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A dual-enzyme cascade is developed for asymmetric hydroxyazidation of alkenes
Regiodivergent and stereoselective hydroxyazidation of alkenes is achieved
Various enantiomerically pure 1,2-azidoalcohols are synthesized from alkenes
Chiral β-hydroxytriazoles are prepared from alkenes by a chemo-enzymatic approach
Regiodivergent and stereoselective hydroxyazidation of alkenes by biocatalytic cascades

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SUMMARY

Asymmetric functionalization of alkenes allows the direct synthesis of a wide range of chiral compounds. Vicinal hydroxyazidation of alkenes provides a desirable path to 1,2-azidoalcohols; however, existing methods are limited by the control of stereoselectivity and regioselectivity. Herein, we describe a dual-enzyme cascade strategy for regiodivergent and stereoselective hydroxyazidation of alkenes, affording various enantiomerically pure 1,2-azidoalcohols. The biocatalytic cascade process is designed by combining styrene monooxygenase-catalyzed asymmetric epoxidation of alkenes and halohydrin dehalogenase-catalyzed regioselective ring opening of epoxides with azide. Additionally, a one-pot chemo-enzymatic route to chiral β-hydroxytriazoles from alkenes is developed via combining the biocatalytic cascades and Cu-catalyzed azide-alkyne cycloaddition.

INTRODUCTION

The 1,2-azidoalcohols are useful building blocks for the synthesis of various pharmaceuticals, biologically active molecules, natural products, and synthetic materials (Bräse et al., 2005; Chiba et al., 2009; Meldal and Tornøe, 2008; Sletten and Bertozzi, 2011). Traditional synthetic methods to 1,2-azidoalcohols include the ring opening of corresponding epoxides (Larrow et al., 1996), substitution of vicinal halohydrins (Ohkuma et al., 2007), and reduction of α-azido carbonyl compounds (Patonay et al., 2011). However, these methods are restricted by some drawbacks such as the use of prefunctionalized starting materials. Direct difunctionalization of alkenes has emerged as a powerful strategy in organic synthesis, which has been successfully applied in the conversion of olefins into more structurally diverse 1,2-difunctionalized compounds (Fu et al., 2017; Ge et al., 2020; Koke and Akta, 2018; McDonald et al., 2011; Sauer and Lin, 2018; Yin et al., 2016). Vicinal hydroxyazidation of alkenes offers a simpler and more convenient approach for preparing 1,2-azidoalcohols. So far, several effective approaches have been developed for the synthesis of 1,2-azidoalcohols through direct hydroxyazidation of alkenes (Prasad et al., 2015; Sakurada et al., 2000). However, stoichiometric or excess oxidants must be used, and these methods are restricted by the specified O-sources. Therefore, it is urgent to develop greener and more efficient strategies for vicinal hydroxyazidation of alkenes.

Molecular oxygen (O₂) is regarded as an ideal oxidant in terms of green and sustainable chemistry due to its inexpensive and environmentally benign nature (Shi et al., 2012). Thus, the replacement of chemical oxidants with O₂ or the more advantageous air for the hydroxyazidation of alkenes is a highly desired task. Recently, Jiao and coworkers have developed a convenient Mn-catalyzed aerobic oxidative hydroxyazidation of olefins for the synthesis of 1,2-azidoalcohols using air as oxidant and TMSN₃ as N₃ source (Figure 1A) (Sun et al., 2015). In addition, Lu and Yang in 2017 also reported a facile visible-light-promoted aerobic hydroxyazidation of alkenes to afford 1,2-azidoalcohols using air and TMSN₃ as the terminal oxidant and N₃ source, respectively (Figure 1B) (Yang and Lu, 2017). These elegant strategies feature mild conditions and broad substrate scope, providing efficient approaches to 1,2-azidoalcohols from alkenes. However, there are several important issues that need to be addressed: (i) control of regioselectivity for regiodivergent synthesis of 1,2-azidoalcohols is difficult; (ii) enantioselective hydroxyazidation of alkenes to afford enantipure 1,2-azidoalcohols still remains challenging.

Biocatalysis is an environmentally attractive and sustainable synthetic technology, which has been integrated into mainstream organic synthesis, particularly for the synthesis of chiral molecules (Devine et al., 2017).
Enzymes are likely to be compatible with each other and thus can be applied on “one-pot” sequential organic transformations without isolating intermediates (France et al., 2017; Ricca et al., 2011; Schrittwieser et al., 2018). Over the past years, many non-natural biocatalytic cascades have been developed by combining multiple enzymatic transformations, synthesizing diverse valuable compounds from simple precursors (Both et al., 2016; Chen et al., 2018; Corrado et al., 2019; Mutti et al., 2015; Wu et al., 2014, 2016, 2017; Zhang et al., 2015; Zhou et al., 2016). Styrene monooxygenases (SMOs) are valuable enzymes, which have been used to catalyze the asymmetric epoxidation of styrenes with air as an oxidant to afford styrene oxides in high optical purity (Figure 2A) (Corrado et al., 2018; Heine et al., 2017, 2018; Panke et al., 2000). By combining SMO with two regioselective epoxide hydrolases, Li and coworkers have developed a cascade biocatalysis for dihydroxylation of olefins, affording stereocomplementary chiral diols in high chemical and optical purity (Wu et al., 2014). Halohydrin dehalogenase (HHDH) is another synthetically attractive enzyme with catalytic promiscuity, which performs in the dehalogenation of vicinal halohydrins with the production of epoxides (Haak et al., 2008; Schallmey and Schallmey, 2016; van Hylckama Vlieg et al., 2001) and the formation of β-substituted alcohols via ring opening of epoxides in the presence of several anionic nucleophiles such as azide (Calderini et al., 2019; de Jong et al., 2005; Hasnaoui-Dijoux et al., 2008; Koopmeiners et al., 2017). HHDHs also have been used to construct biocatalytic cascades for the synthesis of enantiopure 1,2-azidoalcohols and 1,2-hydroxynitriles from α-chloroketones (Schrittwieser et al., 2009). For a long time, HHDHs also have been considered to catalyze ring opening of styrene oxides with azide in favor of β-regioselectivity (Figure 2B) (Lutje Spelberg et al., 2001; Molinaro et al., 2010), while we recently identified the HheG, a HHDH from Ilumatobacter coccineus with high α-regioselectivity (Figure 2C) (An et al., 2019). In this context, we herein develop a practical one-pot biocatalytic cascade strategy for regiodivergent and stereoselective hydroxyazidation of alkenes by combining SMO and two regiocomplementary HHDHs (Figure 2D), affording various enantiopure 1,2-azidoalcohols (Figure 1C).

RESULTS AND DISCUSSION

We initially constructed a recombinant Escherichia coli (SMO-GDH) strain for co-expression of a styrene monooxygenase and a glucose dehydrogenase. Whole-cell bioconversion of 20 mM styrene (1a) with E. coli (SMO-GDH) in a biphasic system showed the specific activity of 12.7 U (g cdw)^{-1} and produced (S)-styrene oxide (2a) with 91% yield and 99% (enantiomeric excess (ee) after 4 hr (Figure S1). We then screened biocatalytic cascades with the model reaction of transformation of 1a into...
2-azido-2-phenylethan-1-ol (3a) and 2-azido-1-phenylethan-1-ol (4a) (Figure 3). By combining with SMO, more than twenty HHDHs were evaluated for asymmetric hydroxyazidation of 1a in one-pot cascade process, and the results are summarized in Figure 3 (see Table S1 for details). As the SMO-catalyzed asymmetric epoxidation step is highly S-enantioselective, both (R)-3a and (S)-4a are produced in high ee in these reactions.
cascades. The control reaction (Figure 3, column 1) in the absence of HHDH indicates that spontaneous formation of 3a and 4a is observed, while the yields (<2%) and regioselectivity (3a:4a = 65:35) are really low. Notably, the SMO-HheG cascade (Figure 3, column 24) generates (R)-3a in relatively good yield, as well as excellent α-regioselectivity (3a:4a = 96:4). Interestingly, the β-regioselectivity in the production of (S)-4a (3a:4a = 60:40) is not high in the SMO-HheC cascade (Figure 3, column 11), although the HheC exhibits good β-regioselectivity in the azide-mediated kinetic resolution of epoxides (Lutje Spelberg et al., 2001). To our delight, when we tried to construct the SMO-HheCM cascade (Figure 3, column 12) by using an S-enantioselective variant HheCM (P84V/F86P/T134A/N176A) mutated from HheC (Guo et al., 2015), (S)-4a was produced in relatively good yield and high β-regioselectivity (3a:4a = 4:96). Therefore, the HheG and HheCM were chosen as two regiocomplementary HHDHs for combining with SMO, constructing biocatalytic cascades SMO-HheG (BCa) and SMO-HheCM (BCb) for synthesizing (R)-3a and (S)-4a through asymmetric hydroxyazidation of 1a, respectively. Subsequently, systematical investigation of the reaction conditions of BCa and BCb was carried out based on the model reaction (see Tables S2–S7 for details). Under the optimized conditions, (R)-3a was formed in 90% yield and >99% ee by BCa, and (S)-4a was produced in 96% yield and >99% ee in the case of BCb (Figure S2).

