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SYNTHESIS OF (2R,3R)-, (2S,3S)-, (2R,3S)- AND (2S,3R)-IMIDAZOLE GLYCEROL PHOSPHATES (IGP): SUBSTRATES FOR IGP-DEHYDRATASE (IGPD)

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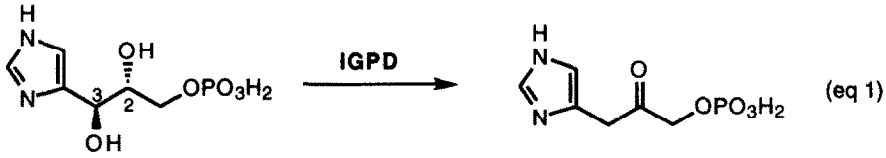
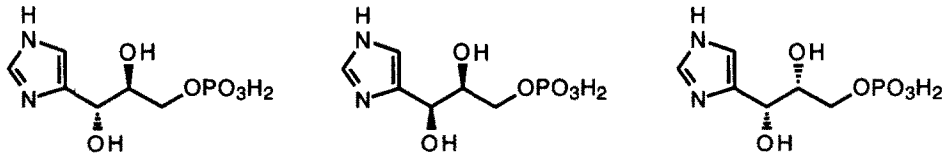
Abstract: D-*erythro*-(2R,3S)-Imidazole glycerol phosphate (IGP) and its stereoisomers [(2S,3R), (2R,3R), (2S,3S)] have been synthesized via the Evans aldol reaction. The stereochemical assignments of these isomers and their IGPD-substrate activities were studied.

Imidazole glycerol phosphate dehydratase (IGPD) (E.C. 4.2.1.19) is the dehydrating enzyme involved in the biosynthetic pathway of histidine. It catalyzes the conversion of D-*erythro*-(2R,3S)-imidazole glycerol phosphate (IGP) (**1**) to imidazole acetol phosphate (IAP) (**2**) (eq 1).[1,2] In spite of increasing interest in the enzymes involved in amino acid biosynthesis,[3] less attention has been paid to this enzyme and very little information is available on its enzymology and reaction mechanism.[4,5] As one aspect of complete understanding of the reaction mechanism, the substrate specificity of IGPD and especially the requirement for stereochemistry of the two hydroxy groups in the substrate (IGP) is of great interest. Therefore, we have synthesized all of the four stereoisomers of IGP (**1**[6] and **3-5**) by using the Evans aldol reaction[7]. Herein we report synthesis and stereochemical assignment of these IGP isomers as well as their substrate activities in the IGPD-catalyzed reaction.

