Brief Articles

Design and Synthesis of a Novel L-Dopa−Entacapone Codrug

Jukka Leppänen,*[,†] Juhani Huuskonen,[†] Tapio Nevalainen,[†] Jukka Gynther,[†,‡] Hannu Taipale,[†] and Tomi Järvinen[†,‡]

Department of Pharmaceutical Chemistry, University of Kuopio, P.O. Box 1627, 70211, Kuopio, Finland, and Finncovery Ltd., Kuopio, Finland

Received July 10, 2001

A novel codrug, in which L-Dopa and entacapone are linked via a biodegradable carbamate spacer to form a single chemical entity, was synthesized and studied kinetically. This carbamate codrug provides adequate stability [$t_{1/2}$ = 12.1 h (pH 1.2); 1.4 h (pH 5.0); 1.1 h (pH 7.4)] against chemical hydrolysis but rapidly hydrolyzes to L-Dopa and entacapone in liver homogenate ($t_{1/2}$ = 7 min; pH 7.4) at 37 °C. The therapeutical potential of this novel codrug is discussed.

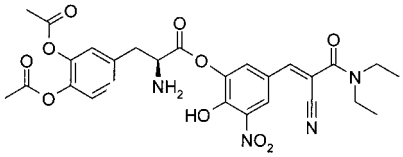
Introduction

The prodrug approach is commonly used to improve physicochemical, biopharmaceutical, and drug delivery properties of therapeutic agents. Ideally, an inactive promoiety is covalently attached to the parent molecule, and the resulting prodrug is converted to the parent drug in the body before it exhibits its pharmacological effect.[1−3] Many diseases are treated by a combination of therapeutic agents that are coadministered in separate dosage forms. However, there are potential advantages in giving the coadministered agents as a single chemical entity (e.g., improved delivery properties and targeting drugs to specific sites of action). As codrugs, at least two different synergistic drugs are linked together and designed to release the parent drugs at the desired site of action.[4−6]

L-Dopa (3,4-dihydroxyphenyl-L-alanine, **1**) is a precursor to dopamine, which is deficient in the brains of patients suffering from Parkinson's disease (PD). Conventional PD treatment consists of L-Dopa combined with an AADC (amino acid decarboxylase) inhibitor, such as carbidopa. During treatment, COMT (catechol-*O*-methyltransferase)[7] remains the main enzyme for metabolizing L-Dopa. Entacapone [(*E*)-2-cyano-*N*,*N*-diethyl-3-(3,4-dihydroxy-5-nitrophenyl)propenamide] is a new, potent inhibitor of COMT, which is currently used as a clinical adjunct to L-Dopa therapy in PD.[8] The administration of entacapone, together with L-Dopa and an AADC inhibitor, leads to increased L-Dopa bioavailability and its prolonged duration of action.[9] However, even after combination therapy of entacapone and L-Dopa, the bioavailability of L-Dopa is low (5−10%).[10] In addition, the bioavailability of entacapone after oral administration is also low (29−46%).[11]

The codrug approach was considered to be a productive way for combining the therapeutic effects of L-Dopa and entacapone, because their delivery route and rate should be identical to improve L-Dopa brain delivery, and they have similar doses on a molar bases. In the present study, we describe a straightforward route of synthesis and preliminary in vitro studies for a novel codrug of L-Dopa and entacapone.

Scheme 1



Results and Discussion

L-Dopa (**1**) has four functional groups (two hydroxyls, one primary amine, and one carboxylic acid) that can link to entacapone via a spacer. Due to potential risk of intramolecular hydrolysis by an unsubstituted hydroxyl group, there were no attempts to link L-Dopa by its hydroxyl groups to entacapone. Moreover, the chemical similarity of the two L-Dopa hydroxyl groups could lead to a difficult separation of the resulting 3- and 4-substituted L-Dopa derivatives.