With the optimum conditions in hand, we next explored the scope of the two biocatalytic cascades for asymmetric hydroxyazidation of alkenes. A series of styrenes 1a-1l bearing electron-withdrawing groups (R = F, Cl, Br) or electron-donating groups (R = Me, OMe) were tested, and the results are summarized in Figure 4. In the case of BCa (Figure 4A), all styrenes perform the transformation to produce the desired enantiopure 1,2-azidoalcohols 3a-3l in high stereoselectivity and regioselectivity. Halo substituents on the phenyl ring are well tolerated (3b-3d, 3g-3i, and 3l). The fluorine group on ortho-, meta-, and para-positions of styrene 1a with different steric hindrance is also tolerated, yielding the corresponding 1,2-azidoalcohols with 70%, 89%, and 95% yields, respectively. As expected, a broad scope was also found in the case of BCb (Figure 4B). Styrenes 1a-1k are smoothly transformed into the enantiopure 1,2-azidoalcohols 4a-4k. The resulting low yields of 1,2-azidoalcohols 4d and 4f are caused by the poor regioselectivity of HheCM. In addition, conversion of the ortho-fluorine-substituted styrene 1l to the 1,2-azidoalcohol 4l is unsuccessful because the sterically hindered epoxide intermediate is not tolerated by HheCM. In general, both BCa and BCb-catalyzed asymmetric hydroxyazidations of alkenes are basically completed after reaction for 6 h.
affording the corresponding 1,2-azidoalcohols in high yields. The formed enantiopure epoxides in the first step are rapidly converted into the corresponding chiral 1,2-azidoalcohols by the subsequent azide-mediated regioselective ring-opening reaction. Therefore, the by-product vicinal diols generated from the epoxide intermediates by water activation are trace in the biocatalytic cascades. It is noteworthy that all the tested styrenes are converted into the corresponding chiral 1,2-azidoalcohols (except for 4l) in excellent optical purity.

Subsequently, we turned our attention to the more sterically hindered substrates, a-methylstyrene 1m and trans-β-methylstyrene 1n. Gratifyingly, both BCα and BCβ are able to catalyze the conversion of 1m into the chiral corresponding 1,2-azidoalcohols (R)-3m and (S)-4m in good yields and excellent ee (Figure 5A). These results reveal that a-methyl substitution of 1a does not influence the stereoselectivity and regioselectivity of the biocatalytic cascades. To our delight, enantiopure 1,2-azidoalcohols (R,S)-3n and (S,R)-4n that contain two chiral centers are also smoothly synthesized from 1n in 95% and 78% yields catalyzed by BCα and BCβ, respectively (Figure 5B). More importantly, both BCα and BCβ exhibit good stereoselectivity as well as diastereoselectivity in asymmetric hydroxyazidation of 1n, yielding (R,S)-3n in 91% ee, >99:1 dr and (S,R)-4n in >99% ee and >99:1 dr, respectively. Absolute configurations of (R,S)-3n and (S,R)-4n were determined by single-crystal X-ray diffraction analysis of the corresponding derivatives (R,S)-5n and (S,R)-6n, respectively. These results clearly demonstrate that the more challenging internal styrene is also well tolerated, highlighting the applicability of these biocatalytic cascades.

The combination of chemocatalysis and biocatalysis for multistep syntheses shows many advantages such as environmental benefits and high selectivity (Huang et al., 2020; Rudroff et al., 2018). The Cu-catalyzed alkyne-azide cycloaddition “click reaction” occurs in aqueous condition (Tiwari et al., 2016), which is suitable for combining with biocatalysis due to the compatibility of reaction systems. Several chemoenzymatic systems have been developed for the synthesis of chiral β-hydroxytriazoles from epoxides
or α-haloketones (Campbell-Verduyn et al., 2010; Szymanski et al., 2010). Here, we tried to synthesize chiral β-hydroxytriazoles from styrenes through a one-pot chemo-enzymatic system. In this system, after asymmetric hydroxyazidation of styrenes by biocatalytic cascades, a subsequent step is carried out via Cu(I)-catalyzed [2 + 3]-dipolar cycloaddition of the enantiopure 1,2-azidoalcohol with phenylacetylene. To demonstrate the concept, transformations of styrenes 1a and 1n to the corresponding chiral β-hydroxytriazoles were investigated. As shown in Figure 6A, chiral β-hydroxytriazoles (R)-5a and (S)-6a are smoothly prepared from 1a in >99% ee. In addition, trans-β-methylstyrene 1n is also converted into corresponding β-hydroxytriazoles (R,S)-5n and (S,R)-6n, both of which are formed in excellent ee and dr (Figure 6B). To the best of our knowledge, it is the first report of preparing enantiopure β-hydroxytriazoles from alkenes.

Since enantiopure 1,2-azidoalcohols are synthesized by the biocatalytic cascades, a variety of chiral molecules could be prepared by further transformations. For example, chiral 1,2-amino alcohols are important precursors of many chiral drugs (Legnani and Morandi, 2016; Perricos and Wenzl, 2017) and serve as important chiral ligands and auxiliaries in asymmetric synthesis (Ager et al., 1996). Herein, facile synthesis of chiral 1,2-amino alcohols (R)-7a (41%, >99% ee) and (S)-8a (58%, >99% ee) was achieved via a simple reduction reaction of (R)-3a and (S)-4a, respectively (Figure 7). In addition, many other useful chiral heterocyclic scaffolds could be obtained according to previous studies of transformations of 1,2-azidoalcohols (Ariza et al.,...
Conclusions

In summary, we have developed an efficient method for regiodivergent and stereoselective hydroxyazidation of alkenes by two novel biocatalytic cascades, providing a direct and green approach to various enantiopure 1,2-azidoalcohols. The reaction is featured by its high regioselectivity, excellent stereoselectivity, good efficiency, broad substrate scope, easy operation, and mild conditions. We also demonstrated that direct preparation of chiral $\beta$-hydroxytriazoles from alkenes is feasible by a one-pot chemo-enzymatic synthesis. We anticipate that this biocatalytic cascade strategy could impact the development of asymmetric difunctionalization of alkenes.

STAR METHODS

Detailed methods are provided in the online version of this paper and include the following:

- **KEY RESOURCES TABLE**
- **RESOURCE AVAILABILITY**
  - Lead contact
  - Materials availability
  - Data and code availability
- **METHOD DETAILS**
  - General experimental information and materials
  - Safety concerning statements
  - Enzymes preparation
  - Chemical synthesis of racemic 1,2-azidoalcohols
  - Biocatalytic synthesis of chiral 1,2-azidoalcohols from alkenes
  - Chemical synthesis of racemic $\beta$-hydroxytriazoles
  - Chemoenzymatic synthesis of chiral $\beta$-hydroxytriazoles from alkenes
  - Transformations of chiral 1,2-azidoalcohols

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.isci.2021.102883.

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AUTHOR CONTRIBUTIONS
J.-F.W., N.-W.W., Y.-N.L., and Q.-P.W. performed the experiments, analyzed the results, and participated in writing the paper. N.-W.W. and Y.-Z.C. supervised the project. B.-D.C. and W.-Y.H. helped to perform some of the analytic experiments related to this study.

DECLARATION OF INTERESTS
The authors declare no competing interests.

