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Synthesis of *N*-Diisopropyl Phosphoryl Benzyltetrahydroisoquinoline, a New Class of Mitochondrial Complexes I and III Inhibitors

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Abstract—The synthesis of *N*-(*O*,*O*-diisopropyl phosphoryl)-benzyltetrahydroisoquinoline (**3**) has been achieved in a 'one pot' procedure from imine (**2**) and diisopropyl-phosphorochloridate (**1**) generated in situ ($POCl_3$ + iPrOH). Compound **3** is the first benzyltetrahydroisoquinoline derivative found to be a potent inhibitor of mitochondrial complexes I and III, and therefore it opens a new perspective with this series of compounds as they can be considered as new class of antitumor agents.

Introduction

The diisopropyl phosphoryl (DIPP) group has been used recently as an amino acid protecting group.[1] Indeed, under classical Atherton–Todd conditions,[2] when an α-amino acid is treated by a dialkylphosphorochloridate (e.g., obtained by treatment of a dialkylphosphite with sodium hypochlorite), the *N*-protected α-amino acid is obtained in high yield.[1] Thus diisopropyl- and various dialkylphosphorochloridates have been used as phosphorylating agents.[3]

We recently accomplished the syntheses of *N*-alkyl-benzyltetrahydroisoquinolines (BTHIQ) by a new method incorporating a 'one pot' cyclization–reduction–alkylation sequence.[4] Following this procedure, herein we described an original method for the preparation of *N*-protected BTHIQ, through the generation of the DIPP protecting group from $POCl_3$ and isopropanol, by a rapid, efficient and unexpensive procedure.

In our studies of new antitumor inhibitors of the mitochondrial electron-transport chain we decided to study, for the first time, the BTHIQ class as inhibitors of cell respiration. Potency found for these compounds was in accordance with the one observed for other commercial agrochemical agents, as well as with previously described cytotoxic and antitumor natural compounds.[5] Having the possibility to study the relevance of a DIPP group in the interaction of these inhibitors with the respiratory enzymes, we have provided the synthesis of *N*-(*O*,*O*-diisopropyl phosphoryl)-1-benzyl-6-benzyloxy-7-methoxy-1,2,3,4-tetrahydroisoquinoline (**3**).

Chemistry

For these reasons a 'one pot' sequence under acid conditions in isopropanol, starting from imine **2**,[6] and subsequent reduction with $NaBH_4$ after 4 h reflux, was proposed and conducted. The *N*-phosphorylation was achieved with success in a 'one-pot' procedure under acid medium via the reaction of $POCl_3$ with isopropanol to give diisopropyl phosphorochloridate (**1**) formed in situ. The reaction of **1** with BDHIQ (**2**) and consecutive $NaBH_4$ reduction afforded a 1:1 mixture of compounds **3** and **4** in 95% overall yield.[7] The DIPP protecting group can be easily cleaved, together with *O*-debenzylation,[4,6] by treatment with ethanol and concd HCl, to afford the corresponding amine (**5**) in good yield (80%)[8] (Scheme 1).

It is interesting to note that when isopropanol is used as a solvent in this reaction sequence, *N*-(*O*,*O*-diisopropyl phosphoryl) derivative was obtained. However, if this alcohol is substituted by EtOH or MeOH, the

