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Redox targeting of LY231617, an antioxidant with potential use in the treatment of brain damage

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Abstract

Several brain-targeting chemical delivery systems (CDS) based on a dihydropyridine ⇌ pyridinium salt-type targetor were synthesized and evaluated for LY231617 (**1**), a di-tert-butylated phenolic amine antioxidant with potential use in the treatment of brain injuries. The dihydropyridine moiety was chemically attached to the amine (by either amide or various substituted carbamate linkages) or to the phenolic hydroxyl (by carboxylic ester linkage) functionalities of LY231617. In vitro stability and in vivo tissue distribution studies (in the rat) were performed with the novel derivatives. The results indicated that a simple amide-type CDS demonstrated efficient delivery of LY231617-targetor conjugate to the CNS. This derivative which contains the intact pharmacophore might possess intrinsic pharmacological antioxidant activity. Favorable in vitro properties suggested that a substituted carbamate-type CDS might be a better delivery modality for LY231617.

Keywords: LY231617; Brain damage; Neuroprotectant; Antioxidant; Chemical delivery systems

1. Introduction

Treatment of acute neurological disorders which include head and spinal cord injury, ischemic and hemorrhagic stroke and brain damage after cardiac arrest is a major goal of medicinal chemistry. Mechanisms which lead to tissue injury in these circumstances and the corresponding therapeutic interventions based on these mechanisms are complicated and include: NMDA antagonists, calcium channel blockers, inhibitors of lipid peroxidation, corticosteroids, inhibitors of arachidonic acid cascade, and diuretics (McCall and Panetta, 1992). Since reactive oxygen species (e.g. free radicals) are important in the pathogene-

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sis of acute neurologic injury (Beckman et al., 1989), the use of antioxidants in their treatment has been considered (Hall, 1988; Martz et al., 1989; McCall and Panetta, 1992). Phenols are important antioxidants which have proven to be effective in protecting the brain against cerebral ischemia in animal models. A series of antioxidants that contains the di-tert-butylated hydroxytoluene substructure has been recently evaluated in models of cerebral ischemia (Clemens et al., 1993). LY231617, the 2,6-bis (1,1-dimethylethyl)-4-[[(1-ethyl)amino]methyl]phenol hydrochloride (**1**), has been reported as effective in protection against CA1 hippocampal damage when it is administered either orally or intravenously 30 min after global ischemia in rats (Beckman et al., 1989; Clemens et al., 1993). Besides reducing histopathological damage, LY231617 also preserved the functional electrophysiological integrity of the hippocampal neuronal circuitry after ischemic insult. In spite of these promising properties, peripheral toxic side-effects such as hypotensive and direct myocardial suppression hamper the development of **1** as a successful neuropharmacological agent.

The improvement of the therapeutic index of **1** would therefore be beneficial. In this context, a dihydropyridine $\rightleftharpoons$ pyridinium salt redox targetor-based chemical delivery systems (CDS) (Bodor, 1981, 1987) was applied. Indeed, this redox system has been shown to enhance specific accumulation of drugs in the central nervous system (CNS) in the form of a quaternary salt derivative which is rapidly eliminated from the systemic circulation. As a result, many studies have suggested that the dosage of a drug could be lowered and toxic side-effects reduced while maintaining or even potentiating the central effects (Pop et al., 1990; Bodor and Brewster, 1991; Pop and Bodor, 1992; Brewster et al., 1993).

The application of the CDS approach to LY231617 has been examined. Since **1** contains both amine and phenolic hydroxyl functionalities, CDSs of various types can be configured by appropriate structural manipulations. Synthesis and evaluation of CDSs in which the redox targetor is attached to the amino or to the hydroxy group are discussed herein.

2. Materials and methods

2.1. Chemistry and supplies

Uncorrected melting points were determined using an electrothermal melting-point apparatus (Fisher Scientific). Elemental microcombustion analyses were performed by Atlantic Microlabs, Inc., Atlanta, GA. Ultraviolet (UV) spectra were determined using a Hewlett-Packard 8451A diode array spectrophotometer. Infrared (IR) spectra were determined on a Perkin-Elmer FTIR spectrometer in KBr pellets or liquid film. Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded on a Varian EM 300 (90-MHz) spectrometer. Samples were dissolved in an appropriate deuterated solvent and chemical shifts were reported in parts per million (δ) relative to tetramethylsilane, the internal standard. Mass spectra (MS) were recorded on a Kratos MS 80-RFA instrument. Fast atom bombardment (FAB) ionization was performed via xenon beam (6 KeV) impingement and the samples were dissolved in a glycerol or a 3-nitrobenzyl alcohol + NaI matrix (CDSs), as described in the literature (Prokai et al., 1989). Thin-layer chromatography (TLC) was performed on EM Reagents DC-aluminum foil plates coated to a thickness of 0.2 mm with silica gel 60 F_{254} using various phases. All chemicals were reagent grade. LY231617 was obtained from Lilly Research Laboratories (Eli Lilly and Company, Indianapolis, IN).

2.1.1. N-[(3,4-Di-tert-butyl-4-hydroxyphenyl)-methyl]-N-ethyl-3-pyridinecarboxamide (2)

To a solution of **1** (3.0 g, 10 mmol) in dry pyridine (30 ml) nicotinic anhydride (2.5 g, 11 mmol) was added by stirring and cooling at 0–5°C and the resultant solution was stirred at 20–25°C for 18 h. The reaction mixture was poured into water (1.2 l) and the precipitated white solid was filtered off and washed thoroughly with water. After drying in a vacuum oven (70°C, 25–30 mm Hg), 2.4 g (65% yield) of **2** was obtained, mp 95–95°C; Rf (EtOAc): 0.3; MS (FAB) $[M-H]^+$ m/z: 369; UV_{max} (MeOH): 222,

270 nm; ^{1}H NMR ($CDCl_3$): δ 1.10 (t, 3H, CH_3); 1.45 (s, 18H, t-Bu); 3.10–3.40 (m, 2H, CH_2-CH_3); 4.40 (m, 2H, Ar-CH_2), 5.10 (s, 1H, OH); 6.90 (s, 2H, C_6H_2), 7.20 (m, 1H, pyridine C-5 proton), 7.60 (d, 1H, pyridine C-4 proton), 8.50–8.60 (m, 2H, pyridine C-6 and C-2 protons).

Anal. — Calc. for $C_{23}H_{32}N_2O_2$: C, 74.96; H, 8.75; N, 7.60. Found C, 74.88; H, 8.78; N, 7.57.

*2.1.2. 3-[N-[(3,5-Di-tert-butyl-4-hydroxyphenyl) methyl]-N-ethyl]carbamoyl]-1-methyl pyridinium iodide (**3**)*

To a solution of **2** (2.3 g, 6.25 mmol) in dry nitromethane (30 ml), methyl iodide (5.7 g, 2.5 ml, 40 mmol) was added and the mixture was stirred at 20–25°C for 48 h (TLC indicated complete reaction). The solvent was removed in vacuo and the residue slurried with ether, filtered, dried, then crystallized from acetone/hexane and dried in a vacuum oven to provide 2.96 g (93%) of **3** as an off-white solid, mp 211–14°C, Rf (EtOAc): 0; MS (FAB) C^+ *m/z*: 383; UV $_{max}$ (MeOH): 194, 274 nm; ^{1}H NMR ($CDCl_3$): δ 1.15 (t, 3H, CH_3), 1.60 (s, 18H, t-Bu), 3.40 (q, 2H, CH_2-CH_3), 4.40 (m, 5H, Ar-CH_2, N-CH_3), 5.20 (s, 1H, OH), 6.90 (m, 2H, C_6H_2), 8.20 (m, 2H, pyridinium C-4 and C-5 protons), 8.90 (m, 1H, pyridinium C-6 proton), 9.40 (s, 1H, pyridium C-2 proton).