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STAR★METHODS

KEY RESOURCES TABLE

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| **Bacterial and virus strains** |
| *Escherichia coli* BL21 (DE3) Competent Cells | Sangon Biotech | Cat#BS28414 |
| Recombinant *E. coli* (SMO-GDH) strain (see Table S8) | This paper | N/A |
| Recombinant *E. coli* (HHDH) strains (see Table S8) | This paper | N/A |
| **Chemicals, peptides, and recombinant proteins** |
| Styrene (1a) | TCI | Cat#10289A |
| 1-fluoro-4-vinylbenzene (1b) | Innochem | Cat#A31520 |
| 1-chloro-4-vinylbenzene (1c) | Acros | Cat#110090100 |
| 1-bromo-4-vinylbenzene (1d) | Adams | Cat#35574D |
| 1-methyl-4-vinylbenzene (1e) | TCI | Cat#71373F |
| 1-methoxy-4-vinylbenzene (1f) | Ark Pharm | Cat#AK-46470 |
| 1-fluoro-3-vinylbenzene (1g) | TCI | Cat#F0409 |
| 1-chloro-3-vinylbenzene (1h) | aladdin | Cat#C140515 |
| 1-bromo-3-vinylbenzene (1i) | Innochem | Cat#A76415 |
| 1-methyl-3-vinylbenzene (1j) | aladdin | Cat#M158347 |
| 1-methoxy-3-vinylbenzene (1k) | Sigma-Aldrich | Cat#5630 |
| 1-fluoro-2-vinylbenzene (1l) | aladdin | Cat#F121723 |
| prop-1-en-2-ybenzene (1m) | Alfa aesar | Cat#L03609 |
| (E)-prop-1-en-1-ybenzene (1n) | Acros | Cat#1501500010 |
| **Deposited data** |
| Crystallographic data of (R,S)-5n | This paper | CCDC: 2074402 |
| Crystallographic data of (S,R)-6n | This paper | CCDC: 2074403 |

RESOURCE AVAILABILITY

Lead contact
Further information and requests for resources should be directed to and will be fulfilled by the lead contact, Nan-Wei Wan (nanweiwan@zmu.edu.cn).

Materials availability
All other data supporting the findings of this study are available within the article and the supplemental information or from the lead contact upon reasonable request.

Data and code availability
Crystallographic data (see Tables S9 and S10) of (R,S)-5n (CCDC: 2074402) and (S,R)-6n (CCDC: 2074403) can be obtained free of charge from The Cambridge Crystallographic Data Center (http://www.ccdc.cam.ac.uk/structures). All other data are available from the authors upon reasonable request.

METHODDETAILS

General experimental information and materials
$^1$H-NMR (400 MHz) and $^{13}$C-NMR (100 MHz) were recorded on Agilent Technologies 400 MR. Chemical shifts were reported in parts per million (ppm) with respect to the residual solvent peak. Signal shapes and splitting patterns were expressed as follows: s = singlet, d = doublet, t = triplet, m = multiplet, dd = doublet of doublets, br. s = broad single. High-resolution mass spectra (HRMS) were recorded by
Isopropyl-β-D-thiogalactopyranoside (IPTG), ampicillin (Amp), streptomycin sulfate (Sm), and kanamycin sulfate (Kan) were purchased from Solarbio (Beijing, China). Unless otherwise noted, all the other reagents and solvents were obtained from commercial suppliers and used without further purification.

Chiral HPLC analysis was performed on Shimadzu LC-20A, equipped with Chiralcel OD-H chiral column (4.6 mm× 250 mmL, particle size 5 μm), Chiralcel OJ-H chiral column (4.6 mm× 250 mmL, particle size 5 μm), Chiralpak AD-H chiral column (4.6 mm× 250 mmL, particle size 5 μm), Chiralpak AS-H chiral column (4.6 mm× 250 mmL, particle size 5 μm), and Chiralpak AS-3 chiral column (4.6 mm× 250 mmL, particle size 3 μm).

**Safety concerning statements**

Organic azides are potentially explosive substances that can decompose with the slight input of energy from external sources. We always keep in mind the following equation when preparing and utilizing organic azides. The equation takes into account all nitrogen atoms in the organic azide, not just those of azido group. We should be careful when handling the organic azides and sodium azide. In addition, we have never experienced a safety problem with these experiments.

\[
\frac{n(C) + n(C)}{n(N)} \geq 3, \text{ n signifies the number of atoms}
\]

**Enzymes preparation**

The *E. coli* (SMO-GDH) strain was constructed by co-expression of styrene monoxygenase (SMO) and glucose dehydrogenase (GDH) using two plasmids pETDuet-1 (Amp\textsuperscript{R}) and pCDFDuet-1(Sm\textsuperscript{R}). The recombinant plasmids pETDuet-styA-styB contained two subunit genes styA (*Nco*I/*HindIII) and styB (*Nde*I/*XhoI) of SMO, and the pCDFDuet-GDH contained GDH gene (*Nde*I/*XhoI). The two plasmids were transformed into *E. coli* BL21(DE3), and screened on LB plate containing 100 μg/mL Amp and 50 μg/mL Sm. All the recombinant *E. coli* (HHDH) strains were constructed using pET-28b (+) (Kan\textsuperscript{R}). The HHDH genes were inserted into the plasmid to construct recombinant plasmids pET-28-HHDH, and followed by transformation into *E. coli* BL21(DE3). All the enzyme genes were synthesized after codon optimization (see Table S8).

Cultivation was carried out using TB medium containing the corresponding resistance (100 μg/mL Amp and 50 μg/mL Sm for *E. coli* (SMO-GDH), 50 μg/mL Kan for *E. coli* (HHDH). After growing at 37°C to an OD\textsubscript{600} of 0.6–0.8, IPTG was added to the final concentration of 0.2–0.5 mM. The culture was incubated at 25°C for another 12 hr for enzyme expression. Expression analysis of twenty-four recombinant *E. coli* (HHDH) strains was analyzed by SDS-PAGE gels (see Figure S3). The recombinant *E. coli* cells that containing recombinant enzymes were harvested by centrifugation at 8,000 × g at 4°C for 5 min. The freshly prepared *E. coli* cells were resuspended for biotransformation with reaction buffer.

**Chemical synthesis of racemic 1,2-azidoalcohols**

**Synthesis of racemic 1,2-azidoalcohols 3a-3n.**  

Racemic 1,2-azidoalcohols 3a-3n were synthesized from alkenes by two reaction steps (Bernasconi et al., 2004; Wang et al., 2016). Step 1: To a 100 mL round bottomed flask, 15 mL CH\textsubscript{2}Cl\textsubscript{2} containing 3.0 mmol alkene and NaHCO\textsubscript{3} (1.5 g in 15 mL H\textsubscript{2}O) was added. Then 2 mL CH\textsubscript{2}Cl\textsubscript{2} containing 3.3 mmol 3-chloroperbenzoic acid (CPBA) was cautiously added to this solution (ice bath). The reaction mixture was stirred at room temperature for 3 hr. After washing with aqueous Na\textsubscript{2}SO\textsubscript{3} (1.95 g in 10 mL) for 20 min, the aqueous phase was then extracted with CH\textsubscript{2}Cl\textsubscript{2} (3 × 15 mL). Afterward, the organic phase was dried over anhydrous Na\textsubscript{2}SO\textsubscript{4}, evaporated at reduced pressure and the resulting mixture was purified by flash chromatography to afford epoxides. Step 2: To a 500 mL round bottomed flask, 89.6 mL ethanol and 22.4 mL dd H\textsubscript{2}O were added. Then epoxides (17.4 mmol), NaN\textsubscript{3} (34.8 mmol) and NH\textsubscript{4}Cl (34.8 mmol) were added to this solution. The reaction mixture was stirred and refluxed at 60°C for 12 hr. Ethyl acetate (3 × 110 mL) was used to extract the reaction mixture, and the organic phases were combined and washed with saturated NaCl solution (2 × 200 mL). Afterward the organic phase was dried over anhydrous Na\textsubscript{2}SO\textsubscript{4}, evaporated at reduced pressure and the resulting mixture was purified by flash chromatography to afford racemic 1,2-azidoalcohols 3a-3n.
Synthesis of racemic 1,2-azidoalcohols 4a-4n. Racemic 1,2-azidoalcohols 4a-4l and 4n were synthesized from α-bromoketones by two reaction steps (Rocha et al., 2015). Step 1: To a 50 mL round bottomed flask, 5 mL DMSO containing 5 mmol α-bromoacetophenones was added. Then 15 mmol NaN₃ (0.99 g) was added to this solution for reaction at room temperature, and the reaction was monitored by TLC (about 20 min). The mixture was poured into 15 mL water and extracted with ethyl acetate (3 × 20 mL). The organic phases were combined, dried over anhydrous Na₂SO₄ and evaporated at reduced pressure to afford crude α-azido ketones. Step 2: To a 50 mL round bottomed flask, 5 mL methanol and crude α-azido ketones were added. Then 6 mmol NaBH₄ (1.2 eq. to α-bromoacetophenones) was added gradually with stirring and cooling (ice bath). The mixture was stirred at ice bath and monitored by TLC (about 30 min). Afterward the reaction was quenched with 25 mL saturated NH₄Cl solution. Ethyl acetate (3 × 30 mL) was used to extract the mixture, and the organic phases were combined, dried over anhydrous Na₂SO₄, and evaporated at reduced pressure. The resulting mixture was purified by flash chromatography to afford racemic 1,2-azidoalcohols 4a-4l and 4n. The racemic 4m was obtained accompanying with the racemic 3m.

