure of 250 psi, several powers (from 10 to 200 W) and reaction times (from 5 to 15 min) were studied, but in some cases the formation of by-products was still detected or incomplete transformations were achieved, so finally 10 W during 15 min were chosen as the most appropriate

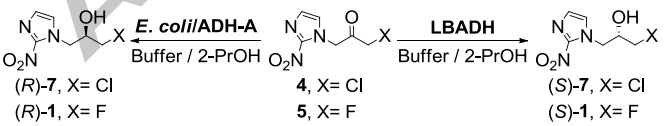
conditions to obtain ketone **10** after extraction with diethyl ether in excellent yield (>95%).



Scheme 4. One-pot two-step synthesis of ketone **5** under MW conditions.

The subsequent nucleophilic substitution of ketone **10** with 2-nitroimidazole **2** was carried out following the reaction conditions shown in entry 19 from Table 2 obtaining, by NMR, a conversion of 63% of fluorinated ketone **5**. A longer time (30 min) and a higher number of K_2CO_3 equivalents (7), improved the conversion until 70%. Due to the fact that both processes followed similar MW conditions and since, at first, no reagent incompatibilities were envisaged, the one-pot two-step procedure at 1 mmol scale was performed avoiding the isolation of volatile ketone **10**, thus synthesizing derivative **5** with 60% of isolated yield (Scheme 4). As can be noted, the reaction times were longer to achieve similar conversions than at smaller scale.

Having in hand α-fluoro ketone **5**, precursor of both F-MISO enantiomers, and α-chloro ketone analogue **9**, we decided to use different alcohol dehydrogenases with opposite stereopreference to synthesize both alcohol antipodes. Thus, among the different ADHs we have in our laboratory, alcohol dehydrogenase from *Rhodococcus ruber* ADH-A overexpressed in *E. coli* (*E. coli*/ADH-A),[16] and commercially available ADH from *Lactobacillus brevis* (LBADH)[17] were used. The transformations were performed in Tris.HCl buffer 50 mM pH 7.5 1 mM NAD(P)H using 2-propanol (5% v v^{-1}) to recycle the nicotinamide cofactor in a 'coupled-substrate' approach[11] and to solubilize the hydrophobic substrate in the reaction medium. Thus, ketone **5** was reduced at 30 mM concentration by *E. coli*/ADH-A and LBADH (Scheme 5) affording, respectively, enantiopure (*R*)- and (*S*)-**1** with >99% and 92% of conversion after 24 h. The bioreduction was especially fast with ADH-A, achieving 86% of conversion after 1 h (see Supplementary Data). On the other hand, chlorinated substrate **4** was reduced by both biocatalysts giving rise to enantiopure (*R*)- and (*S*)-**7** with quantitative conversion. These transformations were performed at higher substrate concentration (100 mM) allowing the synthesis of both antipodes of F-MISO with high to excellent conversions (75% for LBADH and >99% for *E. coli*/ADH-A) while the chlorinated analogue showed also high conversions (75% for LBADH and 86% for *E. coli*/ADH-A) after 24 h.



Scheme 5. ADH-catalyzed synthesis of both enantiopure antipodes of alcohols **1** and **7**.

Herein we have described an efficient protocol to synthesize both enantiopure fluoromisonidazole antipodes *via* one-pot two-step microwave protocol synthesis of ketone **5** plus bioreduction catalyzed by two stereocomplementary ADHs under the 'coupled-substrate' approach using 2-propanol as hydrogen donor. Moreover, after extensive optimization, the chlorinated analogue **4** could also be synthesized through MW reaction and was an excellent substrate for ADH-A and LBADH, affording both chlorohydrin enantiomers in enantiomerically pure form.

Acknowledgments

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Supplementary Material

Supplementary data (general details, procedures for the preparation of intermediates and final compounds, enzymatic protocols, analytics and ^{1}H-, ^{13}C-, and ^{19}F-NMR spectral data) associated with this article can be found, in the online version, at http://xxxx.