Anal. — Calc. for $C_{24}H_{35}IN_2O_2$: C, 56.47; H, 6.91; I, 24.86; N, 5.49. Found C, 56.41; H, 6.95; I, 24.92; N, 5.47.

*2.1.3. 1,4-Dihydro-1-methyl-N[[(3,5-di-tert-butyl-4-hydroxyphenyl)methyl]-N-ethyl]-3-pyridinecarboxamide (**4**)*

To a solution of **3** (1.5 g, 2.9 mmol) in deaerated water (100 ml) and ethyl acetate (75 ml), a mixture of sodium bicarbonate (1.5 g, 17.4 mmol) and sodium dithionite (2.05 g, 11.6 mmol) was added by stirring at 0.5°C. The system was maintained under a nitrogen stream and was stirred for 5 h. The organic layer was separated and the aqueous layer extracted with ethyl acetate (2 × 50 ml). The combined organic layers were washed with cold deaerated water (2 × 50 ml) and cold deaerated brine (2 × 50 ml), dried over $MgSO_4$ then filtered over silica gel and evaporated under reduced pressure to give 0.66 g (59%) of a yellow solid **4**. Rf: 0.3 (EtOAc); MS (FAB) $[M\text{-}Na]^+$ *m/z*: 407; UV$_{max}$ (MeOH): 220, 280, 348 nm; ^{1}H NMR ($CDCl_3$): δ 1.15 (t, 3H, CH_2), 1.45 (s, 18H, t-Bu), 2.80 (s, 3H, N-CH_3), 3.15 (s, 2H, pyridine C-4 protons), 3.35 (q, 2H, CH_2-CH_3), 4.65 (s, 3H, Ar-CH_2 + pyridine C-5 proton), 5.15 (s, 1H, OH), 5.70 (d, 1H, pyridine C-6 proton), 6.10 (s, 1H, pyridine C-2 proton), 7.05 (s, 2H, C_6H_2).

Anal. — Calc. for $C_{24}H_{36}N_2O_2$: C, 74.96; H, 9.44; N, 7.29. Found C, 75.26; H, 9.73; N, 7.58.

*2.1.4. Chloromethyl N-[(3,5-Di-tert-butyl-4-hydroxyphenyl)methyl]-N-ethyl] carbamate (**5a**)*

To a solution of **1** (3.0 g, 10 mmol) in chloroform (20 ml), triethylamine (2.23 g, 3.07 ml, 22 mmol) then, by cooling at 0–5°C and stirring, chloromethyl chloroformate (1.29 g, 10 mmol) dissolved in chloroform (10 ml) was added. After stirring for 75 min, the solution was extracted with water, 1 N hydrochloric acid, water and brine and dried on Na_2SO_4. The solvent was removed in vacuo to provide 2.06 g (57%) of **5a** as an off-white solid, mp 87–90°C, Rf (EtOAc): 0.63; MS (FAB) $[M\text{-}H]^+$ *m/z*: 355; UV$_{max}$ (MeOH): 210, 278 nm; ^{1}H NMR ($CDCl_3$): δ 1.15 (m, 3H, CH_2-CH_3), 1.40 (s, 18H, t-Bu), 3.20–3.40 (m, 2H, CH_2-CH_3), 4.40 (bs, 2H, Ar-CH_3), 5.80 (s, 2H, OCH_2O), 7.10 (d, 2H, C_6H_2).

Anal. — Calc. for $C_{19}H_{30}ClNO_3$: C, 64.12; H, 8.50; Cl, 9.95; N, 3.94. Found C, 64.14; H, 8.55; Cl, 9.92; N, 3.89.

*2.1.5. (±)-1-Chloroethyl N-[[(3,5-Di-tert-butyl-4-hydroxyphenyl)methyl]-N-ethyl]carbamate (**5b**)*

To a solution of **1**, HCl (1.50 g, 5 mmol) and triethylamine (1.21 g, 12 mmol) in chloroform (20 ml) 1-chloroethyl chloroformate (0.86 g, 6 mmol) in chloroform (5 ml) was added at ice cooling. The solution was stirred for 0.5 h at ice cooling and then for 3 days at room temperature. The solution was washed with water, 1 N hydrochloric acid and with water, dried ($MgSO_4$), and evaporated in vacuo to provide 1.78 g (96%) pure **5b** as an oil. Rf (cyclohexane/triethylamine, 8:2): 0.64; MS (FAB) $[M\text{-}H]^+$ *m/z*: 370; ^{1}H NMR ($CDCl_3$): δ 1.15 (t, 3H, CH_3-CH_2), 1.45 (s, 18H, t-Bu), 1.80 (d, 3H, CH_3-CH), 3.10-3.40 (m, 2H, CH_3-CH_2), 4.40 (bs, 2H, Ar-CH_2), 5.20 (s, 1H, OH), 6.70 (q, 1H, CH), 7.05 (s, 2H, C_6H_2).

Anal. — Calc. for $C_{20}H_{32}ClNO_2$: C, 64.93; H, 8.72; Cl, 9.58; N, 3.79. Found C, 65.21; H, 8.94; Cl, 9.39; N, 4.01.

*2.1.6. [[N-[(3,5-Di-tert-butyl-4-hydroxyphenyl)methyl]-N-ethyl]-aminocarbonyloxy]methyl 3-pyridinecarboxylate (**6a**)*

To a solution of **5a** (1.8 g, 5 mmol) in dimethylformamide (DMF) (20 ml) was added a solution prepared from nicotinic acid (0.7 g, 6 mmol) and triethylamine (0.6 g, 0.83 ml, 6 mmol) in DMF (10 ml). The mixture was stirred at 20–25°C for 5 days, then ethyl acetate (200 ml) was added and the precipitated salts were filtered off. The solution was washed with water (4 × 50 ml) and brine (4 × 10 ml), dried ($MgSO_4$) and evaporated in vacuo to give 2 g of a viscous solution. The product was purified by column chromatography: 60 g silica gel Davisol grade 634, 100–200 Mesh, 60 Å, eluent ethyl acetate (5-ml fractions collected, the product was contained as fractions 22–28). By concentration, 1.7 g (77%) of colorless oil resulted in **6a**. Rf (EtOAc): 0.51; MS (FAB) $[M-H]^+$ *m/z*: 443 for $C_{25}H_{34}N_2O_5$; UV_{max} (MeOH): 224, 266 nm; ^1H NMR ($CDCl_3$): 1.05–1.19 (m, 3H, CH_2-CH_3), 1.47 (s, 18H, t-Bu), 3.24–3.34 (q, 2H, CH_2-CH_3), 4.40 (d, 2H, $ArCH_3$), 5.20 (s, 1H, OH), 6.10 (s, 2H, OCH_2O), 7.05 (d, 2H, C_6H_2), 7.41 (*t*, 1H, pyridine C-5 proton), 8.34 (d, 1H, pyridine C-4 proton), 8.81 (d, 1H, pyridine C-6 proton), 9.28 (s, 1H, pyridine C-2 proton).