Biocatalytic synthesis of chiral 1,2-azidoalcohols from alkenes

General procedure for BC-a-catalyzed synthesis of chiral 1,2-azidoalcohols 3a-3n from alkenes 1a-1n: To a 250 mL round bottomed flask, 6 mL n-hexadecane and 48 mL KPB (100 mM, pH 8.0) containing resting cells E. coli (SMO-GDH) (10 g cdw/L), E. coli (HheG) (5 g cdw/L) and 2% W/V glucose were added. To this solution, alkenes 1a-1n was added to a final concentration of 10 mM using DSMO as cosolvent. Then NaN₃ (2.16 mmol) was added to a final concentration of 40 mM, and the mixture was stirred at 30 ºC for 6 h. The reaction mixture was then extracted with ethyl acetate (3 × 55 mL), and the organic phases were separated by centrifugation (7000 rpm × 2 min), combined, dried over anhydrous Na₂SO₄, and evaporated at reduced pressure. The resulting mixture was purified by flash chromatography (ethyl acetate: petroleum ether = 1:10) to afford chiral 1,2-azidoalcohols 3a-3n.

(R)-2-azido-2-phenylethan-1-ol (3a) (Wang et al., 2016)

Light yellow liquid, 79.3 mg, 90% yield, 95.2% ee; [α]₂⁵ = -163.75 (c = 0.74, MeOH); The ee was determined by HPLC (Chiralpak AD-H, Hexane/i-PrOH = 95/5, flow rate 0.5 mL/min, λ = 210 nm, t(R)-3a = 23.8 min, t(S)-3a = 25.7 min). ¹H NMR (400 MHz, CDCl₃) δ 7.44 – 7.31 (m, 5H), 4.67 (dd, J = 7.1, 5.7 Hz, 1H), 3.74 (t, J = 5.6 Hz, 2H), 2.70 (br. s, 1H). ¹³C NMR (100 MHz, CDCl₃) δ 136.3, 129.0, 128.8, 127.2, 67.9, 66.5. HRMS (ESI): calcd. for C₁₈H₁₈N₃O₃Na [M + Na]⁺ 314.1066; found 314.1066.

(R)-2-azido-2-(4-fluorophenyl)ethan-1-ol (3b) (Wang et al., 2016)

Light yellow liquid, 92.9 mg, 95% yield, 99.2% ee; [α]₂⁵ = -125.36 (c = 0.41, MeOH); The ee was determined by HPLC (Chiralpak AD-H, Hexane/i-PrOH = 95/5, flow rate 0.5 mL/min, λ = 210 nm, t(R)-3b = 23.7 min, t(S)-3b = 27.5 min). ¹H NMR (400 MHz, CDCl₃) δ 7.35 – 7.27 (m, 2H), 7.12 – 7.04 (m, 2H), 4.66 (dd, J = 7.3, 5.4 Hz, 1H), 3.77 – 3.66 (m, 2H), 2.23 (br. s, 1H). ¹³C NMR (100 MHz, CDCl₃) δ 162.9 (d, J = 247.6 Hz, 1C), 132.3 (d, J = 3.2 Hz, 1C), 129.0 (d, J = 8.3 Hz, 1C), 116.0 (d, J = 21.6 Hz, 1C), 67.2, 66.5. HRMS (ESI): calcd. for C₁₈H₁₈FN₃O₃Na [M + Na]⁺ 332.0544; found 332.0544.

(R)-2-azido-2-(4-chlorophenyl)ethan-1-ol (3c) (Wang et al., 2016)

White solid, 96.0 mg, 90% yield, 97.5% ee; [α]₂⁵ = -246.69 (c = 0.16, MeOH); mp 76.4-78.1 ºC; The ee was determined by HPLC (Chiralpak AD-H, Hexane/i-PrOH = 95/5, flow rate 0.5 mL/min, λ = 210 nm, t(R)-3c = 24.7 min, t(S)-3c = 27.5 min). ¹H NMR (400 MHz, CDCl₃) δ 7.76 – 7.40 (m, 5H), 4.66 (dd, J = 7.3, 5.4 Hz, 1H), 3.77 – 3.66 (m, 2H), 2.23 (br. s, 1H). ¹³C NMR (100 MHz, CDCl₃) δ 162.9 (d, J = 247.6 Hz, 1C), 132.3 (d, J = 3.2 Hz, 1C), 129.0 (d, J = 8.3 Hz, 1C), 116.0 (d, J = 21.6 Hz, 1C), 67.2, 66.5. HRMS (ESI): calcd. for C₁₈H₁₈FClN₃O₃Na [M + Na]⁺ 348.0454; found 348.0454.
0.5 mL/min, λ = 210 nm, t(R,3c) = 24.9 min, t(S,3c) = 19.7 min. \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta 7.35 – 7.31\) (m, 2H), 7.25 – 7.20 (m, 2H), 4.60 (dd, \(J = 7.7, 5.0\) Hz, 1H), 3.75 – 3.60 (m, 2H), 2.31 (br. s, 1H). \(^13\)C NMR (100 MHz, CDCl\(_3\)) \(\delta 135.0, 134.8, 129.3, 128.7, 67.2, 66.5\). HRMS (ESI): calcd. for C\(_8\)H\(_8\)ClN\(_3\)ONa \([M + Na]^+\) 220.0246; found 220.0246.

\((R)-2\text{-azido-2-(4-bromophenyl)ethan-1-ol}\) (3d) (Wang et al., 2016)

White solid, 107.2 mg, 82% yield, 98.2% ee; \([\alpha]_25 = -105.51\) (c = 0.36, MeOH); mp 95.9 – 97.5 °C; The ee was determined by HPLC (Chiralpak AD-H, Hexane/i-PrOH = 95/5, flow rate 0.5 mL/min, \(\lambda = 210\) nm, \(t(R,3d) = 26.4\) min, \(t(S,3d) = 29.3\) min). \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta 7.59 – 7.48\) (m, 2H), 7.23 – 7.18 (m, 2H), 4.63 (dd, \(J = 7.7, 4.9\) Hz, 1H), 3.77 – 3.67 (m, 2H), 2.16 (br. s, 1H). \(^13\)C NMR (100 MHz, CDCl\(_3\)) \(\delta 135.5, 132.2, 128.9, 122.8, 67.2, 66.4\). HRMS (ESI): calcd. for C\(_8\)H\(_8\)BrN\(_3\)ONa \([M + Na]^+\) 263.9740; found 263.9739.

\((R)-2\text{-azido-2-(p-tolyl)ethan-1-ol}\) (3e) (Wang et al., 2016)

Light yellow liquid, 90.9 mg, 95% yield, 94.3% ee; \([\alpha]_25 = -163.29\) (c = 0.42, MeOH); mp 104.5 – 106.2 °C; The ee was determined by HPLC (Chiralpak AD-H, Hexane/i-PrOH = 95/5, flow rate 0.5 mL/min, \(\lambda = 210\) nm, \(t(R,3e) = 23.6\) min, \(t(S,3e) = 29.3\) min). \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta 7.22\) (s, 4H), 4.70 – 4.57 (m, 1H), 3.77 – 3.68 (m, 2H), 2.36 (s, 3H), 2.20 (br. s, 1H). \(^13\)C NMR (100 MHz, CDCl\(_3\)) \(\delta 138.7, 133.3, 129.7, 127.2, 67.8, 66.5, 21.3\). HRMS (ESI): calcd. for C\(_9\)H\(_{11}\)N\(_3\)O\(_2\) \([M]^+\) 200.0795; found 200.0796.

\((R)-2\text{-azido-2-(4-methoxyphenyl)ethan-1-ol}\) (3f) (Wang et al., 2016)

White solid, 74.1 mg, 71% yield, 99.4% ee; \([\alpha]_25 = -119.51\) (c = 0.25, MeOH); mp 104.5 – 106.2 °C; The ee was determined by HPLC (Chiralpak AS-3, Hexane/i-PrOH = 95/5, flow rate 0.5 mL/min, \(\lambda = 210\) nm, \(t(R,3f) = 34.9\) min, \(t(S,3f) = 42.6\) min). \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta 7.36 – 7.30\) (m, 2H), 7.02 – 6.96 (m, 2H), 4.70 (dd, \(J = 7.3, 5.7\) Hz, 1H), 3.89 (s, 3H), 3.81 – 3.76 (m, 2H), 2.09 (br. s, 1H). \(^13\)C NMR (100 MHz, CDCl\(_3\)) \(\delta 159.9, 128.6, 128.3, 114.4, 67.5, 66.5, 55.4\). HRMS (EI): calcd. for C\(_9\)H\(_{11}\)N\(_3\)O\(_2\) \([M]^+\) 193.0846; found 193.0845.

