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*Original Article*

# Investigation of Intestinal Absorption of Pyridinones in Rat

Masihalah Taher*[a], Lotfollah Saghaie[b] and Maryam Abrahilmi[b]

[a]*Department of Biochemistry, Faculty of Pharmacy and Pharmaceutical Sciences. Isfahan University of Medical Sciences, Isfahan, Iran.* [b]*Department of Medicinal Chemistry, Faculty of Pharmacy and Pharmaceutical Sciences. Isfahan University of Medical Sciences, Isfahan, Iran.*

**Abstract**

Recently, it has been shown that a number of hydroxypyridinones such as 1,2-dimethyl-3-hydroxypyridin-4-one (L1) are useful for the treatment of iron overload in place of desferrioxamine in thalassaemic patients. In this study, the intestinal absorption (I.A.) of L1 and one of its analogues namely 2-methy-3-hydroxypyridin-4-one (L2), which possesses a higher partition coefficient ($K_{part}$) than L1, have been determined.

The ligands L1 and L2, used in the present study, were synthesized from maltol and methylamine or ammonia, respectively in a three step reaction method. Identification and purity of compounds were achieved by spectroscopy and elemental analysis. The I.A. of drugs was determined using the Everted Gut Sac method at different concentrations and time intervals. The concentrations of samples were measured by a UV/Vis spectrophotometer ($\lambda_{max}$=280 nm).

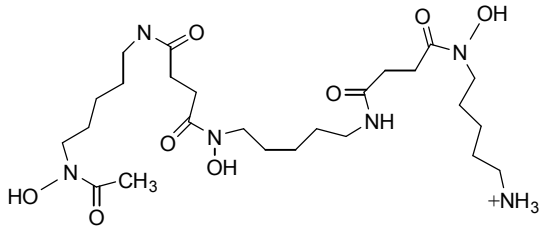
The results showed that the rate and I.A. of L2 are not statistically different from those of L1. At a concentration of 60 mg/lit, and after 45 min, the absorption reached a maximum for both ligands. It is clear that for the prediction of I.A. of a new drug a simple measurement of $K_{part}$ is not sufficient and other factors such as the number of hydrogen bonds between drug molecules and the surrounding molecules should also be taken into account due to their possible interferences. It could be concluded that from the point of I.A, the drug $L_2$ has no advantage over the $L_1$.

**Keywords:** 1.2-dimethyl-3-hydroxypyridin-4-one; Intestinal absorption; Partition coefficient; Everted Gut Sac.

## Introduction

There are a number of inherited disease states which are associated with the gradual accumulation of iron; β-thalassaemia and thalassaemia intermedia being particularly well characterized (1). In some regions of the world genes are relatively common; For instance in South East Asia, approximately 100000 children are born each year suffering from thalassaemia. The normal total body iron is 4-5 g per adult, whereas some thalassaemic patients may accumulate 50-70 g. Iron, by virtue of its facile redox chemistry, is toxic when present in excess (2). Desferrioxamine (DFO) (Scheme 1, **l**) a natural siderophore, has been used for the treatment of iron overload for over 30 years (3), and currently it is the only clinically useful drug available for this purpose. However, DFO suffers from the disadvantage that it is inactive when administered orally, and only causes sufficient iron excretion to keep pace with the transfusion regimes when given either subcutaneously or intravenously over 12-18 h several times per week. For this reason, many patients find it difficult to comply with the treatment, and some

* Corresponding author:
E-mail: taher@pharm.mui.ac.ir



**Scheme 1.** Desferrioxamine

even stop taking the drug altogether, subsequently developing the complications of iron overload. There is, therefore, no doubt that an orally active chelating agent is needed to treat patients on lifelong transfusion programs. The development of an oral iron chelator might also allow the extension of the therapeutic use of red-cell transfusions in sickle-cell anemia.

3-Hydroxypyridin-4-ones (HPOs) are currently one of the main candidates for the development of orally active iron chelators, being alternative to DFO (4). Indeed, the 1,2-dimethyl derivatives CP2O (Deferiprone, $L_1$) (**5a**) is currently in clinical trials. Unfortunately, the dose required to keep a previously well-chelated patient in negative iron balance appears to be relatively high. Not surprisingly, side effects have been observed in some patients receiving $L_1$ (3). One of the major reasons for the limited efficacy of L1 in clinical use is that it undergoes extensive phase II metabolism in the liver (5).

