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Highly Stereoselective Chemoenzymatic Synthesis of the 3*H*-Isobenzofuran Skeleton. Access to Enantiopure 3-Methylphthalides

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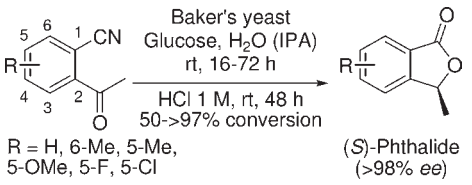
ABSTRACT



A straightforward synthesis of (*S*)-3-methylphthalides has been developed, with the key asymmetric step being the bioreduction of 2-acetylbenzonitriles. Enzymatic processes have been found to be highly dependent on the pH value, with acidic conditions being required to avoid undesired side reactions. Baker's yeast was found to be the best biocatalyst acting in a highly stereoselective fashion. The simple treatment of the reaction crudes with aqueous HCl has provided access to enantiopure (*S*)-3-methylphthalides in moderate to excellent yields.

In recent decades, the syntheses of heterocyclic compounds have attracted the attention of a vast audience of organic chemists,[1] with oxygenated and nitrogenated systems being the most common synthetic targets. In particular, phthalides, also known as 1-(3*H*)-isobenzofuranones, are five-membered ring lactones frequently found as scaffolds in basic bioactive molecules that have traditionally been used as medicines or fragrances.[2] Access to racemic phthalides has been reported with high success in recent years and applied to the synthesis of more complex organic structures.[3] In contrast, development of asymmetric routes remains as a highly demanding goal.[4]

Examples of metal-catalyzed transformations,[5] chemical enantioresolutions,[6] or organocatalytic processes have been extensively reported.[7] Alternatively biocatalytic methods have been scarcely studied for the production of optically active 3-alkylphthalide derivatives[8] through the selective action of oxidoreductases toward adequate intermediates such as 2′-iodoacetophenone[8a] or methyl 2-acetylbenzoate,[8b] while lipases have been efficiently used in the hydrolysis of esters[8a] or the acetylation of alcohols.[8c] Unfortunately a lack of general methods is currently

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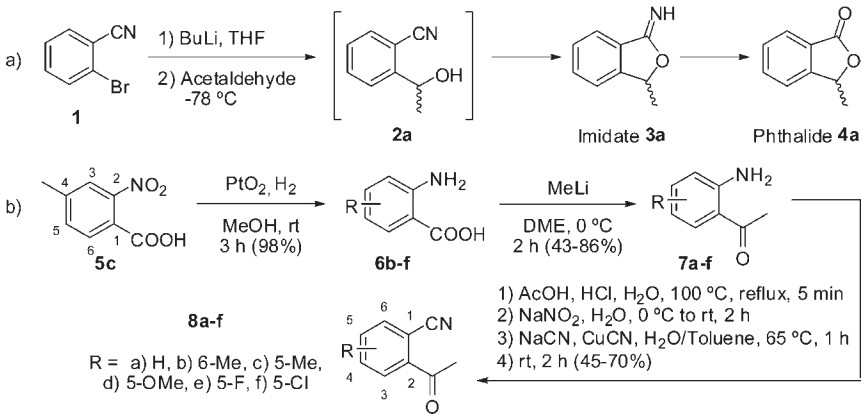
Scheme 1. Preparation of 3-Methylphthalide Precursors[a]



[a] (a) Synthesis and spontaneous intramolecular cyclization of 2-(1-hydroxyethyl)benzonitrile; (b) Chemical synthesis of 2-acetylbenzonitriles **8a**–**f** from the adequate precursor.

noticed. Herein we wish to report a general and straightforward chemoenzymatic asymmetric approach for the synthesis of 3-methylphthalides in enantiopure form based on the synthesis and bioreduction of substituted 2-acetylbenzonitriles.

On the basis of our previous experience in the chemoenzymatic synthesis of optically active 2,3-dihydrobenzofuranes,[9] we decided to react 2-bromobenzonitrile (**1**) with acetaldehyde in order to develop a synthetic pathway for the preparation of 2-(1-hydroxyethyl)-benzonitrile (**2a**, Scheme 1a). As previously observed by other research groups,[10] the strongly basic reaction medium promoted the spontaneous intramolecular cyclization of the alcohol. The corresponding imidate **3a** was isolated as the sole product, which slowly evolved to the complete formation of the phthalide **4a** under atmospheric conditions impeding the use of enzymatic transformations. This fact was clearly demonstrated by infrared data of the nonsubstituted imidate C=NH band (around 1680 cm^{-1}) and the phthalide C=O band (around 1760 cm^{-1}). IR spectra are shown in Figure 1a. As the racemic alcohol was not accessible, an alternative synthetic approach based on the chemical preparation of ketonitriles **8a**–**f** and latter stereoselective reduction of the carbonyl group with alcohol dehydrogenases was designed (Scheme 1b).

