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Practical Synthesis of 3-Carboxy-(2*R*)-[[hydroxy[(tetradecyl)oxy]phosphinyl]oxy]-*N*,*N*,*N*-trimethyl-1-propanaminium Hydroxide Inner Salt (CPI975): A Carnitine Palmitoyltransferase I Inhibitor

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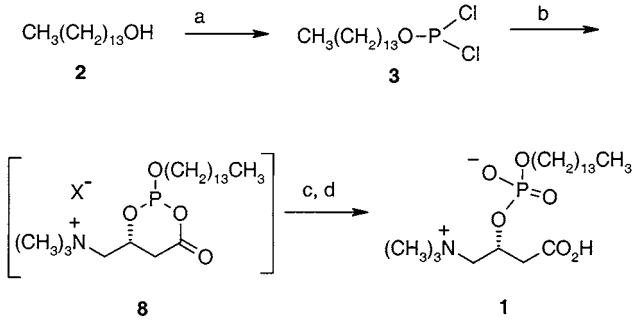
Abstract:

An efficient, safe, and cost-effective synthesis of 3-carboxy-(2*R*)-[[hydroxy[(tetradecyl)oxy]phosphinyl]oxy]-*N*,*N*,*N*-trimethyl-1-propanaminium hydroxide inner salt (1, CPI975), a carnitine palmitoyltransferase I inhibitor, is described. The reaction of 1-tetradecanol (2) with stoichiometric amounts of PCl_3 in THF at −15 to −20 °C furnished 1-tetradecyl phosphorochloridate (3). Treatment of 3 directly with L-carnitine (7) in THF in the presence of 2,4,6-collidine afforded 8, which was oxidized with bromine to afford a crude aqueous solution of 1. Desalting was done using a cheap, stable, and recyclable resin Amberlite XAD-4. The drug substance was purified by recrystallization from a mixture of ethanol and THF. The yield of 1 was 65% with 99.7% purity. Alternatively, instead of desalting with Amberlite XAD-4 resin, 1 can be isolated by an extraction with 1-decanol, followed by precipitation with acetone and recrystallization from ethanol and THF mixture.

Introduction

3-Carboxy-(2*R*)-[[hydroxy[(tetradecyl)oxy]phosphinyl]-oxy]-*N*,*N*,*N*-trimethyl-1-propanaminium hydroxide inner salt (**1**, CPI975) represents a new series of reversible and competitive inhibitors of carnitine palmitoyltransferase I (CPT I), designed based on transition-state analogue theory.[1] It is an orally active hypoglycemic agent in noninsulin-dependent diabetes mellitus (NIDDM) model.[2,3] The Discovery synthesis of **1** is depicted in Scheme 1. 1-Tetradecyl phosphorodichloridate (**3**) was prepared by a reaction of 1-tetradecanol (**2**) with an excess of PCl_3 in a mixture of diethyl ether and toluene. Use of toluene was necessary to remove the excess of PCl_3 azeotropically. Thus, distillative removal of an excess of PCl_3 and toluene and the coupling of resulting **3** with L-carnitine tetrafluoroborate salt (**5**, Figure 1) was accomplished in acetonitrile in the presence of 2,4,6-collidine to afford **8**. Oxidation of **8** with sodium periodate gave crude **1**, which was purified by two chromatographies. L-Carnitine tetrafluoroborate salt (**5**) was prepared by the treatment of L-carnitine hydrochloride salt (**4**, Figure 1) with sodium tetrafluoroborate in a mixture of acetonitrile and ethanol, followed by purification by recrystallization from dichloromethane and diethyl ether.