In order to investigate further ligands, which are able to scavenge iron effectively at low ligand concentrations, it was decided to synthesize another analogue of this type, namely 2-methyl-3-hydroxypyridin-4-one ($L_2$) (**5b**).

In this work, we describe the synthesis, partition coefficients ($K_{part}$) and the intestinal absorption (I.A) of both ligands $L_1$ and $L_2$.

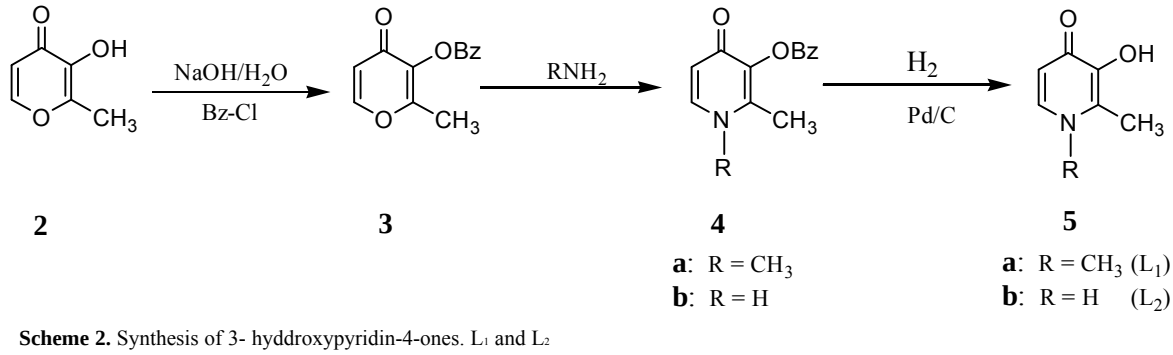
## Experimental

All the chemicals used in this work were obtained from Aldrich (Gillingham, UK). Melting points are uncorrected. IR spectra were recorded on a Perkin-Elmer 1420. Proton NMR spectra were determined with EM-390 (80 MHz). Mass spectra were take using a Vacuum Generaters 16F (35ev). Elemental analyses were performed by micro analytical laboratories, University of Manchester, Manchester. The optical absorbance spectra of the hydroxypyridine ligands were measured with a Perkin-Elmer model 551 UV/Vis spectrophotometer.

2-Alkyl-3-hydroxypyridinenes ($L_1$ and $L_2$) in this study were synthesized utilizing the methodology of Harris (6) (Schema 2).

*Synthesis of 2-methyl-3-benzyloxypran-4-one (Benzyl maltol) (**3**)*

To a solution of maltol (2) (12 g, 0.1 mol) in methanol (100 ml) 10 ml of an aqueous solution of sodium hydroxide (4.4 g, 0.11 mol) was added dissolved in water (10 ml) followed by benzyl chloride (13.9 g, 0.11 mol) and the mixture was refluxed for 6 h. After removal of the solvent by rotary evaporation, the residue was mixed with water (50 ml) and extracted into dichloromethane (3×50 ml). The combined extracts were washed with 5% sodium hydroxide (3×150 ml) and then with water (2× 150 ml). The organic fraction was dried over anhydrous sodium sulphate, filtered and rotary-evaporated to yield an orange-colored oil, which solidified on cooling. Recrystallization form diethyl ether gave the pure product, as colorless needles. 17.7 g (82%). mp 52-53°C, $^1$H NMR




**Scheme 2.** Synthesis of 3- hyddroxypyridin-4-ones. $L_1$ and $L_2$

(DMSO-$d_6$): 2.10 (s, 3H, 2-CH3), 5.10 (s, 2H, O-CH2-Ph), 7.94(d, 1H, 6-H): MS (EI): m/z=216 (M), IR (KBr): 1640 (C=O) Cm-1. Anal. CalCd. for $C_{13}H_{12}O_3$: C, 72.21; H, 5.59%. Found: C, 72.31; H, 5.65%.

