

Conversion of 2-(4-carboxyphenyl)-6-nitrobenzothiazole to 4-(6-amino-5-hydroxybenzothiazol-2-yl)benzoic acid by a recombinant *E. coli* strain†

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E. coli C43(DE3)pNbzAHabA, expressing nitroreductase and mutase enzymes, converts 2-(4-carboxyphenyl)-6-nitrobenzothiazole to 4-(6-amino-5-hydroxybenzothiazol-2-yl)benzoic acid.

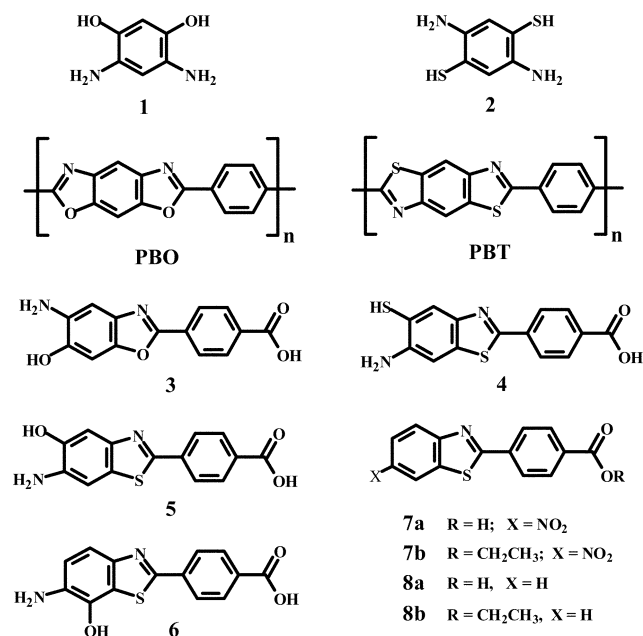
4,6-Diaminoresorcinol (**1**) and 2,5-diamino-1,4-benzenedithiol (**2**) (usually stored as dihydrochloride salts) are key co-monomers for the synthesis of strong, thermally resistant rigid-rod poly(*p*-phenylenebenzobisoxazole) (PBO) and poly(*p*-phenylenebenzobisthiazole) (PBT) polymers (Scheme 1) for lightweight structural, nonlinear optical and electronic applications.^{1,2} More recently, PBO has been considered for use in the proton-exchange membranes of fuel cells.³ While an AB-monomer 4-[5-amino-6-hydroxybenzoxazol-2-yl]benzoic acid (**3**) has been prepared and utilized in the synthesis of the corresponding PBO polymer,⁴ the analogous AB-monomer for PBT (**4**) has not been synthesized. In addition to having an intrinsically perfect stoichiometry that helps to promote high molecular weight polymers in polycondensation processes, AB-monomers are also useful starting materials for the synthesis of AB diblock copolymers, ABA triblock copolymers and star polymers. They can also be grafted onto appropriately functionalized surfaces. The availability of the above aminophenol derivative is limited because the synthesis is complex and yields are low. Recent advances in biologically converting aromatic nitro compounds to the corresponding *o*-aminophenols suggest the possibility of synthesizing novel AB-monomers such as 4-(6-amino-5-hydroxybenzothiazol-2-yl)benzoic acid (**5**) and in turn, the corresponding rigid-rod polymer that is a hybrid of both PBO and PBT with respect to the chemical structure.

Although the requisite starting nitro compounds (either as carboxylic acid, **7a** or ethyl ester **7b**) are relatively simple molecules, they have not been reported to our knowledge. In principle, **5** could be synthesized in two steps: (i) a condensation reaction between 2-aminothiophenol and 4-carboxybenzaldehyde, followed by (ii) nitration of the resulting 2-(4-carboxyphenyl)benzothiazole (**7a**). In practice, the poor solubility of **7a** and **8a** has

necessitated additional esterification/de-esterification steps in order to rigorously establish the identity and purity of the target nitrobenzothiazolecarboxylic acid. The detailed preparative procedures for **7a–8b** are provided as supplementary information.

Previously, we reported that nitroarenes are converted to *o*-aminophenols by nitroreductase and mutase enzymes.⁵ The nitroreductase reduces nitroaromatic compounds to hydroxylaminoarenes⁶ and hydroxylaminobenzene mutase catalyzes a regio-specific reaction converting hydroxylaminoarenes to the corresponding *o*-aminophenols by an intramolecular transfer of the hydroxyl group.⁷ Furthermore, an *E. coli* containing nitroreductase and mutase converts simple nitroarenes regio-specifically to *o*-aminophenols at high yields.⁸

In preliminary experiments, cells of *E. coli* C43(DE3)-pNbzAHabA (*i.e.* strain JS995) were grown and induced as described previously.⁸ 2-(4-Carboxyphenyl)-6-nitrobenzothiazole **7a** (49 μ M) was incubated with the cells and HPLC analysis of the reaction mixture revealed a transformation rate for the parent compound of 0.9 nmoles min⁻¹ mg⁻¹ protein and the accumulation of one product (Fig. 1). LC/MS (+APCI) analysis of the product revealed a 287 *m/z* as expected of an aminophenol. The conversion efficiency was 100%. The transformation was scaled up



Scheme 1 Chemical structures of pertinent monomers, polymers, substrate and intermediates.