*2.1.7. (±)-1-[[N-[(3,5-Di-tert-butyl-4-hydroxyphenyl)methyl)]-N-ethyl]-aminocarbonyloxy]-ethyl 3-pyridinecarboxylate (**6b**)*

A solution of **5b** (1.78 g, 4.8 mmol) in dimethylformamide (7.5 ml) was added to a solution prepared from nicotinic acid (0.71 g, 5.8 mmol) and triethylamine (0.58 g, 5.8 mmol) in dimethylformamide (7.5 ml). The mixture was stirred for 40 h at room temperature then evaporated in vacuo. The residue was treated with ethyl acetate (50 ml) then washed with aqueous sodium chloride solution (3 × 25 ml), dried, ($MgSO_4$), decolorized with activated carbon, and evaporated in vacuo. The residue (1.57 g) was purified by column chromatography on silica gel (200 g, cyclohexane/diethylamine 8:2 v/v, Rf: 0.47) to give 0.89 g (41%) of pure **6b** as a viscous, light brown oil. MS (FAB) $[M-H]^+$ *m/z*: 457 for $C_{26}H_{36}N_2O_5$: ^1H NMR ($CDCl_3$): δ 1.05 (t, 3H, CH_3-CH), 1.40 (s, 18H, t-Bu), 1.65 (d, 3H, CH_3-CH_2), 3.10–3.40 (m, 2H, CH_3-CH_2), 4.30 (s, 2H, Ar-CH_2), 5.10 (s, 1H, OH), 7.00 (s, 2H, C_6H_2), 7.00–7.40 (m, 2H, CH + pyridine C-5 proton), 8.20 (d, 1H, pyridine C-4 proton), 8.70 (d, 1H, pyridine C-6 proton), 9.10 (s, 1H, pyridine C-2 proton).

*2.1.8. [[N-[(3,5-Di-tert-butyl-4-hydroxyphenyl)-methyl]-N-ethyl]-aminocarbonyloxy]-methyl-6-methyl-pyridine-3-carboxylate (**6c**)*

To a solution of **5a** (1.24 g, 6 mmol) in dimethylformamide (20 ml) was added a solution prepared from triethylamine (0.98 g, 7.15 mmol) in DMF (10 ml) by cooling at 0–5°C. The mixture was stirred at 20–25°C for 3 days, then ethyl acetate (200 ml) was added and the precipitated salts were filtered off. The solution was washed with water (2 × 50 ml) and brine (2 × 50 ml), dried ($MgSO_4$) and evaporated in vacuo to give 2.1 g (77%) of viscous oil. The purity of the product permitted its use in the following step of the synthesis: Rf (EtOAc): 0.54 MS (FAB) $[M-H]^+$ *m/z*: 457 for $C_{26}H_{36}N_2O_5$: ^1H NMR ($CDCl_3$): δ 1.05–1.19 (m, 3H, CH_2-CH_3), 1.47 (s, 18H, t-Bu), 2.58 (s, 3H, pyridine 6-CH_3), 3.24–3.34 (q, 2H, Ar-CH_2), 4.40 (m, 2H, CH_2-CH_3), 5.20 (s, 1H, OH), 6.10 (s, 2H, OCH_2O), 7.00 (d, 2H, C_6H_2), 7.20 (d, 1H, pyridine C-5 proton), 8.20 (d, 1H, pyridine C-4 proton), 9.15 (s, 1H, pyridine C-2 proton).

*2.1.9. 3-[[[1-[N-[(3,5-Di-tert-butyl-4-hydroxyphenyl)methyl]-N-ethyl]-aminocarboxyloxy]methoxy]carbonyl]-1-methylpyridinium iodide (**7a**)*

To a solution of **6a** (1.15 g, 2.6 mmol) in nitromethane (20 ml), methyl iodide (2.28 g, 1.5 ml, 1.6 mmol) was added and the mixture was stirred for 3 days at 25–30°C. The solvent was removed in vacuo and the residue was stirred with ether, filtered, rinsed with ether and dried providing 1.35 g (89%) of **7a** as a yellow solid, mp 113–115°C (dec); Rf (EtOAc): 0; MS (FAB) C^+ *m/z*: 457; UV_{max} (MeOH): 222, 279 nm; ^1H NMR

($CDCl_3$) δ: 1.05–1.20 (m, 3H, CH_2-CH_3), 4.70 (s, 3H, N-CH_3), 5.10 (s, 1H, OH), 6.10 (s, 2H, OCH_2O), 7.05 (s, 2H, C_6H_2), 8.30 (d, 1H, pyridinium C-5 proton), 8.90 (d, 1H, pyridinium C-4 proton), 9.35 (s, 1H, pyridinium C-2 proton), 9.75 (d, 1H, pyridinium C-6 proton).

Anal. — Calc. for $C_{26}H_{37}IN_2O_5$: C, 53.43; H, 6.38; I, 21.71; N, 4.79. Found C, 53.18; H, 6.44; I, 21.94; N, 4.76.

*2.1.10. (±)-3-[[1-[[N-[(3,5-Di-tert-butyl-4-hydroxyphenyl)methyl]-N-ethyl]-aminocarbonyl-oxy]ethoxy]carbonyl]-1-methylpyridinium iodide (**7b**)*

A solution of **6b** (0.89 g, 1.95 mmol) in nitromethane (10 ml) was reacted with methyl iodide (1.11 g, 7.8 mmol, 0.49 ml) at room temperature for 6 days. The solvent was removed in vacuo and the residue was crystallized from dichloromethane to provide 0.88 g (75%) of **7b** as yellow powder, mp 160–163°C (decomp). By recrystallization from 2-propanol/ether, pure compound was obtained: mp, 165–167°C (decomp) Rf (EtOAc): 0; MS (FAB) C^+ *m/z*: 471; ^{1}H NMR ($CDCl_3$): δ 1.10 (t, 3H, CH_3-CH_2), 1.40 (s, 18H, t-Bu), 1.60–1.80 (m, 3H, CH_3-CH), 3.30 (q, 2H, CH_3-CH_2), 4.00–4.60 (m, 2H, Ar-CH_2), 4.70 (s, 3H, CH_3-N^+), 5.10 (s, 1H, OH), 6.90 (s, 2H, C_6H_2), 7.10 (q, 1H, CH), 8.30 (t, 1H, pyridinium C-5 proton), 8.80 (d, 1H, pyridinium C-4 proton), 9.25 (s, 1H, pyridinium C-2 proton), 9.70 (d, 1H, pyridinium C-6 proton).

Anal. — Calc. for $C_{27}H_{39}IN_2O_5$ 1.6% H_2O: C, 53.31; H, 6.64; I, 20.86; N, 4.61. Found C, 53.30; H, 6.65; I, 20.80; N, 4.60.

*2.1.11. 3-[[[1-[N-[(3,5-Di-tert-butyl-4-hydroxyphenyl)methyl]-N-ethyl]-aminocarbonyloxy]methoxy]carbonyl]-1,6-dimethylpyridinium iodide (**7c**)*

A solution of **6c** (2 g, 4.4 mmol) and methyliodide (7.6 g, 5 ml) in nitromethane (60 ml) was heated at 60–70°C for 6 h. The solvent was removed in vacuo and the residue was stirred with ether, filtered, rinsed with ether and dried, providing 2.3 g (87%) of **7c** as a yellow solid. Rf (EtOAc): 0; MS (FAB) C^+ *m/z*: 471; UV_{max} (MeOH) 208, 222, 274 nm; ^{1}H NMR ($CDCl_3$) δ 1.05–1.20 (m, 3H, CH_2-CH_3), 1.45 (s, 18H, t-Bu), 3.15 (s, 3H, pyridine 6-CH_3), 3.20–3.30 (q, 2H, CH_2-CH_3), 4.40 (s, 2H, Ar-CH_2), 4.50 (s, 3H, N^+-CH_3), 5.15 (s, 1H, OH), 6.10 (s, 2H, OCH_2O), 7.00 (d, 2H, C_6H_2), 8.20 (d, 1H, pyridinium C-5 proton), 8.75 (d, 1H, pyridinium C-4 proton), 9.40 (s, 1H, pyridinium C-2 proton).