Synthesis of 1,2-dimethyl-3-benzyloxypyridin-4-one hydrochloride (**4a**)

To a solution of compound 3 (25 g, 0.12 mol) in ethanol (200 ml)/water (200 ml) a 40% aqueous methylamine (14 g, o.18 mol) was added, followed by 2N sodium hydroxide solution (10 ml), and mixture refluxed for 12 h. After pH adjustment with HCl, the volume was reduced to 200 ml by rotary evaporation prior to addition of water (200 ml) and washing with diethyl ether (400 ml). Subsequent adjustment of the aqueous fraction to pH 7 with 10 N NaOH solution was followed by extraction into dichloromethane (3×400 ml); the organic layers were then dried over anhydrous sodium sulphate, filtered and rotary-evaporated to give an orange oil. This oil was dissolved in ethanol/hydrochloric acid and rotary-evaporated, the resulting white solid was recrystallized from ethanol/diethyl ether to give a white powder (24.2 g, 76%) mp 206-207°C. 1H NMR (DMSO-$d_6$): 2.21 (s, 3H, 2-CH3), 3.94 (s, 3H, N-CH3), 5.03 (s, 2H, O-CH2-Ph), 6.18 (d, 1H, 5-H) 7.25-7.52 (m, 5H, Ph), 7.58 (d, 1H, 6-H): MS (EI)): m/z=265 (M-HCl). Anal. Calcd. for $C_{14}H_{16}NO_2Cl$: C, 63.27; H, 6.08; N,5.27; Cl,13.34%. Found: C, 63.15; H, 6.11; N, 5.21; Cl 13.43%.

*Synthesis of 1,2-dimethyl-3-hydroxypyridin-4-one hydrochloride (**5a**)*

Compound **4a** (20 g, 0.075 mol) was dissolved in ethanol (270 ml)/water (30 ml) and subjected to hydrogenolysis in the presence of Pd/C catalyst. Filtration, followed by rotary evaporation gave a white solid; Recrystallization from ethanol/diethyl ether yielded a white powder (11.6 g, 88%); mp 190-191°C, $^1$H NMR (DMSO-$d_6$): 2.55 (s, 3H, 2-CH3), 4.05 (s, 3H, N-CH3), 7.4(d, 1H, 5-H) 8.25 (d, 1H, 6-H): MS (EI): m/z=139 (M), IR (KBr): 3120 (OH), 1632 (C=O, for free base) Cm-1. Anal. Calcd. for $C_7H_{10}NO_2Cl.H2O$: C, 43.42; H, 6.26; N, 7.24; Cl, 18.31%. Found: C, 43.58; H, 6.18; N, 7.31; Cl, 18.22%.

*Synthesis of 2-methyl-3-benzyloxypyridin-4-one (**4b**).*

To a solution of 3 (25 g, 0.12 mol) in ethanol (200 ml) a 35% aqueous ammonia solution (400 ml) was added and the mixture was refluxed for 18 h. Removal of the solvent by rotary evaporation gave an oil which solidified on addition of acetone and cooling; recrystallization from ethanol yielded colorless prisms (22 g, 85%); mp 164-165°C, $^1$H NMR (DMSO-$d_6$): 2.10 (s, 3H, 2-CH3), 5.01 (s, 2H, O-CH2-Ph), 6.1(d, 1H, 5-H), 7.20-7.41 (m, 5H, Ph), 7.45 (d, 1H, 6-H): MS (EI): m/z=215 (M), IR (KBR): 1635 (C=O) Cm-1. Anal. Calcd. for $C_{13}H_{13}NO_2$: C, 72.53; H, 6.01; N, 6.51%. Found: C, 72.41; H, 6.09 N, 6.43%.

*Synthesis of 2-methyl-3-hydroxypyridin-4-one hydrochloride (**5b**)*

A solution of **4b** (20 g, 0.093 mol) in ethanol (270 ml)/$H_2O$ (30 ml) was adjusted to pH 1 with HCl prior to hydrogenolysis in the presence of Pd/C catalyst. Filtration, followed by rotary evaporation gave a white solid; recystallization from ethanol/diethyl ether yielded a white powder (13.5 g, 90%): mp 173-174°C, $^1$H NMR (DMSO-$d_6$): 2.4 (s, 3H, 2-CH3), 7.3 (d, 1H, 5-H), 8.0 (d, 1H, 6-H). MS (EI): m/z=125 (M-HCl), IR (KBr): 3255 (OH), 1640 (C=O, for free base) $Cm^{-1}$. Anal. Calcd. for $C_6H_8NO_2Cl$: C, 42.24; H, 4.74; N, 8.21; Cl, 20.78%. Found: C, 42.14; H, 4.69; N, 8.29; Cl, 20.85%.