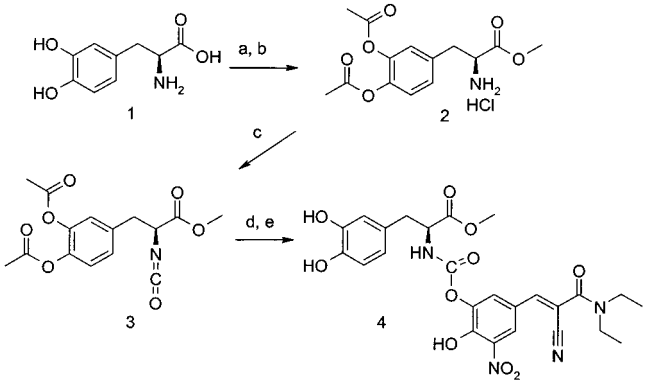
The simplest codrug, a L-Dopa ester of entacapone (Scheme 1), was unstable after deprotection of the amino group, and thus the desired codrug could not be prepared. An attempt was made to stabilize the L-Dopa and entacapone ester codrug by inserting a methylene spacer between entacapone and the carboxylic moiety of L-Dopa.[12,13] An iodomethylene linker was introduced to the carboxylic group of a protected L-Dopa (2xPg_1-O, Pg_2-N) by treating it with chloromethyl chlorosulfonic acid and sodium iodide.[14] Unfortunately, entacapone reacted poorly with the resulting L-Dopa derivative ($RCOOCH_2I$).

Since carbamate ester prodrugs of entacapone are chemically stable and release entacapone enzymatically in vitro,[15] carbamate was evaluated as a possible linker for a L-Dopa−entacapone codrug. The synthesis of the carbamate codrug (**4**) is outlined in Scheme 2.

* Corresponding author: Department of Pharmaceutical Chemistry, University of Kuopio, P.O. Box 1627, FIN-70211, Kuopio, Finland. Tel: +358 17 162 460. Fax: +358 17 162 456. E-mail: jukka.leppanen@uku.fi.
† Department of Pharmaceutical Chemistry.
‡ Finncovery Ltd.

10.1021/jm010980d CCC: $22.00
Published on Web 02/12/2002

Scheme 2[a]



[a] Reagents: (a) MeOH, $SOCl_2$; (b) AcCl, TFA; (c) diphosgene, EtOAc; (d) entacapone, CH_3CN; (e) 3 N HCl in acetone.

To prevent cyclization of isocyanate to *N*-carboxylic anhydride,[16] the carboxylic group of L-Dopa was esterified by treating with thionyl chloride in dry methanol. The methyl ester was treated with trifluoroacetic acid and acetyl chloride to give 2-amino-3-(3,4-diacetoxyphenyl)-propionic acid **2** in quantitative yields. The protected L-Dopa (**2**) was treated with a convenient phosgene source, diphosgene, to yield the isocyanate **3**,[17] which was used immediately for the next step without purification, due to its rapid decomposition on exposure to humidity.

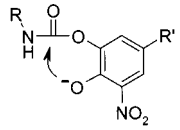
The isocyanate **3** and entacapone were dissolved in dry acetonitrile and refluxed overnight under a N_2 atmosphere in the absence of light to produce the carbamate ester **4**. The reaction proceeded slowly due to the poor nucleophilicity of entacapone, resulting from the electron withdrawing effect of the nitro group and the conjugated structure of entacapone. After flash chromatography purification of **4**, the acetate protecting groups were removed by treatment with 3 N HCl in acetone. Purification attempts by flash chromatography on dry silica gel resulted in rapid degradation of **4**. Thus, **4** was purified by preparative HPLC on reversed-phase silica gel, providing the codrug in moderate yields (46%). Less than 5% of **4** was converted to the *Z*-form during the purification step, probably due to heat or exposure to light.

Instability of **4** in even mildly basic conditions excludes the use of protective groups that are cleaved under basic conditions. Similarly, the reductive cleavage of protecting groups may lead to an unwanted reduction of the functional groups present on **4**. Thus, the acetate was a simple and easily introduced protective group, although the carbamate function was slightly hydrolyzed when the acetate groups were cleaved under acidic conditions.

