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Research Article

Synthesis of deuterated naproxens

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Abstract: A general scheme for the synthesis of (*S*)-6-methoxy-2-propanoic acid (naproxen) deuterated on the naphthyl ring, methoxy group, or both, is described. The resulting labeled naproxen derivatives are useful probes for examining atypical kinetics displayed by cytochrome P450 2C9. Copyright © 2007 John Wiley & Sons, Ltd.

Keywords: naproxen; deuterated; synthesis

Introduction

The cytochrome P450 enzymes metabolize the majority of new chemical entities and currently sold drugs.[1] The CYP2C9 isoform plays a significant role in the metabolism of NSAIDs, endogenous steroids, and is the major clearance pathway for the low therapeutic index drugs warfarin and phenytoin.[2] Due to the role of CYP2C9 in drug metabolism, it is important to evaluate the kinetic behavior of CYP2C9 substrates. Some CYP2C9 substrates display atypical kinetics and certain chemicals (effectors) may accelerate the rate of metabolism of a substrate.[3] When the effector and substrate are different, effector–substrate kinetics can be determined by independently varying substrate and effector concentration. However, some substrates are their own effectors and the kinetics cannot be determined by the methods normally used.

Naproxen is an important example of a CYP2C9 substrate that might catalyze its own metabolism.[4] Because of our interest in the atypical kinetics of CYP2C9, we wish to determine its kinetic parameters. A potential approach to accomplish this has been published by Rock *et al.*[5] and utilizes an isotope effect on product ratios to demonstrate the simultaneous binding of two substrates in the active site of a P450 enzyme. The method requires (1) isotopic labeling of the site of metabolism and (2) that metabolites of the labeled and unlabeled substrate be distinguishable.[5] Naproxen is demethylated by CYP2C9 and the determination of kinetic parameters utilizing this methodology requires labeling the methoxy group and either the aromatic ring or the propionic acid side chain. Alternatively, labeling the methoxy group only and labeling naproxen at a position that is not metabolized can be used. Here, we describe a route whereby naproxen can be labeled on the methoxy group, the aromatic ring, or both.

Results and discussion

The synthetic approach used is shown in Scheme 1. Naproxen (1) was demethylated by treating with a mixture of hydrochloric and acetic acid (2). Hydrogen–deuterium exchange of 2 was accomplished by modification of the method used by Hafferl and Hary.[6] Exposure of 2 to a mixture of phosphorus pentoxide, boron trifloride etherate, and deuterium oxide results in the deuterium labeled 3. NMR studies demonstrated that the exchange occurred at 1, 4, and 7 positions, in agreement with the results of Hafferl and Hary. The conditions used for exchange were based on the previously described mixture of phosphorus pentoxide, boron trifloride, and deuterium oxide[7] but preparation of this reagent requires specialized equipment. In contrast, replacing boron trifluoride with boron trifluoride etherate requires no specialized equipment and is only slightly less reactive (conversion of 2 to 3 requires 24 h at 45°C while Hafferl and Hary found that their reagent achieved exchange in 3 h at 25°C).

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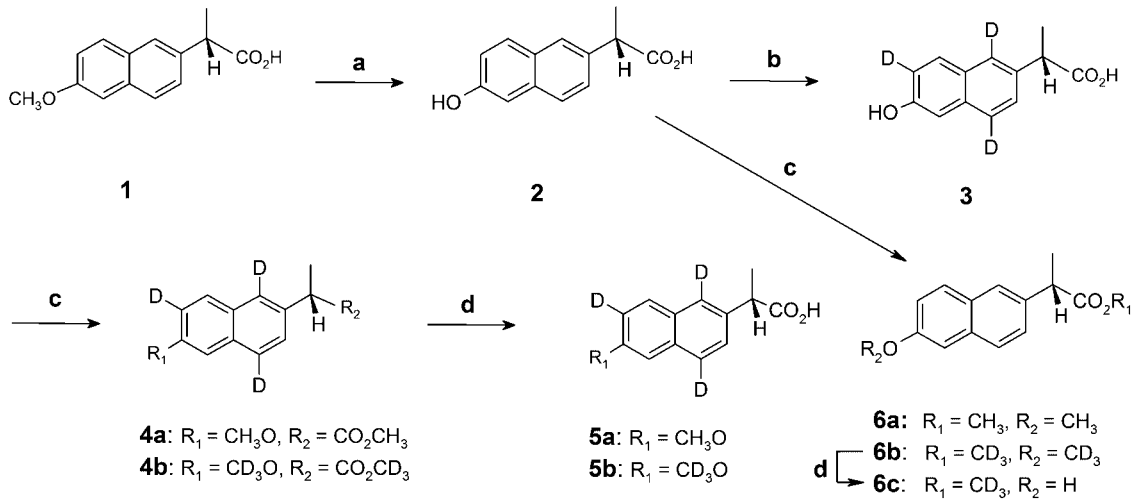

Scheme 1

The H–D exchange reaction displayed the same regioselectivity but caused partial racemization 3. Enantiomeric excess (ee) was determined by NMR[8] using α-phenethylamine as a chiral shift reagent and yielded a value of 63% ee for 3. Subsequent synthetic procedures did not change this value; thus, this was the only step that caused racemization. Resolution was effected on the labeled naproxen products.