\((R)-2\text{-azido-2-(3-fluorophenyl)ethan-1-ol}\) (3g)

Light yellow liquid, 87.1 mg, 89% yield, 99.9% ee; \([\alpha]_25 = -124.82\) (c = 0.63, MeOH); mp 247.2 Hz, 1C), 139.0 (d, \(J = 7.0\) Hz, 1C), 130.7 (d, \(J = 8.3\) Hz, 1C), 122.9 (d, \(J = 3.0\) Hz, 1C), 115.8 (d, \(J = 21.1\) Hz, 1C), 114.3 (d, \(J = 22.3\) Hz, 1C), 67.3 (d, \(J = 1.9\) Hz, 1C), 66.5. HRMS (ESI): calcd. for C\(_8\)H\(_8\)FN\(_3\)ONa \([M + Na]^+\) 204.0542; found 204.0542.

\((R)-2\text{-azido-2-(3-chlorophenyl)ethan-1-ol}\) (3h)
Light yellow liquid, 102.4 mg, 96% yield, 99.9% ee; $\alpha^{25} = -122.92$ (c = 0.51, MeOH); The ee was determined by HPLC (Chiralpak AS-3, Hexane/i-PrOH = 95/5, flow rate 0.5 mL/min, $\lambda = 210$ nm, $t_{R,3i} = 29.4$ min, $t_{S,3i} = 25.9$ min). $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.37 – 7.28 (m, 3H), 7.22 (s, 1H), 4.64 (dd, $J = 7.9, 4.7$ Hz, 1H), 3.77 – 3.67 (m, 2H), 2.43 (br. s, 1H). $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 138.5, 135.0, 130.3, 129.0, 127.4, 125.4, 67.2, 66.5. HRMS (Fli): calcd. for C$_8$H$_8$N$_3$OCl [M$^+$] $^{197.0350}$; found 197.0354.

(R)-2-azido-2-(3-bromophenyl)ethan-1-ol (3i) (Wang et al., 2016)

Yellow liquid, 124.2 mg, 95% yield, 99.9% ee; $\alpha^{25} = -122.49$ (c = 0.72, MeOH);

The ee was determined by HPLC (Chiralpak OD-H, Hexane/i-PrOH = 95/5, flow rate 0.5 mL/min, $\lambda = 210$ nm, $t_{R,3i} = 26.5$ min, $t_{S,3i} = 29.1$ min). $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.49 – 7.42 (m, 2H), 7.26 – 7.21 (m, 2H), 4.60 (dd, $J = 7.9, 4.7$ Hz, 1H), 3.75 – 3.61 (m, 2H), 2.75 (br. s, 1H). HRMS (Fli): calcd. for C$_8$H$_9$BrN$_3$O $[M^+]$ $^{241.9924}$; found 241.9929.

(R)-2-azido-2-(m-toly)ethan-1-ol (3j) (Wang et al., 2016)

Yellow liquid, 90.0 mg, 94% yield, 99.5% ee; $\alpha^{25} = -155.69$ (c = 0.50, MeOH);

The ee was determined by HPLC (Chiralpak OJ-H, Hexane/i-PrOH = 95/5, flow rate 0.5 mL/min, $\lambda = 210$ nm, $t_{R,3j} = 21.2$ min, $t_{S,3j} = 23.5$ min). $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 7.28 – 7.23 (m, 1H), 7.16 – 7.10 (m, 3H), 5.34 – 5.30 (m, 1H), 4.66 (dd, $J = 8.2, 4.5$ Hz, 1H), 3.69 – 3.55 (m, 2H), 2.30 (s, 3H). HRMS (Fli): calcd. for C$_9$H$_{11}$N$_3$O $[M^+]$ $^{177.0897}$; found 177.0903.

(R)-2-azido-2-(3-methoxyphenyl)ethan-1-ol (3k) (Wang et al., 2016)

Yellow liquid, 78.3 mg, 75% yield, 99.9% ee; $\alpha^{25} = -109.85$ (c = 0.57, MeOH);

The ee was determined by HPLC (Chiralpak OD-H, Hexane/i-PrOH = 95/5, flow rate 0.5 mL/min, $\lambda = 210$ nm, $t_{R,3k} = 31.9$ min, $t_{S,3k} = 34.8$ min). $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.34 – 7.25 (m, 1H), 6.95 – 6.84 (m, 3H), 4.63 (dd, $J = 5.3$, 1H), 3.81 (s, 3H), 3.76 – 3.70 (m, 2H), 2.53 (br. s, 1H). $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 160.0, 137.9, 130.0, 119.4, 114.0, 112.9, 67.8, 66.5, 55.3. HRMS (ESI): calcd. for C$_9$H$_{11}$N$_3$O$_2$Na $[M + Na]^+$ $^{216.0743}$; found 216.0741.

(R)-2-azido-2-(2-fluorophenyl)ethanol (3l)

Yellow liquid, 68.5 mg, 70% yield, 98.5% ee; $\alpha^{25} = -101.99$ (c = 0.87 MeOH); The ee was determined by HPLC (Chiralpak AS-3, Hexane/i-PrOH = 95/5, flow rate 0.5 mL/min, $\lambda = 210$ nm, $t_{R,3l} = 25.9$ min, $t_{S,3l} = 23.3$ min). $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.44 – 7.38 (m, 1H), 7.36 – 7.30 (m, 1H), 5.02 (dd, $J = 8.1, 4.2$ Hz, 1H), 3.85 – 3.69 (m, 2H), 2.76 (br. s, 1H). $^{13}$C NMR (100 MHz,
CDCl₃: δ 160.1 (d, J = 247.2 Hz, 1C), 130.2 (d, J = 8.3 Hz, 1C), 128.5 (d, J = 3.6 Hz, 1C), 124.7 (d, J = 3.7 Hz, 1C), 123.6 (d, J = 13.8 Hz, 1C), 115.8 (d, J = 21.7 Hz, 1C), 65.3 (d, J = 1.4 Hz, 1C), 61.4 (d, J = 1.8 Hz, 1C). HRMS (F1): calcd. for C₈H₈FN₃O[M]⁺ 181.0646; found 181.0652.

(R)-2-azido-2-phenylpropan-1-ol (3m) (Yukio et al., 2003)

Yellow liquid, 75.6 mg, 79% yield, 99.9% ee; [α]D = -49.10 (c = 0.58, MeOH); The ee was determined by HPLC (Chiralpak AD-H, Hexane/i-PrOH = 95/5, flow rate 0.5 mL/min, λ = 210 nm, t(R)-3m = 24.6 min, t(S)-3m = 25.6 min). ¹H NMR (400 MHz, CDCl₃) δ 7.48 – 7.29 (m, 5H), 3.71 (d, J = 11.5 Hz, 1H), 3.63 (d, J = 11.5 Hz, 1H), 1.93 (br. s, 1H), 1.74 (s, 3H). ¹³C NMR (100 MHz, CDCl₃) δ 140.9, 128.9, 128.1, 126.1, 70.7, 68.0, 21.5. HRMS (FI): calcd. for C₉H₁₁N₃O[M]⁺ 177.0897; found 177.0900.

(1R,2S)-1-azido-1-phenylpropan-2-ol (3n) (Sayyed and Sudalai, 2004)

Yellow liquid, 90.9 mg, 95% yield, > 99:1 dr, 91.3% ee; [α]D = -123.23 (c = 0.68, MeOH); The dr and ee were determined by HPLC (Chiralpak OD-H, Hexane/i-PrOH = 95/5, flow rate 0.5 mL/min, λ = 210 nm, t(1R,2S)-3n = 20.1 min, t(1S,2R)-3n = 22.1 min). ¹H NMR (400 MHz, CDCl₃) δ 7.44 – 7.32 (m, 5H), 4.47 (d, J = 5.6 Hz, 1H), 4.00 – 3.92 (m, 1H), 2.05 (br. s, 1H), 1.17 (d, J = 6.3 Hz, 3H). ¹³C NMR (100 MHz, CDCl₃) δ 136.3, 128.9, 128.7, 127.9, 71.5, 70.6, 18.6. HRMS (FI): calcd. for C₉H₁₁N₃O[M]⁺ 177.0897; found 177.0904.

