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Permalink
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Journal
ACS omega, 3(7)

ISSN
2470-1343

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Publication Date
2018-07-12

DOI
10.1021/acsomega.7b01899

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

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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 Marley’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. Sulfatases have found use for biotransformation, mainly for analytical chemistry, and some examples of enantioconvergent syntheses employing sulfatases with secondary O-alkyl sulfate esters have been reported. 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 Ca2+ channel from the marine sponge Ianthella basta. Macrocycles pR-(+)-2a exhibits planar chirality and, like bastadin-5, stimulates Ca2+ release (EC50 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 (−)-pS-2a is almost 4 times more potent (EC50 = 6 μM) than (+)-pR-2a.

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

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 mono-methyl 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 molluscs, Helix pomatia and Patella vulgata, were unreactive under the same conditions.

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, ArN2+, and substitution of N3 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 SnAr cyclization (K2CO3, dimethyl sulfoxide (DMSO)) of the 4-fluoro-3-nitroaryl precursor, as previously described. Initial attempts at sulfation of (+)-2a using conventional reagents (e.g., 1 equiv Me3N·SO3, pyridine·SO3, 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
Nitro-substituted macrocycle $(\pm)-1a$ underwent reaction (Table 1) with sulfatase A (22 °C) to give $(\pm)-2a$ 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 $(\pm)-1b$ 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 $(\pm)-1b$ with enzymes B and C, at either temperature, were unsuccessful and gave no product.

Assignment of absolute stereostructure of the product $(\pm)-2a$ (Scheme 3) was achieved through chemical correlation. An independent synthesis of the “syn” atropisomer $(\pm)-6a$ (X-ray crystal structure) followed by enzymatic kinetic resolution (Novozyme 435, vinyl acetate, wet CH$_3$CN, 70 °C, 5 days) gave benzylic O-acetate $(\pm)-pS-7a$, which was easily separated from $(\pm)-pS-2a$ by flash chromatography (silica). Acetate ester 7a was ammoniolyzed (NH$_3$, MeOH) to benzylic alcohol $(\pm)-6a$, which was converted (deoxygenation and deprotection, as described elsewhere) to $(\pm)-pS-2a$ (>99% ee), which was identical, except for optical purity, to that obtained from sulfatase-promoted hydrolysis of $(\pm)-1a$. Therefore, the enantioselection of Halitosis-derived sulfatase A toward $(\pm)-1a$ is for the $(\pm)-pS$-enantiomer.

A comparison of the three sulfatasises, A, B, and C, showed that only A exhibited hydrolytic activity for kinetic resolutions of $(\pm)-1a.b$. No simple trend was observed in enantioselectivity of the nitro- versus azido-substituted substrates, $(\pm)-1a.b$, 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 enantioselectivity or factors that determine enantioselectivity in atropisomeric kinetic resolution of $(\pm)-1a.b$.

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 archeon, Sulfolobus acidocaldarious (Crenarchaea) DSM 639 (pH 2–3), with excellent enantioselectivity (>99% ee). Interestingly, for racemic secondary alkyl O-sulfates, hydrolysis is enantiospecific and selects for the $R$ enantiomer by SN$_2$ reaction with H$_2$O 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 enanitooenriched products (>97% ee R). In contrast, sulfatase-mediated hydrolysis of aryl O-sulfates, of necessity, is hydrolyzed by attack of H$_2$O at S.