Methylation of 3 was accomplished by treatment of 3 with methyl iodide or methyl iodide–d_3 to yield 4a or 4b, respectively, and the esters were hydrolyzed with sodium hydroxide yielding 5a and 5b. Methylation of 2 also occurred and gave 6a or 6b and subsequent hydrolysis of these esters gave 1 or 6c, respectively. NMR confirmed the positions of deuterium incorporation and isotopic incorporation was determined by mass spectrometry for compounds 5a, 5b, and 6c (2.9, 6, and 3 D, respectively).

Experimental

Reagents and solvents were used as received unless otherwise noted (Aldrich, Milw, WI). NMR spectra were acquired using a Varian Inova 300 MHz broadband spectrometer. Proton assignments are based on ^{1}H, ^{13}C, COSY, HETCOR, and NOESY NMR spectra. Mass spectra were acquired from a Thermo Electron Finnigan LCQ Deca mass spectrometer. Reactions were monitored by TLC (silica gel, 1:1 hexane/ethyl acetate).

2-(6-Hydroxy-2-naphthyl)propanoic acid (2)

(*S*)-(+)-Naproxen (1) (2.46 g, 10.7 mmol) was added to a 3:2 mixture of conc. HCl/glacial acetic acid (30 mL) and heated at reflux for 24 h. The mixture was then cooled to room temperature and the resulting precipitate was isolated by filtration. Yield 2 (1.94 g, 85%). IR (KBr) cm^{-1} 2500–3600, 1740, 1620, 1240; ^{1}H NMR (dmso-d_6) δ ppm 1.414 (3H, d, $J = 7.5$ Hz), 3.752 (1H, q, $J = 7.5$ Hz), 7.058 (1H, dd, $J = 2.7$, 8.7 Hz), 7.072 (1H, s), 7.313 (1H, dd, $J = 1.4$, 8.9 Hz), 7.62 (1H, d, $J = 7.8$ Hz) 7.633 (1H, s), 7.712 (1H, d, $J = 8.1$ Hz), 9.645 (1H, s), 12.239 (1H, s); ^{13}C NMR (dmso-d_6) δ ppm 18.4, 44.54, 108.4, 118.7, 125.5, 126.09, 126.13, 127.6, 129.1, 133.5, 135.4, 155.1, 175.5; UV (MeOH) λ nm (log (ε)) 230 (4.87), 264 (3.73), 270 (3.79); MS *m/z* (intensity) 215 (1 0 0), 171 (9).

2-[6-Hydroxy(1,4,7-$^2D_3$)-2-naphthyl]propanoic acid (3)

P_2O_5 (3.1 g, 22 mmol), $BF_3 \cdot O(C_2H_5)_2$ (5.4 mL, 44 mmol), and D_2O (1.3 mL, 65 mmol) were mixed in the order listed, with the addition of D_2O at 0°C. Once the exchange reagent was a homogeneous solution, 2 (0.28 g, 1.3 mmol) was added and heated at 45°C for 24 h. The reaction mixture was quenched by pouring onto ice with stirring, 10% NaOH (20 mL) and CH_2Cl_2 (20 mL) were added to dissolve all solids. The layers were separated, the aqueous layer extracted with CH_2Cl_2 (3 × 20 mL) and acidified with concentrated HCl (pH 1). The aqueous layer was extracted with CH_2Cl_2 (3 × 20 mL), dried over $MgSO_4$, filtered, concentrated *in vacuo*, and dried to yield 3 (0.25 g, 88%). IR (KBr) cm^{-1} 2400–3600, 1710, 1600, 1220; ^{1}HNMR (dmso-d_6) δ ppm 1.41 (3H, d, $J = 7.2$ Hz), 3.748 (1H, q, $J = 7.2$ Hz), 7.08 (s), 7.308 (s), 7.65 (s), 7.707 (s) 9.63 (1H, bs), 12.234 (1H, bs); ^{13}C NMR (dmso-d_6) δ ppm 18.4, 44.54, 108.4, 118.17 (t), 125.5 (t), 126.09,

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126.13 (t), 127.6, 129.1, 133.5, 135.4, 155.1, 175.5; UV (MeOH) λ nm (log (ε)) 229 (4.63), 265 (3.56), 274 (3.59); MS *m/z* (intensity) 220 (9), 219 (36), 218 (100), 217 (57), 216 (7) (Total $D = 2.95$); $[\alpha]_D = 66.1$.

