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Resolution of Atropisomeric Cyclic Catechol Monoether O-Sulfate Esters by a Molluscan Sulfatase

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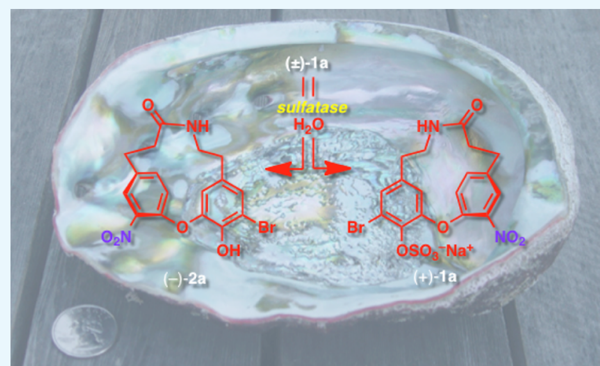
Supporting Information

ABSTRACT: Atropisomeric cyclic catechol ethers are notoriously difficult to resolve by classical chiral phase high-performance liquid chromatography. Here, we show the first application of sulfatase enzymes for the kinetic resolution of *O*-sulfato-catechol ethers with enantioselectivities ranging from 30 to 65% ee, as determined by preparation of their Marfey's ether derivatives. Substrate-structure dependence was briefly explored.



1. INTRODUCTION

Enzymes are used extensively in organic synthesis for the kinetic resolution of racemates and desymmetrization of meso-compounds to gain access to enantiomerically enriched starting materials. Most commonly, esterases (lipases) are used for either the hydrolysis of esters or acylation of alcohols, although occasionally other enzyme−substrate pairs have been exploited (e.g., reductase-ketone or aldehyde, oxidase-alcohol, hydrolase-nitrile, and so on) to procure enantioenriched precursors.[1] Sulfatases have found use for biotransformation,[2] mainly for analytical chemistry, and some examples of enantioconvergent syntheses employing sulfatases with secondary *O*-alkyl sulfate esters have been reported.[3] To the best of our knowledge, there are no reported examples of kinetic resolution of atropisomeric *O*-sulfate esters by sulfatases.

Recently, we were tasked with the resolution of a series of racemic, atropisomeric, cyclic catechol monoethers (±)-**2** that are mimics of the natural product bastadin-5, a potent agonist of the RyR1-FKBP12 Ca^{2+} channel from the marine sponge *Ianthella basta*.[4] Macrocycle p*R*-(+)-**2a** exhibits planar chirality and, like bastadin-5, stimulates Ca^{2+} release (EC_{50} 21 μM)—the last stage of excitation-contraction in striated muscle—through the RyR1-FKBP12 channel from stores in the sarcoplasmic reticulum. Significantly, the enantiomer (−)-p*S*-**2a** is almost 4 times more potent (EC_{50} = 6 μM) than (+)-p*R*-**2a**.[5a]

Racemic (±)-**2a** was resistant to separation by chiral phase high-performance liquid chromatography (HPLC) under a variety of conditions[a] and failed to undergo efficient resolution by acylation in the presence of commercially available lipases.[b]

We found, however, that the corresponding monosulfate esters, (±)-**1**, underwent enantioselective hydrolysis by sulfatases derived from a marine mollusc. Here, we report sulfatase-mediated resolution of atropisomeric *O*-sulfato-catechol monomethyl ethers (±)-**1a**,**b** by the aryl sulfatase from abalone, *Haliotis* sp., in modest to good enantiomeric excess. The latter result was somewhat surprising as the well-studied aryl sulfatases from other mollusc, *Helix pomatia* and *Patella vulgata*, were unreactive under the same conditions.[c]

2. RESULTS AND DISCUSSION

A survey of kinetic resolution was carried out with commercially available sulfatases on two substrates: the nitro- and azido-substituted macrocyclic catechol monoethers (±)-**1a**,**b** (Scheme 1). Both of the latter compounds are masked aniline derivatives that would be suitable precursors for reductive conversion to diazonium salts, ArN_2^+, and substitution of N_2 with a variety of groups (e.g., Sandmeyer reaction: Cl, Br, or CN) to explore modulation of RyR1-FKBP12.