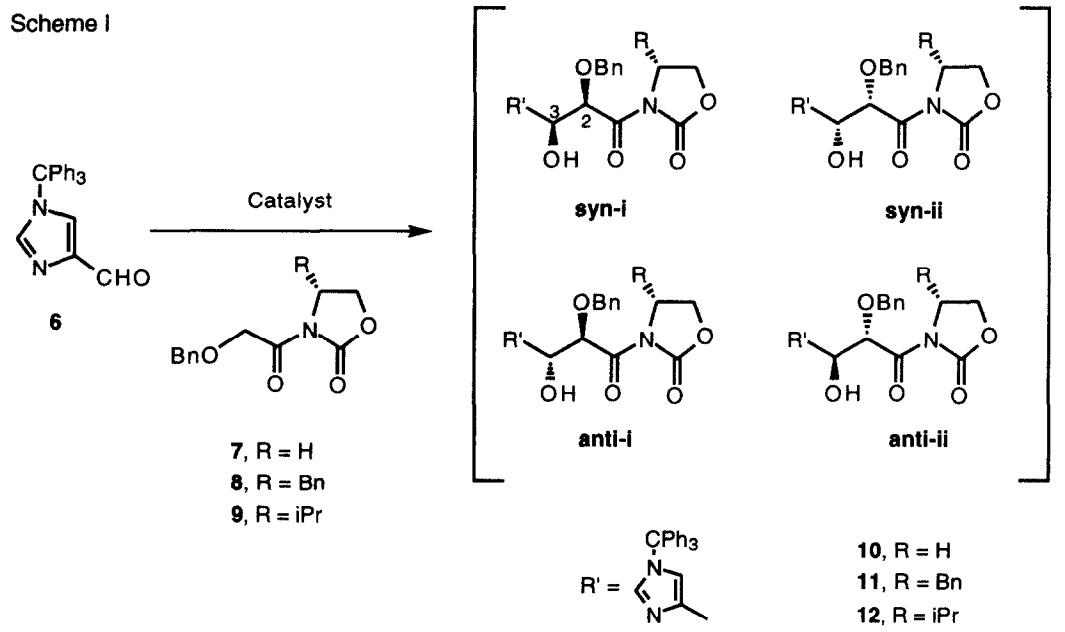


1 (2R,3S) (IGP) **2**



3 (2S,3R) **4** (2S,3S) **5** (2R,3R)

Scheme I



The synthesis began with the aldol reaction[7,8] of 1-tritylimidazole-4-carboxaldehyde (**6**)[9] and the enolate of benzyloxyacetimides (**7-9**) (Scheme I). The reactions were carried out under various conditions using Lewis acid catalysts. The results are summarized in Table 1. Reactions of the achiral oxazolidinone **7** led to the formation of a mixture of racemates, syn-**10** and anti-**10,** which were easily isolated by column chromatography after silylation (TBDMSCl, imidazole, DMF). In the case of reactions using chiral oxazolidinones, **8** and **9**, a mixture of four diastereomers of the adducts (**11** and **12,** respectively) were obtained. All these isomers were isolated in pure form by HPLC using a silica gel column [Shim-pack Shimadzu)] with the solvent system of hexane/$CHCl_3$/iPrOH (18:2:1). While the observed diastereoselectivities are diverse, each isomer could be obtained in a reasonable amount by choosing appropriate conditions. The stereochemical assignments were made on the basis of spectroscopic data, chemical conversion as well as identification with an authentic sample of **1**[6] (*vide infra*).

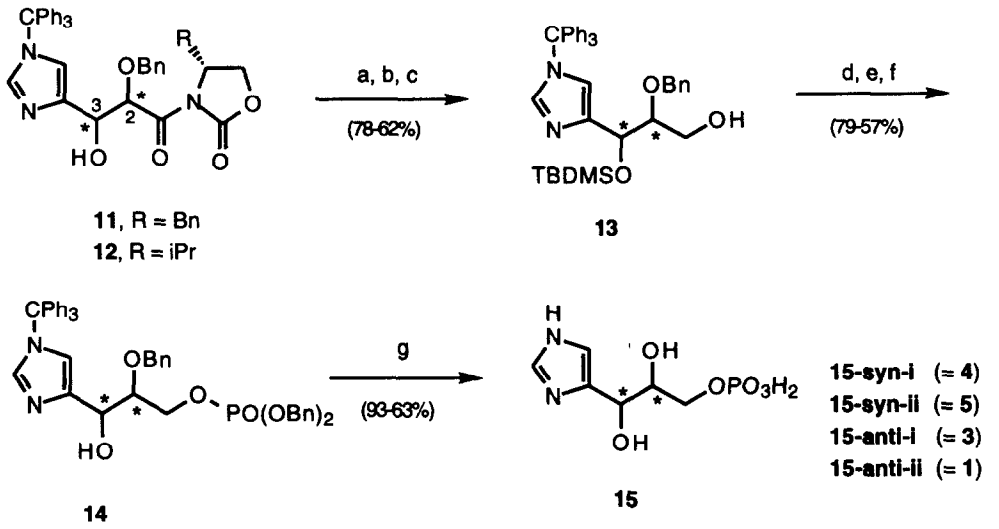
Each diastereomer of **11** and **12** was separately transformed into the IGP isomer **15** as shown in Scheme II. The reductive cleavage of oxazolidinone moiety of **11** (or **12**) was accomplished by a successive treatment[10] with $LiAlH_4$ (-78 °C) and $NaBH_4$ (0 °C), and the resulting alcohol **13** was phosphorylated by the method of Bannwarth and Trzeciak[11] to give **14**. Catalytic hydrogenation of **14** under acidic conditions (AcOH) afforded fully deprotected **15**.[12] Among four isomers thus prepared, the compound **15-anti-ii** derived from **11-anti-ii** (or **12-anti-ii**) was found to be identical with authentic IGP **1**[6] in all aspects including the biological data. Thus, the absolute stereochemistry of the **anti-ii** isomer was concluded to be 2R,3S.

