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Facile Syntheses of L-α-Glycerophosphorylcholine

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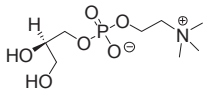
As people age, numerous disorders involving brain metabolism, regional blood supply and neurotransmitter availability often occur.[1] These conditions lead to progressive deterioration of memory formation and retention representing the main attributes of Alzheimer's disease (AD), the most common adult-onset cognitive disorder[2] and major public health problem.[3] Alzheimer's disease is the most common type of dementia and is characterized by a progressive decline in cognitive function due to damage or disease in the brain beyond what may be expected from normal aging. The oldest hypothesis for AD suggests that the disease occurs due to reduced biosynthesis of the neurotransmitter acetylcholine. In view of this, several clinical studies[4] have shown the therapeutic usefulness and efficacy of choline alfoscerate, also known as L-α-glycerophosphorylcholine (GPC) (**1**), as a cholinergic precursor. GPC was examined for its use in the treatment of cerebrovascular disease, reduction of senile dementia (cognitive dysfunction), improvement in learning ability and promotion of non-REM sleep.[5] The broad application of GPC in medical treatments, as well as its application in cosmetics as an active whitening ingredient, make its synthesis medicinally and industrially important.



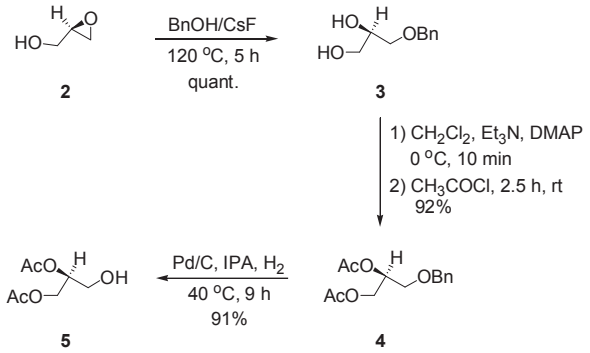
L-α-Glycerophosphorylcholine, **1**

Choline alfoscerate has been successfully isolated from the bovine pancreas.[6] However, this method is not suitable for large-scale production, making chemical synthesis of GPC necessary. There have been many published papers[7] and patents[8] for GPC synthesis, but each of these methods has drawbacks. Some processes are lengthy and cumbersome, use expensive and toxic reagents, require heavy metals during purification, or result in poor yield. To address these issues, we report two new and facile methods for synthesis and purification of choline alfoscerate utilizing relatively cheap, commercially available starting materials with overall yields comparable to known methods.

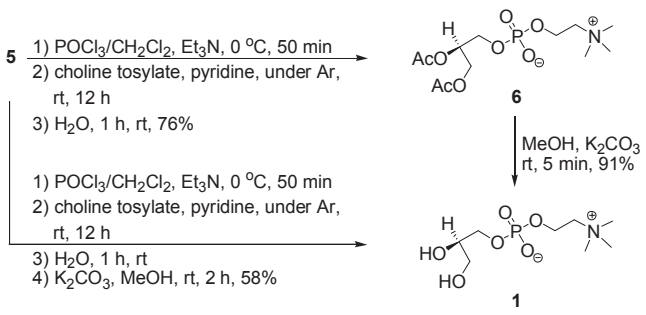
Results and Discussion

Our aim was to develop a synthetic route for L-α-glycerophosphorylcholine (GPC) that was simple and industrially feasible. The challenge was based on the fact that choline alfoscerate has labile bonds and is soluble in water, making its synthesis difficult. Retrosynthetically, we considered that a triol was a candidate intermediate that could possibly come from a substituted epoxide. Therefore, we decided on (*R*)-glycidol, **2** as the starting material. At first, protection of the 1^0 alcohol followed by ring opening was considered; however, it led to a different configuration. Thus, we considered a regioselective nucleophilic reaction of alcohol with oxirane to obtain **3** (Scheme 1) resulting in the desired configuration.[9] This was followed by protection of the diol to produce **4** and debenzylation to obtain **5** using a known method.[10]

