Available online at www.sciencedirect.com
ScienceDirect
Tetrahedron Letters
Tetrahedron Letters 48 (2007) 711–714

Synthesis and activity of two trifluorinated analogues of 1-deoxy-D-xylulose 5-phosphate

Odile Meyer, Catherine Grosdemange-Billiard, Denis Tritsch and Michel Rohmer*

Université Louis Pasteur – CNRS (UMR 7177 LC3), Institut Le Bel, 4 rue Blaise Pascal, 67070 Strasbourg Cedex, France

Received 21 September 2006; revised 13 November 2006; accepted 14 November 2006
Available online 8 December 2006

Abstract—An improved synthesis of 1,1,1-trifluoro-1-deoxy-D-xylulose 5-phosphate and an access to the reduced diastereomer mixture analogues 1,1,1-trifluoro-1-deoxy-D-xylitol 5-phosphate and 1,1,1-trifluoro-1-deoxy-D-lyxitol 5-phosphate are described. Inhibitor activity of all compounds on the MEP pathway for isoprenoid biosynthesis was evaluated.
© 2006 Elsevier Ltd. All rights reserved.

In animals, fungi, plant cytoplasm, archaebacteria and some eubacteria, isopentenyl diphosphate and dimethylallyl diphosphate, the precursors of isoprenoids, are synthesized through the mevalonate pathway,[1] while they derive from the more recently discovered methylerythritol phosphate (MEP) pathway in most bacteria, in the chloroplasts of all phototrophic organisms and in some unicellular eukaryotes, including *Plasmodium falciparum*, the parasite responsible for malaria.[2] As the inhibition of the biosynthesis of essential isoprenoids is lethal, each enzyme of the MEP pathway is a potential target for antibacterial or antiparasitic drugs.[3] We focused our attention on the second enzyme of the MEP pathway, the DXP reductoisomerase (DXR), which mediates the conversion of 1-deoxy-D-xylulose 5-phosphate (DXP) **1** into 2-*C*-methyl-D-erythritol 4-phosphate (MEP) **2** (Fig. 1).[4] This enzyme is a promising target to inhibit microbial growth. Indeed, fosmidomycin blocks the DXR in *Escherichia coli*,[5] *Zymomonas mobilis*[6] and *P. falciparum*[3a] and leads to the inhibition of the MEP pathway in higher plants.[7] Its structural analogue FR900098 inhibits the DXR of *P. falciparum*, and ester prodrugs thereof showed an improved in vivo antimicrobial activity against *Plasmodium vinckei* in mice.[8] Fosmidomycin or its prodrugs have also been shown to present some positive effects for the treatment of urinary infections[9] and simple malaria cases[10] in humans, indicating that DXR is a valuable antimicrobial target.

Compounds containing fluorine are useful tools for the elucidation of enzyme mechanisms and often used for the design of enzyme inhibitors. In this context, Bouvet and O'Hagan synthesized the 1-fluoro and 1,1-difluoro analogues of DX as the potential inhibitors of enzymes involved in the DX and DXP metabolism, but these compounds did not show any antibacterial properties against *E. coli* and *Staphylococcus aureus*,[11] perhaps because of the absence of an efficient phosphorylating system. To circumvent this problem, monofluorinated DXP analogues have been synthesized and tested on the DXR from *E. coli*.[12] The C-1 fluorinated analogue was found to behave as a substrate, whereas the C-3

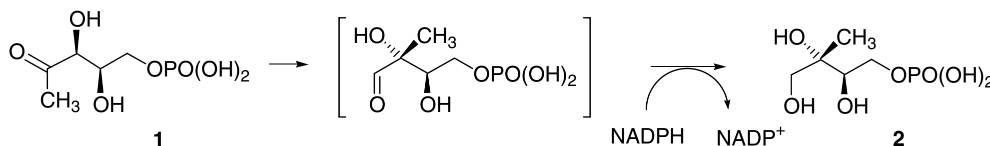

Figure 1. Reaction catalyzed by the deoxyxylulose phosphate reductoisomerase (DXR) in the MEP pathway: 1-deoxy-D-xylulose 5-phosphate **1**, 2-*C*-methyl-D-erythritol 4-phosphate **2**.

