# Reaction Stereochemistry of 2-Deoxy-*scyllo*-inosose Synthase, the Key Enzyme in the Biosynthesis of 2-Deoxystreptamine

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The reaction stereochemistry of 2-deoxy-*scyllo*-inosose (DOI) synthase (DOIS) derived from butirosin-producing *Bacillus circulans*, the key starter enzyme of 2-deoxystreptamine biosynthesis, was closely analyzed by use of the purified recombinant DOIS. The stereochemical pathway of the DOIS reaction is common in the aminoglycoside-producing *Bacillus* and *Streptomyces*, but is distinct from a related dehydroquinate synthase reaction in the shikimate pathway. Evolutionary relationship between these enzymes is also discussed.

2-Deoxystreptamine (DOS)-containing aminoglycoside antibiotics such as neomycin, kanamycin, gentamicin, and ribostamycin are clinically important antibacterial agents.[1,2] A key enzyme in the biosynthesis of DOS is 2-deoxy-*scyllo*-inosose (DOI) synthase (DOIS), which catalyzes multistep cyclization of D-glucose-6-phosphate (G-6-P) into the 6-membered carbocycle DOI in the first step of DOS biosynthesis (Figure 1).[1,3–10]

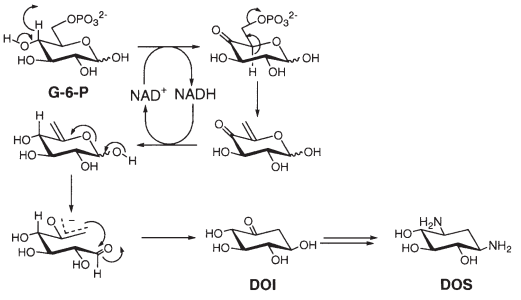


**Figure 1.** Reaction mechanism of DOI synthase.

The stereochemistry of DOIS reaction in ribostamycin-producing *Streptomyces ribosidificus* was previously elucidated by in vivo deuterium labeling experiments and $^2$H-NMR spectroscopy. The results suggested similarity of the DOIS reaction to that of dehydroquinate synthase (DHQS) in terms of overall chemistry and difference in stereochemistry of their crucial C-C bond forming reactions.[5,6,8] DHQS is among well-known enzymes in the primary metabolism and catalyzes a similar carbocycle-forming reaction in the shikimate pathway.

Recently, we isolated DOIS from butirosin-producing *Bacillus circulans* SANK72073 and the structural gene (*btrC*) was subsequently cloned and over-expressed in *Escherichia coli*.[11,12] Certain homology (25-29%) was observed between the *B. circulans* DOIS and several microbial DHQSs on the amino acid level. Since the crystallographic structure of fungal modular DHQS was elucidated recently,[13] it appears extremely intriguing to figure out chemical similarity and dissimilarity of this class of enzymes. In addition, we have successfully demonstrated promising short-step conversion of D-glucose into catechol by chemoenzymatic approach with the overexpressed BtrC,[14] and for this potential usefulness, it appears to be beneficial to have closer look into the reaction mechanism of DOIS. This communication describes precise stereochemistry of the DOIS reaction, difference between DOIS and DHQS, and evolutionary aspect.

The cyclization stereochemistry of the DOIS reaction had been assigned previously by the whole cell experiments using *Streptomyces ribosidificus* as mentioned above.[5,6] In the present study, the DOIS reaction was analyzed by the use of the purified recombinant BtrC derived from *Bacillus*. This study thus allowed to compare the *Bacillus* DOIS reaction with that of *Streptomyces*. The enzyme reaction was carried out by a method as described recently.[14] Thus, labeled precursors,[15–18] D-(*6S*)-[6-$^2H_1$]glucose (containing 20% (*6R*)-isomer) and D-(*6R*)-[6-$^2H_1$]glucose (containing 17% (*6S*)-isomer), were separately incubated with a mixture of the recombinant BtrC and hexokinase in the presence of ATP, $NAD^+$ and $Co^{2+}$, which yielded monodeuterated DOI in up to 78% yield.