Methyl 2-(6-methoxy-2-naphthyl)propanoate (6a)

To DMSO (3.0 mL) and crushed KOH pellets (0.318 g, 5.68 mmol) was added a solution of 2 (0.32 g, 1.44 mmol), and iodomethane (0.375 mL, 6 mmol) in DMSO (2.25 mL), dropwise. The reaction mixture was stirred at room temperature for 24 h and then concentrated to dryness *in vacuo*. The dried product was dissolved in distilled water (20 mL), filtered and the filter cake dissolved in CH_2Cl_2, dried ($MgSO_4$), filtered, and concentrated *in vacuo* to give 6a (0.23 g, 69%). The NMR of the proto material, 6a, is given here. For the remaining isotopically labeled compounds (4a, 4b, 6b) the proton, carbon, and UV data were identical to 6a except as would be predicted based on the isotopic substitution. IR (KBr) cm^{-1} 2980, 1730, 1605, 1200; ^1H NMR (dmso-d_6) δ ppm 1.465 (3H, d, $J = 7.5$ Hz), 3.584 (3H, s), 3.853 (3H, s), 3.911 (1H, q, $J = 7.5$ Hz), 7.143 (1H, dd, $J = 2.7$, 9 Hz), 7.277 (1H, d, $J = 2.7$ Hz), 7.373 (1H, d, $J = 9$ Hz), 7.696 (1H, $J = 1.8$ Hz), 7.788 (1H, d, $J = 9$ Hz), 7.965 (1H, d, $J = 9$ Hz); ^{13}C NMR (dmso-d_6) δ ppm 19.13, 45.02, 52.46, 55.86, 106.4, 119.5, 126.3, 126.9, 127.7, 129.1, 129.8, 134.0, 136.4, 157.9, 175.1; UV (MeOH) λ nm (log (ε)) 231 (4,91), 261 (3.83), 272 (3.82).

(S)-(+)-2-(6-methoxy-2-naphthyl)propanoic acid (1)

Hydrolysis of 6a was achieved by heating 4 (200 mg, 0.82 mmol) in 10% NaOH (7 mL) at reflux for 3 h and then stirred at room temperature during which a precipitate was formed. The entire mixture was acidified with conc. HCl (3 mL, pH 1). The mixture was then filtered and dried *in vacuo* to yield 5 (97%). The product was treated with D-L-amino-1-(1′-naphthyl)-ethane (0.21 mg, 1.23 mmol) in boiling ethanol–acetone (9:1, 5 mL) and cooled. The precipitate was recrystallized twice from methanol, acidified, extracted into CH_2Cl_2, dried, filtered, and concentrated *in vacuo*. NMR analysis in the presence of α-phenyethylamine indicated that the product was enantiomerically pure. The NMR of the proto material, 6a, is given here. For the remaining isotopically labeled compounds the proton, carbon, and UV data were identical to 6a except as would be predicted based on the isotopic substitution. Consequently, for the isotopically labeled compounds, only the C–D absorptions observed in the IR and the mass spectrometry data are reported. 1: IR (KBr) cm^{-1} 2500–3500, 1715, 1600, 1230; ^1H NMR (dmso-d_6) δ ppm 1.430 (3H, d, $J = 7.2$ Hz), 3.809 (1H, q, $J = 7.2$ Hz), 3.891 (3H, s), 7.137 (1H, dd, $J = 2.8$, 8.9 Hz), 7.275 (1H, d, $J = 2.8$ Hz), 7.392 (1H, dd, $J = 2.1$, 8.5 Hz), 7.698 (1H, d, $J = 2.2$ Hz), 7.760 (1H, d, $J = 8.5$ Hz), 7.789 (1H, d, $J = 8.9$ Hz), 12.28, (1H, bs); ^{13}C NMR (dmso-d_6) δ ppm 18.4, 44.55, 55.12, 108.4, 118.7, 125.5, 126.3, 126.8, 128.4, 129.1, 133.2, 136.3, 157.1, 175.5; UV (MeOH) λ nm (log (ε)) 232 (4.81), 261 (3.42), 271 (3.39); MS *m/z* (intensity) 231 (93), 217 (19), 180 (1 0 0).

2-[6-(1,4,7-$^2D_3$)-2-naphthyl]propanoic acid (5a)

IR (KBr) cm^{-1} 2210, 2070; MS *m/z* (intensity) 234 (7), 233 (36), 232 (1 0 9), 231 (31), 230 (6) (2.91 D).

2-(6-[($^2D_3$)methyloxy](1,4,7-$^2D_3$)-2-naphthylpropanoic acid (5b)

IR (KBr) cm^{-1} 2230, 2215, 2070; MS *m/z* (intensity) 237 (9), 236 (36), 235 (100), 234 (57), 233 (12) (6.01 D).

2-(6-[($^2D_3$)methyloxy]-2-naphthyl)propanoic acid (6c)

IR (KBr) cm^{-1} 2230, 2215, 2070; MS *m/z* (intensity) 234 (4), 233 (26), 232 (100), 231 (14), 230 (6) (3.05 D).

Conclusions

The modification of the hydrogen–deuterium exchange reagent reported here and originally developed by Yavorsky and Gorin is more conveniently used and yet as effective, though somewhat less reactive. While the reaction conditions caused racemization, chiral resolution resulted in optically pure deuterium-labeled products. The methods described here can be used to prepare naproxen labeled on the aromatic ring, methoxy group or both and provide optically pure products.

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