2-Acetylbenzonitriles **8a**–**f** were prepared from commercially available 2′-amino-acetophenone (**7a**), amino acids **6b**,**d**–**f**, or 4-methyl-2-nitro-benzoic acid (**5c**) respectively. Therefore, 4-methyl-2-nitro-benzoic acid was hydrogenated in the presence of platinum(IV) oxide obtaining the amino acid **6c** in quantitative yield. Treatment of **6b**–**f** with a methyl lithium solution afforded aminoacetophenones **7b**–**f** in moderate to good yields,[11] being lower when electronegative atoms were bonded to the C-4 position (OMe, F, and Cl, 43–53%).

Finally the amino ketones were converted into the desired 2-acetylbenzonitriles by Sandmeyer's reaction,[12] obtaining **8a**–**f** in moderate yields, attaining in 70% yield the methoxy and the chlorinated derivatives **8d**,**f** while the others, **8a**,**b**,**c**,**e**, were isolated in 45–50% yields.

With 2-acetylbenzonitrile (**8a**) selected as a model substrate, the first focus was on the development of bioreduction experiments catalyzed by a panel of commercially available alcohol dehydrogenases in Tris-HCl buffer working at their optimum pH (around 7.5), but in all cases 3-hydroxy-3-methyl-2-benzofuran-1(3*H*)-imine (**9a**) was observed as the unique final product. Product formation may be explained by the instability of the ketone at pH > 7 as outlined in Scheme 2a. First, the formation of the hemiacetal is highly favored at high pH's. Then, the intramolecular cyclization followed by the protonation of the imide anion led to the formation of the imidate **3a**. At the same time a sample of the racemic alcohol **2a** was searched for analytical purposes, so the chemical reduction of ketone **8a** was attempted with sodium borohydride (Scheme 2b). Nevertheless, the imidate **3a** was obtained as a unique product instead of the alcohol **2a** due to the instability of the alcohol at basic pH, which lately evolved to the racemic phthalide **4a** under atmospheric conditions or more rapidly with acidic catalysis.

At this point we moved forward to the use of other oxidoreductases capable of reacting at neutral or acidic pH. Among them Baker's yeast (*Saccharomyces cerevisiae*) is possibly one of the most frequently employed microorganisms in C=O or C=C bond reductions due to its easy

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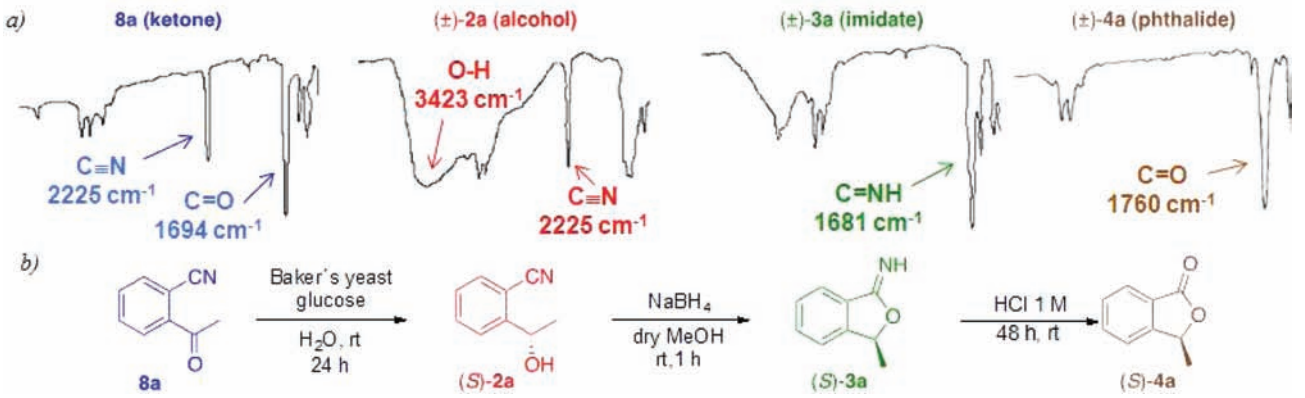
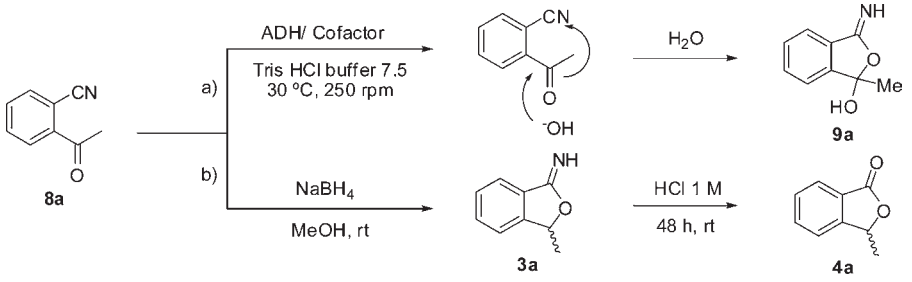


