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Synthesis of L-(+)-3-(3-Hydroxy-4-pivaloyloxybenzyl)-2,5-diketomorpholine as Potential Prodrug of L-Dopa

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Abstract—The synthesis and in vitro chemical and enzymatic stability of L-(+)-3-(3-hydroxy-4-pivaloyloxybenzyl)-2,5-diketomorpholine (**9**) as L-Dopa prodrug are described. Prodrug **9** possesses a good lipophilicity (log $P = 2.153 \pm 0.017$), is stable in aqueous buffer solutions (pH 1.3 and 7.4), and in 80% rat and human plasma it is turned into L-Dopa.

L-(−)-3-(3,4-Dihydroxyphenyl)alanine (L-Dopa, **1**), the immediate biological precursor of dopamine, is still considered the drug of choice in the treatment of Parkinson's disease. Substitution therapy with L-Dopa is, however, associated with a number of acute problems. The drug undergoes extensive decarboxylation to dopamine by amino acid decarboxylase (AADC) in the gastrointestinal tract before entering the systemic circulation and is converted by catechol-*O*-methyltransferase (COMT) into the inactive metabolite 3-*O*-methyldopa before crossing the blood-brain barrier.

The peripheral conversion of L-Dopa to dopamine is responsible for the typical gastrointestinal (nausea, emesis) and cardiovascular (arrhythmia, hypotension) side effects. To minimize the conversion to dopamine outside the central nervous system L-Dopa is usually given in combination with peripheral inhibitors of AADC (carbidopa and benserazide). In spite of that other central nervous side effects such as dyskinesia, on-off phenomenon and end-of-dose deterioration still remain. These effects might be reduced by attenuating peaks and rapid fluctuations of L-Dopa plasma levels.[1,2]

The main factors responsible for the poor bioavailability and the wide range of inter- and intra-patient variations of plasma levels are the drug's physical-chemical properties: low water and lipid solubility, resulting in unfavorable partition, and the high susceptibility to chemical and enzymatic degradation. In order to improve the bioavailability the prodrug approach appeared to be the most promising and some L-Dopa prodrugs have been prepared in an effort to solve these problems.[3–6] An ideal prodrug of L-Dopa should be soluble in water and in lipids, completely absorbed by the gastrointestinal tract without any chemical degradation or metabolism, and thus deliver intact L-Dopa in the blood stream at a reproducible therapeutic level.

In this study we designed and synthesized the L-3-(3-hydroxy-4-pivaloyloxybenzyl)-2,5-diketomorpholine (**9**) as L-Dopa prodrug. In this compound the carboxylic and amino groups were masked in the 2,5-diketomorpholine skeleton as amide and ester groups, respectively. The 2,5-diketomorpholine system has been chosen in order to obtain a lipophilic prodrug with enhanced absorption and to protect the L-Dopa aminoacidic moiety toward amino acid decarboxylase (AADC). Moreover, enzymatic hydrolysis by esterase and peptidases could restore L-Dopa. The catechol was protected by the pivaloyl group considering that the L-3-(3-hydroxy-4-pivaloyloxyphenyl)alanine produces a sustained L-Dopa plasma level and large L-Dopa bioavailability after oral dosing in rats and dogs.[7]

The synthesis of compound **9** was carried out as shown in Scheme 1. The *N*-Boc derivative **2** was transformed into the benzyl derivative **3**. The carboxylic and the amino groups were deprotected with NaOH and trifluoroacetic acid, respectively. The trifluoroacetate salt **5** was acylated