Table 1[a] Aldol Reactions of **6** with **7-9**

entry	R (7-9)	catalyst	base	solvent	product	yield (%)[b]	diastereomer ratio[c]			
							syn-i	syn-ii	anti-i	anti-ii
1	H	9-BBNOTf	iPr_2NEt	CH_2Cl_2	**10**	76	70[d]		30[d]	
2	H	$(C_5H_9)_2BOTf$	iPr_2NEt	CH_2Cl_2	**10**	88	93[d]		7[d]	
3	H	$Sn(OTf)_2$	1-ethylpiperidine	CH_2Cl_2	**10**	46	56[d]		44[d]	
4	CH_2Ph	9-BBNOTf	iPr_2NEt	CH_2Cl_2	**11**	74	24.5	4.2	69.6	1.7
5	CH_2Ph	$(C_5H_9)_2BOTf$	iPr_2NEt	CH_2Cl_2	**11**	97	93.7	0.5	5.1	0.7
6	CH_2Ph	$Sn(OTf)_2$	1-ethylpiperidine	CH_2Cl_2	**11**	54	18.9	8.0	63.5	9.6
7	iPr	9-BBNOTf	iPr_2NEt	CH_2Cl_2	**12**	89	26.4	4.2	67.4	2.0
8	iPr	$(C_5H_9)_2BOTf$	iPr_2NEt	CH_2Cl_2	**12**	98	--	30.0	--	70.0
9	iPr	$Sn(OTf)_2$	1-ethylpiperidine	CH_2Cl_2	**12**	47	9.5	15.4	65.1	10.0

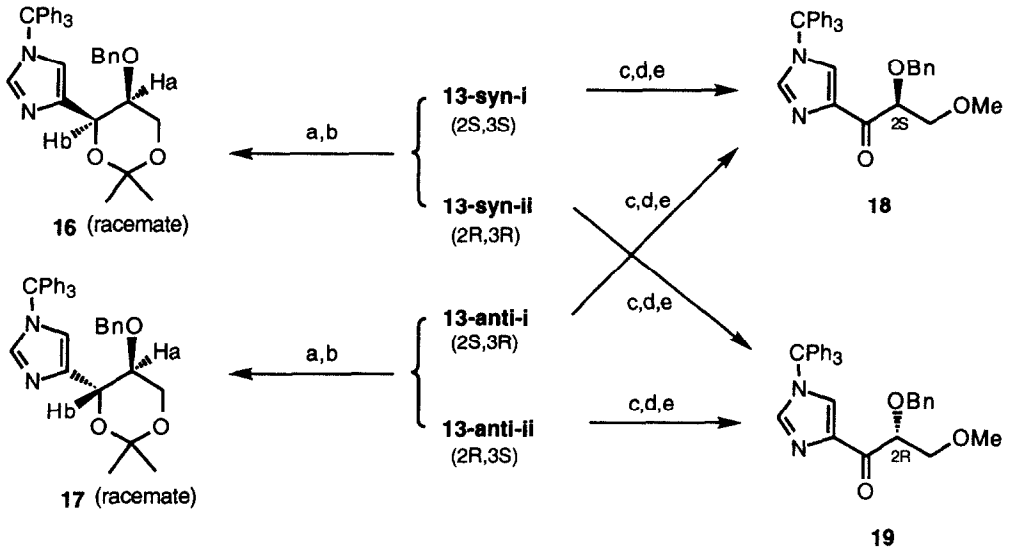
[a]All reactions were carried out at -78 → -10 °C under Ar. [b]Isolated yields. [c]Determined by HPLC (see text). [d]The syn/anti ratio was determined on the basis of isolated yields of aldol products (racemates) after silylation (TBDMSCl, imidazole, DMF).

Scheme II[a]



[a]Reagents and conditions: (a) TBDMSCl, imidazole, DMF, 0 → 25 °C; (b) $LiAlH_4$, THF, -70 °C; (c) $NaBH_4$, MeOH, 0 °C; (d) $(iPr)_2NP(OBn)_2$, tetrazole, CH_3CN, 25 °C; (e) m-CPBA, CH_2Cl_2, -40 °C; (f) Bu_4NF, THF, -10 → 25 °C; (g) 10% Pd/C, H_2, AcOH/MeOH (8:1).