Figure 1. (a) Infrared spectra of ketone **8a** and alcohol (±)-**2a**, imidate (±)-**3a**, and phthalide (±)-**4a**. (b) Chemoenzymatic route for the preparation of (*S*)-**4a**.

Scheme 2. Approach for the Preparation of 3-Methyl Phthalide (**4a**)[a]



[a] (a) Instability of ketone **8a** at pH above 7; (b) synthesis of racemic **4a**.

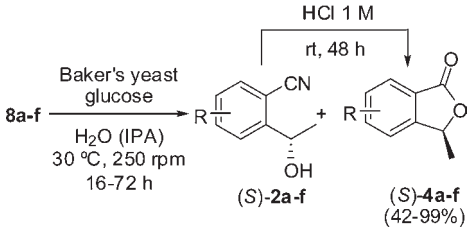
handling and high availability.[13] The bioreduction of **8a** was conducted in an aqueous medium, and the complete disappearance of the starting material was observed after 16 h, obtaining the (*S*)-alcohol **2a** as sole product and in quantitative yield. Then Baker's yeast was identified as a suitable biocatalyst within this context. Notably, when the reaction was carried out in the absence of glucose, the alcohol was also obtained although in lower isolated yield (83%). Undesired products were not detected in any case as the bioreduction occurs at the pH range 3.9−4.4 due to the Baker's yeast metabolism. The treatment of (*S*)-**2a** with $NaBH_4$ afforded the enantiopure imidate (*S*)-**3a**, a very interesting compound with potential applications in asymmetric catalysis.[14] Subsequently the imidate was transformed into the phthalide in the presence of hydrochloric acid during 48 h at room temperature. In this manner the (*S*)-phthalide **4a** was finally isolated in quantitative yield and in enantiopure form. The complete reaction pathway is represented in Figure 1b together with the IR spectrum of each of the species involved in the sequential strategy.

Once the best experimental conditions were found, we decided to explore the bioreduction of other 2-acetylbenzonitriles **8b**−**f** previously synthesized following the strategy showed in Scheme 1. For this study 5- and 6-substituted derivatives were considered due to the commercial availability of amino acids **6b,d**−**f** and benzoic acid **5c**. Satisfyingly, a similar reactivity compared to that with **8a** (Table 1, entry 1) was observed for the 6-methyl

(14) *N*-Protected chiral imidates have been recently synthesized from chiral amines and later applied as ligands in asymmetric aziridination reactions or diethylzinc additions to benzaldehyde: Noël, T.; Vandyck, K.; Robeyns, K.; Van Meervelt, L.; Van der Eycken, J. *Tetrahedron* **2009**, *65*, 8879.

(15) **General procedure for the synthesis of phthalides (*S*)-4a−f.** Baker's yeast (2.2 g) was added to a solution of glucose (282 mg) in H_2O (19 mL) with stirring of the resulting suspension for 15 min at 30 °C and 250 rpm. After this time the corresponding ketone **8a**−**f** (0.29 mmol) was added (dissolved in 453 μL of IPA for **8d**), and the suspension stirred for the required time (see Table 1). Then the reaction was centrifuged, and the supernatant was extracted with Et_2O (3 × 20 mL). Organic phases were combined, dried over Na_2SO_4, and filtered, and the solvent was evaporated under reduced pressure affording a reaction crude containing a mixture of alcohol (*S*)-**2a**−**f** and phthalide (*S*)-**4a**−**f**. The reaction crude was dissolved in HCl 1 M (2.0 mL), and the solution was stirred at room temperature for 48 h. After this time the solution was extracted with CH_2Cl_2 (3 × 5 mL). Organic layers were combined, dried over Na_2SO_4, and filtered, and the solvent was evaporated by distillation under reduced pressure. Finally the reaction crude was purified by flash chromatography (30% EtOAc/hexane) affording the corresponding enantiomerically pure phthalides (*S*)-**4a**−**f** (42−98%).