Anal. — Calc. for $C_{27}H_{39}IN_2O_5$: C, 54.18; H, 6.57; I, 21.20; N, 4.68. Found C, 54.40; H, 6.81; I, 21.08; N, 4.52.

*2.1.12. [[N-[(3,5-Di-tert-butyl-4-hydroxyphenyl)methyl]-N-ethyl]-aminocarbonyloxy]methyl 1,4-dihydro-1-methyl-3-pyridinecarboxylate (**8a**)*

By reducing **7a** (1 g, 1.7 mmol) dissolved in water (50 ml) and ethyl acetate (100 ml), with sodium bicarbonate (0.84 g, 10.2 mmol) and sodium dithionate (1.17 g, 6.8 mmol) (50 min at 0–5°C), 0.7 g (90%) of **8a** resulted, as a yellow hygroscopic solid, Rf (EtOAc): 0.64 MS (FAB) $[M\text{-}Na]^+$ *m/z*: 481 for $C_{26}H_{38}N_2O_5$; UV_{max} (MeOH): 220, 270, 362 nm. ^{1}H NMR ($CDCl_3$): δ 1.15 (t, 3H, CH_2-CH_3), 1.45 (s, 18H, t-Bu), 2.90 (s, 3H, N-CH_3), 3.15 (bs, 2H, pyridine C-4 protons), 3.35 (q, 2H, CH_2-CH_3), 4.35 (s, 3H, Ar-CH_2 + pyridine C-5 proton), 5.15 (bs, 1H, OH), 5.80 (d, 1H, pyridine C-6 proton), 6.10 (s, 1H, pyridine C-2 proton), 7.05 (s, 2H, C_6H_2).

*2.1.13. (±)-1-[[N-[(3,5-Di-tert-butyl-4-hydroxyphenyl)methyl]-N-ethyl]-aminocarbonyl-oxy]ethyl 1,4-dihydro-1-methyl-3-pyridinecarboxylate (**8b**)*

To a solution of **7b** (0.12 g, 0.2 mmol) in a mixture of toluene (20 ml) and water (10 ml) that was cooled to 1°C and deaerated by purging nitrogen (30 min), a mixture of sodium dithionite (0.139 g, 0.8 mmol) and sodium bicarbonate (0.101 g, 1.2 mmol) was added. The mixture was stirred in the same conditions for 10 h, and the layers were separated. The organic layer was washed with ice-cold deaerated water, dried over magnesium sulfate and evaporated in vacuo at 30°C, giving 0.089 g (94%) of **8b** as a pale brown glasslike material, Rf (cyclohexane/ethyl acetate 1:1): 0.66; MS (FAB) $[M\text{-}Na]^+$ *m/z*: 495 for $C_{27}H_{40}N_2O_5$; ^{1}H NMR ($CDCl_3$): δ 1.10 (t, 3H, CH_3-CH_2), 1.40 (s, 18H, t-Bu), 1.50 (d, 3H, CH_3-CH), 2.85 (s, 3H, CH_3-N), 3.00–3.50 (m, 4H,

CH_3-CH_2 + pyridine C-4 protons), 4.35 (s, 2H, Ar-CH_2), 4.60–4.85 (m, 1H, pyridine C-5 proton), 5.10 (s, 1H, OH), 5.60 (d, 1H, pyridine C-6 proton), 6.90–7.25 (m, 1H, CH), 7.00 (s, 3H, C_6H_2 + pyridine C-2 proton).

*2.1.14. [[N-[(3,5-Di-tert-butyl-4-hydroxyphenyl)-methyl]-N-ethyl]-aminocarbonyloxy] methyl 1,4-dihydro-1,6-dimethyl-3-pyridinecarboxylate (**8c**)*

By reducing **7c** (0.6 g, 1 mmol) dissolved in water (60 ml) and ethyl acetate (60 ml) with sodium bicarbonate (0.6 g, 7 mmol) and sodium dithionite (0.7 g, 4 mmol) (45 min, at 0–5°C), 0.4 g (85%) of **8c** resulted as a yellow solid. Rf (EtOAc): 0.67; MS (FAB) $[M\text{-}Na]^+$ *m/z*: 495 for $C_{27}H_{40}N_2O_5$; UV_{max} (MeOH); 222, 274, 360 nm, ^1H NMR ($CDCl_3$): δ 1.05–1.20 (m, 3H, CH_2-CH_3), 1.45 (s, 18H, t-Bu), 1.80 (s, 3H, pyridine 6-CH_3), 2.90 (s, 3H, N-CH_3), 3.15 (bs, 2H, pyridine C-4 protons), 3.25 (q, 2H, CH_2-CH_3), 4.35 (s, 3H, Ar-CH_2 + pyridine C-5 proton), 5.15 (s, 1H, OH), 5.80 (s, 2H, OCH_2O), 7.05 (d, 2H, C_6H_2), 7.10 (s, 1H, pyridine C-2 proton).

*2.1.15. Benzyl N-[(3,5-Di-tert-butyl-4-hydroxy-phenyl)methyl]-N-ethylcarbamate (**9**)*

To a solution of 1 N HCl (1.50 g, 5 mmol) and triethylamine (1.26 g, 12.5 mmol) in ethanol-free chloroform (20 ml), benzyl chloroformate (1.13 g, 6.6 mmol) in ethanol-free chloroform (5 ml) was added at 0–5°C. The solution was stirred for 1 h at 0–5°C and then for 4 days at room temperature. The solution was washed with water (5 ml), 1 N hydrochloric acid (2 × 5 ml) and water (2 × 5 ml), dried over $MgSO_4$, and evaporated in vacuo to provide 1.97 g (99%) of **9** as a viscous oil; Rf: (cyclohexane/triethylamine 9:1) 0.53: MS (FAB) $[M\text{-}H]^+$ *m/z*: 398 for $C_{25}H_{35}NO_3$; IR (liquid film): cm^{-1} 3660 (OH, sharp), 2980 (CH_3), 1700 (C = O), 1160 (C-O-C), 770 (phenyl); ^1H NMR ($CDCl_3$): δ 1.10 (t, 3H, CH_3-CH_2), 1.40 (s, 18H, t-Bu), 3.25 (q, 2H, CH_3-CH_2), 4.35 (s, 2H, Ar-CH_2-N), 5.05 (s, 1H, OH), 5.15 (s, 2H, CH_2-O), 6.95 (s, 2H, C_6H_2), 7.30 (s, 5H, C_6H_5).