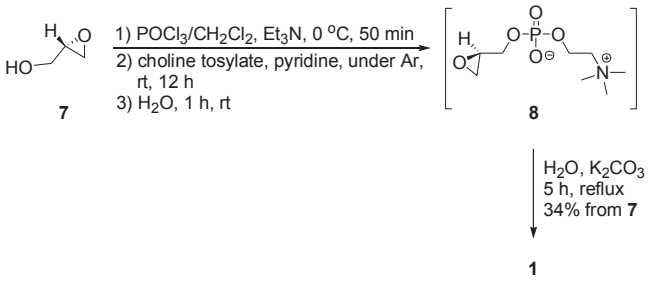
The next major step was coupling of the choline group *via* phosphorylation using phosphorus oxychloride and choline tosylate under standard conditions to produce **6** (Scheme 2).[11] Subsequent deacetylation afforded the target compound **1**. We found it was possible to phosphorylate, cholinate and deacetylate *in situ*;[12] however, the yield was slightly lower compared



Scheme 1



Scheme 2



Scheme 3

to that when compound **6** was first isolated.

Knowing that phosphorylation successfully occurs using the aforementioned procedure, we devised a simpler synthetic route for direct phosphorylation of the epoxide ring (Scheme 3). This method was previously reported for the synthesis of various phospholipids.[11b] Upon direct phosphorylation[11b] of (*S*)-glycidol, **7** and *in situ* epoxide ring opening of **8**, we obtained the correct compound **1** wherein all the data were in good agreement with those found in the literature.[13]

Conclusions

We developed two facile synthetic routes for L-α-glycerophosphorylcholine. The present route is shorter and has comparable overall yields with that of syntheses reported previously. Notably, this method utilized readily available and non-toxic reagents, making it feasible for industrial production.

Experimental Section

General. All reagents were purchased and used without modification from Aldrich and Alfa. All reactions requiring inert conditions were conducted under Ar. Analytical TLC was conducted on E. Merck 60 F254 aluminum-backed silica gel plates (0.2 mm). Developed plates were visualized using UV light or a 2.0% phosphomolybdic acid stain. Flash column chromatography was performed using Merck silica gel 60 (230 - 400 mesh). ^{1}H and ^{13}C NMR spectra were obtained using Varian 300 and Bruker 300 spectrometers (300 and 75 MHz, respectively) with TMS as the internal standard. Coupling constants (*J*) are given in Hz and all chemical shifts are in ppm. IR spectra were recorded on a Bio-Rad FTS 6000 FT-IR spectrophotometer as a KBr pellet. HRMS were obtained on a JMS 700 spectrometer. Optical rotations were measured at the sodium D line on an ATAGO 300 digital polarimeter at ambient temperature.

(*R*)-3-Benzyloxy-1,2-propanediol (3).[9,14] (*R*)-Glycidol (0.25 g, 3.37 mmol), CsF (0.01g, 0.067 mmol), and BnOH (1.75 mL, 16.85 mmol) were sealed under vacuum in a round bottom flask and heated for 5 h at 120 °C using an oil bath. The solution was allowed to cool to room temperature and was purified using flash column chromatography with a hexane:ethyl acetate (1:2, v/v, R_f = 0.13) eluent system to give compound **3** as a colorless oil (quant.). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.32 (m, 5H), 4.54 (s, 2H), 3.88 (m, 1H), 3.60 (m, 4H); $[\alpha]_D^{20}$ = +4.7 (*c* 4.7 in $CHCl_3$); $[\alpha]_D^{25,\,lit.}$ = +5.5 (*c* 10 in $CHCl_3$).[15]

