THE SYNTHESIS OF (-)-4-METHYL-8-CHLORO-*trans*-1,2,3,4,4a,5,6,10b-OCTAHYDROBENZO-[f]-QUINOLIN-3-ONE-[3-^{14}C] (LY300502-^{14}C]) *VIA* A CIRCUITOUS ROUTE

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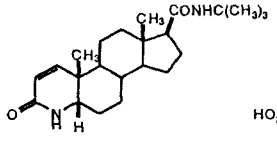
SUMMARY

The synthesis of the C-14 labeled isotopomer of LY300502, a potent 5α-reductase inhibitor has been accomplished in four radiochemical steps. The route involves the synthesis of LY300502-[^{14}C] from LY300502 *via* a circuitous route; the label was introduced with ethyl chloroformate-[carbonyl-^{14}C]

Key words: selective type I 5α-reductase inhibitor, LY300502, carbon 14

INTRODUCTION

Andersson and Russell recently discovered that the reduction of testosterone to dihydrotestosterone (DHT) is catalysed by two different 5α-reductases.[1] The elevation of DHT has been implicated as the cause of such maladies as benign prostatic hypertrophy[2], acne[3], hirsutism[4], and androgenic alopecia.[5] Finasteride (**1**, Proscar™, MK-906) has been shown to be rather non-selective inhibiting both Type I and Type II enzymes[6], while SKF105687 (**2**) has been shown to be selective against the Type II enzyme.[7] Jones *et al.* reported on the synthesis and SAR of a series of benzoquinolinones, which are selective inhibitors of the Type I enzyme.[8] One member of this series, (-)-4-methyl-8-chloro-*trans*-1,2,3,4,4a,5,6,10b-octahydrobenzo-[f]-quinolin-3-one (**3**, LY300502) has been selected for further evaluation. In order to support ADME studies in laboratory animals, radiolabeled material was required. Opportunities to label this material in a metabolically stable position, without extensive effort were few (especially since a resolution needed to



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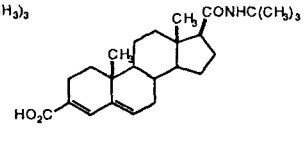

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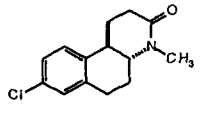

3, LY300502

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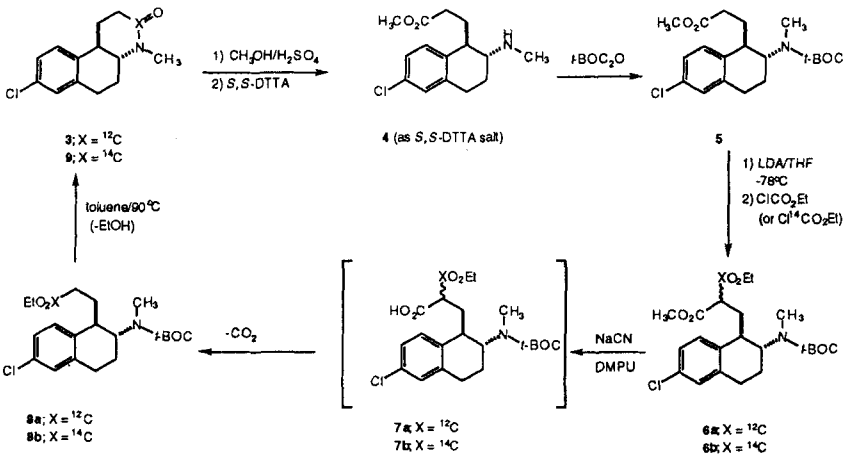
take place near the end of the synthesis); however, we envisioned LY300502 (**3**)[9] as a potential starting material. Herein are the details of a circuitous synthesis of C-14 labeled LY300502.

DISCUSSION

Methanolysis of lactam **3**, followed by salt formation by reaction with (+)-di-*p*-toluoyl-*S,S*-tartaric acid, provided amino ester **4** (as its *S,S*-DTTA salt)[9], which upon reaction with *t*-BOC_2O yielded the *t*-BOC protected amino ester **5**. Following treatment of **5** with lithium *iso*-propylcyclohexylamide (LICA)/THF at -78°C, the resulting anion was quenched with ethyl chloroformate (or ethyl chloroformate-[carbonyl-^{14}C]) to afford diester **6a,b**. Selective cleavage of the methyl ester with NaCN/1,3-dimethyl-3,4,5,6-tetrahydro-2(1H)-

SCHEME I



pyrimidinone (DMPU) and concomitant decarboxylation of the resulting acid **7a,b** yielded the corresponding ethyl ester **8a,b**. Deprotection of **8a,b** by treatment with TFA/anisole in CH_2Cl_2, followed by re-lactamization by heating in toluene at 90°C for 24 hr yielded **3** (or its ^{14}C isotopomer **9**). Thus, **9** was prepared from **3** in six total steps (four radiochemical steps). The radiochemical yield was 48%.