Scheme 1[a]



[a] **Reagents:** a) PCl_3 (excess), diethyl ether, toluene; b) L-carnitine tetrafluoroborate salt (**5**), 2,4,6-collidine, CH_3CN; c) $NaIO_4$; d) two chromatographies **or** a) PCl_3 (3.0 equiv), heptane; b) L-carnitine tetraphenylborate salt (**6**), THF, 2,4,6-collidine; c) $NaIO_4$; d) (i) C-8 reverse phase silica gel, (ii) ethanol−acetone, (iii) *n*-butanol, (iv) acetone, (v) ethanol−acetone **or Method 1:** a) PCl_3 (1.1 equiv), THF; b) L-carnitine (**7**), THF, 2,4,6-collidine; c) Br_2; d) (i) Amberlite XAD-4 resin, (ii) ethanol−THF **or Method 2:** a) PCl_3 (1.08 equiv), THF; b) L-carnitine (**7**), THF, 2,4,6-collidine; c) Br_2; d) (i) 1-decanol, (ii) acetone, (iii) ethanol−THF.

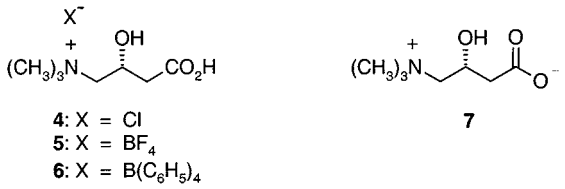


Figure 1.

Results and Discussion

The conditions used in the Discovery synthesis of **1** were deemed unsuitable for large scale. The shortcomings included the use of diethyl ether in the reaction of **2** with PCl_3, and the use of diethyl ether and dichloromethane for the purification of **5**. The tetrafluoroborate salt (**5**) was soluble in acetonitrile but **3** had only slight solubility in this solvent, and the oxidation of **8** with sodium periodate presented operational difficulties. Furthermore, the isolation and purification of **1** by two chromatographies was cumbersome. Our goal was to eliminate the undesirable solvents, find

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another salt form of L-carnitine that has similar solubility properties as **3**, and simplify the purification of **1**. We found that L-carnitine tetraphenylborate salt (**6**, Figure 1) was highly soluble in organic solvents and was selected for further process development work. It was readily prepared by a reaction of L-carnitine with sodium tetraphenylborate in water and HCl.[4] The reaction of alcohols with 7.0 equiv of PCl_3 in acetonitrile was known,[5] but we found that **2** reacted with only 3.0 equiv of PCl_3 in heptane (Scheme 1), thus eliminating the use of diethyl ether. The excess PCl_3 was removed during distillation of heptane, which was necessary for solvent exchange to THF, and crude **3** was used in the next coupling step without purification. The coupling of **3** with **6** was carried out in THF in the presence of 2,4,6-collidine at 25−30 °C, and the resulting intermediate **8** was oxidized with sodium periodate.[6] Workup of the reaction mixture furnished an aqueous solution of **1**, which also contained inorganic salts. The inorganic salts were removed by filtration (desalting) of this solution over C-8 reverse-phase silica gel using water, ethanol−water mixture, and ethanol sequentially as the eluant. The desired fractions were concentrated and purified by sequential treatment with ethanol−acetone mixture, *n*-butanol, acetone, and ethanol−acetone mixture. The isolated yield of **1** was 41% with 98.8% purity.

While this process was suitable for kilogram scale (1 kg), further improvements were necessary to improve the safety, economy, and ecology by addressing the following problems. Removal of excess of PCl_3 and solvent exchange from heptane to THF required distillation. This distillation was not desirable because intermediate **3** was thermally unstable and it generated a large amount of waste. Tetraphenylborate salt (**6**) of L-carnitine required preparation, thus adding an extra step. Additionally, sodium tetraphenylborate is an expensive reagent. Intermediate **6** was also found to be toxic. The solid waste, after the sodium periodate oxidation and sodium thiosulfate quench, as well as the aqueous solution containing the product exhibited low thermal stability. The aqueous solution had to be concentrated to distill off THF, making the process hazardous. C-8 reverse-phase silica gel, used for desalting, was expensive and unstable under acidic conditions, with limited recycling potential. Finally, isolation of **1** utilized several steps and was cumbersome. Thus, our goals were to use stoichiometric amounts of PCl_3 for **3**, thus avoiding solvent exchange, to use L-carnitine (**7**) directly instead of its salt, to find another oxidizing agent for **8** to replace sodium periodate, to find a cheaper desalting agent and eliminate this operation, and to simplify purification of **1**.