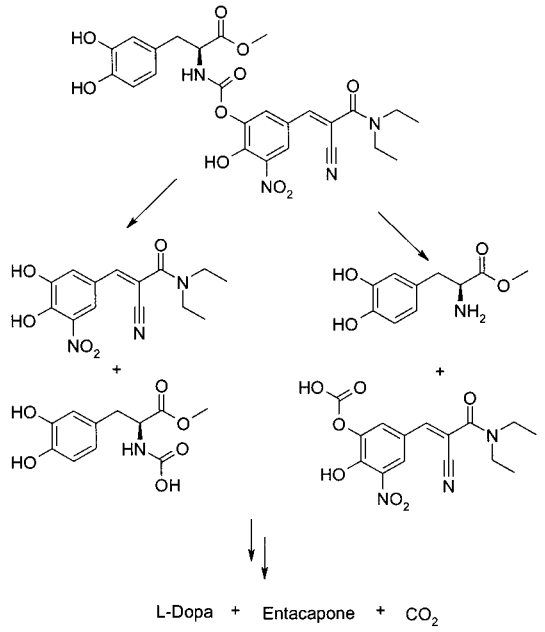
The chemical stability of **4** increased with decreasing pH of the solution. At pH 7.4, codrug **4** demonstrated only adequate stability ($t_{1/2}$ = 66 min; 37 °C), which was probably a result of entacapones's low pK_a. The ionized hydroxyl group of entacapone may assist hydrolysis of the carbamate ester (Scheme 3).

However, codrug **4** was more stable at acidic pH [$t_{1/2}$ = 84 min (pH 5.0); $t_{1/2}$ = 726 min (pH 1.2)], which is a desirable property for increased stability in the stomach and small intestine.

Scheme 3



Scheme 4



Compound **4** was readily hydrolyzed enzymatically ($t_{1/2}$ = 7 min) to entacapone and L-Dopa in a liver homogenate at pH 7.4, which fulfilled the criteria of a bioreversible codrug. Unfortunately, the direct quantitation of the parent drugs was difficult, due to the low aqueous solubility of **4** (i.e., low concentrations of the parent compound in liver homogenate) and rapid metabolism of the released L-Dopa in liver homogenate.[18]

Two alternative biodegradation routes of **4** are outlined in Scheme 4. During the hydrolysis of the carbamate spacer, the carboxylic residue remains either with entacapone or L-Dopa. The subsequent release of carbon dioxide is spontaneous.[19] The carbamate may also hydrolyze back to the unstable isocyanate,[20] which rapidly decomposes in an aqueous environment to primary amine and carbon dioxide.

Our results suggest that **4** may release L-Dopa and entacapone in the body after absorption. Furthermore, biodegradation of **4** and the simultaneous release of both L-Dopa and entacapone results in the inhibition of COMT, which is expected to facilitate a decrease in metabolism of L-Dopa more efficiently than if either therapeutic agents were administered in separate dosage forms. Thus, the novel L-Dopa−entacapone codrug approach warrants further studies.

Conclusions

A novel L-Dopa−entacapone codrug was synthesized by linking the parent compounds together via the carbamate spacer. The presented synthetic route is straightforward and produces the desired endproduct in moderate yields. Codrug **4** was adequately stable against chemical hydrolysis and released the parent drugs by enzymatic hydrolysis in liver homogenate, thus

fulfilling the codrug criteria. The yield could be improved if a more easily cleaved protecting group were introduced to L-Dopa or if such a group was replaced by biolabile groups, making the deprotection step redundant.

Experimental Section

Reagents. All solvents and reagents were of the highest purity and used without further purification. Diphosgene was purchased from Fluka Chemie AS. *Care must be excercised in the handling of diphosgene due to release of phosgene when heated or activated on charcoal.*

Chemistry. ^{1}H and ^{13}C NMR analyses were recorded on a Bruker Avance 500, operating at 500.1 and 125.6 MHz, respectively. Chemical shifts are reported in parts per million (δ) using TMS as the internal standard. The splitting pattern abbreviations are as follows: s = singlet, d = doublet, t = triplet, q = quartet, br = broad. Electrospray ionization mass spectra were acquired by an LCQ ion trap mass spectrometer equipped with an electrospray ionization source (Finnigan MAT, San Jose, CA). The samples were diluted with methanol to 20 μg/mL and injected directly into the eluent flow via a 5 μL loop injector (the total amount of sample was approximately 100 ng), and full scan mass spectra were recorded. Elemental analyses were carried out on a ThermoQuest CE Instruments EA 1110-CHNS-O elemental analyzer. The synthetic reactions were monitored by TLC (Kieselgel 60 F 254, DC-Alufolien, Merck). HPLC purification was accomplished by using a Beckmann System Gold Programmable Solvent Module 116 on reversed phase silica (Kromasil 100 Å spherical silica, C8, 16 μm, Eka Chemicals AB).