General procedure for BC₃F₄-catalyzed synthesis of chiral 1,2-azidoalcohols 4a-4n from alkenes 1a-1n: To a 250 mL round bottomed flask, 6 mL n-hexadecane and 48 mL Tris-H₂SO₄ (100 mM, pH 9.0) containing resting cells E. coli (SMO-GDH) (10 g cdw/L), E. coli (HheCM) (5 g cdw/L) and 2% W/V glucose were added. To this solution alkenes 1a-1n was added to a final concentration of 10 mM using DSMO as cosolvent. Then NaN₃ (2.16 mmol) was added to a final concentration of 40 mM, and the mixture was stirred at 30 °C for 6 h. The reaction mixture was then extracted with ethyl acetate (3 × 55 mL), and the organic phases were separated by centrifugation (7000 rpm, 3 min), combined, dried over anhydrous Na₂SO₄, and evaporated at reduced pressure. The resulting mixture was purified by flash chromatography (ethyl acetate: petroleum ether = 1:30) to afford chiral 1,2-azidoalcohols 4a-4n.

(S)-2-azido-1-phenylethan-1-ol (4a) (Tae Cho et al., 2002)

Light yellow liquid, 79.3 mg, 90% yield, 99.9% ee; [α]D = +65.76 (c = 0.93, MeOH); The ee was determined by HPLC (Chiralpak OD-H, Hexane/i-PrOH = 95/5, flow rate 0.5 mL/min, λ = 210 nm, t(S)-4a = 37.3 min, t(R)-4a = 32.7 min). ¹H NMR (400 MHz, CDCl₃) δ 7.43 – 7.30 (m, 5H), 4.85 (dd, J = 8.0, 4.0 Hz, 1H), 3.51 – 3.38 (m, 2H), 2.69 (br. s, 1H). ¹³C NMR (100 MHz, CDCl₃) δ 140.7, 128.9, 128.7, 127.9, 71.5, 70.6, 18.6. HRMS (F1): calcd. for C₈H₉N₃O[M]⁺ 163.0740; found 163.0740.

(S)-2-azido-1-(4-fluorophenyl)ethan-1-ol (4b) (Tae Cho et al., 2002)

Light yellow liquid, 79.2 mg, 81% yield, 99.6% ee; [α]D = +43.16 (c = 0.72, MeOH); The ee was determined by HPLC (Chiralpak OD-H, Hexane/i-PrOH = 95/5, flow rate 0.5 mL/min, λ = 210 nm, t(S)-4b = 26.8 min, t(R)-4b = 23.4 min). ¹H NMR (400 MHz, CDCl₃) δ 7.41 – 7.32 (m, 2H), 7.12 – 6.97 (m, 1H), 4.00 – 3.92 (m, 1H). ¹³C NMR (100 MHz, CDCl₃) δ 136.3, 128.9, 128.4, 126.0, 73.5, 58.1. HRMS (F1): calcd. for C₈H₇F₂N₃O[M]⁺ 177.0897; found 177.0904.
(S)-2-azido-1-(4-chlorophenyl)ethan-1-ol (4c) (Tae Cho et al., 2002)

White solid, 96.2 mg, 90% yield, 99.0% ee; [α]25 = +106.56 (c = 0.24, MeOH); mp 46.2-47.8°C. The ee was determined by HPLC (Chiralpak OD-H, Hexane/i-PrOH = 95/5, flow rate 0.5 mL/min, λ = 210 nm, tR(4c) = 31.9 min, tR(4c) = 26.4 min). 1H NMR (400 MHz, CDCl3) δ 7.38 – 7.33 (m, 2H), 7.33 – 7.27 (m, 2H), 4.85 (dd, J = 7.1, 4.7 Hz, 1H), 3.47 – 3.40 (m, 2H), 2.71 (br. s, 1H). 13C NMR (100 MHz, CDCl3) δ 139.1, 134.2, 128.9, 127.4, 72.8, 58.0. HRMS (FI): calcd. for C8H8ClN3O [M]+ 181.0646; found 181.0644.

(S)-2-azido-1-(4-bromophenyl)ethan-1-ol (4d) (Hoff et al., 2020)

White solid, 66.7 mg, 51% yield, 99.7% ee; [α]25 = +57.95 (c = 0.22, MeOH); mp 65.4-67.1°C. The ee was determined by HPLC (Chiralpak OD-H, Hexane/i-PrOH = 95/5, flow rate 0.5 mL/min, λ = 210 nm, tR(4d) = 33.7 min, tR(4d) = 29.3 min). 1H NMR (400 MHz, CDCl3) δ 7.61 – 7.53 (m, 2H), 7.37 – 7.28 (m, 2H), 4.94 – 4.88 (m, 1H), 3.53 – 3.47 (m, 2H), 2.56 (br. s, 1H). 13C NMR (100 MHz, CDCl3) δ 139.6, 131.9, 127.8, 122.4, 72.9, 58.1. HRMS (ESI): calcd. for C8H8BrN3ONa [M + Na]+ 263.9743; found 263.9749.

(S)-2-azido-1-(p-tolyl)ethan-1-ol (4e) (Tae Cho et al., 2002)

Yellow liquid, 71.8 mg, 75% yield, 94.6% ee; [α]25 = +39.22 (c = 0.19, MeOH); The ee was determined by HPLC (Chiralpak OD-H, Hexane/i-PrOH = 95/5, flow rate 0.5 mL/min, λ = 210 nm, tR(4e) = 33.7 min, tR(4e) = 27.8 min). 1H NMR (400 MHz, CDCl3) δ 7.28 – 7.22 (m, 2H), 7.22 – 7.16 (m, 2H), 4.84 (dd, J = 8.2, 3.9 Hz, 1H), 3.52 – 3.38 (m, 2H), 2.35 (s, 3H), 1.67 (br. s, 1H). 13C NMR (100 MHz, CDCl3) δ 138.3, 137.7, 129.5, 126.0, 73.4, 58.2, 21.3. HRMS (FI): calcd. for C9H11N3O [M]+ 177.0897; found 177.0898.

(S)-2-azido-1-(4-methoxyphenyl)ethan-1-ol (4f) (Ankati et al., 2008)

Yellow liquid, 39.6 mg, 38% yield, 94.6% ee; [α]25 = +57.78 (c = 0.71, MeOH); The ee was determined by HPLC (Chiralpak AD-H, Hexane/i-PrOH = 95/5, flow rate 0.5 mL/min, λ = 210 nm, tR(4f) = 39.2 min, tR(4f) = 40.7 min). 1H NMR (400 MHz, CDCl3) δ 7.33 – 7.27 (m, 2H), 7.22 – 7.16 (m, 2H), 4.86 – 4.78 (m, 1H), 3.83 (s, 3H), 3.52 – 3.35 (m, 2H), 2.72 (br. s, 1H). 13C NMR (100 MHz, CDCl3) δ 159.6, 132.9, 127.3, 114.1, 73.0, 58.0, 55.4. HRMS (EI): calcd. for C9H11N3O2 [M]+ 193.0846; found 193.0846.

(S)-2-azido-1-(3-fluorophenyl)ethanol (4g)
[S]-2-azido-1-(3-chlorophenyl)ethan-1-ol (4h) (Tae Cho et al., 2002)

Yellow liquid, 98.2 mg, 92% yield, 99.9% ee; [α]_25 = +80.95 (c = 0.72, MeOH); The ee was determined by HPLC (Chiralpak OD-H, Hexane/i-PrOH = 95/5, flow rate 0.5 mL/min, λ = 210 nm, t_{S,4h} = 34.5 min, t_{R,4h} = 28.4 min). ^1H NMR (400 MHz, CDCl_3) δ 7.36 (s, 1H), 7.31–7.27 (m, 2H), 7.25–7.19 (m, 1H), 4.81 (dd, J = 6.8, 5.1 Hz, 1H), 3.44–3.40 (m, 2H), 2.64 (br. s, 1H). ^13C NMR (100 MHz, CDCl_3) δ 142.6, 134.7, 130.1, 128.5, 126.2, 124.1, 72.8, 57.9. HRMS (FI): calcd. for C_8H_8ClN_3O [M]⁺ 197.0350; found 197.0357.

(S)-2-azido-1-(3-bromophenyl)ethan-1-ol (4i)

Yellow liquid, 108.5 mg, 83% yield, 99.9% ee; [α]_25 = +66.24 (c = 0.35, MeOH); The ee was determined by HPLC (Chiralpak OD-H, Hexane/i-PrOH = 95/5, flow rate 0.5 mL/min, λ = 210 nm, t_{S,4i} = 40.6 min, t_{R,4i} = 30.8 min). ^1H NMR (400 MHz, CDCl_3) δ 7.52 (s, 1H), 7.47–7.40 (m, 1H), 7.30–7.18 (m, 2H), 4.81 (dd, J = 6.8, 5.1 Hz, 1H), 3.45–3.33 (m, 2H), 2.59 (br. s, 1H). ^13C NMR (100 MHz, CDCl_3) δ 142.9, 131.5, 130.4, 129.2, 124.6, 122.9, 72.8, 58.0. HRMS (FI): calcd. for C_8H_8BrN_3O [M]⁺ 240.9845; found 240.9843.