The resulting monodeuterated DOI was further converted to a *p*-nitrobenzyl oxime derivative as described previously,[9] and the oxime derivative was characterized by $^1$H- and $^2$H-NMR. As a result, the axial proton signal at C-2 of the oxime derived from D-(*6S*)-[6-$^2H_1$]glucose appeared as a major broad doublet at 1.88 ppm (in $CD_3OD$, $J = 10.7$ Hz) and the equatorial proton was observed only as a weak signal at 3.49 ppm ($J = 4.8$ Hz) in the $^1$H-NMR spectrum. In the $^2$H-NMR spectrum (in $CH_3OH$), an intense signal due to the equatorial $^2$H was observed at 3.5 ppm and a minor signal was observed at 1.9 ppm (Figure 2b). Therefore, it appears that the *pro-S* hydrogen at C-6 of G-6-P was incorporated stereospecifically into the equatorial position at C-2 of DOI. Reverse was true for the deuterated DOI derivative formed from D-(*6R*)-[6-$^2H_1$]glucose. A major signal due to the axial $^2$H at C-2 was observed at 1.9 ppm and a minor signal was observed 3.5 ppm. (Figure 2c) The reaction stereochemistry of the DOIS (BtrC) derived from *Bacillus* is completely identical with that in the whole cell system of *Streptomyces ribosidificus* and is different from DHQS. The *pro-S* hydrogen at C-7 of D-3-deoxy-*arabino*-heptulosonate 7-phosphate (DAHP) is known to be incorporated into the axial position of the corresponding methylene group of DHQ by the DHQS reaction as shown in Figure 3.[19,20]

The stereochemical mechanism for these incorporation patterns in the DOI reaction can be explained in two possible ways (Figure 3). Namely, the phosphate elimination reaction may take place in *anti*-fashion, followed by cyclization of the inter-

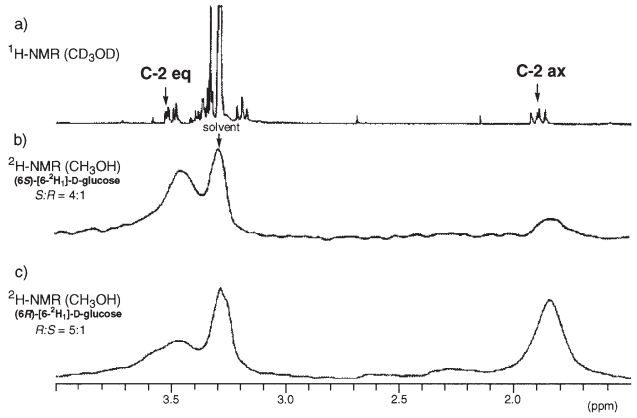


**Figure 2.** Pertinent region of $^1$H- and $^2$H-NMR spectra of DOI-oxime derivatives. a) nonlabeled DOI, b) DOI derived from D-(*6S*)-[6-$^2H_1$]glucose, c) DOI derived from D-(*6R*)-[6-$^2H_1$]glucose.

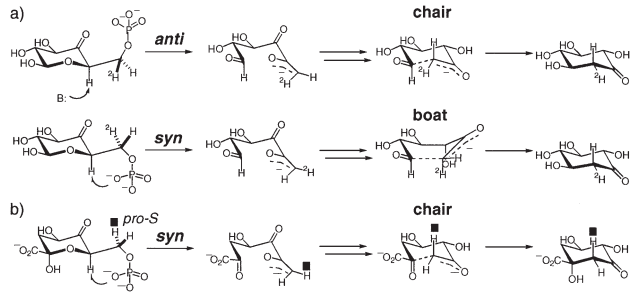


**Figure 3.** Comparison of the stereochemistry in the enzyme reaction. a) DOIS, b) DHQS.

mediate through a *chair*-conformation, or the phosphate is eliminated in *syn*-fashion and the following ring closure proceeds through a *boat*-conformation. However, the former appears to be more plausible because an intermediate in the enzyme active site tends to change its conformation in the least motion. Much severe conformational change should be involved in the latter case, which cannot be completely ruled out at the moment though.

In conclusion, the present study clearly demonstrated that the stereochemical pathway of the DOIS reaction is common both in *Bacillus* and *Streptomyces*, but is distinct from the DHQS reaction in the shikimate pathway. The evolution of the DOIS enzymes involved in microbial secondary metabolism is closely related as sister enzymes, but may be a bit apart from a cousin enzyme (DHQS) in a primary metabolism.

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