(*S*)-1,2-Diacetoxy-3-benzyloxypropane (4).[16] Compound **3** (3.0 g, 16.46 mmol) was dissolved in dichloromethane (50 mL) in a round bottom flask sealed with a rubber septum. Triethylamine (5.5 mL, 39.50 mmol) and 4-dimethylamino pyridine (DMAP, 0.58 g, 4.77 mmol) were added to solution **3**. The reaction mixture was stirred for 10 min at 0 °C, with subsequent drop wise addition of acetyl chloride (2.6 mL, 36.21 mmol). The reaction mixture was stirred for 2.5 h at room temperature. The mixture was washed with 5% HCl (12 mL twice) and the phases were separated. The organic phase was washed with 10% Na_2CO_3 (12 mL) followed by saturated $NaHCO_3$ (12 mL). The organic layer was collected, dried over anhydrous $MgSO_4$, filtered and concentrated using a rotary evaporator. The residue was purified by silica gel column chromatography with a hexane: acetone (5:1, v/v, R_f = 0.35) eluent system to give (*S*)-1,2-diacetoxy-3-benzyloxypropane, **4** as a colorless oil (4.04 g, 92%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.32 (m, 5H), 5.22 (m, 1H), 4.57 (AB type, 1H), 4.51 (AB type, 1H), 4.34 (dd, *J* = 11.7, 3.9 Hz, 1H), 4.19 (dd, *J* = 11.7, 6.3 Hz, 1H), 3.59 (m, 2H), 2.08 (s, 3H), 2.04 (s, 3H); $[\alpha]^{20}$ = +16.3 (*c* 1.63 in $CHCl_3$); $[\alpha]^{20,\,lit.}$ = +16.3 (*c* 1.64 in $CHCl_3$).[16]

(*S*)-1,2-Diacetyl glycerol (5).[10a] Isopropyl alcohol (IPA, 20 mL) and 10 wt % Pd/C (0.3 g, 0.28 mmol) were mixed in a flask equipped with a H_2 balloon. Compound **4** (2.2 g, 8.26 mmol) was added to the mixture and stirred at 40 °C for 9 h. The mixture was filtered and the solvent was evaporated. The residue was purified by silica gel column chromatography with a hexane:ethyl acetate (3:2, v/v, R_f = 0.2) eluent system to give 1,2-diacetyl glycerol, **5** (1.32 g, 91%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.08 (m, 1H), 4.23 (m, 2H), 3.74 (m, 2H), 2.11 (s, 3H), 2.08 (s, 3H); $[\alpha]_D^{20}$ = −4.6 (*c* 1 in MeOH); $[\alpha]_D^{20,\,lit.}$ = +1.8 (*c* 1 in MeOH, 40% ee).[10a]

(*R*)-1,2-Diacetoxy glycerylphosphoryl choline (6). Triethylamine (0.65 mL, 4.7 mmol) and compound **5** (0.61 g, 3.5 mmol) were added to a solution of phosphorous oxychloride (0.41 mL, 4.4 mmol) in dichloromethane (50 mL). The mixture was stirred for 50 min at 0 °C. Choline tosylate (1.93 g, 7.0 mmol) and pyridine (2.25 mL, 2.8 mmol) were then added and the mixture was stirred for 12 h at room temperature. The reaction mixture was hydrolyzed with distilled water (2 mL) for 1 h. A vacuum rotary evaporator removed the solvent, and the residue was purified by silica gel column chromatography with a CH_2Cl_2:MeOH:H_2O (7:6:1 to 2:3:1, v/v, R_f = 0.3) eluent system to give a gummy solid, (*R*)-1,2-diacetoxy glyceryl phosphorylcholine (0.908 g, 76%). IR (KBr pellet) 2949, 1739, 1246, 1096, 1059, 971 cm^{-1}; ^{1}H NMR (300 MHz, CD_3OD) δ 5.22 (m, 1H), 4.40 (dd, 1H, *J* = 12.0, 3.7 Hz) 4.29 (m, 2H), 4.20 (dd, 1H, *J* = 12.0, 6.4 Hz), 4.03 (m, 2H), 3.66 (m, 2H) 3.24 (s, 9H), 2.08 (s, 3H), 2.06 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 170.7, 170.4, 71.1 (d, *J* = 7.7 Hz), 65.8 (d, *J* = 6.2 Hz), 63.0 (d, *J* = 5.1 Hz), 62.9, 58.9 (d, *J* = 5.2 Hz), 53.5, 21.3, 21.0; $[\alpha]_D^{20}$ = +12.11 (*c* 1.8 in MeOH); HRMS (FAB, *m*/*z*): calc. 342.1318, found 342.1315 for $C_{12}H_{25}NO_8P$.