Keywords: 1-Deoxy-D-xylulose 5-phosphate reductoisomerase; Isoprenoid biosynthesis: 1,1,1-trifluoro-1-deoxy-D-xylulose 5-phosphate.
* Corresponding author. Tel.: +33 3 90 24 13 46; fax: +33 3 90 24 13 45; e-mail: mirohmer@chimie.u-strasbg.fr

0040-4039/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetlet.2006.11.091

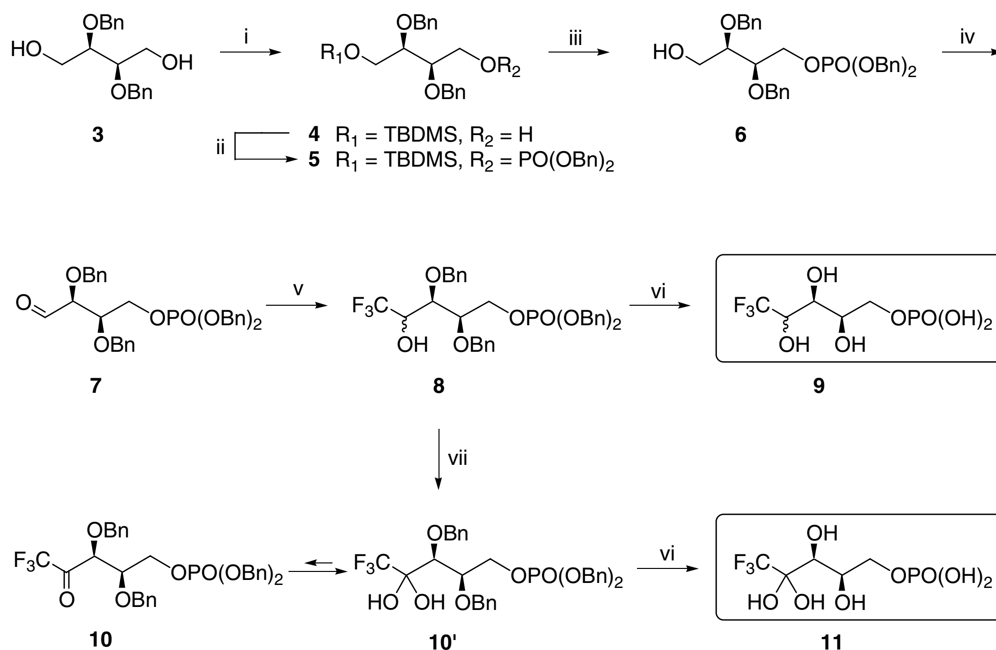

Figure 2. Synthesis of 1,1,1-trifluoro-1-deoxy-D-xylulose 5-phosphate **11** and the diastereomer mixture of 1,1,1-trifluoro-1-deoxy-D-xylitol 5-phosphate/1,1,1-trifluoro-1-deoxy-D-lyxitol 5-phosphate **9**. (i) TBDMSCl, NaH, THF (87%); (ii) $(BnO)_2PNEt_2$, tetrazole, *m*CPBA, CH_2Cl_2 (85%); (iii) Bu_4NF, THF, (87%); (iv) PCC, CH_2Cl_2, 3 Å molecular sieves (82%); (v) (a) $TMSCF_3$, CsF, (b) TBAF, THF (72%); (vi) H_2, 10% Pd/C, MeOH/H_2O (9:1) (quantitative); (vii) Dess–Martin, DMPI, CH_2Cl_2 (54%).

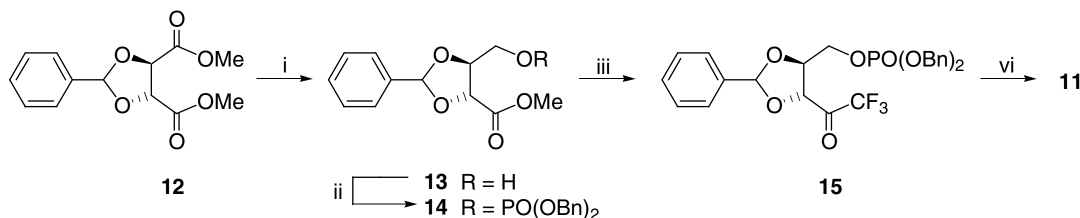

Figure 3. Improved synthesis of 1,1,1-trifluoro-1-deoxy-D-xylulose 5-phosphate **11**. (i) $NaBH_4$, MeOH, 88% (conversion: 55%); (ii) $(BnO)_2PNEt_2$, tetrazole, *m*CPBA, CH_2Cl_2 (81%); (iii) $TMSCF_3$, CsF, TBAF, THF (70%); (iv) H_2, 10% Pd/C, MeOH/H_2O (9:1) (quantitative).