EXPERIMENTAL

The ethyl chloroformate-[carbonyl-^{14}C] was purchased from American Radiolabeled Chemicals, Inc. The NMR spectra were obtained on a General Electric QE-300 spectrometer at 300 (^{1}H) and 75 (^{13}C) MHz. Chemical shifts are reported in parts per million (ppm) downfield from tetramethylsilane. Direct chemical ionization mass spectra (DCI-MS) were recorded on a Nermag R30-10 triple stage quadrapole mass spectrometer; field desorption mass spectra were recorded on a Varian Associates MAT 731 mass spectrometer. High resolution fast atom bombardment mass spectra were obtained from a VG Analytical VG-ZAB-3F mass spectrometer.[10] The optical rotations were determined on a Perkin Elmer 241 polarimeter. Microanalytical data were provided by the Computational Chemistry and Molecular Structure Research Department of the Lilly Research Laboratories.

Flash chromatography was performed as described by Still *et al.*, using E.M. Science silica gel 60 (230-400 mesh).[11] Thin-layer chromatography was conducted on E. Merck silica gel F_{254} plates. Unless otherwise noted, the organic extracts were dried over anhydrous magnesium sulfate.

Radiochemical purity (RCP) was assessed by TLC/autoradiography employing E. Merck silica gel F-254 TLC plates and Kodak BB-5 x-ray film. The radioactive lane was divided, suspended in methanol, and after sonication, the mixture was diluted with DuPont Aquassure scintillation cocktail and counted. Radiochemical Purity was further assessed by HPLC by collecting the eluant at 30 sec intervals; the samples were diluted with DuPont Aquassure and counted.

Methyl 3-[(2-*R*-*N*-*tert.*-Butyloxycarbonylmethylamino-6-chloro-*S*-1,2,3,4-tetrahydro-naphthalen)-1-yl]-propionate, 5: A mixture of **4** as its (-)-*di*-*p*-tolyl-*L*-(*R,R*)-tartaric acid salt (3.62 g, 7.97 g, 11.93 mmol) and *di-tert.*-butyl dicarbonate (6.48 g, 49.3 mmol) in toluene (120 mL) was treated in a dropwise fashion with 1% aqueous $NaHCO_3$ (400 mL) over 1 hr. After the addition was complete, stirring was continued for an additional 2 hr. TLC ($CHCl_3$/MeOH/NH_4OH) showed the loss of starting material and the formation of **5** and a small amount of **3**. The layers were separated and the aqueous layer was extracted with EtOAc (4 x 55 mL). The combined organic extracts were washed with brine, dried and concentrated *in vacuo*. The residue was purified by flash chromatography, eluting first with CH_2Cl_2 to remove the *di-tert.*-butyl dicarbonate. Further elution with CH_2Cl_2/ Et_2O (10:1) yielded **5** (4.126 g, 90.8%): ^{1}H-NMR (acetone/d_6) δ 1.43 (9H, s, *t*-Bu), 1.84-2.00 (4H, m), 2.40 (1H, m), 2.70-3.00 (2H, m), 2.74 (3H, s, N-CH_3), 3.16 (1H, m, ArCH), 3.53 (3H, s, CH_3), 4.28 (1H, m, CHN), 7.11 (1H, br. s, H-5), 7.16 (1H, dd, J = 8.4, 2 Hz, H-7), and 7.20 (1H, d, J = 8.4 Hz, 8-H); DCI-MS $[M+H]^+$ 382; TLC CH_2Cl_2/MeOH (50:1) (R_f = 0.52). Anal. calc'd for $C_{20}H_{28}ClNO_4$: C, 62.90; H, 7.39; and N, 3.67. Found: C, 63.18; H, 7.23; and N, 3.86.