(*R*)-Choline alfoscerate (1).

Procedure 1: Compound **6** (0.31 g. 0.91 mmol) was dissolved in MeOH (10 mL) and K_2CO_3 (0.30 g, 2.17 mmol) was added to the solution. The mixture was stirred for 5 min and neutralized with 1% HCl. A vacuum rotary evaporator removed the solvent and the residue was purified by silica gel column chromato-

graphy with a CH_2Cl_2:MeOH:H_2O (7:6:1 to 2:3:1, v/v, R_f = 0.2) eluent system to give compound **1** (0.21 g, 91%) as a colorless gummy oil.

Procedure 2: Triethylamine (0.65 mL, 4.7 mmol) and compound **5** (0.61 g, 3.5 mmol) were added to a solution of phosphorous oxychloride (0.41 mL, 4.4 mmol) in dichloromethane (50 mL). The mixture was stirred for 50 min at 0 °C. Choline tosylate (1.93 g, 7 mmol) and pyridine (2.25 mL, 2.8 mmol) were added and the mixture was stirred for 12 h at room temperature. The reaction mixture was hydrolyzed with distilled water (2 mL) for 1 h. A vacuum rotary evaporator removed the solvent and the residue was mixed with potassium carbonate (K_2CO_3, 2.41 g, 17.4 mmol) and methanol (20 mL) for 2 h. A vacuum rotary evaporator removed the solvent and the residue was purified by silica gel column chromatography with a CH_2Cl_2:MeOH:H_2O (7:6:1 to 2:3:1, v/v, R_f = 0.23) eluent system to give compound **1** (0.52 g, 58%) as a colorless gummy oil.

Procedure 3: Triethylamine (0.65 mL, 4.7 mmol) and (*S*)-glycidol (**7**) (0.23 mL, 3.5 mmol) were added to a solution of phosphorous oxychloride (0.41 mL, 4.4 mmol) in dichloromethane (50 mL). The mixture was stirred for 50 min at 0 °C. Choline tosylate (1.93 g, 7.0 mmol) and pyridine (2.25 mL, 2.8 mmol) were added and the mixture was stirred for 12 h at room temperature. The reaction mixture was hydrolyzed with distilled water (2 mL) for 1 h. The solvent was removed by vacuum rotary evaporator and the crude (*S*)-glycidyl phosphorylcholine, **8** (0.68 g, 80%) was obtained. Without further purification, the crude compound **8** (0.28 g, 1.17 mmol) was dissolved in H_2O (6 mL) and K_2CO_3 (0.1 g, 0.72 mmol) was added. The mixture was refluxed for 5 h and neutralized with 1% HCl upon cooling. The solvent was removed by a vacuum rotary evaporator and the residue was purified by silica gel column chromatography with a CH_2Cl_2:MeOH:H_2O (7:6:1 to 2:3:1, v/v, R_f = 0.2) eluent system to give compound **1** (0.13 g, 43%) as a colorless gummy oil. ^{1}H NMR (300 MHz, CD_3OD) δ 4.29 (m, 2H), 3.89 (m, 2H), 3.77 (m, 1H), 3.64 (m, 2H), 3.56 (m, 2H), 3.22 (s, 9H); $[\alpha]_D^{20}$ = −2.5 (*c* 2.6 in MeOH); −2.8 (*c* 2.6 in H_2O); $[\alpha]_D^{18,\,lit.}$ = −2.7 (*c* 2.7 in H_2O),[13a] $[\alpha]_D^{23,\,lit.}$ = −2.85 (*c* 2.2 in H_2O).[13b]

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