2-Amino-3-(3,4-diacetoxy-phenyl)-propionic Acid (2). L-Dopa methyl ester was prepared by treatment of L-Dopa (2 g, 10 mmol) with thionyl chloride (5 mL) in dry methanol (10 mL).[21] The resulting white solid was stirred with trifluoroacetic acid (4 mL) and acetyl chloride (1.5 mL) at room temperature to give the desired product with quantitative yield and high purity.[22] The procedures are described in detail in the references. ^{1}H NMR (CD_3OD) δ: 2.32 (6H, s, CH_3CO), 3.24 (1H, q, J = 7.5 and 14.2 Hz, CH_ACH), 3.34 (1H, q, J = 5.8 and 14.2 Hz, CH_BCH), 3.86 (3H, s, OCH_3), 4.24 (1H, q, J = 5.8 and 7.5 Hz, CH_2CH), 7.14 (1H, d, J = 1.3 Hz, ArH), 7.20 (1H, d, J = 1.3 Hz, ArH), 7.21 (1H, d, ArH). ESI-MS: 296.3 (M+1).

3-(3,4-Diacetoxy-phenyl)-2-isocyanato-propionic Acid (3). The HCl salt of compound **2** (1.5 g, 4.5 mmol) was dissolved in dry ethyl acetate, and diphosgene (1.1 mL, 9.0 mmol) was added while stirring at −10 °C under N_2 atmosphere. The mixture was allowed to warm to room temperature, then refluxed for 5 h and evaporated to dryness under high vacuum. The product was used immediately in the following reaction without further purification.

(*S*)-2-[5-((*E*)-2-Cyano-2-diethylcarbamoyl-vinyl)-2-hydroxy-3-nitro-phenoxycarbonylamino]-3-(3,4-dihydroxy-phenyl)-propionic Acid Methyl Ester (4). The isocyanate **3** was dissolved in dry acetonitrile (10 mL) with entacapone (553 mg, 1.81 mmol) under N_2 atmosphere in the absence of light. The mixture was refluxed for 20 h and evaporated to dryness. The product was purified by flash chromatography on silica gel using dichloromethane/methanol (100:1) as an eluent. Acetate protecting groups were removed by treating with an acetone/3 N HCl (20:1) solution for 2 h at 50 °C. The resulting clear yellow mixture was evaporated to dryness and purified by preparative HPLC using acetonitrile/water (50:50) as an eluent. Evaporation of solvents yielded a yellow solid (436 mg, 46%). mp (decomposed). ^{1}H NMR ($CDCl_3$) δ: 1.26 (6H, br, CH_2CH_3), 2.95 (1H, q, J = 6.1 and 13.7 Hz, CH_ACH), 3.11 (1H, q, J = 4.7 and 13.7 Hz, CH_BCH), 3.50 (4H, br, CH_2CH$_3$), 3.77 (3H, s, OCH_3), 4.59 (1H, q, J = 5.9 and 7.0 Hz, CH_2CH), 6.14 (1H, d, J = 7.5 Hz, NH) 6.15 (1H, d, J = 8.0 Hz, ArH), 6.66 (1H, s, ArH) 6.72 (1H, d, J = 8.0 Hz, ArH), 7.52 (1H, s, CH=C), 7.92 (1H, s, J = 1.8 Hz, ArH), 8.32 (1H, s, J = 1.8 Hz, ArH). ^{13}C NMR (125.8 MHz, CD_3OD) δ: 12.5, 13.6, 37.0, 41.1, 43.6, 52.7, 55.4, 107.0, 115.5, 116.6, 121.4, 122.9, 124.9, 127.6, 130.0, 134.5, 141.3, 143.3, 143.9, 144.0, 148.1, 151.1, 152.7, 162.9, 171.4. ESI-MS: 543.1 (M+1). Anal. ($C_{25}H_{26}N_4O_{10}\cdot 0.25CH_2Cl_2$) C, H, N.

HPLC Analysis. The HPLC system used for the determination of in vitro samples consisted of a Beckman System Gold Programmable Solvent Module 126, Beckman System Gold Detector Module 166 with variable wavelength UV detector (set at 254 nm), and a Beckman System Gold Autosampler 507e. Separations were accomplished on a Purospher RP-18 reverse-phase column (12.5 cm × 4.0 mm i.d., 5 μm) (Merck, Darmstadt, Germany). The chromatographic conditions were as follows: injection volume, 50 μL; column temperature, 40 °C; flow rate, gradient/isocratic at 1.0 mL/min. The mobile phase consisted of various proportions of methanol/water mixture (90:10) and a citrate/phosphate buffer pH 2.2.