Methyl 1-Ethoxycarbonyl-3-[(2-*R*-*N*-*tert.*-butyloxycarbonylmethylamino-6-chloro-*S*-1,2,3,4-tetrahydro-naphthalen)-1-yl]-propionate, 6a: A THF solution (3 mL) of *N*-*i*-propylcyclohexylamine (0.8 mL, 4.85 mmol, ICA) at -10-0°C under nitrogen was treated dropwise with *n*-BuLi (2.5 mL, 1.6*M*) over a 10 min period. The resulting mixture was cooled to -70°C and a THF solution (8 mL) of **5** (0.93 g, 2.44 mmol) was transferred *via* cannula to the stirred solution. The mixture was stirred for 1 hr, whereupon ethyl chloroformate (0.25 mL, 2.6 mmol) was added quickly in one portion. After 30 min, the reaction was quenched by the addition of a few drops of saturated aqueous $NaHCO_3$. The mixture was allowed to warm to room temperature and was diluted with 1:1 Et_2O/brine (100 mL). The aqueous layer was extracted twice more with Et_2O (55 mL). The combined Et_2O layers were washed with brine, dried, and concentrated. The residue was purified by flash chromatography, eluting with Et_2O/hexanes (1:4) in 25 mL fractions. Fractions 54-92 were combined and concentrated to yield **6a** (1.056 g, 95.5%): ^{1}H-NMR (acetone/d_6) δ 1.14 and 1.24 (3H, t, $CH_2\underline{CH_3}$), 1.48 (9H, s, *t*-BOC), 1.84 (2H, m), 2.29 (2H, t), 2.64 (3H, s, NCH_3), 2.84 (2H, m), 3.09 (1H, m), 3.45 and 3.70 (3H, s, CO_2CH_3), 3.88 and 4.17 (2H, q, OCH_2), 4.34 (1H, m, CHN), 7.12 (1H, br.s, h-5), 7.18 (1H, br.d, H-7), and 7.30 (1H, d, H-8); DCI-MS $[M+H]^+$ 454, [M-*t*-BOC+H] 354 (base).

Anal. calc'd for $C_{23}H_{32}ClNO_6$: C, 60.85; H, 7.11; and N, 3.09. Found: C, 60.85; H, 6.97; and N, 3.21.

Methyl 1-Ethoxycarbonyl-[carbonyl-^{14}C]3-[(2-*R*-*N*-*tert.*-butyloxycarbonylmethyl-amino-6-chloro-*S*-1,2,3,4-tetrahydronaphthalen)-1-yl]-propionate, 6b: A THF solution (3 mL) of LiICA (prepared as described above from 0.85 mL, 5.08 mmol of ICA and 2.6 x 1.6M *n*-BuLi) was coold to -78°C under nitrogen and was treated dropwise with **5** (0.97g, 2.54 mmol) in 8 mL of THF. Ethyl chloroformate-[carbonyl-^{14}C] (50 mCi, 55 mCi/mmol, 96 μL/mmol in toluene, 0.91 mmol) was added in one portion, followed by ethyl chloroformate (0.180 mL, 1.88 mmol). The reaction was worked up as described above to yield **6b** (1.023 g, 88.9%). This material co-eluted with **6a** on TLC in Et_2O/hexanes (1:4) $R_f = 0.13$.

Ethyl 3-[(2-*R*-*N*-*tert.*-butyloxycarbonylmethylamino-6-chloro-*S*-1,2,3,4-tetrahydro-naphthalen)-1-yl]-propionate, 8a: To **6a** (1.01 g, 2.23 mmol) in DMPU (38 mL) was added NaCN (0.153 g, 3.12 mmol) and the mixture was heated at 85°C with stirring for 2 hr. Additional NaCN (0.050 g) was added and stirring was continued for 6 hr. TLC (3:1 hexanes/Et_2O) showed the formation of the product ($R_f = 0.29$) and loss of starting material ($R_f = 0.2$). The reaction mixture was diluted with water (450 mL) and extracted with Et_2O (4 x 60 mL). The combined Et_2O extracts were washed with brine, dried, and concentrated to a light brown oil. This material was purified by flash chromatography, eluting with 25 mL fractions of hexanes/Et_2O (4:1). Fractions 36-56 were combined and concentrated *in vacuo* to yield **8a** as a colorless oil (0.604 g, 66.6%): ^{1}H-NMR (acetone/d_6) δ 1.16 (3H, t, J = 7.0 Hz, $CH_2\underline{CH_3}$), 1.45 (9H, s, t-Bu), 1.84-2.02 (4H, m), 2.20 (1H, m), 2.40 (1H, m), 2.76 (3H, s, NCH_3), 2.86-2.96 (2H, m), 3.15 (1H, m, ArCH), 4.03 (2H, d, J = 7.0 Hz, OCH_2), 4.30 (1H, m, CHN), 7.11 (1H, s, H-5), 7.16 (1H, dd, J = 8.4, 2.0 Hz, H-7), and 7.30 (1H, d, J = 8.4 Hz, H-8); DCI-MS $[M+H]^+$ 396, $[M+1-CO_2CMe_3+H]$ 296 (base). Anal. calc'd for $C_{21}H_{30}ClNO_4$: C, 63.71; H, 7.64; and N, 3.54. Found: C, 63.92; H, 7.53; and N, 3.54.