New conditions were developed for the preparation of **3** by a reaction of **2** with 1.1 equiv of PCl_3 in THF, the solvent of choice for the next step, at −15 to −20 °C (Scheme 1). The reaction was clean and was monitored by ^{31}P NMR (chemical shift for phosphorus in PCl_3 was δ 182.0, and 178.2 for intermediate **3**). These conditions avoided the use of excess of PCl_3 and solvent exchange, and eliminated additional operations. Interestingly, reaction of **3** directly with L-carnitine (**7**) in the presence of 2,4,6-collidine furnished intermediate **8** as confirmed by ^{31}P NMR, which exhibited a characteristic chemical shift for phosphorus at δ 129.6. Direct use of L-carnitine made the process economical and improved safety. We next focused our attention on the oxidation of **8**. Oxidation of phosphites with iodine,[7−10] hydrogen peroxide,[11,12] and *tert*-butyl peroxide[13,14] was known. Because of safety reasons, we investigated iodine and bromine as possible oxidants for **8**. Both oxidants gave satisfactory results. Bromine, which has not been reported for this purpose, was selected for further scale-up because it is cheaper than iodine. Use of bromine instead of sodium periodate afforded the aqueous solution of **1** which had no thermal stability problems.

Having defined safer, economical, and ecologically cleaner conditions for all the chemical steps, our next goal was to find a cheaper desalting agent and simplify the purification of **1**. Cheaper nonionic resins such as Amberlite XAD-2, XAD-4, and XAD-7 were investigated as desalting agents to replace C-8 reverse-phase silica gel. Amberlite XAD-4 gave satisfactory results and was used for further scale-up. Amberlite XAD-4 was highly recyclable as it was stable at pH 1−14. It was also much cheaper ($25/kg) compared to the C-8 reverse-phase silica gel. Thus, the aqueous solution containing **1** was loaded onto Amberlite XAD-4 resin. Elution with water eluted the inorganic salts as monitored by measuring the conductance of fractions. The product was then eluted with ethanol. Fractions containing the product were mixed and concentrated to afford crude **1**. The final challenge was to develop an easy isolation method for **1** by recrystallization. We found that **1** can be purified by only one recrystallization from a mixture of ethanol and THF to afford **1** in 65% overall yield from **2** with 99.7% purity (Method 1). Although this new method of desalting with Amberlite XAD-4 and recrystallization from ethanol and THF were practical, it was highly desirable to develop an alternative method for the isolation of **1** that avoided the desalting operation. After extensive studies we found that desalting with Amberlite XAD-4 resin can be eliminated by an extraction of the product from the aqueous layer with 1-decanol at 75 °C. Concentration of the organic layer followed by precipitation with acetone furnished crude **1**, which was then recrystallized from ethanol and THF to afford 43% yield of **1** with 100% purity (Method 2). Even though the yield of **1** was lower than in the previous process, this was a preferred method due to the ease of processing on a

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large scale. At the same time, the coupling of **3** with L-carnitine (**7**) was further optimized by increasing the amount of 2,4,6-collidine and reversing the order of addition of L-carnitine and 2,4,6-collidine. With these modifications, the coupling was performed at lower temperature (32−37 °C) instead of 48−57 °C.