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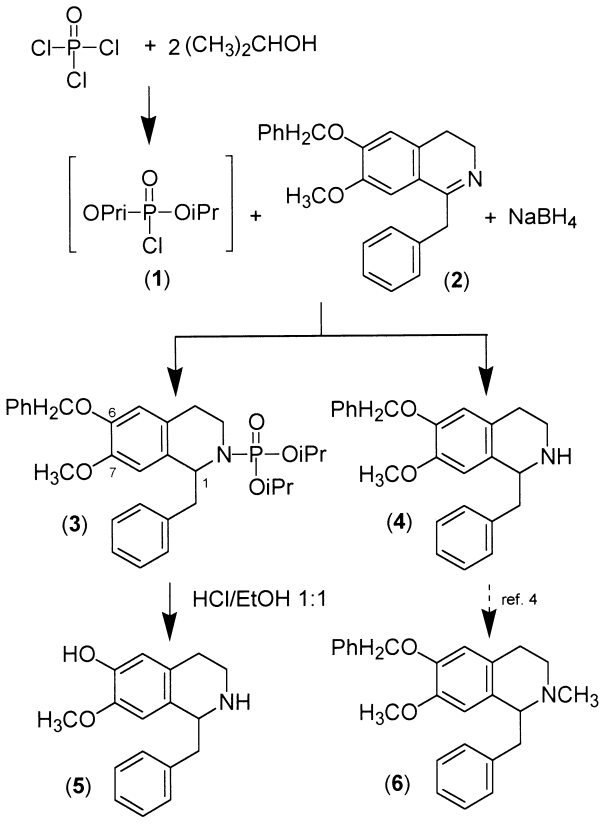
corresponding *N*-ethyl and *N*-methyl derivatives were obtained due to in situ formation of $PO(OEt)_3$ or $PO(OMe)_3$, respectively.[4,9]

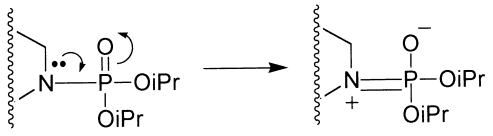
The molecular weight for compound **3** was determined by low resolution LSIMS (Cs^+ at 30 kV) mass spectrometry (*m*/*z* 524 for $[MH]^+$ corresponding to $C_{30}H_{38}NO_5P$). Under EI conditions the molecular ion fragments were quickly detected. High resolution mass spectrometry (HREIMS) allowed us to confirm the proposed structure for compound **3** by exactly measuring the masses of the significant daughter ions formed.[10] Losses of benzyl groups, propene molecules and phosphorous acid are involved in the observed fragmentation pattern. Compound **3** was characterized on the basis of its spectroscopic data (1D and 2D NMR experiments: ^1H, ^{13}C, DEPT, NOEDIFF, NOESY, COSY 45, COSY LR and HM QC),[11] which indicated that the *N*-phosphoryl group should be disposed as a phosphorimonium form (Scheme 2).

The ^{31}P NMR ($CDCl_3$, 100 MHz) spectrum of **3** showed an absorption at $\delta_P = 7.04$, characteristic of a phosphoryl moiety. In the ^{13}C NMR ($CDCl_3$, 100 MHz) spectrum of **3**, three methyne carbons appear as doublets ($^2J_{CP} = 6$, $^2J_{CP} = 5.5$ and $^2J_{CP} = 5$ Hz), at $\delta_C = 70.7$, $\delta_C = 70.1$ (2 CH, isopropyl) and $\delta_C = 55.7$ (CH-1), respectively, just as four isopropyl–methyl groups appear as four doublets ($^3J_{CP} = 6$ Hz), between $\delta_C = 23.8$ and $\delta_C = 23.5$.

In addition, the NOEDIFF's observed for **3** were in accordance with an *anti* conformation of 1-benzyl group



Scheme 1.



Scheme 2.