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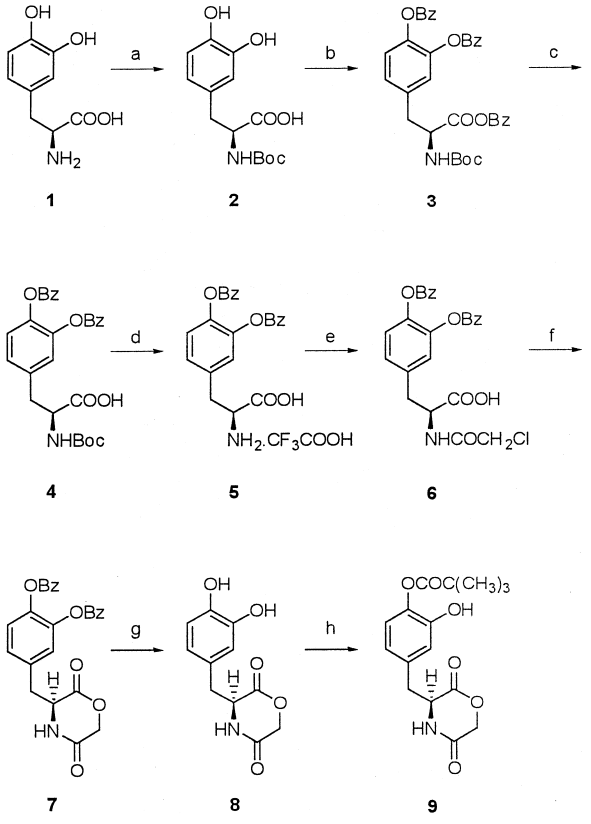
with chloroacetylchloride. Cyclization of the *N*-chloroacetyl derivative **6** was accomplished using triethylamine to give the L-3-(3,4-dibenzyloxy-benzyl)-2,5-diketomorpholine **7**. By treatment with H_2-Pd/C 10% the benzyl groups were removed. The final conversion of the catechol **8** into **9** was obtained by selective acylation with pivaloyl chloride.[5] In no synthesis step was racemization observed and optical activity was retained in the final compounds **8** and **9**.[8]

Lipophilicity is an important factor controlling the interaction of drugs with biological membranes. It is generally accepted that good absorption of an orally administered drug could be obtained when the value of the octanol/water partition coefficient is $\geq$100 (log $P \geq 2$) and the aqueous solubility is more than 10 μg/mL.[9] To assess this potential, the apparent partition coefficient (log P) of the studied prodrug **9** was determined in *n*-octanol/phosphate buffer of pH 7.4. The concentration of compound **9** in octanol and buffer layer was determined by correlating the peak areas in HPLC to a known concentration of the compound. The mean value obtained from experiments conducted in triplicate was: log $P = 2.153 \pm 0.017$ ($\pm$SEM; $n = 3$).

The aqueous solubility was 50 μg/mL. Thus, the data indicate that **9** possesses the requirements for good oral absorption.



Scheme 1. Reagents: (a) $(t\text{-BuCO}_2)_2O$, H_2O-dioxane, Et_3N, 0 °C, then rt, 10 h, 95%; (b) $C_6H_5CH_2Br$, K_2CO_3, acetone, reflux 5 h; (c) NaOH 1N, H_2O-dioxane, rt, 24 h; (d) CH_2Cl_2, CF_3COOH, rt, 15 min, 41%; (e) NaOH, $ClCH_2COCl$, 0 °C, 77%; (f) DMF, Et_3N, 100 °C, 12 h, 38%; (g) EtOH, HCl 36%, H_2-Pd/C 10%, 20 psi, 40 min, 95%; (h) CF_3COOH, $(CH_3)_3CCOCl$, 1.2 equiv, rt, 6 h, 29%.

The prodrug **9** was also assayed in vitro to evaluate its chemical and enzymatic stability. The kinetic of chemical hydrolysis was studied at 37 °C in aqueous buffer solutions of pH 1.3 and pH 7.4 (Fig. 1) as well as in 80% rat and human plasma.[10]

The results obtained showed pseudo-first-order kinetics, and the decomposition product was the catechol **8**, unequivocally identified by HPLC and NMR analysis.

Figure 2 shows the time course for disappearance of **9** and for appearance of **8** in phosphate of pH 7.4. The rate constant (K_{obs}) for chemical and enzymatic hydrolysis and the corresponding half-life times are listed in Table 1. The hydrolysis of pivaloyl ester moiety in compound **9** at pH 7.4 is faster than that in buffer of pH 1.3. The half-life time of **9** at pH 7.4 ($t_{1/2} = 92.8$ min) is higher than that reported for L-3-(3-hydroxy-4-pivaloyloxyphenyl)alanine ($t_{1/2} = 22.3$ min) which seem to be stable at pH 2.[7]

Data show that the prodrug **9** is stable in aqueous buffer solution of pH 1.3 ($t_{1/2} = 52.2$ h). This stability implies that the compound passes unhydrolyzed through the