*Determination of partition coefficients using the shake flask method*

A solution of ligands with a concentration of $10^{-4}$ M was prepared in tris buffer (pH 7.4) and the absorbance of solution was measured in the ultraviolet region at a wavelength of approximately 280 nm, using the buffer as the blank. A 50 ml sample of the solution was stirred vigorously with 50 ml of 1-octanol in a glass vessel for 1 h. The two layers were separated by centrifugation for 5 min. An aliquot of the aqueous layer was then carefully removed, using a glass Pasteur pipette, ensuring that the sample was not contaminated with 1-octanol. The absorbance of the sample was measured as above and the partition coefficient, $K_{part}$, was then calculated using the following formula:

$$K_{part} = \frac{A_1 - A_2}{A_2} \times \frac{V_W}{V_0} \qquad \text{(Equation 1)}$$

Where

$A_1$=Absorbance reading in the aqueous layer before partitioning

$A_2$=Absorbance reading in the aqueous layer after partitioning

$V_W$=Volume of aqueous layer used in partitioning

$V_0$=Volume of 1-octanol layer used in partitioning

For each sample, the experiment was repeated at least four times which led to calculation of a mean $K_{part}$ value and standard deviation (Table 1).

*Intestinal absorption (Everted Gut Sac)*

In this study, the intestinal absorption (I.A) of bidentate ligands was determined using Everted Gut Sac (E.G.S) method (7). For this purpose, male wistar rats were purchased from Tehran Pasteur Institute and kept in the animal house under standard conditions and fed until their weights reached 250-300 grams.

Animals were killed by cervical dislocation. Small intestine was removed, cleaned from debris, washed, bottle-dried and weighed. The intestine was cut into small pieces (between 7-8 cm) and the segments were everted. The everted gut sacs were filled with 200 ml tris buffer (pH 7.4) and suspended in the tris buffer medium with a ligand concentration of 0-100 mg/lit. The incubated mixture was capped and gassed with 0.2/CO2=95/5 on water bath shaker at 37°C. At set time intervals, absorbance of the ligands present within the sacs was measured using spectrophotometrically at maximum wavelength ($\lambda_{max}$) of 280 nm; the corresponding concentrations were then determined by elaborating the Beer-Lambert standard curves.

## Results and Discussion

The general methodology (6) adopted for the synthesis of $L_1$ and $L_2$, is summarized in Schema 1. The commercially available maltol 2 was benzylated to give 3. Reaction of 3 with methylamine or ammonia, gave the benzylated pyridinenes 4a and 4b, which were subsequently subjected to catalytic hydrogenation to remove the protecting group, yielding the corresponding bidentate chelaters $L_1$ and $L_2$ as hydrochloride salts.

**Table 1.** Partition coefficient values of ligands and their corresponding iron (III) complexes between 1-octanol and tris buffer at pH 7.4. n=4.

| Compound | $K_{part}$ of Ligand | $K_{part}$ of Fe-Complex |
|---|---|---|
| $L_1$ | 0.18±0.01 | 0.0015 |
| $L_2$ | 0.35±0.01 | 0.0020 |

Conversion of maltol to the corresponding pyridinones can be achieved without the protection of the 3-hydroxyl group (8). However, the yield of this synthetic route is less than 40% (9).

The partition coefficients of ligands between 1-octanol and tris buffer (pH 7.4) were determined using the shake flask method (10). The partition coefficients of their Fe-complexes ($K_{part}$ Fe-complex) can be calculated from equation 2 (11). The resulting values are presented in table 1.

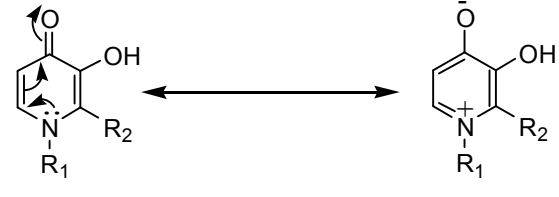
$$\text{Log } K_{part} \text{ Fe-complex} = 0.49 \log K_{part} \text{ Ligand} - 2.45 \qquad \text{(Equation 2)}$$

Surprisingly, the unsubstituted pyridine $L_2$ possesses a higher $K_{part}$ value than the corresponding N-methyl pyridinene $L_1$. This trend also holds for their iron (III) complexes. A likely explanation for this observation is the change in the balance of the relative contribution of the canonical forms (Figure 1, 6 and 7).