Table 1. Enzymatic Reduction of Ketones **8a**–**f** Using Baker's Yeast in H_2O at 30 °C and 250 rpm for the Asymmetric Synthesis of Enantiopure Phthalides (*S*)-**4a**–**f**[15]



entry	ketone	*t* (h)	*c* (%)[a]	(*S*)-**2** (%)[b]	(*S*)-**4** (%)[b]	(*S*)-**4** (% *ee*)[c]
1	**8a** (H)	16	>97	>97	<3 (99)	>99
2	**8b** (6-Me)	72	85	85	<3 (61)	99
3	**8c** (5-Me)	48	>97	88	12 (81)	>99
4[d]	**8d** (5-OMe)	67	50	–	50 (42)	>99
5	**8e** (5-F)	54	95	45	50 (92)	>99
6	**8f** (5-Cl)	48	>97	29	71 (72)	>99

[a] Conversion value of the reaction related to the dissapearance of starting material and the formation of alcohol and phthalide (unique reaction products). [b] Ratio of alcohol and phthalide calculated by ^{1}H NMR of the reaction crude. Isolated yields of phthalides in brackets after acidic treatment. [c] Enantiomeric excess determined by HPLC. [d] 2-Propanol (IPA, 453 μL) used to dissolve the starting ketone (0.29 mmol).

substituted ketone **8b**, yielding preferentially the alcohol although with a lower reaction rate (entry 2).

Different trends were observed for the 5-substituted derivatives in terms of alcohol/phthalide ratio although in all cases phthalides (*S*)-**4c**–**f** were obtained in enantiopure form. Weak electron-donating groups led preferentially to the formation of the alcohol (entry 3), while strong electron-donating groups as the 5-methoxy group (entry 4) favored the formation of the phthalide although with moderate yields due to the low solubility of the ketone in water. On the other hand weak electron-withdrawing groups such as fluoride or chloride led to the isolation of mixtures, where the formation of phthalides was slightly favored (entries 5 and 6). The (*S*)-stereochemistry for the phthalide chiral center was assigned in accordance with the data already reported in the literature.[16]

In summary a straightforward synthesis of (*S*)-3-methylphthalides has been designed from commercially available nitro acids, amino acids, or amino ketones where the key step is the bioreduction of 2-acetylbenzonitriles in a highly stereoselective fashion. Baker's yeast was found to be the optimal biocatalyst displaying excellent degrees of activity and stereoselectivity, fullfilling the prerequisites to act at acidic pH's toward these panels of substrates. Different treatments of the reaction crudes enable the production of highly valuable compounds in enantiopure form, such as the alcohol (*S*)-**2a**, the imidate (*S*)-**3a**, or the phthalides (*S*)-**4a**–**f**, the last ones being isolated in moderate to excellent yields depending on the ring pattern substitution.

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Supporting Information Available. General methods, experimental procedures, characterization data for new compounds, and copies of ^{1}H, ^{13}C, and DEPT NMR experiments. This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.

(16) (a) Optical rotation power obtained for (*S*)-**4a**: $[\alpha]^{20}_D$ −39.5 (*c* 1, $CHCl_3$) (>99% *ee*). Described $[\alpha]^{20}_D$ −42.1 (*c* 0.95, $CHCl_3$) in ref 5f. (b) Optical rotation power obtained for (*S*)-**4c**: $[\alpha]^{20}_D$ −36.0 (*c* 1, $CHCl_3$) (>99% *ee*). Described $[\alpha]^{20}_D$ −35.0 (*c* 1, $CHCl_3$) in ref 5e. (c) Optical rotation power obtained for (*S*)-**4f**: $[\alpha]^{20}_D$ −33.7 (*c* 1, $CHCl_3$) (>99% *ee*). Described $[\alpha]^{20}_D$ −36.1 (*c* 0.3, $CHCl_3$) in ref 5e.