Ethyl 3-[(2-*R*-*N*-*tert.*-butyloxycarbonylmethylamino-6-chloro-*S*-1,2,3,4-tetrahydro-naphthalen)-1-yl]-propionate-[carbonyl-^{14}C], 8b: To **6b** (1.023 g, 2.26 mmol) in DMPU (30 mL) at 60°C was added NaCN (0.155 g, 3.16 mmol). The mixture was heated with stirring at 85°C. After 2 hr, an additional 0.05 g of NaCN was added and heating was continued for 6 hr. The reaction was worked up and the product was purified as described above to yield **8b** (0.583 g, 65.4%) as a viscous, colorless oil:

(-)-4-Methyl-8-chloro-*trans*-1,2,3,4,4a,5,6,10b-octahydrobenzo-[f]-quinolin-3-one, 3: TFA (4 mL) was added to a CH_2Cl_2 solution (4 mL) of **8a** (1.587 g, 1.48 mmol) with vigorous stirring. After 1 hr, TLC (2:1 hexanes/Et_2O) showed no remaining starting material. The mixture was concentrated and the residue was re-dissolved in toluene (50 mL). The toluene solution was washed with saturated aqueous $NaHCO_3$ (3 x 25 mL), water (2 x 25 mL), and concentrated *in vacuo*. The residue was dissolved in toluene (50

mL) and evaporated once again. The residue was re-dissolved in toluene (50 mL) and stirred at 95°C for 24 hr under a nitrogen atmosphere. TLC ($CHCl_3$/MeOH/NH_4OH 100:1:0.25 showed the loss of starting material and the formation of a product which co-eluted with authentic 3.[9] The toluene was removed and the residue was purified by flash chromatography eluting with $CHCl_3$/MeOH/NH_4OH (100:1:0.25) to yield 3 (0.328 g, 88.2%) as a colorless oil which crystallized upon standing. This material was identical in all respects to that prepared by Astleford *et al.*[9]

(-)-4-Methyl-8-chloro-*trans*-1,2,3,4,4a,5,6,10b-octahydrobenzo-[f]-quinolin-3-one-[carbonyl-^{14}C], 9: The *t*-BOC moiety was removed from **8b** (0.583 g, 1.48 mmol) in the manner described above. The resulting aminoalcohol was dissolved in toluene (28 mL) and stirred under nitrogen at 95°C for 24 hr. A small amount of unreacted starting aminoalcohol remained as evidenced by TLC ($CHCl_3$/MeOH, 100:1), so the bath temperature was raised to 100°C and heating was continued for an additional 8 hr. The reaction mixture was allowed to cool to room temperature and the toluene was removed in vacuo. The residue was purified by flash chromatography. Eluting with CH_2Cl_2/MeOH, two yellow bands of non-radioactive material were removed, followed by the desired product. The fractions were combined and evaporated; the residue was crystallized from Et_2O/hexanes (1:1) to yield **9** (0.309 g, 80.6%). A portion of this material (0.209 g) was retained at the higher specific activity. The remaining 0.100 g was mixed with carrier (**3**, 0.350 g) and dissolved in EtOAc (5 mL). The solution was concentrated and the residue was crystallized from Et_2O/hexanes (1:1) to yield diluted **9** (0.433 g, 96.2%) as a white crystalline solid: specific activity 18.1 μCi/mg (4.52 mCi/mmol); DCI-MS $[M+H]^+$ 250/252 This material co-eluted with authentic **3** by TLC (CH_2Cl_2/MeOH, 40:1, R_f = 0.286). Radiochemical purity by TLC-autoradiography using this same TLC system was 99.8%. Radiochemical purity as determined by radio-HPLC using a Zorbax C8 column (4.6 x 250 mm) was 99.3% (R_T = 42.02 min). A binary gradient elution system was used consisting of the following solvents:

Solvent A: 34:66 MeCN/H_2O with 250 μL TFA/L solvent

Solvent B: 80:20 MeCN/H_2O with 250 μL TFA/L solvent

The flow rate was 1 mL/min; UV detection was at 220 nm. The sample was dissolved in 30:70 MeCN/H_2O. The gradient was as follows:

100% A 0-36 min

100% A to 100%B 37-50 min

100% B to 100% A 50-51 min

100%A 51-66 min

The diastereotopic purity was 99.3% as determined by HPLC[9] using a ChiralCel OD column (4.6 x 250 mm), eluting with 90:10 hexanes/IPA at 1 mL/min (R_T (+)-enantiomer = 16.94 min; R_T **9** = 18.75 min).

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