or C-4 fluorinated derivatives were weak non-competitive inhibitors. In the search of novel drugs acting on the MEP pathway, DXP analogues containing a trifluoromethyl group were also synthesized. A synthesis and the evaluation of 1,1,1-trifluoro-1-deoxy-D-xylulose 5-phosphate **11** were recently reported by Fox and Poulter.[13] In this contribution, an improved synthesis of the latter compound and an access to the reduced diastereomer mixture analogues **9**, 1,1,1-trifluoro-1-deoxy-D-xylitol 5-phosphate and 1,1,1-trifluoro-1-deoxy-D-lyxitol 5-phosphate, are described (Figs. 2 and 3).

The trifluorinated compounds **9** and **11** were synthesized from the commercially available (2*R*,3*R*)-2,3-*O*,*O*-dibenzyl-threitol **3** following the reaction sequence outlined in Figure 2. The first step of the synthesis is the phosphorylation of a single hydroxyl group of the starting threitol derivative **3**. Direct phosphorylation of compound **3** with dibenzylphosphorochloridate was unsatisfactory since it resulted in a mixture of the desired monophosphate **6** (40% yield), the corresponding 1,4-bisphosphate and the starting material.[14] To avoid the formation of the bisphosphate, one of the two primary alcohol groups was protected as silylether using sodium hydride and *t*-butyldimethylsilyl chloride to afford **4** (87% yield). The remaining hydroxyl group was phosphorylated by the phosphoramidite method to give **5** in an 85% yield. Removal of the *O*-silyl group with tetrabutylammonium fluoride led to compound **6** in an 87% yield from **5**, corresponding to a 65% global yield from threitol derivative **3**. Due to the sensitivity of the phosphate group in basic conditions, the Swern oxidation could not be used, and aldehyde **7** was obtained by the oxidation of **6** with PCC (82% yield). The key step of this route, the introduction of the trifluoromethyl group, was performed with (trifluoromethyl)trimethylsilane (Ruppert's reagent). This nucleophilic trifluoromethylating agent leads to trifluoromethyl alcohols from aldehydes and ketones.[15] A modified procedure using cesium fluoride as the catalyst was employed. Aldehyde **7** was mixed with Ruppert's reagent and cesium fluoride. When the starting material was totally consumed, the addition of tetrabutylammonium fluoride allowed the formation of the secondary alcohol **8** in a 72% yield.[16] Removal of the benzyl protecting groups by catalytic hydrogenolysis in the presence of Pd/C provided quan-

titatively the fluorinated derivative **9** as a 1:2 mixture of two diastereomers in six steps and a 38% global yield.[17] Separation of the diastereomer mixture by silica gel chromatography was not possible, neither for the protected alcohol **8**, nor for the free compound **9**. Dess–Martin conditions[18] oxidized alcohol **8** into intermediate **10/10′** in a rather modest yield (54%). Other oxidation reagents (PCC, TPAP) did not increase the yield of this reaction. NMR spectra showed that trifluoromethylketone **10** was, as expected,[19] only in the hydrate form **10′**. The final deprotection by catalytic hydrogenolysis in the presence of Pd/C gave quantitatively and without epimerization at C-3 the enantiomerically pure trifluorinated analogue **11** of DXP in seven steps and a 21% overall yield. Its spectroscopic data were identical with those reported for the same compound by Fox and Poulter.[13]