Conclusions

In summary, an efficient, safe, and cost-effective synthesis of 3-carboxy-(2*R*)-[[hydroxy[(tetradecyl)oxy]phosphinyl]-oxy]-*N*,*N*,*N*-trimethyl-1-propanaminium hydroxide inner salt (**1**, CPI975) was developed. The reaction of 1-tetradecanol (**2**) with stoichiometric amounts of PCl_3 in THF at −15 to −20 °C furnished 1-tetradecyl phosphorochloridate (**3**). Treatment of **3** directly with L-carnitine (**7**) in THF in the presence of 2,4,6-collidine afforded **8**, which was oxidized with bromine to afford a crude aqueous solution of **1**. Desalting was done using a cheap, stable, and recyclable resin Amberlite XAD-4. The drug substance was purified by recrystallization from a mixture of ethanol and THF. The yield of **1** was 65% with 99.7% purity. Alternatively, instead of desalting with Amberlite XAD-4 resin, **1** can be isolated by an extraction with 1-decanol, followed by precipitation with acetone and recrystallization from ethanol and THF mixture.

Experimental Section

All the melting points are uncorrected. ^{1}H, ^{13}C, and ^{31}P NMR spectra were recorded on a Bruker DPX300 instrument. Amberlite XAD-4 nonionic polymeric adsorbent (20−60 mesh polystyrene adsorbent having a high surface area of 725 m^2/g) was purchased from Rohm and Haas. L-Carnitine was available from Lonza, and 1-tetradecanol, from Tokyo Kasei.

3-Carboxy-(2*R*)-[[hydroxy[(tetradecyl)oxy]phosphinyl]-oxy]-*N*,*N*,*N*-trimethyl-1-propanaminium Hydroxide Inner Salt (1). Method 1. A 5-L, four-necked round-bottomed flask, equipped with a mechanical stirrer, digital thermometer, addition funnel, nitrogen inlet−outlet, and cooling bath was charged with THF (1.75 L) and cooled to an internal temperature of −15 to −20 °C. PCl_3 (55.9 mL, 0.64 mol) was added over 5−10 min, while maintaining the same internal temperature. A solution of 1-tetradecanol (**2**, 125.0 g, 0.583 mol) in THF (250 mL) was added over 30−35 min while maintaining an internal temperature of −15 to −20 °C. The reaction mixture was warmed to room temperature (21−23 °C) over 40−45 min, and L-carnitine (**7**, 103.38 g, 0.641 mol) was then added. The suspension was heated to an internal temperature of 48−50 °C, and a solution of 2,4,6-collidine (330 mL, 2.5 mol) in THF (250 mL) was added over a period of 75 min while maintaining an internal temperature of 48−57 °C. The resulting white slurry was stirred overnight (16 h) and cooled to 0−5 °C, and water (750 mL) was added over 30 min while maintaining an internal temperature of 5 to 10 °C. Bromine (95.03 g, 0.5946 mol) was then added over 1 h while maintaining an internal temperature of 5−10 °C. The addition funnel was rinsed with THF (10 mL) and added to the reaction mixture. The reaction mixture was warmed to room temperature (21−23 °C) in 30 min and stirred at this temperature for additional 1 h. The mixture was cooled to 5 to 10 °C, and a solution of sodium thiosulfate (11.0 g) in water (20 mL) was added over a period of 6−8 min while maintaining the same internal temperature. It was followed by the addition of water (480 mL) in 6−8 min at the same temperature. A solution of sodium hydroxide (150.0 g, 3.75 mol) in water (330 mL) was then added over 30 min while maintaining an internal temperature of 5−10 °C. The reaction mixture was warmed to room temperature (21−23 °C) in 30 min. A two-phase mixture was obtained. This mixture was added to ethyl acetate (3.0 L) in a 10-L separatory funnel and mixed for 20 min. Minor solids were removed by filtration and washed with water (100 mL). The layers were separated, and the organic layer was discarded. The aqueous layer was cooled to an internal temperature of 0−5 °C, and concentrated HCl (69.0 mL) was added over 15 min while maintaining an internal temperature of 5−10 °C (pH 3.31). The solution was warmed to room temperature (21−23 °C) and diluted with water (1.98 L) to bring the total volume to 4.2 L. This solution was loaded onto a column containing Amberlite XAD-4 resin (2.2 kg). The column was prepared using a slurry of Amberlite XAD-4 resin (2.2 kg) in water (1.7 L). The dimensions of the resin bed were 36.0 cm (length) and 11.5 cm (diameter). The volume of the bed was 3.25 L. The resin was washed with water (12.0 L) at a flow rate of 150 mL/min. This was followed by washing with 200-proof ethanol (4.0 L). The bed volume increased to 4.0 L due to swelling of the resin. Finally, the resin was washed with water (15.0 L). The bed volume decreased back to 3.25 L. The first three fractions (combined volume 4.2 L) contained the product and were reapplied onto the column. After collecting five fractions of 1 L each, none of which contained any product, the column was eluted with water (16.0 L) until the conductance of the fraction reached <100 μS/cm (the fraction was colorless). The column was then eluted with 200-proof ethanol (7.0 L), and the fractions were checked for the product by TLC. The first 1.0-L fraction (from the point of switching to ethanol) was discarded. The resin was regenerated by washing with additional 200-proof ethanol (2.0 L) and water (12.0 L) to shrink the bed volume back to 3.4 L. The next 6.0-L fraction was concentrated under reduced pressure (25−30 mbar, bath temperature 40 to 45 °C) to collect 5.5 L of solvent. The resulting thick slurry was dissolved in 200-proof ethanol (0.5 L) by heating to 55 to 60 °C, and the resulting solution was filtered into a 5-L, four-necked round-bottomed flask equipped with a heating mantle, digital thermometer, mechanical stirrer, and addition funnel. The solution was heated to an internal temperature of 55 to 60 °C, and THF (1.8 L) was added over a period of 40 min while maintaining an internal temperature of 50 to 55 °C. The mixture was cooled to 22 to 23 °C over a period of 2 h and stirred at this temperature for additional 12 h. The solids were collected by filtration with suction and were washed with THF (300 mL) in three equal portions of 100 mL each, followed by acetone (150 mL). The solids were dried under reduced pressure (25−30 mbar) at 45 to 50 °C