Macrocyclic catechol ethers (±)-**2a**−**c** were prepared (Scheme 1), starting with atropisomeric (±)-**3**, obtained from intramolecular S_NAr cyclization (K_2CO_3, dimethyl sulfoxide (DMSO)) of the 4-fluoro-3-nitroaryl precursor, as previously described.[5d] Initial attempts at sulfation of (±)-**2a** using conventional reagents (e.g., 1 equiv $Me_3N\cdot SO_3$, pyridine$\cdot SO_3$, and sulfamic acid, Δ) failed to provide any detectable product (±)-**1a**. Because, in our hands, sulfation of 4-nitrophenol was successful under all of the above conditions, we surmised that the

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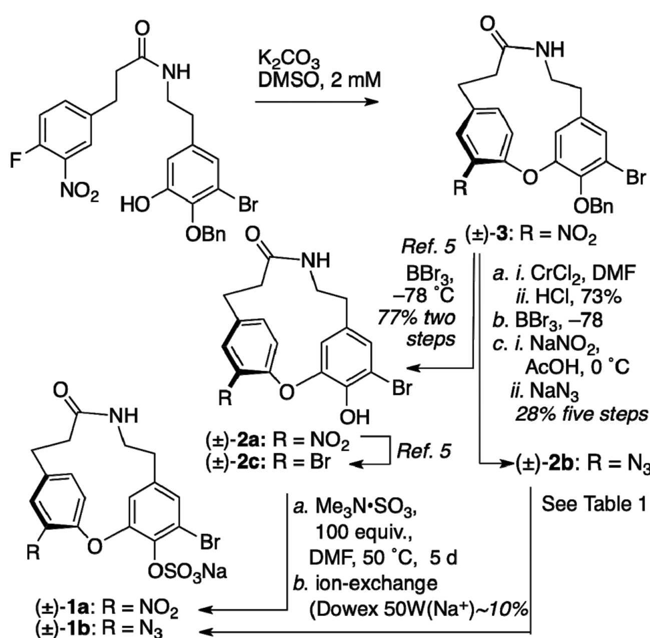
Scheme 1. Preparation of Atropisomeric Cyclic Catechol Monoether Sulfate Esters

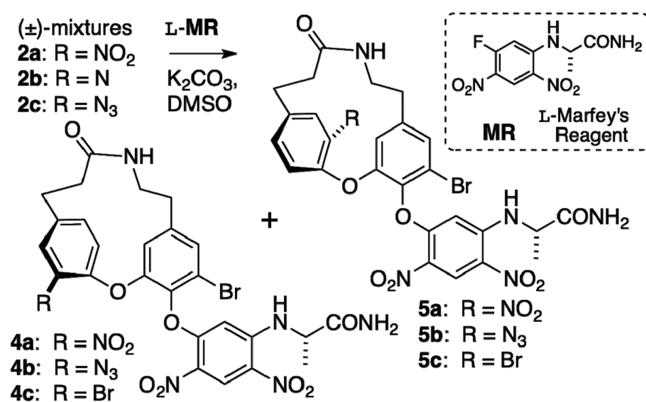


failure of (±)-**2a** to react was due to the highly hindered nature of the phenolic OH. Finally, when (±)-**2a** or (±)-**2b** was treated under forcing conditions (100 equiv $Me_3N{\cdot}SO_3$, dimethylformamide, 50 °C, 5 days), acceptable yields (∼10%) of (±)-**1a**,**b** were obtained for enzyme studies.[e] The Me_3NH^+ counterion in each product was exchanged with Na^+ (Dowex AG 50 W-X8, Na^+ form) prior to enzymatic reactions.

The susceptibility of the sulfate esters toward enzyme-promoted hydrolysis was examined at two different temperatures (acetate buffer, pH 6.8) with commercially available sulfatases obtained from three molluscan sources: A, from abalone, *Haliotis* sp.; B, from the intertidal limpet, *P. vulgata;* and C, from the Burgundy snail, *H. pomatia*.