*2.1.16. Benzyl N-[(3,5-Di-tert-butyl-4-nicotin-oyloxyphenyl)methyl]-N-ethyl-carbamate (**10**)*

To a mixture of **9** (1.65 g, 4.15 mmol), tetrabutylammonium hydrogen sulfate (0.1 g), dichloromethane (35 ml), and powdered sodium hydroxide (1.66 g, 4.15 mmol), freshly prepared nicotinoyl chloride hydrochloride (1.77 g, 9.96 mmol) were added under argon in five portions over 20 min at room temperature. After 3 h of vigorous stirring, the salts were filtered and rinsed with methylene chloride (2 × 5 ml), then the solution was evaporated in vacuo. The residue (1.91 g) was purified by column chromatography on silica gel (200 g, hexane: isopropanol 9:1 v/v, Rf: 0.31) to provide 0.81 g (39%) of pure **10** as a viscous oil. IR (liquid film): cm^{-1} 3750–3100 (broad, moisture), 2960 (CH_3), 1760 (C = O, ester), 1700 (C = O, urethane), 1180 (C-O-C), 740 (phenyl); MS (FAB) $[M\text{-}H]^+$ *m/z*: 503 for $C_{31}H_{38}N_2O_4$: ^1H NMR ($CDCl_3$): δ 1.10–1.14 (m, 21H, CH_3-CH_2 + t-Bu), 3.40 (q, 2H, CH_3-CH_2), 4.50 (s, 2H, N-CH_2-Ar), 5.20 (s, 2H, CH_2-O), 7.20 (s, 2H, C_6H_2), 7.30 (s, 5H, C_6H_5), 7.40–7.55 (dd, 1H, pyridine C-5 proton), 8.45 (dd, 1H, pyridine C-4 proton), 8.85 (dd, 1H, pyridine C-6 proton), 9.40 (s, 1H, pyridine C-2 proton).

*2.1.17. 3-[2,6-Di-tert-butyl-4-((N-benzyloxycarbonyl-N-ethyl)-aminomethyl)-phenoxy-carbonyl]-1-methylpyridinium iodide (**11**)*

To a solution of **10** (0.428 g, 0.85 mmol) in nitromethane (4.5 ml), methyl iodide (1.21 g, 0.53 ml, 8.5 mmol) was added and the mixture was heated at 75–77°C for 6 h. The solvent was then removed in vacuo and the residue was treated with ether (4 × 5 ml) and then dried at 40°C (2 mm Hg), affording 0.503 g (92%) of **11** as a pale, yellow foam. Rf: (MeOH/EtOAc, 9:1) 0.34; ^1H NMR ($CDCl_3$): δ 1.15 and 1.30 (t and s, overlapped, 21H, CH_3-CH_2 and t-Bu), 3.40 (q, 2H, CH_3-CH_2), 4.45 (s, 2H, N-CH_2-Ar), 4.80 (s, 3H, N^+-CH_3), 5.20 (s, 2H, O-CH_2), 7.20 (s, 2H, C_6H_2), 7.30 (s, 5H, C_6H_5), 8.50 (dd, 1H, pyridinium C-5 proton), 9.05 (d, 1H, pyridinium C-4 proton), 9.40 (s, 1H, pyridinium C-2 proton), 10.05 (d, 1H, pyridinium C-6 proton).

Anal. — Calc. for $C_{32}H_{41}IN_2O_4$: C, 59.63; H, 6.41; I, 19.69; N, 4.35. Found C, 59.92; H, 6.58; I, 19.75; N, 4.22.

2.1.18. 3-[2,6-Di-tert-butyl-4-ethylaminomethyl-phenoxycarbonyl]-1-methyl-pyridinium iodide hydrobromide (12)

To a solution of **11** (0.465 g, 0.72 mmol) in dry methylene chloride (10 ml), a 0.5 M solution of hydrogen bromide (7.2 ml, 3.6 mmol) in dry methylene chloride was added over 30 min at room temperature by stirring. After 4 h the crystalline product was filtered, washed with dry methylene chloride (3 × 2 ml) and dried in vacuo providing 0.413 g (97%) of **12** as light yellow crystals; mp: 245–247°C (decomp); Rf: (MeOH/AcOH 9:1) 0.26; ^{1}H NMR ($CDCl_3$ + DMSO-d_6): δ 1.35 and 1.45 (s and t, overlapped, 21 H, t-Bu and CH_3-CH_2), 2.95–3.30 (m, 2H, CH_3-CH_2), 4.00–4.25 (m, 2H, CH_2-Ar), 4.75 (s, 3H, N^+-CH_3), 7.70 (s, 2H, C_6H_2), 8.45 (dd, 1H, pyridium C-5 proton), 9.05–9.50 (m, 3H, N^+H_2 and pyridinium C-4 proton), 9.70 (d, 1H, pyridinium C-6 proton), 9.85 (s, 1H, pyridinium C-2 proton).

Anal. — Calc. for $C_{24}H_{36}BrIN_2O_2$: C, 48.72; H, 6.14; Br (total halogen), 27.02; N, 4.74. Found C, 49.01; H, 6.23; Br (total halogen), 26.85; N, 4.53.

2.1.19. 2,6-Di-tert-butyl-4-ethylaminomethyl-phenyl 1,4-dihydro-1-methyl-3-pyridinecarboxylate (13)

To an ice-cooled solution of **12** (0.367 g, 0.623 mmol) in a mixture of water (15 ml) and ethyl acetate (30 ml) deaerated by purging argon, a mixture of sodium dithionite (0.434 g, 2.49 mmol) and sodium hydrogen carbonate (0.366 g, 4.36 mmol) was added. The mixture was stirred in the same conditions for 1 h, the layers separated and the aqueous layer was extracted with ethyl acetate (5 × 25 ml), a mixture of ethyl acetate and dichloromethane (8:2 v/v, 3 × 25 ml), and ethyl acetate (2 × 15 ml). The combined layers were washed with ice-cooled deaerated water (25 ml), dried over $MgSO_4$ and evaporated in vacuo at room temperature. The residue was treated with dry ether (2 × 5 ml) and dried (2 mmHg) at room temperature to provide 0.174 g (73%) of pure **13** as a yellow solid. Rf (benzene-triethylamine 9:1 v/v): 0.40, MS (FAB) $[M-H]^+$ m/z: 385 for $C_{24}H_{36}N_2O_2$; UV_{max} (MeOH): 206, 366 nm; ^{1}H NMR ($CDCl_3$): δ 1.10–1.45 (m, 21H, CH_3-CH_2 and t-Bu), 2.75 (q, 2H, CH_3-CH_2), 2.95 (s, 3H, N-CH_3), 3.15–3.35 (m, 2H, pyridine C-4 protons), 3.80 (s, 2H, Ar-CH_2), 4.80 (dd, 1H, pyridine C-5 proton), 5.70 (d, 1H, pyridine C-6 proton), 7.10 (s, 1H, pyridine C-2 proton), 7.35 (s, 2H, C_6H_2).

2.2. Analytical methodology

High performance liquid chromatography (HPLC) was used for quantitative analysis of the various dihydropyridines, pyridinium salts and LY231617. The HPLC system included a SpectraPhysics Model SP8800 ternary solvent pump, a SpectraPhysics Model SP 8490 variable wavelength detector, a SpectraPhysics Model SP 4270 integrator and a SpectraPhysics Model SP 8780 refrigerated autosampler. Analysis was completed using an Alltech Spherisorb C8, 5 μM particle size, 25 cm × 4.6 mm i.d. analytical column. The mobile phase consisted of 75:25 acetonitrile: 0.05 M KH_2PO_4 buffer and the flow rate was 1.0 ml/min. All analyses were conducted at 5°C temperature to avoid degradation and analytes were detected at 274 (LY231617), 268 (quaternary salts) and 366 nm (CDS), respectively. Using the system described above, LY231617 eluted with a retention time of 5.9 min. Compounds **3**, **7a**, **8a** and **12** eluted with retention times of 6.8, 8.4, 12.5 and 6.7 min, respectively. For the analysis of compounds **7c** and **8c**, the mobile phase consisted of 75:25 acetonitrile: 0.05 M KH_2PO_4; 5 μmol/l 1-hexanesulfonic acid Na salt (0.94 g/l), LY231617 and **7c** was detected at 274 nm (retention times: 7.5 and 10 min, respectively) while **8c** was detected at 360 nm (retention time: 7.5 min). Quantitations were performed by external calibration standards over a concentration range of 0.05–100 μg/ml. Calibration curves were linear over the range of concentrations examined (r = 0.999).