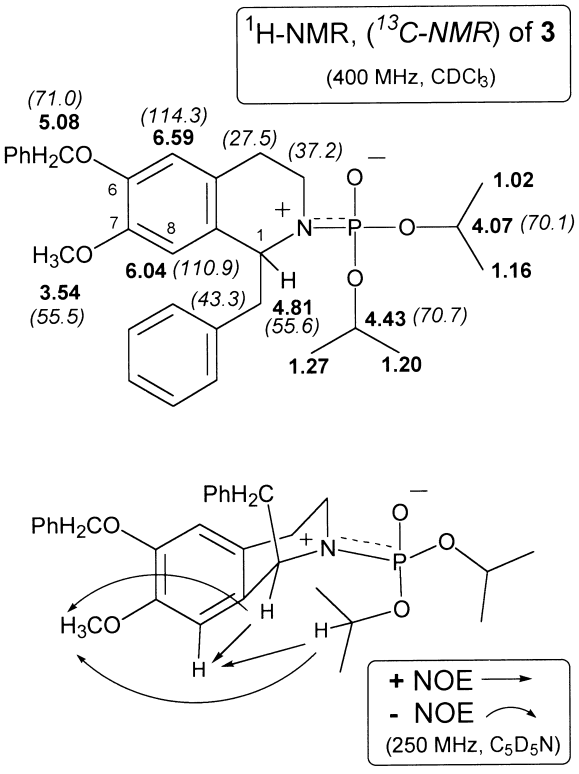
with regard to the *N*-diisopropyl phosphoryl moiety. Two important NOE's were observed between H-8 and both H-1 and one of the CH-isopropyl protons, as well as negative NOE's which were observed between the H-1/OMe-7 and the CH-isopropyl/OMe-7. All these data revealed an out-of-plane orientation of the 1-benzylic moiety, and a spatial proximity of one of the isopropyl groups to the isoquinoline skeleton (Schemes 3 and 4).

Bioactivities

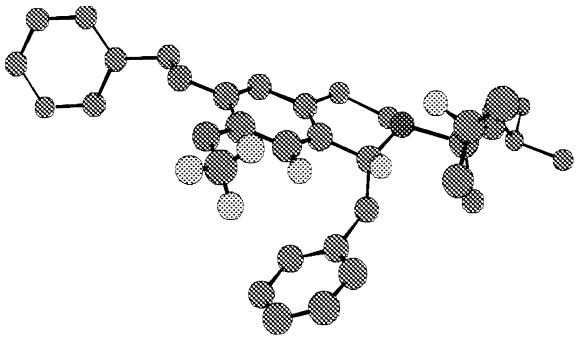
As phosphate esters and *N*-(*O*,*O*-diisopropyl phosphoryl) derivatives can be considered as good transport systems in several lipid membranes,[12] and due to their ability of dislocating electron charges, we decided to assay **3** and their related compounds (**2** and **4**) as well as the corresponding *N*-methyl analogue (**6**),[13] as inhibitors of the electron transport in the mitochondrial respiratory chain.[14]

Synthesized **2**–**4**, and **6** were initially found to be inhibitors of the integrated respiratory chain NADH oxidase enzymatic activity that includes respiratory chain complexes I, III and IV (see IC_{50} in Fig. 1).[15]

Once we observed that BTHIQ's inhibited the respiratory transport-chain at a micromolar range, we decided to



Scheme 3.



Scheme 4. Fully energy-minimized structure perspective of **3** by MM2 calculations.

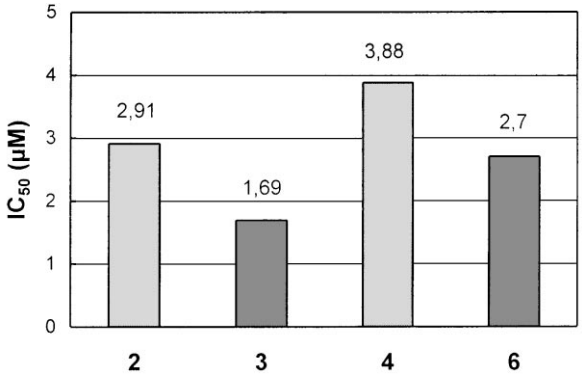


Figure 1. IC_{50} of NADH oxidase activity for compounds **2**–**4** [**2** (2.91 ± 0.23) μM, **3** (1.69 ± 0.27) μM, **4** (3.88 ± 0.54) μM, **6** (2.70 ± 0.28) μM].

perform a series of time-course assays to find out the target enzyme inhibited within the respiratory complexes. For these purposes we selected the most potent inhibitor of the series, the *N*-diisopropyl phosphoryl-BTHIQ (**3**) at a fixed high-concentration of 75 μM.