In the course of this investigation, an analytical method was required to quantitate the otherwise inseparable enantiomers of (±)-**2a**−**c**. The latter need was met with the development of an HPLC method (Scheme 2), on the basis of conversion of the product catechols, **2a**−**c**, to the corresponding diastereomeric diaryl ethers **4a**−**c** and **5a**−**c** with L-Marfey's reagent (1-fluoro-2,4-dinitrophenyl-5-L-alaninamide,[6] K_2CO_3, DMSO, 1 h, 23 °C) and peak integration.

Scheme 2. Marfey's Derivatives of (±)-2a−c. HPLC Analysis



Nitro-substituted macrocycle (±)-**1a** underwent reaction (Table 1) with sulfatase A (22 °C) to give (±)-**2a** with modest enantioselectivity (55% ee, k_{rel} = 5.2), which diminished at a higher temperature (37 °C, 45% ee, k_{rel} = 3.7). The azido-substituted macrocycle (±)-**1b** was partially resolved in good enantioselectivity at 22 °C by enzyme A (entry 8, 65% ee, k_{rel} = 7.9) but poorly so at 37 °C (30% ee, k_{rel} = 2.3). Attempted resolutions of (±)-**1b** with enzymes B and C, at either temperature, were unsuccessful and gave no product.

Assignment of absolute stereostructure of the product (−)-**2a** (Scheme 3) was achieved through chemical correlation. An independent synthesis of the "*syn*" atropisomer (±)-**6a** (X-ray crystal structure[5]) followed by enzymatic kinetic resolution (Novozyme 435, vinyl acetate, wet CH_3CN, 70 °C, 5 days) gave benzylic *O*-acetate (−)-p*S*-**7a**, which was easily separated from (+)-p*S*-**2a** by flash chromatography (silica). Acetate ester **7a** was ammoniolysed (NH_3, MeOH) to benzylic alcohol (−)-**6a**, which was converted (deoxygenation and deprotection, as described elsewhere[5]) to (−)-p*S*-**2a** (>99% ee), which was identical, except for optical purity, to that obtained from sulfatase-promoted hydrolysis of (±)-**1a**.[f] Therefore, the enantioselection of *Haliotis*-derived sulfatase A toward (±)-**1a** is for the (−)-p*S*-enantiomer.[g]

A comparison of the three sulfatases, A, B, and C, showed that only A exhibited hydrolytic activity for kinetic resolutions of (±)-**1a**,**b**. No simple trend was observed in enantioselectivity of the nitro- versus azido-substituted substrates, (±)-**1a**,**b**, and we conclude that the π-acidity of the aryl ether ring is less important than steric properties of the substituents that impose rigidity on the macrocycle. Unlike sulfatase-promoted hydrolysis of secondary linear alkyl *O*-sulfate esters, the current enzyme active site models are inadequate at explaining either the enantiopreference or factors that determine enantioselectivity in atropisomeric kinetic resolution of (±)-**1a**,**b**.

Wallner and co-workers showed that *O*-*n*-alkyl sulfate esters of long-chain secondary alcohols undergo hydrolysis in the presence of sulfatase from the archean, *Sulfolobus acidocaldarious* (Crenarchaea) DSM 639 (pH 2−3), with excellent enantioselectivity (>99% ee).[7] Interestingly, for racemic secondary alkyl *O*-sulfates, hydrolysis is enantiospecific and selects for the *R* enantiomer by S_N2 reaction with H_2O at C, with inversion, to give the corresponding (S) secondary alcohol. Consequently, this so-called "inverting" enzyme carries out highly enantioselective kinetic resolutions (E > 200) of a limited range of secondary racemic *O*-sulfates to deliver enantioenriched products (>97% ee *R*).[7h] In contrast, sulfatase-mediated hydrolysis of aryl *O*-sulfates, of necessity, is hydrolyzed by attack of H_2O at S.