Scheme III[a]



[a]Reagents and conditions: (a) Bu_4NF, THF, -10 → 25 °C (97-98%); (b) 2,2-dimethoxypropane, p-TsOH, 25 °C (55-64%); (c) NaH, MeI, THF, 0 °C (50-61%); (d) Bu_4NF, THF (86-100%) ; (e) MnO_2, CH_2Cl_2, 0 → 25 °C (58-71%).

With the knowledge of the absolute configuration of **anti-ii** isomer (same as the natural IGP[6]), stereochemical assignment of the other isomers was done on the basis of the chemical transformations as outlined in Scheme III. The relative (syn/anti) stereochemistry at carbon C-2 and C-3 was confirmed by converting racemic alcohols syn-**13** and anti-**13** into the cyclic acetonides **16** and **17**, respectively (Scheme III). In the ^1H NMR spectra, the coupling between two vicinal protons (Ha, Hb) of **16** (cis) (Ja,b = 4.2 Hz) is smaller than that of **17** (trans) (Ja,b = 5.7 Hz) and only **16** shows a NOE effect (2.4%) between these two protons. On the other hand, the absolute configuration at C-2 position was determined by the following chemical transformations. The above-mentioned anti isomer **13-anti-ii** (2R,3S) was converted into a 3-keto compound **19** (2R): $[\alpha]_D$ +32°, by the sequential reactions: (1) methylation of the C-1 alcohol, (2) desilylation, and (3) oxidation of the C-3 hydroxyl group to a carbonyl. The similar treatment of **13-syn-ii** afforded the same **19**, whereas **13-syn-i** and **13-anti-i** were transformed into the enantiomer **18:** $[\alpha]_D$ -31° (Scheme III). These results clearly indicate that **syn-ii** and **anti-ii** isomers have the R-configuration at C-2 position, while **syn-i** and **anti-i** have the S-configuration as shown in Scheme I. Hence, IGP isomers **15-syn-i**, **15-syn-ii**, **15-anti-i**, and **15-anti-ii** were determined to be **4**, **5**, **3**, and **1** (IGP), respectively.

The substrate activities of these compounds were explored using wheat germ IGPD.[13] As shown in Figure 1, only **1** was a good substrate and all other isomers (**3**, **4**, **5**) did not show any potency as a substrate in

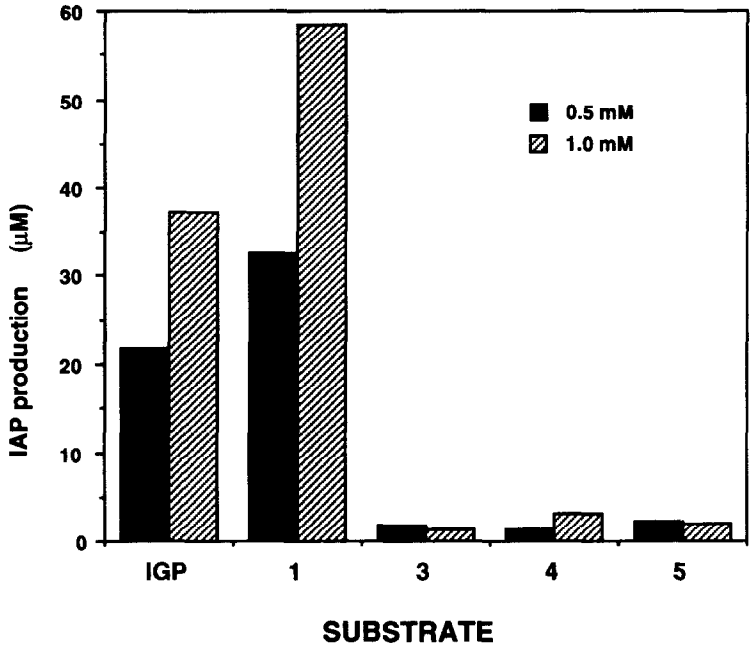


Figure 1. Substrate activities in the IGPD catalysis.[13]

comparison with authentic IGP[15]. In addition, none of these isomers showed any significant inhibitory activity. These results clearly indicate that not only the stereochemistry of the C-3 hydroxy group (a leaving group) but also that of the C-2 hydroxy group is important for specific binding interaction and that the (2R,3S) configuration is required as an active substrate of IGPD.