2.3. In vitro stability studies

The stability of the dihydropyridines **8a**, **8c** and the quaternary salts **7c** and **12** was determined in several biological matrices including 20% rat brain and liver homogenates and 50% rat blood. Homogenates were prepared using freshly harvested tissues from male Sprague–Dawley rats. One gram of brain or liver was homogenized with isotonic phosphate buffer to produce a 20% w/v homogenate. One millilitre of freshly obtained trunk blood was diluted with 1 ml of isotonic phosphate buffer. The matrices were then equilibrated for 5 min in a Dubenoff shaking water bath which was thermostated to 37°C. Solutions of various compounds (5×10^{-3} M in dimethylsulfoxide) were then added to the matrices producing an initial ($t = 0$) concentration of 50 μM. At various times subsequent to compound addition, 200 μl of the matrix were removed and diluted with 800 μl of ice-cold acetonitrile. The system was then centrifuged (7 min) at 12 000 $\times$ g in a Beckman Microfuge 12. The supernatant was then transferred to the autosampler vials and the rate of disappearance determined by HPLC.

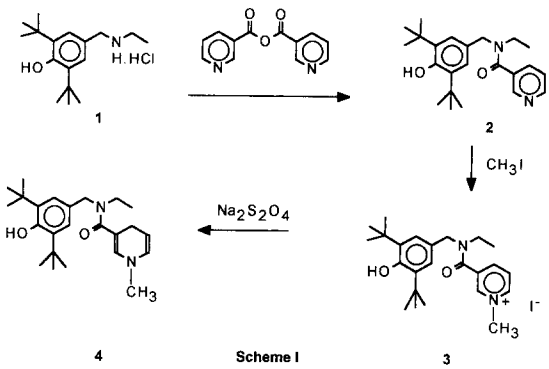
2.4. In vivo distribution studies

CDSs **4** and **13** were tested in vivo for their ability to deliver the parent compound to the CNS. Test compounds were administered by the tail vein to conscious, restrained Sprague–Dawley rats weighing 200–225 g (Harlan Sprague Dawley, Inc., Indianapolis, IN). Rats were housed in a climate-controlled vivarium (temperature 22 $\pm$ 2°C, humidity 40–65%, lights on 07:00–19:00 h) for 1 week prior to the study. The drug vehicle was DMSO and the amount administered was maintained at 0.5 ml/kg. After several prescribed periods of time (5 min, 15 min, 30 min, 1 h, 2 h, 4 h and 6 h, for **4**, 15 min and 1 h for **13**), animals were sacrificed by rapid decapitation. For each compound and at each time point, five animals were used. Trunk blood and various tissues including brain and liver were collected, weighed and frozen on dry ice. All tissues were frozen within 90 s of sacrifice.

In preparing samples for analysis, 1 ml of blood and approximately 1 g of other preweighed tissues were homogenized with 1 ml of phosphate buffer using a Polytron Model PT-1200C homogenizer. The homogenate was then deproteinated with 4 ml of ice-cold acetonitrile and the system vortexed. Concentrated brine (1.0 ml) was then added to the resulting suspension and the system was mixed and allowed to settle at 20°C for 2 h. The organic phase which separated under these conditions, was then removed, filtered through a 0.45-μm membrane and placed in HPLC autosampler vials for analysis. Sample stability was assessed during the storage conditions and no degradation was observed. A standard curve was prepared in the appropriate tissue homogenates for quantitation determination.

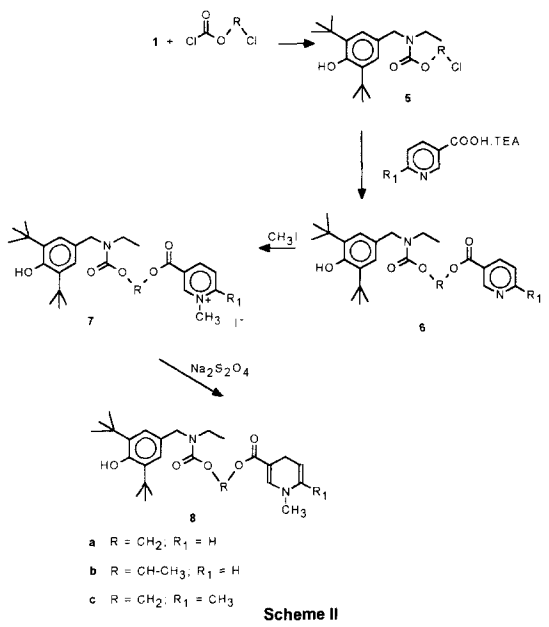
3. Results and discussion

The synthetic procedures used to manipulate the amino functionality of LY231617 are summarized in Schemes 1 and 2. By reacting **1** with nicotinic anhydride in pyridine in mild conditions (0–25°C), the nicotinamide **2** resulted. The hindered phenolic hydroxyl group was inert in these conditions. N-Alkylation of **2** with iodomethane in nitromethane provided the pyridinium quaternary salt derivative **3**, which by reduction with aqueous sodium dithionite in a biphasic solvent system consisting of ethyl acetate and aqueous sodium bicarbonate regioselectively (Eisner and



Scheme I

Scheme 1. Synthesis of an amide-type CDS of LY231617.

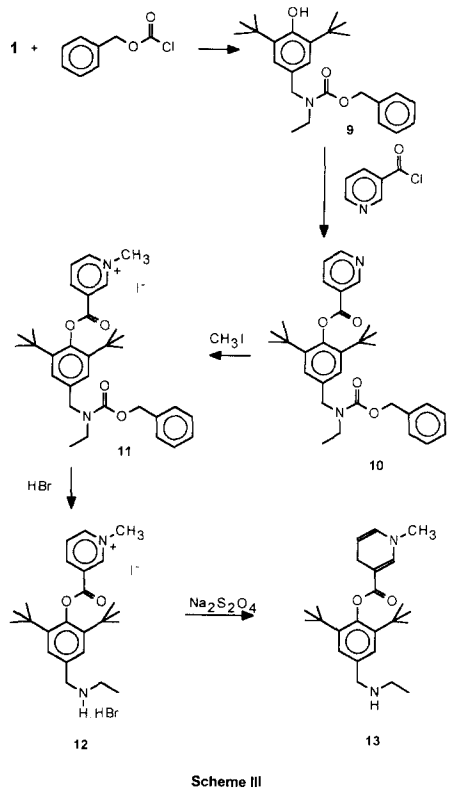


Scheme 2. Synthesis of substituted carbamate-type CDSs of LY231617.

Kuthan, 1972) afforded the 1,4-dihydropyridine **4** (Scheme 1).

The synthesis of a series of CDSs in which the dihydropyridine moiety is attached to the drug through a hydrolytically labile, substituted carbamate linkage is described in Scheme 2. The haloalkyl carbamate intermediates **5** were obtained by acylation of **1** with haloalkyl chloroformates. By reacting **5** with the triethylammonium salt of the nicotinic or 6-methyl nicotinic acids, the carbonyloxyalkyl pyridine carboxylates **6a–c** resulted. N-Methylation of **6a–c**, followed by the dithionite reduction of the resulting quaternary salts **7a–c**, yielded the CDSs **8a–c**.