In the present case of kinetic resolution of (±)-**1** → **2**, the latter mechanism must proceed through a transition state within the enzyme active site that differs considerably from that of *O*-alkyl sulfatases. Molluscan sulfatases (e.g., *H. pomatia*) are known to be of the aryl sulfatase class and operate through a mechanism that involves transesterification of the aryl sulfate, stabilized within the active site by metal coordination and ligation to Lys and His residues, followed by initial attack on S by one hydroxyl of an aldehyde hydrate **i**, derived from Ca-formyl glycine (FG, Figure 1), followed by the successive displacement of ArOH and elimination of bisulfate.

We anticipated that resolution of (±)-**1a** would strongly depend on the rigidified chiral space defined by the macrocycle conformation and enzyme active site. We briefly examined the density functional theory (DFT) optimized geometry of p*S*-**1a** (B3LYP/6-31+G(d), H_2O continuum, Figure 2) to ascertain

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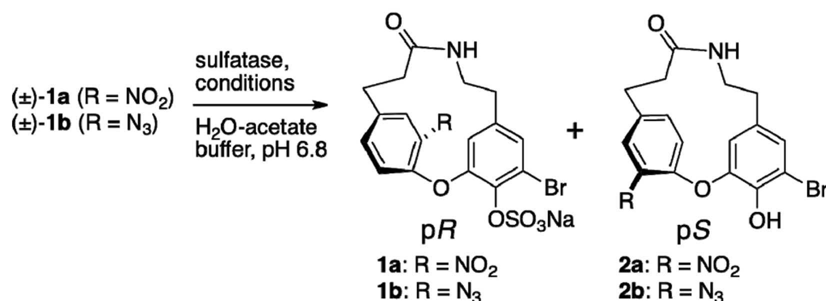
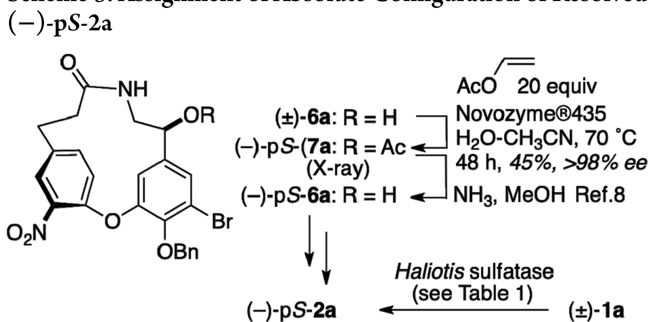
Table 1. Sulfatase-Promoted Kinetic Resolution of (±)-1a,b[a,b]



entry	enzyme[c]	substrate	(−)-p*S*-**2**% ee$_{prod}$[d]	temp (°C)	k_{rel}[b,f]
1	A	(±)-**1a**	45	37	3.7
2	A	(±)-**1a**	55	22	5.2
3	B	(±)-**1a**	[e]	37	
4	B	(±)-**1a**	[e]	22	
5	C	(±)-**1a**	[e]	37	
6	C	(±)-**1a**	[e]	22	
7	A	(±)-**1b**	30	37	2.3
8	A	(±)-**1b**	65	22	7.9
9	B	(±)-**1b**	[e]	37	
10	B	(±)-**1b**	[e]	22	
11	C	(±)-**1b**	[e]	37	
12	C	(±)-**1b**	[e]	22	

[a]Reactions were run with substrate (10 mM) and sulfatase (15 U) dissolved in aqueous buffer (pH = 6.8, 0.25 M, NaOAc−HOAc) within a conical vial sealed with a Teflon-lined cap. [b]k_{rel} was determined at 45% conversion (c = 0.45) measured by UV-HPLC peak integration (C_{18}, isocratic MeOH−H_2O−HCOOH) (60:40:0.25). [c]Enzymes were used as received (Sigma-Aldrich) derived from the following sources: A, abalone (*Haliotis* sp.); B, European limpet (*P. vulgata*); C, Burgundy snail (*H. pomatia*). [d]Determined by HPLC analysis C_{18} (MeOH−H_2O, 60:40) or SiO_2 (CH_2Cl_2-MeOH, 97:3) of Marfey's derivative of **2** (see Scheme 2, L-Marfey's reagent, K_2CO_3, DMSO, room temperature, 1 h). See Scheme 3 for determination of absolute configuration of **2**. [e]No product detected. [f]Calculated using the equation $k_{rel} = \ln(1 - c(1 + ee))/\ln(1 - c(1 + ee))$, where ee is the enantiomeric excess of the product and c is the conversion.