Hydrolysis in Aqueous Solution. The rate of chemical hydrolysis of **4** was determined in aqueous phosphate buffer solution (0.16 M) at pH 7.4, 5.0, and 1.2 at 37 °C. An appropriate amount of **4** was dissolved in 10 mL of preheated buffer, and the solution was placed in a thermostatically controlled water bath at 37 °C. At appropriate time intervals, samples were taken and analyzed for the remaining codrug by HPLC. Pseudo-first-order half-time ($t_{1/2}$) for the hydrolysis of **4** was calculated from the slope of the linear portion of the plotted logarithm of remaining codrug vs time.

Hydrolysis in 10% Rabbit Liver Homogenate. The rabbit liver was homogenized with approximately four equivalent volumes of isotonic phosphate buffer at pH 7.4 using an X-1020 homogenizer (Ystral, Germany). The homogenate was centrifuged for 90 min at 9000*g* and 4 °C with a Biofuge 28 RS-centrifuge (Heraeus Instruments, Germany). The supernatant was stored at −80 °C until analysis. An appropriate amount of **4** was dissolved in one volume of preheated 20% liver homogenate. The solution was then incubated at 37 °C. At appropriate time intervals, samples (300 μL) were withdrawn. Samples were pretreated with 300 μL of methanol to terminate enzymatic activity. After mixing and centrifugation, 400 μL of the supernatant was evaporated to dryness under a stream of air. The residue was redissolved in 400 μL of the mobile phase buffer and analyzed by HPLC.

Acknowledgment. This work was supported by the Academy of Finland and The National Technology Agency of Finland. Orion Pharma is gratefully acknowledged for donation of entacapone. The authors are grateful to Ms. Miia-Liisa Räsänen and Mrs. Helly Rissanen for their skillful technical assistance. A grant from the Emil Aaltonen Foundation to J.L. is gratefully acknowledged.

References

(1) Sinkula, A. A.; Yalkowsky, S. H. Rationale for Design of Biologically Reversible Drug Derivatives: Prodrugs. *J. Pharm. Sci.* **1975**, *64*, 181−210.
(2) Stella, V. J. Low Molecular Weight Prodrugs. *Adv. Drug Delivery Rev.* **1996**, *19*, 111−114.
(3) Fleischer, D.; Bong, D.; Stewart, B. H. Improved Oral Drug Delivery: Solubility Limitations Overcome by the Use of Prodrugs. *Adv. Drug Delivery Rev.* **1996**, *19*, 115−130.
(4) Singh, G.; Sharma, P. D. Mutual Prodrugs − a Recent Trend in Prodrug Design. *Indian J. Pharm. Sci.* **1994**, *56*, 69−79.
(5) Menger, F. M.; Rourk, M. J. Synthesis and Reactivity of 5-Fluorouracil/Cytarabine Mutual Prodrugs. *J. Org. Chem.* **1997**, *62*, 9083−9088.
(6) Silverman, R. B. *The organic chemistry of drug design and drug action;* Academic Press: San Diego, 1992; pp 376−377.
(7) Bäckstrom, R.; Honkanen, E.; Pippuri, A.; Kairisalo, P.; Pystynen, J.; Heinola, K.; Nissinen, E.; Linden, I. B.; Männisto, P. T.; Kaakkola, S. Pohto, P. Synthesis of Some Novel Potent and Selective Catechol *O*-Methyltransferase Inhibitors. *J. Med. Chem.* **1989**, *32*, 841−6.
(8) Männisto, P. T.; Ulmanen, I.; Lundström, K.; Taskinen, J.; Tenhunen, J.; Tilgmann, C.; Kaakkola, S. Characteristics of Catechol *O*-Methyltransferase (COMT) and Properties of Selective COMT inhibitors. *Prog. Drug Res.* **1992**, *39*, 291−350.