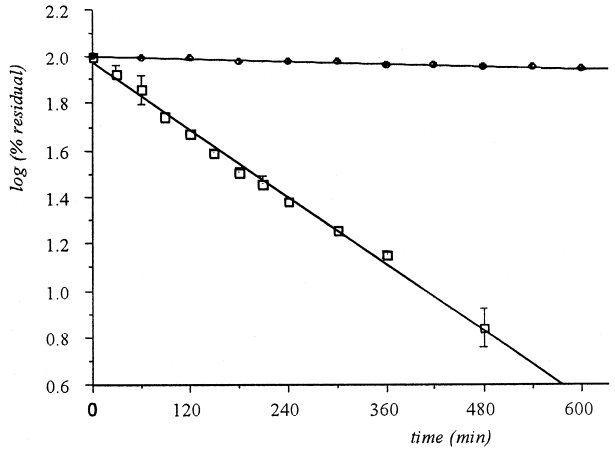


Figure 1. First-order kinetic plots for hydrolysis of prodrug **9** in phosphate buffer of pH 7.4 (□) and in hydrochloric acid buffer of pH 1.3 (◆) at 37 °C.

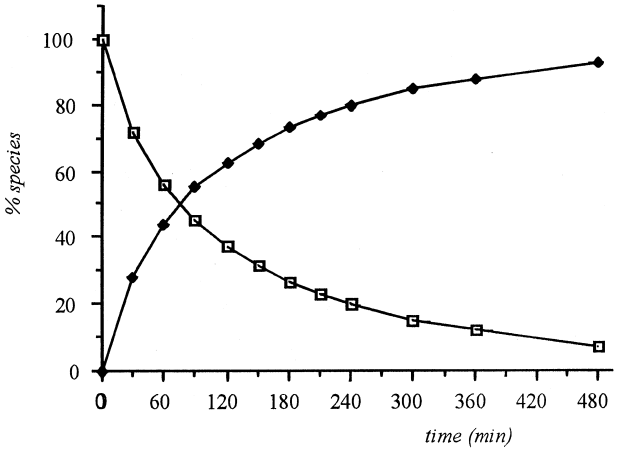


Figure 2. Time courses for disappearance of **9** (□) and appearance of **8** (◆) during hydrolysis in phosphate buffer of pH 7.4 at 37 °C.

Table 1. Kinetic data for chemical and enzymatic hydrolysis of prodrug **9** at 37 °C

Conditions	$t_{1/2}$ (min)[a]	K_{obs} (min^{-1})[a]
Buffer pH 1.3	3135.7 ± 22.5	$2.2\times10^{-4} \pm 1.1\times10^{-5}$
Buffer pH 7.4	92.8 ± 1.49	$7.5\times10^{-3} \pm 1.2\times10^{-4}$
80% rat plasma	2.7 ± 0.09	0.29 ± 0.01
80% human plasma	36.7 ± 1.1[b]	$1.9\times10^{-2} \pm 3.6\times10^{-4}$[b]
80% human plasma	147.6 ± 2.71[c]	$4.7\times10^{-3} \pm 1.4\times10^{-4}$[c]

[a]Values represent the mean±SEM; $n=3$.
[b]$t_{1/2}$ and K_{obs} for the hydrolysis of the prodrug **9** to **8**.
[c]$t_{1/2}$ and K_{obs} for the hydrolysis of **8** to L-Dopa.

stomach after oral administration. At pH 7.4 the prodrug is stable enough ($t_{1/2}=1.5$ h) to be absorbed intact from the intestine.

Figures 3 and 4 show the plots of pseudo-first-order kinetics for the hydrolysis in 80% rat and human plasma at 37 °C; the rate constants (K_{obs}) and the corresponding half-life times are shown in Table 1. In rat and human plasma both the catechol ester and diketomorpholine ring of the prodrug **9** were cleaved and L-Dopa was formed. A rapid conversion of **9** to L-Dopa was observed in rat plasma ($t_{1/2}=2.7$ min). Hydrolysis in 80% human plasma proceeds more slowly by a two steps reaction; at first the prodrug **9** was cleaved to catechol diketomorpholine **8** which afterwards was turned into L-Dopa.