Scheme 3. Assignment of Absolute Configuration of Resolved (−)-p*S*-2a



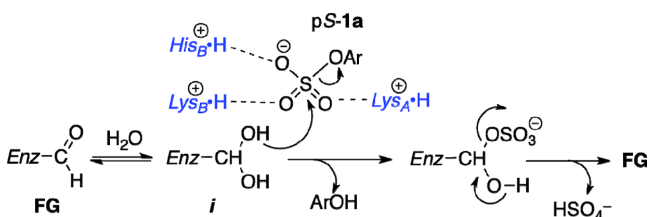


Figure 1. Formyl glycine (FG) active site model for aryl sulfatase enzymes. For clarity, metal ions are not shown. Adapted from ref 2b.

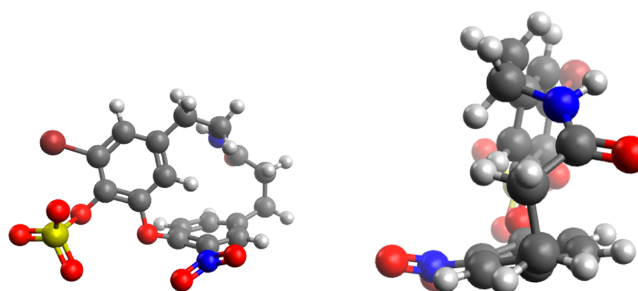


Figure 2. Model of DFT optimized geometry of (−)-p*S*-**1a** (geometries at B3LYP/6-31+G(d) level and energies at B3LYP/6-311++G(2d,p) level, H_2O continuum), (a) and (b) rotated 90°.

intramolecular nonbonded and dipolar interactions that may shed light on the origins of enantioselectivity.

As expected, the two phenyl rings are skewed from each other and subtend a C−O−C−C(NO_2) torsional angle of $\theta = -104.4°$ to relieve ring strain. No dominant bonding interaction appears between the NO_2 group and the OSO_3^- groups, although this will depend on the degree of association with the Na^+ counterion and solvation. Assuming a similar conformation in the sulfatase binding pocket, the geometry of **1a** appears to favor the approach of a nucleophilic hydrate−enzyme complex (Figure 1) from the catechol face opposite the nitroaryl group, which blocks access to the sulfate group. The product, (−)-p*S*-**2a**, is favored likely by contacts within the enzyme active site that largely accommodate (−)-p*S*-**1a** geometry through nonbonded accommodation of the distal linker atoms and nitroaryl group, rather than a conventional three-point binding model, which stabilizes a particular sulfate-transition state geometry.

Despite the widespread use of molluscan sulfatases in analytical chemistry[8] and ease of procurement from *H. pomatia* or *Haliotis* spp., little is known of their enzymology pertaining to kinetic resolution.[3]

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Specific sulfatases have been isolated from whole tissues of *H. pomatia*,[9] and the kinetics of hydrolysis of a- and b-naphthol sulfate esters, with an immobilized form of the enzyme, have been studied.[10] Recently, two sulfastase cDNAs—designated SULF1 and SULF2—have been cloned and characterized from *H. pomatia*,[11] opening a window of opportunity for refinement, and directed evolution and improvement of specific activity and enantioselectivity similar to the advantages accorded to sulfatases derived from prokaryotes and archaea (e.g., *Sulfolobus*). In contrast, similar studies of sulfatases derived from *Haliotis* have not appeared in the literature.