The synthesis of the latter compound **11** was improved by a shorter reaction sequence starting from the commercially available dimethyl 2,3-*O*,*O*-benzylidene-D-tartrate **12** (Fig. 3). The phosphate group was introduced on the primary alcohol **13** obtained in an 88% yield by treating tartrate diester **12** with $NaBH_4$ in methanol.[11] The presence of a participating neighbouring group facilitated the ester reduction.[20] Such conditions did not give a complete conversion of the starting material, but compound **13** was easily isolated by silica gel flash chromatography, and the starting material was recycled. The primary alcohol **13** was phosphorylated using the phosphoramidite method affording the phosphate derivative **14** in an 81% yield. The introduction of the fluorinated group was performed as described above and directly provided trifluoromethylketone **15**, only in the hydrate form as judged from the ^{13}C and ^{19}F NMR spectra, from methyl ester **14** in a 70% yield.[16,21] Finally, the enantiomerically pure trifluorinated DXP analogue **11** was released quantitatively and without epimerization by catalytic hydrogenolysis of all benzyl protecting groups in a global yield of 50% in four steps. Both trifluorinated analogues of DXP **9** and **11** could be stored without decomposition at −18 °C for one year as lyophilized samples.

Fox and Poulter recently described a synthesis of **11** in seven steps in a 45% overall yield from the commercially available 2,3-*O*-isopropylidene-D-threitol.[13] In contrast with our route towards **11**, they first introduced the trifluoromethyl group before the phosphate group. The final removal of the isopropylidene 1,2-diol protecting group after hydrogenolysis of the benzylidene phosphate protections proved, however, to be difficult and required 5 days. In contrast, a single hydrogenolysis removes in our method simultaneously and in one step all benzyl protecting groups of the phosphate as well as of the hydroxyls, does not require purification of the end product and allows an easy scale-up of the synthesis. The hydrogenolysis was performed on small quantities of precursor **15** (up to 150 mg), which is a convenient stock material for compound **11**: it can be readily prepared in a larger amount (i.e., 1 g), stored for several months at −18 °C and hydrogenolyzed into **11** just before use. Our synthesis involving the addition of (trifluoromethyl)trimethylsilane on an ester rather than on an aldehyde is of a general scope and may be applied to the synthesis of other 1,1,1-trifluoro-1-deoxyketose derivatives.

1,1,1-Trifluoro-DXP **11** was not a substrate of the *E. coli* DXR since no oxidation of NADPH was observed. The inhibitory activity of trifluoro-DXP **11** and of diastereomer mixture **9** on the DXR was also investigated.[22] 1,1,1-Trifluoro-DXP **11** revealed as a poor inhibitor of the *E. coli* DXR with a 1.7 mM IC_{50}, quite similar to that reported by Fox and Poulter (2 mM),[13] and to be compared to the K_m found for DXP (97 μM) or MEP (158 μM).[4c] The hydration of the carbonyl group may prevent the binding to the active site of the enzyme. Compound **9** was found to be more efficient on DXR with a 0.6 mM IC_{50}. It was therefore investigated in more details and revealed as a reversible non-competitive inhibitor with a 360 μM K_i value for the 2:1 diastereomer mixture resulting from the synthetic scheme. None of compounds **9** or **11** inhibited the growth of *E. coli* in test on Petri dishes by the agar diffusion method on filter paper discs (6 mm diameter). Even the highest amount tested (100 μg/disc) did not prevent bacterial growth. Despite their low activities, weak inhibitors may be used for screening of inhibitors in pharmaceutical research or for RX studies on enzymes. They are allowed to bind to the enzyme, and compound libraries may be screened to identify those, which displace the weak binders.

Acknowledgments

We thank Mr. R. Huber for the MS analysis. This investigation was supported by a grant to M.R. from the 'Institut Universitaire de France'. O.M. was a recipient of a grant from the 'Ministère de la Jeunesse, de l'Education Nationale et de la Recherche'.