When R1 is a methyl group, the resonance from 7 is stabilized due to the electron donation of methyl group. Such stabilization is not possible with the non-methylated pyridinene.

Thus, the dipole of the N-methyl pyridinone ($L_1$) is predicted to be larger and hence more hydrophilic than that of the non methylated pyridinone ($L_2$).

In order to investigate the intestinal absorption of both ligands, by the E.G.S method, the effect of incubation time on this process was




**6** **7**

**Figure 1.** Resonance forms of N-alkyl hydroxypyridinones

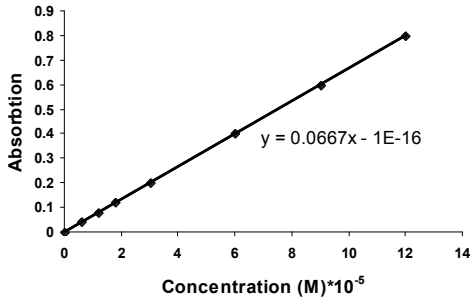



**Figure 2.** The standard absorption curve for ligand 1,2-dimethyl-3-hydroxypyridin-4-one ($L_1$).

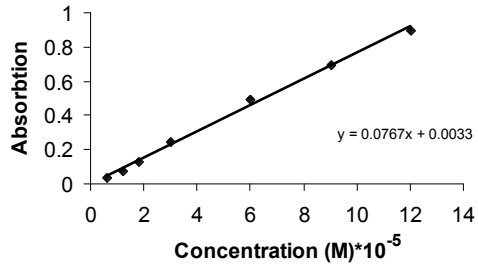



**Figure 3.** The standard absorption curve for ligand 2-methyl-3-hydroxypyridin-4-one ($L_2$).

studied first. To follow this, E.G.S was prepared and incubated in two series of conical flasks in the tris medium containing 100 mg/lit $L_1$ and/or $L_2$. At set intervals, E.G.S was removed from the medium and the concentrations of both ligands within the sacs were determined. The results showed that maximum $L_1$ and $L_2$ uptake occurred after 45 min of incubation time (Figures 4 and 5). The level of ligands uptake was then decreased, suggesting that the mucosal cells gradually loose their ability to take up ligands (Fig 4 and 5).

In order to determine whether ligand uptake by E.G.S was dependent on the concentration of these iron chelators, various concentrations of ligands (0-100 mg/lit) were added to two series of conical flasks. The sample solutions were incubated for 45 min under the same conditions mentioned above. At the end of the incubation time, E.G.S from each flask was removed and ligand concentration within the sacs was determined. The data presented in figures 6 and 7 showed that there was a gradual increase in $L_1$ and $L_2$ uptake by E.G.S up to a value of 60 mg/lit and thereafter the level remained unchanged.

In order for a chelating agent to exert its pharmacological effect, a drug must be able to reach the target sites at a sufficient concentration. Hence, the key property for an orally active iron chelator is its ability to be efficiently absorbed from the gastrointestinal tract and to cross biological membranes, thereby gaining access to the desired target sites, such as the liver. There are several factors which influence the ability of a compound to freely permeate a lipid membrane, three of which are, lipophilicity, ionization state and molecular size.

In order to achieve efficient oral absorption, the chelator should possess appreciable lipid solubility, which could facilitate the molecule to penetrate the gastrointestinal tract (octanol/water partition coefficient greater than 0.1) (12). Membrane permeability can also be affected by the ionic state of the compound. Uncharged molecules penetrate cell membranes more rapidly than charged molecules. As the pKa values for hydroxypyridinones are in the region of 3.6 and 9.9, they are neutral over a wide range of physiological pH values (12). Molecular size is another factor which influences the rate of drug absorption. Generally, molecules with molecular masses>400 Da only poorly penetrate the

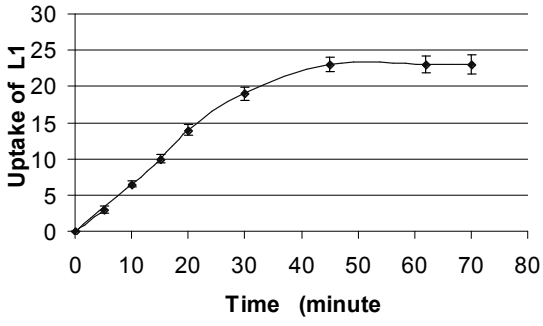



**Figure 4.** Effect of incubation time on $L_1$ uptake at a concentration of 100 mg/lit by E.G.S. (Data are mean±SD, n=3).