The preliminary studies described here, together with recent interest in sulfatases,[2] should provide a foundation for the expanded exploration of sulfatase activity in organic synthesis. Although individual sulfatases have been purified and characterized from the red abalone, *Haliotis rufescens*,[12] and other species, no X-ray crystal structures are available yet.[13] Further studies on organotransformations using sulfatase *Haliotis* spp. may benefit from cloning and "directed evolution" approaches that have seen great success in refining other classes of enzymes as biocatalysts in organic synthesis (e.g., bacterial lipases,[14] hydrolases,[15] oxidases[16]).

3. CONCLUSIONS

In conclusion, the use of *Haliotis*-derived sulfatase for kinetic resolution of atropisomeric cyclic monosulfate esters in modest to good enantioselectivity is described for the first time. The study also resulted in a useful analytical HPLC method for determining % ee in kinetically resolved products—the otherwise inseparable, cyclic atropisomeric catechol monoethers.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsomega.7b01899.

Preparation, characterization, ^{1}H NMR and ^{13}C NMR of (±)-**1a** and (±)-**1b**, HPLC t_R of Marfey's derivatives, and full details of DFT calculations of (−)-p*S*-**1a** (four conformers, 89% of the Boltzmann population) (PDF)

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Notes

The authors declare no competing financial interest.

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■ ADDITIONAL NOTES

[a]Analytical chiral phase columns (4.6 mm × 250 mm) that were investigated included Chiralpak OD, Chiralpak AD, Pirkle-type (Regis (*R*,*R*)-Whelk-O1), and Chirex D-penicillamine.

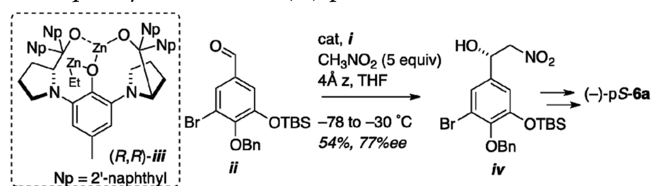
[b]The *O*-acetate ester of (±)-**2a**, in the presence of Novozyme 435 and *n*-butanol (10 equiv, 70 °C, 5 d), underwent transesterification to give (−)-p*S*-**2a** in 50% ee and only 20% conversion. Lipases from *Candida cylindracea* and *Pseudomonas* sp., under the same conditions, gave (−)-**2a** in 0% ee and 5% ee, respectively.

[c]The aryl sulfatase from *H. pomatia*, although capable of hydrolyzing primary sulfate esters, is unreactive to secondary alkyl sulfates. Schober, M. Ph.D. thesis, 2013, Karl-Franzens University, Graz, Austria.

[d]Control studies and brief investigations of the barrier to rotation in (±)-**2a** suggest that spontaneous racemization of the substrates and products under enzyme incubation conditions is negligible.

[e]Compound (±)-**2c** (R = Br; see ref 5b) failed to undergo sulfation under any condition.

[f]The assignment of absolute configuration of p*S*-**7a** derives from the known enantiopreference of Novozyme 435 for hydrolysis of acetates of benzylic alcohols; however, in the present case, it was also corroborated by independent synthesis of p*S*-**7a**, starting with the asymmetric Henry reaction of aldehyde **ii** with CH_3NO_2, using Trost's (*R*,*R*)-catalyst **iii** to give **iv** (54%, 77% ee). Trost, B. M.; Yeh, V. S. C.; Ito, H.; Bremeyer, N. Effect of Ligand Structure on the Zinc-Catalyzed Henry Reaction. Asymmetric Syntheses of (−)-Denopamine and (−)-Arbutamine. *Org. Lett.* **2002**, *4*, 2621−2623. Nitroethanol **iv** was subsequently converted to (−)-p*S*-**6a**. See ref 5b.



[g]Although not rigorously proven here, we assume the sulfatase preference toward (±)-**2b** is also for the p*S* configuration.

[h]It should be noted that the successful kinetic resolutions of Wallner's sulfatase from *Sulfolobus acidocaldarius* DSM 639 are as much the outcome of extensive screening-selection of sulfur-metabolizing archaea as it is refinement of reaction conditions.

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