References and notes

1. (a) Bloch, K. *Steroids* **1992**, *57*, 378–383; (b) Bochar, D. A.; Friesen, J. A.; Stauffacher, C. V.; Rodwell, V. W. In *Comprehensive Natural Product Chemistry*; Cane, D. E., Ed.; Isoprenoids Including Carotenoids and Steroids; Pergamon: Oxford UK, 1999; Vol. 2, pp 15–44.
2. (a) Rohmer, M. *Nat. Prod. Rep.* **1999**, *16*, 565–574; (b) Lichtenthaler, H. K. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **1999**, *50*, 47–65; (c) Eisenreich, W.; Rohdich, F.; Bacher, A. *Trends Plant Sci.* **2001**, *6*, 78–84.
3. (a) Jomaa, H.; Wiesner, J.; Sanderbrand, S.; Altincicek, B.; Weidemeyer, C.; Hintz, M.; Türbachova, I.; Eberl, M.; Zeidler, J.; Lichtenthaler, H. K.; Soldati, D.; Beck, E. *Science* **1999**, *285*, 1573–1576; (b) Rohmer, M.; Grosdemange-Billiard, C.; Seemann, M.; Tritsch, D. *Curr. Opin. Invest. Drugs* **2004**, *5*, 154–162.
4. (a) Kuzuyama, T.; Takahashi, S.; Takagi, M.; Seto, H. *J. Biol. Chem.* **2000**, *275*, 19928–19932; (b) Radykewicz, T.; Rohdich, F.; Wungsintaweekul, J.; Herz, S.; Kis, K.; Eisenreich, W.; Bacher, A.; Zenk, M. H.; Arigoni, D. *FEBS Lett.* **2000**, *465*, 157–160; (c) Hoeffler, J.-F.; Tritsch, D.; Grosdemange-Billiard, C.; Rohmer, M. *Europ. J. Biochem.* **2002**, *269*, 4446–4457.