until a constant weight was obtained to afford pure 3-carboxy-(2*R*)-[[hydroxy[(tetradecyl)oxy]phosphinyl]oxy]-*N*,*N*,*N*-trimethyl-1-propanaminium hydroxide inner salt (**1**, 165.8 g, 65%): mp 189−191 °C; ^{1}H NMR (300 MHz, CD_3OD) δ 0.91 (t, 3H), 1.28 (m, 22H), 1.66 (m, 2H), 2.72 (dd, 1H), 3.0 (dd, 1H), 3.15 (s, 9H), 3.54 (m, 1H), 3.71 (m, 1H), 3.88 (m, 2H), 4.99 (m, 1H); ^{13}C NMR (CD_3OD) δ 173.01, 70.95, 70.84, 68.10, 68.03, 66.85, 66.77, 54.83, 38.90, 33.03, 31.90, 31.79, 30.77, 30.73, 30.44, 26.88, 23.69, 14.52; ^{31}P NMR (CD_3OD) δ 0.177; IR (KBr) 3427, 2927, 2854, 1705, 1468, 1221, 1064 cm^{-1}; MS (FAB) 438.1 (MH^+); $[\alpha]_D$ −12.54 (c = 1, CH_3OH); Anal. Calcd for $C_{21}H_{44}NO_6P$: C, 57.65; H, 10.14; N, 3.20. Found: C, 57.51; H, 10.24; N, 3.05.