The synthesis of a CDS in which the redox carrier is attached at the phenolic hydroxyl group is described in Scheme 3. The more reactive amino group of LY231617 was protected as a benzyloxycarbonyl derivative **9**, obtained by acylation of the parent compound with benzyl chloroformate in chloroform. The acylation of the phenolic OH of **9** through the use of either nicotinate anhydride or nicotinic acid in the presence of dicyclohexylcarbodimide (DCC) as a dehydrating agent failed. This likely occurred as a result of the strong steric hinderance of the bulky tert-butyl groups each ortho to the phenolic hydroxyl. By using phase-transfer conditions (tetrabutylammonium hydrogen sulfate [catalyst], methylene chloride and sodium hydroxide), **9** was acylated by nicotinoyl chloride in poor yield. N-Methylation of the phenyl nicotinate **10** in nitromethane resulted in the quaternary salt **11**. Deprotection of **10** with hydrogen bromide in dichloromethane took place in quantitative yield, and the resulting quaternary salt **12** was reduced with sodium dithionite to produce the CDS **13**. No migration of the acyl group from the phenol to the amine (intramolecular 'transesterification') was registered. As a proof, the methylene protons α to the NH ($N\underline{CH_2}CH_3$ and Ph-$\underline{CH_2}N$) appear at δ 2.75 and 3.80 ppm, respectively in the ^1H NMR spectra of **13** while they are shifted downfield in the spectra of the amides (e.g. **4**) to δ 3.35 and 4.65 ppm, respectively.



Scheme 3. Synthesis of a phenol ester-type CDS of LY231617.

Table 1
Concentration of compound **3** (μg/ml or μg/g) in various tissues after a 15.7 mg/kg dose of compound **4** given i.v. to Sprague–Dawley rats (n = 5)

Time (min)	Blood (μg/ml ± SEM)	Brain (μg/g ± SEM)	Liver (μg/g ± SEM)
0	0.00	0.00	0.00
5	2.55 ± 0.25	4.76 ± 0.37	19.9 ± 4.50
15	1.75 ± 0.17	6.48 ± 1.13	7.17 ± 2.2
30	1.53 ± 0.06	7.56 ± 0.78	5.21 ± 0.44
60	1.48 ± 0.05	8.02 ± 0.89	4.20 ± 0.65
120	1.35 ± 0.01	7.65 ± 0.86	3.61 ± 2.30
180	0.68 ± 0.26	6.85 ± 0.92	2.88 ± 0.30
240	0.00 ± 0.00	6.33 ± 0.52	2.57 ± 0.24
360	0.00	5.20 ± 0.80	2.08 ± 0.16

Note: No levels of **4** were detected in any of the samples.

The synthesized compounds were characterized by elemental analysis, MS, UV and ^{1}H-NMR spectroscopy. Chromatographic techniques (TLC and HPLC) indicated one major component (> 97%) in each case. The dihydropyridines were shown to be predominantly the 1,4-isomers (absorption at ~360 nm), accompanied by small amounts of the 1,2 and 1,6 isomers. All dihydropyridines could be converted to the corresponding quaternary salts via methanolic silver nitrate or H_2O_2 in the presence of catalytic cupric ions.

The in vivo evaluation of two simple delivery systems, **4** (amide type) and **13** (phenolic type), was first considered. The results of the tissue distribution after administration of **4** to rat are given in Table 1. The data show rapid conversion of the dihydronicotinamide **4** to the corresponding quaternary salt **3**. No CDS **4** was detected in the brain even at the first time-point of the determination (5 min). The levels of **3** in liver were high by 5 min but rapidly declined. Brain levels of **3** increased to 60 min and then slowly declined; after 6 h, large amounts of **3** could still be detected in the CNS. The elimination of the quaternary salt from blood was rapid. As compared to the distribution of equimolar administration of LY231617 (Brewster et al., 1995), the CDS produces greater selectivity in brain delivery as defined by lower peripheral levels of the CDS components. Due to low amidase activity of the CNS, the parent drug (**1**) was not readily released from the conjugate **3** and levels of the parent drug were below the assay detection limit (0.05 μg/ml). Because efficacy has not been evaluated, it was not possible to estimate a therapeutic index for the compound. Activity studies of **4** would be of interest since the antioxidant properties of LY231617 induced by the phenolic hydroxyl group should be maintained in **3**, generating an active analog.

Results of the distribution study performed on the CDS **13** are summarized in Table 2. Levels of **13** and the quaternary salt metabolite **12** were determined at 15 and 60 min. In contrast with the CDS **4**, the oxidation process was much slower for **13**. After 1 h, the two compounds were present in the brain in approximately the same concentrations. The oxidative stability of **13** prevented a significant accumulation of compound **12** in the brain. Although no in vitro stability data are available, it appears that **12** and **13** are hydrolytically stable since the parent drug LY231617 was not detected in brain during the in vivo study. Obviously the steric hindrance of the t-butyl groups did not allow for the access of the brain esterases to the reaction site (Pop et al., 1992).

In order to investigate this finding, compound **12** was examined in vitro in rat brain and blood. Both in blood and brain, there were little changes in the concentration of **12** over several hours. In blood, the peak height fell 3% over 5 h and brain levels declined by 6 and 12% over 4 and 23 h,

Table 2
Blood and brain concentration for CDS **13**, its quaternary metabolite (**12**) and LY231617 (**1**) after a 7.85 mg/kg dose of **13** given i.v. to Sprague–Dawley rats (n = 5)

Time (min)	Blood (μg/ml ± SEM)			Brain (μg/g ± SEM)		
	13	**12**	LY231617	**13**	**12**	LY231617
15	0.6 ± 0.1	1.8 ± 0.3	B.D.	1.8 ± 0.7	2.0 ± 0.0	B.D.
60	0.3 ± 0.0	B.D.	B.D.	1.3 ± 0.2	1.5 ± 0.5	B.D.

B.D., below assay detection 0.05 μg/ml.

respectively. The data suggest that compound **12** releases LY231617 too slowly to be a useful delivery system. Moreover, in contrast with the CDS **4** and its metabolite **3**, no antioxidant activity is expected in the case of **13** and **12**, since the phenolic hydroxyl pharmacophore is blocked.

Following these results another category of CDSs, the substituted carbamate-type derivatives **8**, have been investigated. Based on available data (Alexander et al., 1988; Pop et al., 1989a; Prokai-Tátrai et al., 1991), it was expected that, in contrast with the hydrolytically stable amide type derivatives **3** and **4**, compounds **8** and their metabolites **7** would readily release the native drug **1**. Indeed, substituted carbamates are hydrolyzed (by esterases) at the ester site of the acyloxyalkyl linkages. The hydroxyalkoxycarbamates (hemiketals), which result as the first products of hydrolysis, are unstable in an aqueous environment and decompose spontaneously to the parent compound through an intermediary carbamic acid: **7** → R-NHCOO$(CH_2)_n$OH → R-NHCOOH → R-NH_2. The side-products of the reaction are N-methyl (or 1,6-dimethyl) nicotinic acid, an aldehyde and carbon dioxide.