5. Kuzuyama, T.; Shimizu, T.; Takahashi, S.; Seto, H. *Tetrahedron Lett.* **1998**, *39*, 7913–7916.
6. Grolle, S.; Bringer-Meyer, S.; Sahm, H. *FEMS Microbiol. Lett.* **2000**, *191*, 131–137.
7. Fellermeier, M.; Kis, K.; Sagner, S.; Maier, U.; Bacher, A.; Zenk, M. *Tetrahedron Lett.* **1999**, *40*, 2743–2746.
8. (a) Reichenberg, A.; Wiesner, J.; Weidemeyer, C.; Dreiseidler, E.; Sanderbrand, S.; Altincicek, B.; Beck, E.; Schlitzer, M.; Jomaa, H. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 833–835; (b) Ortmann, R.; Wiesner, J.; Reichenberg, A.; Henschker, D.; Beck, E.; Jomaa, H.; Schlitzer, M. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 305–314; (c) Ortmann, R.; Wiesner, J.; Reichenberg, A.; Henschker, D.; Beck, E.; Jomaa, H.; Schlitzer, M. *Arch. Pharm. Chem. Life Sci.* **2005**, *338*, 305–314.
9. (a) Kuemmerle, H. P.; Murakawa, T.; Sakamoto, H.; Sato, N.; Konishi, T.; De Santis, F. *Int. J. Clin. Pharmacol. Ther. Toxicol.* **1985**, *23*, 521–528; (b) Kuemmerle, H. P.; Murakawa, T.; De Santis, F. *Chemioterapia* **1987**, *6*, 113–119.
10. (a) Missinou, M. A.; Borrmann, S.; Schindler, A.; Issifou, S.; Adegnika, A. A.; Matsiegui, P.-B.; Binder, R.; Lell, B.; Wiesner, J.; Baranek, T.; Jomaa, H.; Kremsner, P. G. *Lancet* **2002**, *360*, 1941–1942; (b) Lell, B.; Ruangweerayut, R.; Wiesner, J.; Missinou, M. A.; Schindler, A.; Baranek, T.; Hintz, M.; Hutchinson, D.; Jomaa, H.; Kremsner, P. G. *Antimicrob. Agents Chemother.* **2003**, *47*, 735–738.
11. Bouvet, D.; O'Hagan, D. *Tetrahedron* **1999**, *55*, 10481–10486.
12. Wong, A.; Munos, J. W.; Devasthali, V.; Johnson, K. A.; Liu, H.-W. *Org. Lett.* **2004**, *6*, 3625–3628.
13. Fox, T.; Poulter, D. C. *J. Org. Chem.* **2005**, *70*, 1978–1985.
14. Taylor, S. V.; Vu, L. D.; Begley, T. P.; Schörken, U.; Grolle, S.; Sprenger, G. A.; Bringer-Meyer, S.; Sahm, H. *J. Org. Chem.* **1998**, *63*, 2375–2377.
15. Prakash, G. K. S.; Krishnamurti, R.; Olah, G. A. *J. Am. Chem. Soc.* **1989**, *111*, 393–395.
16. Singh, R. P.; Cao, G.; Kirchmeier, R. L.; Shreeve, J. M. *J. Org. Chem.* **1999**, *64*, 2873–2876.
17. 1,1,1-Trifluoro-1-deoxy-D-xylitol 5-phosphate and 1,1,1-trifluoro-1-deoxy-D-lyxitol 5-phosphate diastereomer mixture **9**. Compound **8** (120 mg, 0.19 mmol) was hydrogenolyzed over 20% Pd/C (25 mg) in MeOH/H_2O (9:1, 5 mL) for 24 h at room temperature and atmospheric pressure. After filtration on Celite and evaporation to dryness, the 2:1 mixture of diastereomers **9** was dissolved in water (1 mL), and the solution was adjusted to pH 6–7 by the addition of 1 M sodium hydroxide and lyophilized. Vitreous solid, which decomposes at 88 °C. $R_f = 0.28$, (*i*-propanol/water/ethyl acetate, 6/3/1). NMR spectroscopy. Signals labelled with the superscript * correspond to those that are differentiated in the spectra of each diastereomer. δ_H (300 MHz, D_2O) 3.67 (2H, m, 5-H), 3.75 (1H, m, 3-H), 3.87 (1H, m, 4-H), 4.04 (1H, d, $J_{2-3} = 6.6$ Hz, 2-H); δ_C (75 MHz, D_2O) 65.6 (CH_2, d, $J_{C-P} = 5.0$ Hz, C-5), 65.8 (CH_2, d, $J_{C-P} = 5.0$ Hz, C-5*), 68.1 (CH, d, $J_{C-P} = 6.0$ Hz, C-4), 68.5 (CH, d, $J_{C-P} = 6.0$ Hz, C-4*), 68.8 (CH, C-3), 68.9 (CH, C-3*), 71.0 (CH, q, $J_{C-F} = 10$ Hz, C-2), 124.6 (quaternary C, q, $J_{C-F} = 282$ Hz, C-1), 125.3 (quaternary C, q, $J_{C-F} = 282$ Hz, C-1*); δ_P (121.5 MHz, D_2O) 2.0 (s); δ_F (282 MHz, D_2O) −76.56 (d, $J_{H-F} = 6.2$ Hz, CF_3), −75.51 (d, $J_{H-F} = 6.2$ Hz, CF_3^*). IR (KBr) ν_{max}/cm^{-1}: 3382, 1648, 1393, 1273, 1177, 1141, 1070, 925. MS (ES^-) *m*/*z*: 269 ($M-H^+$).
18. Dess, D. B.; Martin, J. C. *J. Am. Chem. Soc.* **1991**, *113*, 7277–7287.
19. Gelb, M. H.; Svaren, J. P.; Abeles, R. H. *Biochemistry* **1985**, *24*, 1813–1817.
20. (a) Dalla, V.; Catteau, J. P.; Pale, P. *Tetrahedron Lett.* **1999**, *40*, 5193–5196; (b) Nichols, B. W.; Safford, R. *Chem. Phys. Lipids* **1973**, *11*, 222–227; (c) Giuminiani, A. G.; Tubaro, F. *J. Prakt. Chem.* **1990**, *332*, 755–761.
21. Wiedemann, J.; Heiner, T.; Mloston, G.; Prakash, G. K. S.; Olah, G. A. *Angew. Chem., Int. Ed.* **1998**, *37*, 820–821.
22. The His-tagged *E. coli* DXR was obtained as previously reported.[4c] The enzymatic activity was determined in a 50 mM Tris–HCl, pH 7.5 buffer containing 3 mM $MgCl_2$ and 2 mM DTT at 37 °C. The concentrations of NADPH and DXP were 0.15 and 0.11 mM, respectively. The reaction was initiated by adding His-tagged DXR (2 μg). Initial rates were measured by following the decrease of the absorbance at 340 nm due to the oxidation of NADPH (Uvikon 933, Kontron Instruments). The influence of compounds **9** and **11** on the enzymatic activity was studied by adding them to the reaction medium at various concentrations (0.1–3 mM). The IC_{50} was calculated from a semi-log plot of enzyme residual activity as a function of inhibitor concentration. The K_i of compound **9** was calculated from a double reciprocal plot of enzymatic rate versus DXP concentration (0.05–0.5 mM) in its absence and in its presence at various concentrations (0.3–1 mM) in the reaction medium.