The cytochrome c oxidase activity (complex IV)[5b] was not affected by **3**, and this activity was completely blocked by cyanide (2 mM), a specific inhibitor of this enzyme. This result seems to give evidence that **3** could inhibit the respiratory chain at complex I and/or III levels.

Figures 2A and 2B show the replotted traces of the effect found for **3** on the time-course oxidation of NADH and decylubiquinol, both represent the selective enzymatic activity inhibition of complexes I and III, respectively.[5c,16]

Indeed the target enzymes within the respiratory chain of **3** resulted to be both NADH:decylubiquinone oxidoreductase (complex I) and decylubiquinol:cytochrome c oxidoreductase (complex III). Our results seem to show that the presence of a diisopropylphosphoryl moiety increases the potency inside this new kind of respiratory chain inhibitors. This discovery opens a new field to study this large class of natural and synthetic compounds as inhibitors of cell respiration focusing in their possible development as new antitumor agents.

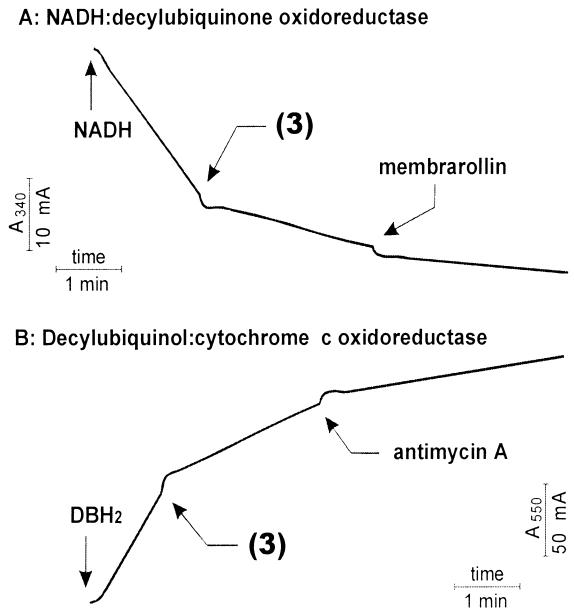


Figure 2.

Acknowledgements

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6. 1-Benzyl-6-benzyloxy-7-methoxy-3,4-dihydroisoquinoline (**2**) was prepared as previously described by Cabedo, N.; Protais, P.; Cassels, B. K.; Cortes, D. *J. Nat. Prod.* **1998**, *61*, 709.
7. *Preparation of compound* **3**: A solution of 1-benzyl-6-benzyloxy-7-methoxy-3,4-dihydroisoquinoline (**2**, 50 mg, 0.14 mmol) in $(CH_3)_2CHOH$ (10 mL) was treated with $POCl_3$ (0.3 mL, 3.21 mmol) and the mixture refluxed for 4 h. $NaBH_4$ (7 mg, 0.18 mmol) was added in small portions to the reaction mixture over 1h at rt, which was then stirred for 15 h. The acid isopropanolic solution, after removing the solvent under reduced pressure, was dissolved in H_2O, basified with NH_3 aq (pH≈9) and extracted with CH_2Cl_2. The organic layer was washed with H_2O and brine, dried over anhydrous Na_2SO_4 and concentrated. The residue was purified through 60 H silicagel column (toluene/EtOAc/DEA 96:2:2) to afford compounds **3** (29.3 mg, 40%) and **4** (28.6 mg, 55%). Compound **4** was previously described in ref 4.

8. *Cleavage of the benzyloxy and DIPP protective groups*: *N*-Diisopropyl phosphoryl BTHIQ **3** (15 mg, 0.028 mmol) was refluxed for 3 h with a mixture of equal volumes of ethanol and concentrated HCl (7 mL). The reaction solution was made basic (pH≈9) and extracted with CH_2Cl_2. The organic layer was washed with H_2O, dried, concentrated and purified over a 60 H silicagel column (CH_2Cl_2/EtOAc/MeOH/NH_4OH 40:50:10:0.1) to afford 1-benzyl-6-hydroxy-7-methoxy-1,2,3,4-tetrahydroisoquinoline (**5**, 6 mg, 80%). The spectroscopic data of this compound have been reported in ref 4.