(9) Kaakkola, S.; Teräväinen, H.; Ahtila, S.; Rita, H.; Gordin, A. Effect of Entacapone, a COMT inhibitor, on Clinical Disability and Levodopa Metabolism in Parkinsonian Patients. *Neurology* **1994**, *44*, 77−80.
(10) Männisto, P. T.; Kaakkola, S. Rationale for Selective COMT Inhibitors as Adjuncts in the Drug Treatment of Parkinson's Disease. *Pharmacol. Toxicol.* **1990**, *66*, 317−323.
(11) Keränen, T.; Gordin, A.; Karlsson, M.; Korpela, K.; Pentikäinen, P. J.; Rita, H.; Schultz, E.; Seppälä, L.; Wikberg, T. Inhibition of Soluble Catechol-O-Methyltransferase and Single-Dose Pharmacokinetics After Oral and Intravenous Administration of Entacapone. *Eur. J. Clin. Pharmacol.* **1994**, *46*, 151−157.
(12) Leppänen. J.; Savolainen, J.; Nevalainen, T.; Forsberg, M.; Huuskonen, J.; Taipale, H.; Gynther, J.; Männistö, P. T.; Järvinen, T. Synthesis and In Vitro/In Vivo Evaluation of Orally Administered Entacapone Prodrugs. *J. Pharm. Pharmacol.* **2001**, *53*, 1489−1498.
(13) Bodor, N.; Sloan, K. B.; Kaminski, J. J.; Shih, C.; Pogany, S. A Convenient Synthesis of (Acyloxy)alkyl α-Ethers of Phenols. *J. Org. Chem.* **1983**, *48*, 5280−5284.
(14) Harada, N.; Hongu, M.; Tanaka, T.; Kawaguchi, T.; Hashiyama, T., and Tsujihara, K. A Simple Preparation of Chloromethyl Esters of the Blocked Amino Acids. *Synth. Commun.* **1994**, *24*, 767−772. The chloromethyl ester of the protected L-Dopa was then treated with NaI in dry acetone to yield the corresponding iodide. The product was purified by flash column chromatography using dichloromethane as an eluent.
(15) Savolainen, J.; Leppänen, J.; Forsberg, M.; Taipale, H.; Nevalainen, T.; Huuskonen, J.; Gynther, J.; Männistö, P. T.; Järvinen, T. Synthesis and *In Vitro*/*In Vivo* Evaluation of Novel Oral N-Alkyl- and N,N-Dialkyl-Carbamate Esters of Entacapone. *Life Sci.* **2000**, *67*, 205−216.
(16) Daly, W. H.; Poche, D. The Preparation of N-Carboxyanhydrides of α-Amino Acids Using Bis(trichloromethyl)carbonate. *Tetrahedron Lett.* **1988**, *29*, 5859−5862.
(17) Sigurdsson, S. T.; Seeger, B.; Kutzke, U.; Eckstein, F. A. A Mild and Simple Method for the Preparation of Isocyanates From Aliphatic Amines Using Trichloromethyl Chloroformate. Synthesis of an Isocyanate Containing an Activated Disulfide. *J. Org. Chem.* **1996**, *61*, 3883−3884.
(18) Wikberg, T. Simultaneous Determination of Levodopa, Its Main Metabolites and Carbidopa in Plasma by Liquid Chromatography. *J. Pharm. Biomed. Anal.* **1991**, *9*, 167−176.
(19) Alexander, J.; Bindra, D. S.; Glass, J. D.; Holahan, M. A.; Renyer, M. L.; Rork, G. S.; Sitko, G. R.; Stranieri, M. T.; Stupienski, R. F.; Veerapanane, H.; Cook, J. J. Investigation of (Oxodioxolenyl)-methyl Carbamates as Nonchiral Bioreversible Prodrug Moieties for Chiral Amines. *J. Med. Chem.* **1996**, *39*, 480−486.
(20) Vigroux, A.; Bergon, M.; Zedde, C. Cyclization-Activated Prodrugs: N−(Substituted 2-Hydroxyphenyl and 2-Hydroxypropyl)-Carbamates Based on Ring-Opened Derivatives of Active Benzoxazolones and Oxazolidinones as Mutual Prodrugs of Acetaminophen. *J. Med. Chem.* **1995**, *38*, 3983−3994.
(21) Bodor, N.; Sloan, K. B.; Higuchi, T.; Sasahara, K. Improved Delivery Through Biological Membranes. 4. Prodrugs of L-Dopa. *J. Med. Chem.* **1977**, *20*, 1435−1445.
(22) Ihara, M.; Tsuchiya, Y.; Sawasaki, Y.; Hisaka, A.; Takehana, H.; Tomimoto, K.; Yano, M. A New Potential Prodrug to Improve the Duration of L-Dopa: L-3-(3- hydroxy-4-pivaloyloxyphenyl)-alanine. *J. Pharm. Sci.* **1989**, *78*, 525−529.

JM010980D