Method 2. A 5-L, four-necked round-bottomed flask, equipped with a mechanical stirrer, digital thermometer, addition funnel, nitrogen inlet−outlet, and cooling bath was charged with THF (0.5 L) and cooled to an internal temperature of −15 °C. PCl_3 (46.5 mL, 0.522 mol) was added over 5 min while maintaining the same internal temperature. A solution of 1-tetradecanol (**2**, 104.0 g, 0.483 mol) in THF (0.8 L) was added over 30 min while maintaining an internal temperature of −13 to −15 °C. The reaction mixture was warmed to 18 °C and 2,4,6-collidine (0.4 L, 3.02 mol) was added over 20 min while maintaining an internal temperature of 20 to 22 °C. L-Carnitine (**7**, 78.6 g, 0.483 mol) was then added. The suspension was heated to an internal temperature of 32 °C in 30 min and then to 37 °C in 10 min. The resulting white slurry was stirred at 32−37 °C for 2 h and then at 23 °C for 16 h. The mixture was cooled to 8 °C, and water (750 mL) was added over 30 min while maintaining an internal temperature of 8−10 °C. Bromine (96.16 g, 0.601 mol) was added over 30 min while maintaining an internal temperature of 13−15 °C. The addition funnel was rinsed with THF (20 mL) and added to the reaction mixture. The reaction mixture was warmed to room temperature (21−23 °C) in 10 min and stirred at this temperature for additional 30 min. A solution of sodium thiosulfate (15.0 g) in water (60 mL) was added in 5 min while maintaining the same internal temperature. The mixture was cooled to an internal temperature of 12 °C, and a solution of sodium hydroxide (167.0 g, 4.17 mol) in water (750 mL) was then added over 20 min while maintaining an internal temperature of 13−15 °C. Ethyl acetate (0.9 L) was then added, and the contents were stirred at 15−17 °C for 5 min. The layers were separated, and the organic layer was extracted with saturated sodium chloride solution (100 mL). The aqueous layers were combined, and concentrated HCl (75.0 mL) was added over 15 min while maintaining an internal temperature of 22−24 °C (pH 3.0−3.2). The aqueous layer was extracted with ethyl acetate (200 mL), and the organic layer was discarded.

The aqueous layer was transferred to a 5-L, four-necked flask and concentrated under reduced pressure (100 mbar) at 46 to 53 °C to collect 160 mL of solvent (THF and ethyl acetate). Concentrated HCl (10 mL) and 1-decanol (660 mL) were added to the aqueous layer (pH 1.7), and the contents were heated to an internal temperature of 75 °C. The organic layer was separated and washed with brine (600 mL), and water (2 × 600 mL) at 75 °C. The organic layer was concentrated under reduced pressure (60−100 mbar) at 55−60 °C until no further solvents distilled. The residue was dissolved in THF (200 mL) and concentrated until no further solvent distilled. This operation was repeated once more with THF (200 mL). To the residue was added acetone (1.8 L) at 50 to 52 °C, and the suspension was cooled to 25 °C in 1 h. The stirring was continued at 22 °C for additional 16 h. The solids were collected by filtration, washed with acetone (400 mL), and dried under reduced pressure (50−100 mbar) at 55 °C for 16 h to obtain a constant weight of 115.2 g. This solid was transferred to a 2-L, four-necked round-bottomed flask and was suspended with Celite (8.0 g) in 200-proof ethanol (300 mL). The contents were heated to an internal temperature of 75 °C in 15 min, and the hot solution was filtered into another 2-L, four-necked round-bottomed flask. THF (600 mL) was added to the filtered solution over a period of 20 min while maintaining an internal temperature of 50−55 °C. The mixture was cooled to 23 °C over 1 h, and the suspension was stirred at this temperature for additional 2 h. The solids were collected by filtration, washed sequentially with THF (300 mL) and acetone (100 mL), and dried under reduced pressure (25−30 mbar) at 45−50 °C until a constant weight to afford pure 3-carboxy-(2*R*)-[[hydroxy[(tetradecyl)oxy]phosphinyl]oxy]-*N*,*N*,*N*-trimethyl-1-propanaminium hydroxide inner salt (**1**, 96.0 g, 43.4%).

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