In conclusion, all four stereoisomers of IGP were synthesized and their stereochemical assignments were achieved. It was revealed that only the (2R,3S)-isomer is a substrate for the IGPD-catalyzed reaction indicating a high degree of substrate specificity of this enzyme.[14]

Acknowledgment. We are grateful to Drs. J. Mano and A. Scheidegger for the helpful discussions. We also thank Mr. K. Kanaori for measurement of 600 MHz NMR spectra of IGP.

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10. The two-step procedure was found to be most effective for reductive cleavage of the oxazolidinone group.
11. Bannwarth, W.; Trzeciak, A. *Helv. Chim. Acta.* **1987**, *70*, 175.
12. All new compounds gave satisfactory spectral data. **15-syn-i** (= **4**): $[\alpha]_D$+10.8° (c = 0.97, H_2O); ^{1}H NMR (400 MHz; D_2O), δ 3.80-3.88 (1H, m), 3.93-4.00 (1H, m), 4.03-4.08 (1H, m), 5.05 (1H, d, J = 5.0 Hz), 7.45 (1H, s), 8.62 (1H, s); ^{31}P NMR (101 MHz; D_2O) δ 1.44 (s); ^{13}C NMR (75 MHz; D_2O) δ 65.56, 65.77, 72.82, 117.10, 133.70, 134.37; FAB-MS, 239 (M^++H). **15-syn-ii** (= **5**): $[\alpha]_D$-10.7° (c = 1.0, H_2O). **15-anti-i** (= **3**): $[\alpha]_D$ +6.8° (c = 0.98, H_2O); ^{1}H NMR (400 MHz; D_2O), δ 3.89-4.03 (2H, m), 4.05-4.10 (1H, m), 5.00 (1H, d, J = 6.0 Hz), 7.46 (1H, s), 8.65 (1H, s); ^{31}P NMR (D_2O) δ 1.73 (s); ^{13}C NMR (D_2O) δ 65.45, 65.88, 72.92, 117.53, 133.43, 134.44; FAB-MS, 239 (M^++H). **15-anti-ii** (= **1**): $[\alpha]_D$ -6.1° (c = 0.99, H_2O). **16**: ^{1}H NMR (90 MHz; $CDCl_3$), δ 1.21 (6H, s), 3.38 (1H, dd, J = 10.2, 5.6 Hz), 3.57 (1H, dd, J = 10.1, 4.4 Hz), 3.95 (1H, m), 4.51 (2H, AB-q, J_{AB} = 11.6 Hz), 4.74 (1H, d, J = 4.2 Hz), 6.80 (1H, s), 6.90 - 7.50 (21H, m). **17**: ^{1}H NMR (90 MHz; $CDCl_3$), δ 1.29 (6H, s) 3.56 - 3.70 (2H, m), 3.77 - 4.04 (1H, m), 4.60 (2H, AB-q, J_{AB} = 12.1 Hz), 4.86 (1H, d, J = 5.7 Hz), 6.88 (1H, s), 6.98 - 7.58 (21H, m). **18**, **19**: ^{1}H NMR (90 MHz; $CDCl_3$), δ 3.35 (3H, s), 3.86 (2H, d, J = 4.5 Hz), 4.50 (1H, d, J = 11.9 Hz), 4.74 (1H, d, J = 11.9 Hz), 5.03 (1H, t, J = 4.2 Hz), 7.00-7.45 (21H, m), 7.77 (1H, s).
13. The enzyme activity was determined by using the partially purified wheat germ IGPD[14] in 100 μl of 50 mM bistris-propane containing 100 mM 2-mercaptoethanol, 0.4 mM $MnCl_2$ and a substrate. After incubation at pH 6.7 at 30°C for 20 min, the formed IAP was dephosphorylated by alkaline phosphatase and quantified by measuring the UV absorption at 370 nm (the enol form of imidazolacetol) on addition of 5 N NaOH phosphate: Ames, B. N.; Mitchell, H. K. *J. Biol. Chem.* **1955**, *212*, 687.
14. Details of purification and characterization of IGPD from wheat germ will be published elsewhere: Mano, J.; Hatano, M.; Koizumi, S.; Tada, S.; Scheidegger, A. (manuscript in preparation)
15. The smaller activity of authentic IGP compared with **1** is due to the partial epimerization taking place during preparation of the former.[6]