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10. HREIMS (20 eV) of **3**: *m*/*z* 432.19428 (432.19399 calcd for $[C_{23}H_{31}NO_5P]^+$); *m*/*z* 390.14580 (390.14704 calcd for $[C_{20}H_{25}NO_5P]^+$); *m*/*z* 348.10158 (348.10009 calcd for $[C_{17}H_{19}NO_5P]^+$); *m*/*z* 257.04465 (257.04531 calcd for $[C_{10}H_{12}NO_5P]^+$); *m*/*z* 176.07072 (176.07115 calcd for $[C_{10}H_{10}NO_2]^+$); *m*/*z* 91.05838 (91.05477 calcd for $[C_7H_7]^+$).

11. Compound **3**: $C_{30}H_{38}NO_5P$; IR (film) ν_{max} 2917, 2849, 1517, 1453, 1384, 1246 (P=O), 1115, 1090, 1011 (P-O-C), 980 cm^{-1}; ^{31}P NMR ($CDCl_3$, 100 MHz) δ 7.04 (phosphoryl group); ^1H NMR* ($CDCl_3$, 400 MHz) δ 1.02, 1.16, 1.20, and 1.27 (12H, 4d, *J* = 6Hz, $CH(C\underline{H}_3)_2$), 2.49 and 2.88 (2H, 2m, CH_2-4), 2.98 and 3.17 (2H, 2 dd, *J* = 14Hz, *J′* = 7 Hz, CH_2-α), 3.23 and 3.50 (2H, 2m, CH_2-3), 3.54 (3H, s, OCH_3-7), 4.07 and 4.43 (2H, 2 sept, *J* = 6Hz, $C\underline{H}(CH_3)_2$), 4.81 (1H, dd, *J* = 14Hz, *J′* = 7Hz, H-1), 5.08 (2H, s, $OC\underline{H}_2$Ph-6), 6.04 (1H, s, H-8), 6.59 (1H, s, H-5), 7.17–7.43 (10H, m, 2Ph); ^{13}C NMR* ($CDCl_3$, 100 MHz) δ 147.0 and 146.9 (C-6 and C-7), 138.8 and 137.2 (C-1′ and C-1″), 130.0–125.4 (2Ph), 129.8 and 125.5 (C-4a and C-8a), 114.3 (C-5), 110.9 (C-8), 71.0 ($O\underline{C}H_2$Ph-6), 70.7 and 70.1 (2CH, $\underline{C}H(CH_3)_2$), 55.6 (C-1), 55.5 ($\underline{O}CH_3$-7), 43.3 (CH_2-α), 37.2 (C-3), 27.5 (C-4), 23.8, 23.7, 23.5, 23.0 (4 CH_3, $CH(\underline{C}H_3)_2$); LSIMS *m*/*z* 524 $[MH]^+$; EIMS *m*/*z* (%) 432 (97), 390 (28), 348 (100), 257 (30), 176 (12), 91 (82). *The assigments were made by COSY 45, COSY LR, NOEDIFF, NOESY, DEPT and HMQC; see also Scheme 3.

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13. *N*-Methyl-1-benzyl-7-methoxy-1,2,3,4-tetrahydroisoquinoline (**6**) was prepared as previously described by Andreu, I. et al.[4]

14. Enzymatic respiratory titrations were performed in beef heart submitochondrial particles (SMP) by addition of increasing concentrations of each inhibitor. IC_{50} values were the final compound concentrations in the assay cuvette that yielded an average of 50% inhibition of the NADH oxidase activity.[5]

15. In all the assayed compounds, full inhibition of the NADH oxidase activity was reached at 10 μM. The residual activity beyond this concentration was the same as that of the nonsensitive NADH oxidation by other inhibitors.[5c]

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