In the present case of kinetic resolution of $(\pm)-1 \rightarrow 2$, the latter mechanism must proceed through a transition state within the enzyme active site that differs considerably from that of O-alkyl sulfatasises. Molluscan sulfatasises (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 $(\pm)-1a$ 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 pS-1a (B3LYP/6-31+G(d), H$_2$O continuum, Figure 2) to ascertain
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 $C_O-C_N$ torsional angle of $\theta = -104.4^\circ$ to relieve ring strain. No dominant bonding interaction appears between the $NO_2$ group and the $OSO_3^-$ groups, although this

| entry | enzyme | substrate | (-)-pS-2% ee prod | temp (°C) | $k_{rel}^{1/2}$ |
|-------|--------|-----------|-------------------|----------|----------------|
| 1     | A      | (±)-1a    |                  | 45       | 3.7            |
| 2     | A      | (±)-1a    |                  | 55       | 5.2            |
| 3     | B      | (±)-1a    |                  | 37       |                |
| 4     | B      | (±)-1a    |                  |          |                |
| 5     | C      | (±)-1a    |                  | 37       |                |
| 6     | C      | (±)-1a    |                  | 22       |                |
| 7     | A      | (±)-1b    |                  | 30       | 2.3            |
| 8     | A      | (±)-1b    |                  | 65       | 7.9            |
| 9     | B      | (±)-1b    |                  | 37       |                |
| 10    | B      | (±)-1b    |                  |          |                |
| 11    | C      | (±)-1b    |                  | 37       |                |
| 12    | C      | (±)-1b    |                  | 22       |                |

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. $k_{rel}$ was determined at 45% conversion ($c = 0.45$) measured by UV-HPLC peak integration (C18 isocratic MeOH–H2O–HCOOH) (60:40:0.25). 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). Determined by HPLC analysis C18 (MeOH–H2O, 60:40) or SiO2 (CH2Cl2-MeOH, 97:3) of Marfey’s derivative of 2 (see Scheme 2, t-Marfey’s reagent, K2CO3, DMSO, room temperature, 1 h). See Scheme 3 for determination of absolute configuration of 2. No product detected. 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 (−)-pS-2a
Specific sulfatases have been isolated from whole tissues of *Haliotis pomatia*,\(^9\) and the kinetics of hydrolysis of \(\alpha\)- and \(\beta\)-naphthol sulfate esters, with an immobilized form of the enzyme, have been studied.\(^{10}\) Recently, two sulfatase cDNAs—designated SULF1 and SULF2—have been cloned and characterized from *Haliotis 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,\(^7\) 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.

### ADDITIONAL NOTES

*Analytical chiral phase columns (4.6 mm \(\times\) 250 mm) that were investigated included Chiralpak OD, Chiralpak AD, Pirkle-type (Regis (R,R)-Whelk-O1), and Chirex \(\beta\)-penicillamine.

*The \(\alpha\)-acetate ester of \((\pm)-2\text{a}\), in the presence of Novozyrne 43S and \(n\)-butanol (10 equiv, 70 °C, 5 d), underwent transesterification to give \((\pm)-pS-2\text{a}\) in 50% ee and only 20% conversion. Lipases from *Candida cylindracea* and *Pseudomonas* sp., under the same conditions, gave \((\pm)-2\text{a}\) in 0% ee and 5% ee, respectively.

*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.

*Control studies and brief investigations of the barrier to rotation in \((\pm)-2\text{a}\) suggest that spontaneous racemization of the substrates and products under enzyme incubation conditions is negligible.

*Compound \((\pm)-2\text{c}\) (R = Br; see ref 5b) failed to undergo sulfation under any condition.

*The assignment of absolute configuration of \(pS-7\text{a}\) derives from the known enantiopreference of Novozyrne 43S for hydrolysis of acetates of bentzyl alcohols; however, in the present case, it was also corroborated by independent synthesis of \(pS-7\text{a}\), starting with the asymmetric Henry reaction of aldehyde \(ii\) with \(\text{CH}_3\text{NO}_2\), 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 \((\pm)-\text{Denopamine and } (\pm)-\text{Arbutamine. Org. Lett.*} 2002, 4, 2621–2623. Nitroethanol \(iv\) was subsequently converted to \((\pm)-pS-6\text{a}\). See ref 5b.

*Although not rigorously proven here, we assume the sulfatase preference toward \((\pm)-2\text{b}\) is also for the \(pS\) configuration.

*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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