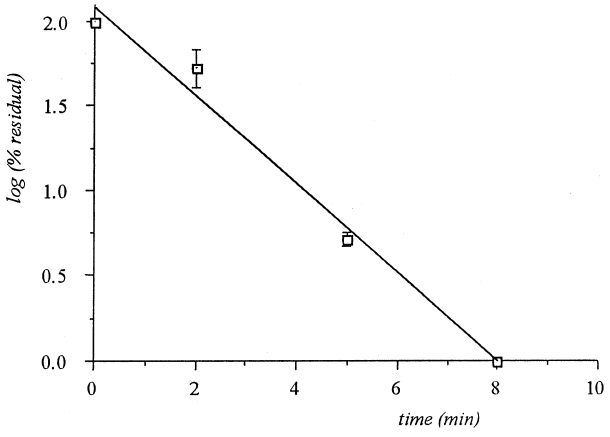


Figure 3. First-order kinetic plot for hydrolysis of prodrug **9** in 80% rat plasma at 37 °C.

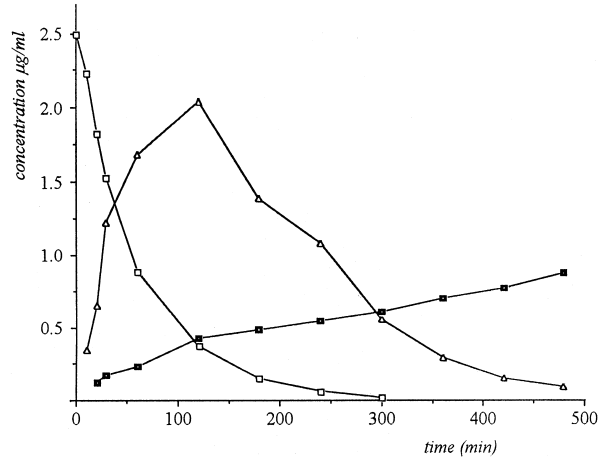


Figure 4. Times courses for hydrolysis of prodrug **9** (□) to L-Dopa (■) via the intermediate formation of **8** (△) in 80% human plasma at 37 °C.

The faster hydrolysis in rat than in human plasma may be ascribed to the different enzyme systems. It is well reported that the esterases are highly efficient in rat plasma.[11]

In conclusion, we designed and synthesized L-(+)-3-(3-hydroxy-4-pivaloyloxybenzyl)-2,5-diketomorpholine (**9**) as L-Dopa prodrug. The present findings indicate that prodrug **9** meets the requirements for gastrointestinal absorption, shows good stability toward gastrointestinal hydrolysis, and releases L-Dopa in human plasma after enzymatic hydrolysis.

The 2,5-diketomorpholine ring seems to be a useful system in the design of L-Dopa prodrugs. Further studies are in progress to investigate the bioavailability and to evaluate the in vivo pharmacological activity of this prodrug.

Acknowledgements

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8. All new compounds gave satisfactory microanalyses and NMR spectra. The purity of compounds **8** and **9** was checked by HPLC using the column Merck Purospher RP-18 endcapped (5 μm) 125–3 with MeOH:H_2O, 60/40 as eluent. The determination of enantiomeric excess was performed by using a commercially available HPLC Chirobiotic reversed-phase chiral column (StepBio, Bologna, Italy) with mobile phase of ethanol:water:methanol (60:40.5, v/v/v), (ee >97%); see ref 12. L-(+)-3-(3,4-dihydroxybenzyl)-2,5-diketomorpholine **8**: oil, purified by column chromatography with AcOEt as eluent, yield 95%, $R_f=0.33$, $[\alpha]_D^{20}=+14.28$ ($c=1$, EtOH abs), ^{1}H NMR (DMSO-d_6) δ 8.80 (br s, 1H, OH), 8.70 (br s, 1H, OH), 7.72 (d, 1H, NH), 6.60 (m, 2H, ArH), 6.42 (m, 1H, ArH), 4.47 (m, 1H, CH-N), 3.80 (s, 2H, CH_2CO), 2.84 (d, 2H, $ArCH_2$). MS *m/e* 237 (M+). L-(+)-

3-(3-hydroxy-4-pivaloyloxybenzyl)-2,5-diketo-morpholine **9**: oil, purified by column chromatography with AcOEt as eluent, yield 32%, $R_f = 0.45$, $[\alpha]_D^{20} = +9.46$ ($c = 1$, EtOH abs), ^{1}H NMR (DMSO-d_6) δ 9.57 (br s, 1H, OH), 7.88 (m, 1H, NH), 6.76 (m, 3H, ArH), 4.50 (m, 1H, CH-N), 3.82 (m, 2H, CH_2CO), 2.98 (m, 2H, CH_2Ar), 1.30 (s, 9H, $(CH_3)_3C$). MS *m*/*e* 309 (M+).

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