(S)-2-azido-1-(m-tolyl)ethan-1-ol (4j)

Yellow liquid, 67.0 mg, 70% yield, 99.9% ee; [α]_25 = +69.64 (c = 0.32, MeOH); The ee was determined by HPLC (Chiralpak OJ-H, Hexane/i-PrOH = 95/5, flow rate 0.5 mL/min, λ = 210 nm, t_{S,4j} = 37.5 min, t_{R,4j} = 28.9 min). ^1H NMR (400 MHz, CDCl_3) δ 7.33–7.27 (m, 1H), 2.94 (br. s, 1H), 2.40 (s, 3H). ^13C NMR (100 MHz, CDCl_3) δ 140.6, 138.4, 129.1, 128.6, 126.6, 123.0, 73.4, 58.0, 21.4. HRMS (FI): calcd. for C_9H_11N_3O [M]⁺ 177.0897; found 177.0894.

(S)-2-azido-1-(3-methoxyphenyl)ethan-1-ol (4k) (Ankati et al., 2008)

Yellow liquid, 101.2 mg, 97% yield, 99.9% ee; [α]_25 = +58.27 (c = 0.38, MeOH); The ee was determined by HPLC (Chiralpak OD-H, Hexane/i-PrOH = 95/5, flow rate 0.5 mL/min, λ = 210 nm, t_{S,4k} = 37.8 min, t_{R,4k} = 29.9 min). ^1H NMR (400 MHz, CDCl_3) δ 7.30–7.21 (m, 1H), 6.93–6.87 (m, 2H), 6.39–6.27 (m, 2H), 3.94–3.88 (m, 3H). ^13C NMR (100 MHz, CDCl_3) δ 148.1, 130.5, 128.7, 125.6, 116.2, 112.5, 38.5, 36.1. HRMS (FI): calcd. for C_10H_11NO_2 [M]⁺ 194.0873; found 194.0874.
6.86–6.79 (m, 1H), 4.81 (dd, \( J = 8.0, 4.0 \) Hz, 1H), 3.78 (s, 3H), 3.46–3.37 (m, 2H), 2.51 (br. s, 1H). \(^{13}\)C NMR (100 MHz, CDCl\(_3\)) \( \delta 160.0, 142.4, 129.9, 118.3, 113.9, 111.6, 73.5, 58.1, 55.4 \). HRMS (FI): calcd. for C\(_9\)H\(_{11}\)N\(_3\)O\(_2\) [M]\(^+\) 193.0846; found 193.0850.

\((S)-2\)-azido-1-(2-fluorophenyl)ethan-1-ol (4L)

1H NMR (400 MHz, CDCl\(_3\)) \( \delta 7.50–7.42 \) (m, 2H), 7.42–7.35 (m, 2H), 7.34–7.27 (m, 1H), 3.61 (d, \( J = 12.3 \) Hz, 1H), 3.45 (d, \( J = 12.3 \) Hz, 1H), 2.39 (br. s, 1H), 1.60 (s, 3H). \(^{13}\)C NMR (100 MHz, CDCl\(_3\)) \( \delta 144.7, 128.6, 127.6, 124.9, 74.7, 62.2, 27.2 \). HRMS (FI): calcd. for C\(_9\)H\(_{11}\)N\(_3\)O [M]\(^+\) 177.0897; found 177.0895.

\((1S,2R)-2\)-azido-1-phenylpropan-1-ol (4n)

1H NMR (400 MHz, CDCl\(_3\)) \( \delta 7.42–7.29 \) (m, 5H), 4.74 (d, \( J = 4.5 \) Hz, 1H), 3.77–3.69 (m, 1H), 2.14 (br. s, 1H), 1.19 (d, \( J = 6.7 \) Hz, 3H). \(^{13}\)C NMR (100 MHz, CDCl\(_3\)) \( \delta 140.2, 128.6, 128.2, 76.5, 62.5, 13.6 \). HRMS (FI): calcd. for C\(_9\)H\(_{11}\)N\(_3\)O [M]\(^+\) 177.0897; found 177.0895.

Chemical synthesis of racemic \( \beta \)-hydroxytriazoles

Racemic \( \beta \)-hydroxytriazoles \( 5a, 6a, 5n \) and \( 6n \) were synthesized from the corresponding racemic 1,2-azidoalcohols (Campbell-Verduyn \textit{et al.}, 2010). General procedure: To a 10 mL flask, 3 mL water containing CuSO\(_4\)·5H\(_2\)O (31.1 mg, 0.123 mmol) and sodium ascorbate (24.6 mg, 0.123 mmol) was added. Then MonoPhos (9.8 mg, 0.027 mmol) was added to this solution, and the mixture was stirred at room temperature for 15 min. Afterward the mixture was transferred to a 100 mL round bottomed flask. To this solution, 76.3 mg (0.49 mmol) 1,2-azidoalcohols \( 3a \), phenylacetylene (111 \( \mu \)L, 0.98 mmol), 9 mL distilled water and 4.0 mL DMSO were added. Then the mixture was stirred at room temperature for 12 hr, and 12 mL cold water was added to the mixture. The precipitate was filtered off, washed with cold water and purified by flash chromatography to afford racemic \( \beta \)-hydroxytriazoles \( 5a \).

Chemoenzymatic synthesis of chiral \( \beta \)-hydroxytriazoles from alkenes

Chemoenzymatic synthesis of chiral \( \beta \)-hydroxytriazoles \( (R)-5a \) and \( (R,S)-5n \): To a 250 mL round bottomed flask, 6 mL \( n \)-hexadecane and 48 mL KPB (100 mM, pH 8.0) containing resting cells \( E. \) coli (SMO-GDH) (10 g cdw/L), \( E. \) coli (HheG) (5 g cdw/L) and 2% W/V glucose were added. To this solution alkene \( 1a \) or \( 1n \) was added to a final concentration of 10 mM using DMSO as cosolvent. Then NaN\(_3\) (2.16 mmol) was added to a final concentration of 40 mM, and the mixture was stirred at 30°C for 6 hr.

To a 10 mL flask, 1 mL KPB containing CuSO\(_4\)·5H\(_2\)O (68.2 mg, 0.27 mmol) and sodium ascorbate (28.0 mg, 0.14 mmol) was added. Then MonoPhos (10.9 mg, 0.03 mmol) was added to this solution, and the mixture was stirred at room temperature for 15 min. Afterward the mixture was transferred into the enzymatic
reaction mixture (250 mL round bottomed flask) and phenylacetylene (125 μL, 1.1 mmol) was added, then the mixture was stirred at 30°C for another 16 hr.

The reaction mixture was extracted with ethyl acetate (3 × 60 mL), and the organic phases were separated by centrifugation (7000 rpm × 2 min), combined, dried over anhydrous Na₂SO₄, evaporated at reduced pressure. The resulting mixture was purified by flash chromatography (ethyl acetate: petroleum ether = 1:3) to afford chiral β-hydroxytriazole (R)-5a or (1R,2S)-5n.

(R)-2-phenyl-2-(4-phenyl-1H-1,2,3-triazol-1-yl)ethan-1-ol (5a)

Light yellow solid, 50.1 mg, 35% yield; [α]²⁵ = −2.75 (c = 0.17, CHCl₃); mp 116.1–117.9°C. The ee was determined by HPLC (Chiralpak OD-H, Hexane/i-PrOH = 80/20, flow rate 1 mL/min, λ = 210 nm, t₁₈ᵣ,₅₈a = 13.1 min, t₁₈ᵣ,₅₈S = 10.8 min). ¹H NMR (400 MHz, CDCl₃) δ 7.86–7.81 (m, 2H), 7.80 (s, 1H), 7.51–7.44 (m, 5H), 7.44–7.40 (m, 1H), 7.39–7.33 (m, 2H), 5.77 (dd, J = 8.4, 3.7 Hz, 1H), 4.73 (dd, J = 12.4, 8.4 Hz, 1H), 4.31 (dd, J = 12.5, 3.7 Hz, 1H), 3.25 (br. s, 1H). ¹³C NMR (100 MHz, CDCl₃) δ 136.1, 130.2, 129.3, 129.2, 129.0, 128.5, 127.3, 125.8, 120.8, 67.5, 65.3. HRMS (ESI): calcd. for C₁₆H₁₆N₃O [M + H]⁺ 266.1288; found 266.1289.