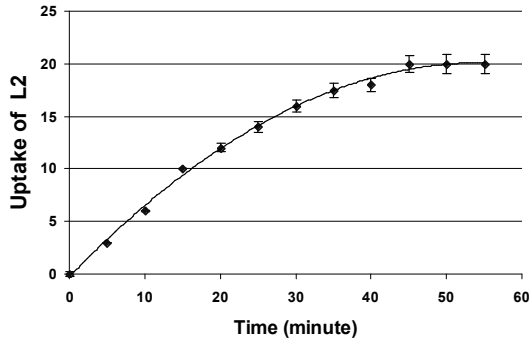



**Figure 5.** Effect of incubation time on $L_2$ uptake at concentration of 100 mg/lit by E.G.S. (Data are mean±SD, n=3).

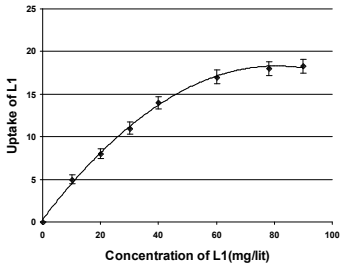



**Figure 6.** Effect of concentration on $L_1$ absorption by E.G.S. (Data are mean±SD, n=3).

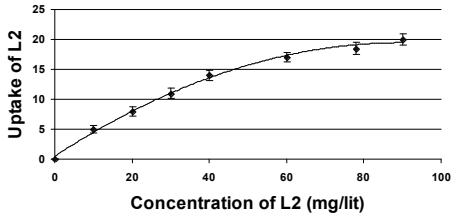



**Figure 7.** Effect of concentration on $L_2$ absorption by E.G.S. (Data are mean±SD, n=3).

biological membranes by simple diffusion (12). Bidentate ligands typically fall within the molecular-mass range of 100-250 Da. Thus, by virtue of their lower molecular masses, bidentate ligands are likely to have a sufficient bioavailability (13).

Data which has been reported in table 1 show that the $K_{part}$ value of $L_2$ is much higher than the $K_{part}$ value of $L_1$. As mentioned above, this factor plays an important role in drug absorption. Thus, we expected $L_2$ to exhibit a higher intestinal absorption. The results presented in figures 4-7, showed that the rate and extent of intestinal absorption of $L_2$ are not statistically different from those of $L_1$. It seems that drug absorption by E.G.S depends on some other factors such as the number of hydrogen bonds between the drug and surrounding molecules.

It is possible that for N-methyl pyridinone ($L_1$), intramolecular hydrogen bonding (Fig 8) restricts the number of hydrogen-bonding sites, which in turn aids the transfer of such molecules across the cell membranes, with H-N derivative ($L_2$). Although the same intramolecular hydrogen bonding will occur, the hydrogen bonding ability of the H-N group is unaffected by such interactions and clearly this functional group has a dominant influence on the penetration of these compounds.

In support of this hypothesis, the introduction

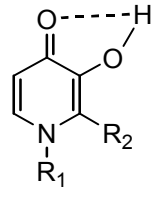



**Figure 8.** Possible interamolecular hydrogen bonding of the hydroxypyridinones.

of a hydroxylalkyl group at the N-position of hydroxypyridinenes is associated with a decrease in blood brain barrier penetration compared with N-alkylated hydroxypyridinones (14). This is due to the formation a hydrogen bond between the hydroxyalkyl group (at N-position) and the adjacent molecules.

In conclusion, it can be said that although from the point of intestinal absorption, the drug $L_2$ has no advantage over $L_1$, but ammonia in comparison to methylamine, which is used as a starting material for the synthesis of $L_2$ is more available than methylamine. The overall yield of the synthesis of $L_2$ is marginally higher than that of $L_1$; this is possibly another advantage of $L_2$ over $L_1$.

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