Three substituted carbamate-type CDSs were synthesized for LY231617: the aminocarbonyloxymethyl- and aminocarbonyloxyethyl-1,4-dihydro-1-methylpyridine carboxylates **8a** and **8b** as well as the aminocarbonyloxymethyl-1,4-dihydro-1,6-dimethylpyridine carboxylate **8c**. Compound **8b** was expected to have an increased hydrolytic stability as compared to **8a** as a result of a sterically hindered environment at the site of enzyme attack. Compound **8c** was synthesized based on previous findings which indicated that electron donating (+ I) groups (such as methyl) at C-6 of 1,4-dihydropyridines increase the rate of their oxidation (Pop et al., 1989b, 1994). The stability of CDSs **8a** and **8c** as well as of the quaternary salt-type derivative **7c** in various biological matrices are indicated in Table 3. The data revealed rapid conversion of the CDS in liver homogenate with a pseudo first-order rate constant of (2.51 ± 0.27) × 10^{-2} min^{-1} and an associated half-life of disappearance of 27 min. The derivative was significantly more stable in blood and brain with degradation rate constants of (2.29 ± 0.83) × 10^{-3} min^{-1} and (3.34 ± 0.62) × 10^{-3} min^{-1} indicating tissue half-lives of approximately 5 and 3.5 h, respectively. The oxidation rate of **8a** in brain appears to be too slow in comparison with other CDSs (Bodor, 1987; Pop et al., 1990); half lives of oxidation, which vary with the conditions of determinations, should not exceed 30–60 min in order to assure efficient accumulation of drugs in the CNS. Evidently the use of the homologue **8b**, originally synthesized with the goal to increase the hydrolytic stability of the CDS cannot solve this problem and was not further investigated. On the other hand, compound **8c** while stable in blood (half life 1.4 h) with degradation resulting in products of both oxidation and hydrolysis

Table 3
Stability (half lives, min) of the CDS **8a** and **8c** and the quaternary salt **7c** in rat biological materials at 37°C

Medium	Half life ($t_{1/2}$) min		
	8a	**8c**	**7c**
Blood homogenate (50%)	300.0	84.1	9.0
Brain homogenate (20%)	210.0	21.0	130.2
Liver homogenate (20%)	27.0	6.6	13.2

proved to be relatively rapidly degraded in brain. While oxidation was the main transformation, the product of hydrolysis was also detected. The 6-methyl group in **8c** resulted in a 10-fold acceleration of the oxidation rate of the CDS in brain tissue (21 min compared to 210 min for **8a**) which empirically correlates with sequestration of the drug in the CNS in the form of **7c**. Importantly, the degradation of **8c** in liver was also rapid (half life 6.6 min) producing both **7c** and **1**. The stability of the quaternary salt **7c** is also of interest. The results presented in Table 3 indicate that while **7c** rapidly hydrolyzed in blood (half life 13.2 min), the process was much slower (half life 2.17 h) in brain. The parent drug LY231617 was in both cases the main product of hydrolysis. These data suggest a sustained release of the drug from **7c** in the brain.

These in vitro results indicate that the CDS **8c** presents all the required properties (stability in blood, fast oxidation in brain, sustained release of the drug from **7c** in brain) for proper delivery of LY231617 to the CNS. Further in vivo studies of **8c** both in rat and dog models are required to support these assumptions.

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References

Alexander, J., Cargill, R., Michelson, S.R. and Schwann, H., (Acyloxy)alkyl carbamates as novel bioreversible prodrugs for amines; Increased permeation through biological membranes. *J. Med. Chem.*, 31 (1988) 318–322.

Beckman, J.S., Liu, T.H., Hogan, E.L., Lindsay, S.L., Freeman, B.A. and Hsu, C.Y., Evidence for a role of oxygen radicals in cerebral ischemic injury. In Ginsberg, M.D. and Dietrich, W.D. (Eds.), *Cerebrovascular Disease*, Raven Press, New York, 1989, pp. 373–380 and references therein.

Bodor, N., Novel approaches in prodrug design. *Drugs Future*, 6 (1981) 165–182.

Bodor, N., Redox drug delivery systems for targeting drugs to the brain. *Ann. New York Acad. Sci.*, 507 (1987) 289–306.

Bodor, N. and Brewster, M.E., Clinical delivery systems. In Juliano, R.L. (Ed.), *Handbook of Experimental Pharmacology*, Vol. 100, Springer, Berlin, 1991, pp. 231–284.

Brewster, M.E., Pop, E. and Bodor, N., Chemical approaches to brain-targeting of biologically active compounds. In Kozikowski, A.P. (Ed.), *Drug Design for the Neurosciences*, Raven Press, New York, 1993, pp. 435–467.

Brewster, M.E., Anderson, W.R., Estes, K.S., Bodor, N.S., Pop, E. and Panetta, J.A., Tissue distribution of LY231617, an antioxidant with neuroprotectant activity, in the rat. *J. Pharm. Sci.*, 84 (1995) 791–794.

Clemens, J.A., Saunders, R.D., Ho, P.P., Phebus, L.A. and Panetta, J.A., The antioxidant LY231617 reduces global ischemic neuronal injury in rats. *Stroke*, 23 (1993) 716–723.

Eisner, U. and Kuthan, J., The chemistry of dihydropyridines. *Chem. Rev.*, 72 (1972) 1–42.

Hall, E.D., Effects of the 21-aminosteroid U74006F on post-traumatic spinal cord ischemia in cats. *J. Neurosurg.*, 68 (1988) 462–465.

Martz, D., Rayos, G., Schielke, G.P. and Betz, A.L., Allopurinol and dimethylthiourea reduces brain infarction following middle cerebral artery occlusion in rats. *Stroke*, 20 (1989) 488–494.

McCall, J.M. and Panetta, J.A., Traumatic and ischemia/reperfusion injury to the CNS. In Bristol, J.A. (Ed.), *Annual Reports in Medicinal Chemistry*, Vol. 27, Academic Press, San Diego, CA, 1992, pp. 31–40 and references therein.

Pop, E. and Bodor, N., Chemical systems for delivery of antiepileptic drugs to the central nervous system. *Epilepsy Res.*, 2 (1992) 457–466.

Pop, E., Brewster, M.E. and Bodor, N., Central nervous system enhanced delivery of β-lactam antibiotics. *Drugs Future*, 15 (1990) 473–492.

Pop, E., Huang, M.-J., Brewster, M.E. and Bodor, N., A theoretical study of the hydrolysis of some sterically hindered phenolic esters. *Int. J. Quantum. Chem. Quantum Biol. Symp.*, 19 (1992) 77–85, and references therein.

Pop, E., Huang, M.-J., Brewster, M.E. and Bodor, N., Stability of 1,3-substituted 1,4-dihydropyridines; Substituent effects on the acid catalyzed hydration and oxidation reactions. *Int. J. Quantum Chem. Quantum Biol. Symp.*, 21 (1994) 173–184.

Pop, E., Wu, W.-M., Shek, E. and Bodor, N., Chemical delivery systems for drugs containing an amino group; syntheses and properties of some pyridine derivatives of desipramine. *Drug Des. Deliv.*, 5 (1989a) 93–115.

Pop, E., Shek, E., Murakami, T. and Bodor, N., Improved anticonvulsant activity of phenytoin by a redox brain delivery system. *J. Pharm. Sci.*, 78 (1989b) 609–616.

Prokai, L., Hsu, B., Farag, H. and Bodor, N., Desorption chemical ionization, thermospray, and fast atom bombardment mass spectrometry of dihydropyridine $\rightleftharpoons$ pyridinium salt-type redox systems. *Anal. Chem.*, 61 (1989) 1723–1728.

Prokai-Tátrai, K., Pop, E., Anderson, W., Liu, J.-L., Brewster, M.E. and Bodor, N., Redox derivatives of tranylcypromine; Syntheses, properties and monoamine oxidase inhibitor activity of some chemical delivery systems. *J. Pharm. Sci.*, 80 (1991) 255–281.