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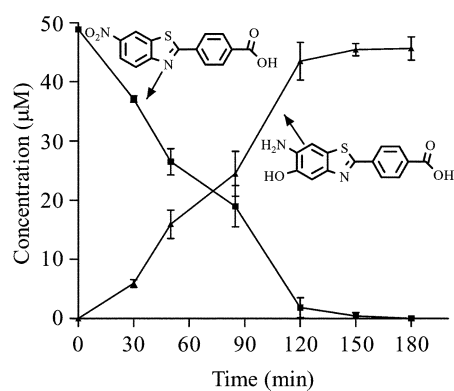


Fig. 1 Transformation of 2-(4-carboxyphenyl)-6-nitrobenzothiazole to 4-(6-amino-5-hydroxybenzothiazol-2-yl)benzoic acid by *E. coli* C43(DE3)pNbzAHabA.

to a 2 L bioreactor containing M9 medium⁹ (30 °C) supplemented with sorbitol (20 g/L), ampicillin (100 µg/L) and isopropylthio-β-D-galactoside (1 mM). Induced cells were added to the bioreactor ($A_{600} = 8.0$) and **7a** (5 mM) dissolved in NH_4OH (2 N) at 65 °C was delivered repeatedly to the reactor. The disappearance of reactant and the accumulation of product were monitored by HPLC. Over a 2 hour period, 200 mg of **7a** were converted. The cells were removed by centrifugation and the product was precipitated by adjusting the pH of the supernatant to 2.7 with HCl. Green crystals were recovered by centrifugation. The crystals were dissolved in NH_4OH , filtered and recrystallized by lowering the pH to 2.7. The pelleted crystals were dried overnight under vacuum, washed with water, and then with acetone. The melting point was 326–328 °C.

The proton NMR spectrum (270 MHz) of the purified compound in DMSO-d_6 showed that in the aromatic proton region, there were three singlet peaks at δ (ppm) 7.117, 7.309, and 8.012 at relative intensities of 1 : 1 : 4. The fact that only two distinct singlets were observed from the two protons on the phenyl ring with tetrasubstitution ruled out the isomeric structure **6** or mixture of **5** and **6** (Fig. 2). The FT-IR (KBr) spectrum (ESI) is consistent with the NMR data, indicating the presence of $\nu(\text{C}=\text{O})$ of carboxylic acid at 1692 cm^{-1} and a strong, broad band centered at $\sim 3431\text{ cm}^{-1}$ that is attributable to the hydroxyl-group vibrations of the carboxylic acid and the phenol moieties. The symmetrical and asymmetrical NH_2 stretches, typically detected as a doublet at ~ 3400 and $\sim 3500\text{ cm}^{-1}$ respectively, are most likely hidden beneath the broad $\nu(\text{OH})$ band. Electron-impact mass spectroscopy gave a molecular ion with $m/z = 285.96$ (100% relative abundance). Thus, all the available spectroscopic data confirm the structure of the product as 4-(6-amino-5-hydroxybenzothiazol-2-yl)benzoic acid, **5**.

The biocatalyst, *E. coli* C43(DE3)pNbzAHabA converts 2-(4-carboxyphenyl)-6-nitrobenzothiazole to a potentially useful *ortho*-aminophenolic synthon for the synthesis of novel polymers. Our previous work indicated that the combination of the reductase and mutase enzymes could catalyze the transformation of very simple nitroaromatic compounds to the corresponding *ortho*-aminophenols. The results presented here indicate that the biocatalyst can transform more complex and potentially useful nitroaromatic compounds stoichiometrically to the *ortho*-aminophenols. Such

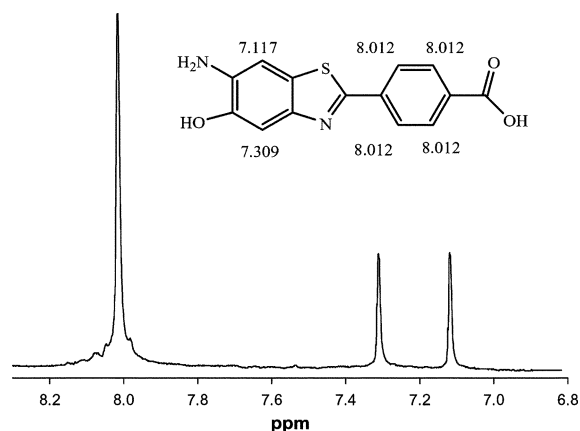


Fig. 2 Proton NMR spectrum of aminophenol product in DMSO-d_6 . For clarity, only the aromatic proton region is shown.

conversions using traditional organic chemistry would be prohibitively complex and expensive. We are currently exploring the ability of the biocatalyst to transform a variety of other complex molecules.

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