(1R,2S)-1-phenyl-1-(4-phenyl-1H-1,2,3-triazol-1-yl)propan-2-ol (5n)

Colorless solid, 61.8 mg, 41% yield; > 99:1 dr, 97.4% ee; [α]²⁵ = +48.94 (c = 0.98, CHCl₃); mp 132.1–133.8°C. The dr and ee were determined by HPLC (Chiralpak OJ-H, Hexane/i-PrOH = 80/20, flow rate 1 mL/min, λ = 210 nm, t₁₈ᵣ,₅₈a = 13.4 min, t₁₈ᵣ,₅₈S = 20.0 min). ¹H NMR (400 MHz, CDCl₃) δ 7.78–7.71 (m, 3H), 7.47–7.42 (m, 2H), 7.41–7.35 (m, 5H), 7.35–7.25 (m, 1H), 5.40–5.36 (m, 1H), 4.91 (dd, J = 6.5, 4.3 Hz, 1H), 3.05 (br. s, 1H), 1.24 (d, J = 6.4 Hz, 3H). ¹³C NMR (100 MHz, CDCl₃) δ 147.5, 134.9, 130.3, 129.1, 129.0, 128.9, 128.4, 125.7, 120.5, 70.5, 68.7, 19.9. HRMS (ESI): calcd. for C₁₇H₁₈N₃O [M + H]⁺ 280.1444; found 280.1446.

Chemoenzymatic synthesis of chiral β-hydroxytriazoles (S)-6a and (S,R)-6n: To a 250 mL round bottomed flask, 6 mL n-hexadecane and 48 mL Tris-H₂SO₄ (100 mM, pH 9.0) containing resting cells E. coli (SMO-GDH) (10 g cdw/L), E. coli (HheCM) (5 g cdw/L) and 2% W/V glucose were added. To this solution alkene 1a or 1n was added to a final concentration of 10 mM using DMSO as cosolvent. Then NaN₃ (2.16 mmol) was added to a final concentration of 40 mM, and the mixture was stirred at 30°C for 6 hr.

To a 10 mL flask, 1 mL Tris-H₂SO₄ containing CuSO₄·5H₂O (68.2 mg, 0.27 mmol) and sodium ascorbate (28.0 mg, 0.14 mmol) was added. Then MonoPhos (10.9 mg, 0.03 mmol) was added to this solution, and the mixture was stirred at room temperature for 15 min. Afterward, the mixture was transferred into the enzymatic reaction mixture (250 mL round bottomed flask) and phenylacetylene (125 μL, 1.1 mmol) was added, then the mixture was stirred at 30°C for another 16 hr.

The reaction mixture was extracted with ethyl acetate (3 × 60 mL), and the organic phases were separated by centrifugation (7000 rpm × 2 min), combined, dried over anhydrous Na₂SO₄, evaporated at reduced pressure.
pressure. The resulting mixture was purified by flash chromatography (ethyl acetate: petroleum ether = 1:3) to afford chiral β-hydroxytriazole (S)-6a or (1S,2R)-6n.

(S)-1-phenyl-2-(4-phenyl-1H-1,2,3-triazol-1-yl) ethan-1-ol (6a)

Light yellow solid, 84.5 mg, 59% yield, 99.9% ee; [a]_{25}^{25} = +9.69 (c = 0.45, CHCl₃); mp 156.5–158.4°C. The ee was determined by HPLC (Chiralpak OJ-H, Hexane/i-PrOH = 80/20, flow rate 1 mL/min, λ = 210 nm, t_{R,S,6a} = 22.7 min, t_{R,S,6a} = 25.1 min). ¹H NMR (400 MHz, CDCl₃) δ 7.80 (s, 1H), 7.69–7.64 (m, 2H), 7.53–7.48 (m, 2H), 7.48–7.42 (m, 2H), 7.42–7.30 (m, 4H), 5.35 (dd, J = 9.2, 3.0 Hz, 1H), 4.69 (dd, J = 13.9, 3.0 Hz, 1H), 4.42 (dd, J = 13.9, 9.2 Hz, 1H), 3.99 (br. s, 1H). ¹³C NMR (100 MHz, CDCl₃) δ 147.2, 140.4, 130.1, 128.9, 128.8, 128.5, 128.2, 126.1, 125.6, 121.5, 72.7, 57.9. HRMS (ESI): calcd. for C₁₆H₁₆N₃O [M+H]⁺ 266.1288; found 266.1288.

(1S,2R)-1-phenyl-2-(4-phenyl-1H-1,2,3-triazol-1-yl) propan-1-ol (6n)

Colorless solid, 55.8 mg, 37% yield, >99:1 dr, 99.9% ee; [a]_{25}^{25} = +10.20 (c = 0.98, CHCl₃); mp 139.9–141.7°C. The dr and ee were determined by HPLC (Chiralpak OJ-H, Hexane/i-PrOH = 80/20, flow rate 1 mL/min, λ = 210 nm, t_{1S,2R,6n} = 13.2 min, t_{1S,2S,6n} = 16.9 min). ¹H NMR (400 MHz, CDCl₃) δ 7.78–7.71 (m, 3H), 7.47–7.27 (m, 8 H), 5.40–5.36 (m, 1H), 4.91 (dd, J = 6.6, 4.5 Hz, 1H), 3.12 (br. s, 1H), 1.23 (d, J = 6.4 Hz, 3H). ¹³C NMR (100 MHz, CDCl₃) δ 140.1, 130.3, 128.9, 128.6, 128.2, 128.1, 126.1, 125.6, 119.4, 75.4, 62.7, 13.5. HRMS (ESI): calcd. for C₁₇H₁₈N₃O [M+H]⁺ 280.1444; found 280.1444.

Transformations of chiral 1,2-azidoalcohols

Synthesis of chiral 1,2-amino alcohols from chiral 1,2-azidoalcohols was performed according to previous method (Tae Cho et al., 2002). In a 100 mL round bottomed flask, a mixture of 1,2-azidoalcohols (R)-3a or (S)-4a (150 mg, 0.92 mmol) and 10% Pd/C (70 mg) in 5 mL methanol was hydrogenated using hydrogen balloon at room temperature for 16 hr. The mixture was filtered, and the filtrate was concentrated and purified by flash chromatography (dichloromethane: methanol = 3:1) to afford chiral 1,2-amino alcohols (R)-7a or (S)-8a. Racemic 1,2-amino alcohols 7a and 8a were also prepared from the corresponding racemic 1,2-azidoalcohols (Tae Cho et al., 2002).

(R)-2-amino-2-phenylethan-1-ol (7a) (Wang et al., 2016)

White solid, 51.7 mg, 41% yield, 99.9% ee; [a]_{25}^{25} = +25.48 (c = 0.85, MeOH); mp 76.1–77.8°C. The ee was determined by HPLC (Chiralpak OJ-H, Hexane/i-PrOH = 95/5, flow rate 0.5 mL/min, λ = 210 nm, t_{R,7a} = 30.2 min, t_{S,7a} = 27.8 min). ¹H NMR (400 MHz, CDCl₃) δ 7.45–7.20 (m, 5H), 4.03 (dd, J = 8.4, 4.1 Hz, 1H), 3.72 (dd, J = 11.0, 4.1 Hz, 1H), 3.56 (dd, J = 11.0, 8.4 Hz, 1H), 2.81 (br. s, 3H). ¹³C NMR (100 MHz, CDCl₃) δ 142.4, 128.6, 127.5, 126.6, 67.8, 57.4. HRMS (ESI): calcd. for C₈H₁₂NO [M+H]⁺ 138.0919; found 138.0912.

(S)-2-amino-1-phenylethan-1-ol (8a) (Tae Cho et al., 2002)
Light yellow solid, 73.2 mg, 58% yield, 99.9% ee; $[\alpha]^\text{D}_{25} = 38.70$ (c = 0.46, MeOH); mp 54.4–56.3°C. The ee was determined by HPLC (Chiralpak IH, Hexane/i-PrOH = 95/5, flow rate 0.5 mL/min, $\lambda = 210$ nm, $t_{\text{R}}$-$\text{aa} = 39.1$ min, $t_{\text{S}}$-$\text{aa} = 41.4$ min). $^1$H NMR (400 MHz, CDCl$_3$) δ 7.38–7.26 (m, 5H), 4.64 (dd, $J = 7.8, 3.9$ Hz, 1H), 2.97 (dd, $J = 12.8, 4.0$ Hz, 1H), 2.81 (dd, $J = 12.8, 7.8$ Hz, 1H), 2.21 (br. s, 3H). $^{13}$C NMR (100 MHz, CDCl$_3$) δ 142.7, 128.5, 127.7, 126.0, 74.4, 49.4. HRMS (ESI): calcd. for C$_8$H$_{12}$NO [M + H]$^+$ 138.0919; found 138.0912.