# Bisphosphonamidate Clodronate Prodrug Exhibits Potent Anticancer Activity in Non-Small Cell Lung Cancer cells.

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**Supporting Information**

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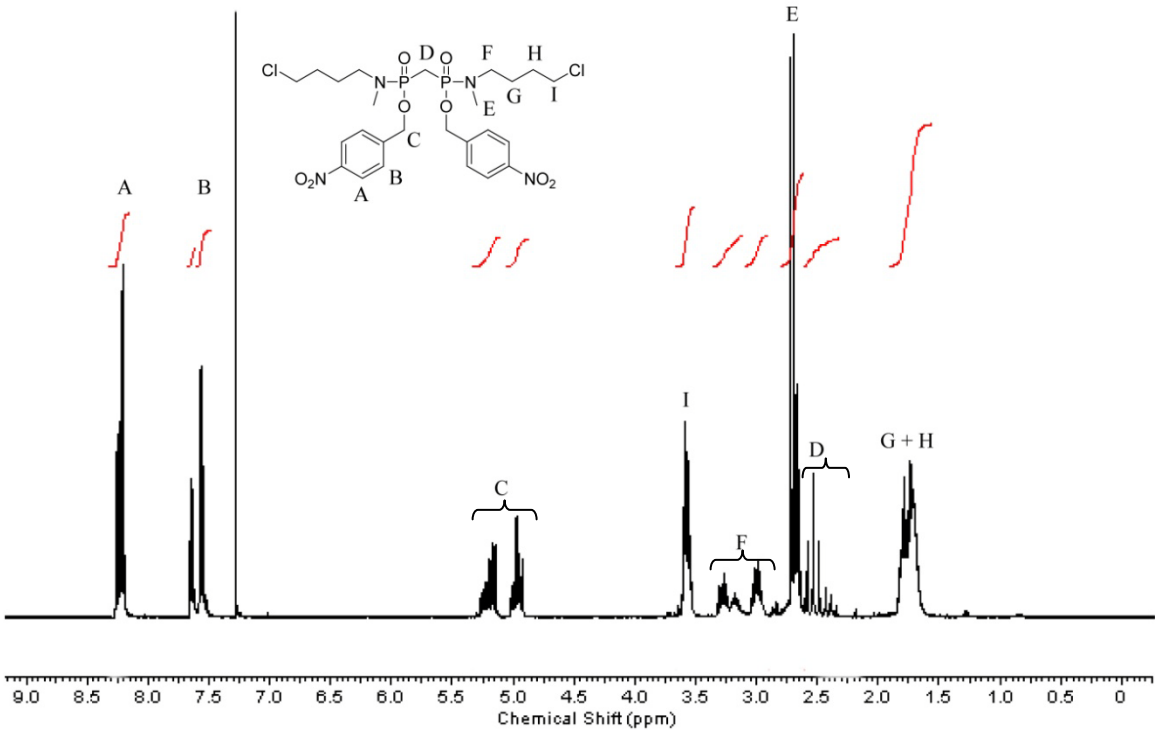


**Figure S1**. $^{1}H$ NMR spectrum of prodrug **14** acquired in $CDCl_3$ on a 400 MHz Bruker NMR. Chemical shifts are reported in the accompanying publication (Experimental Procedures).

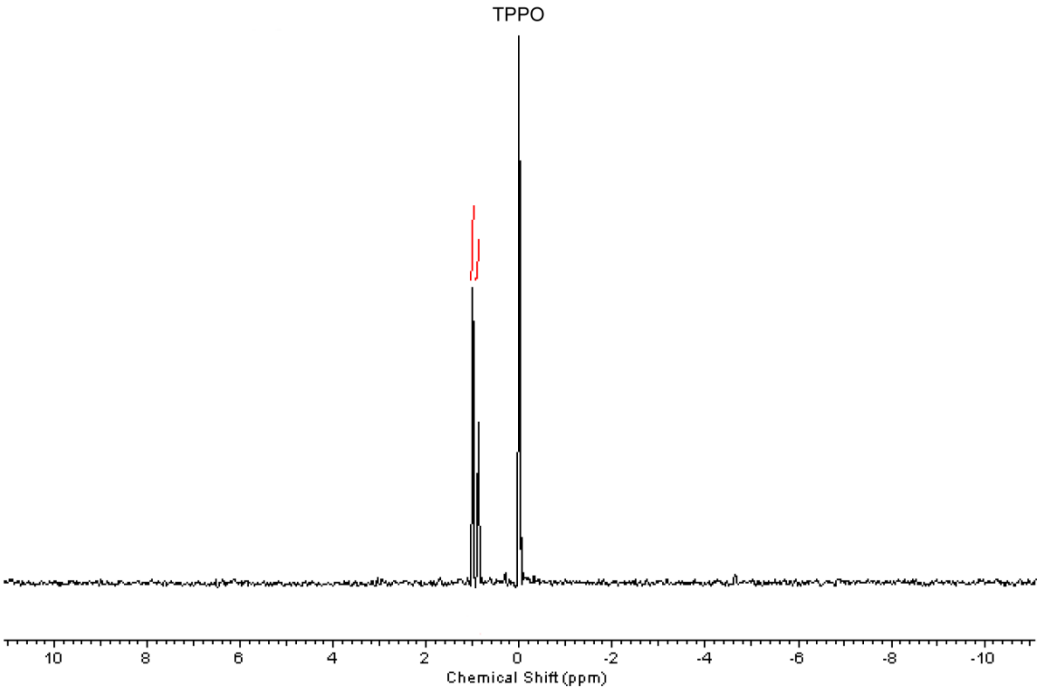


**Figure S2**. $^{31}P$ NMR spectrum of prodrug **14** acquired in $CDCl_3$ on a 400 MHz Bruker NMR, using TPPO as an external standard. Chemical shifts are reported in the accompanying publication (Experimental Procedures).

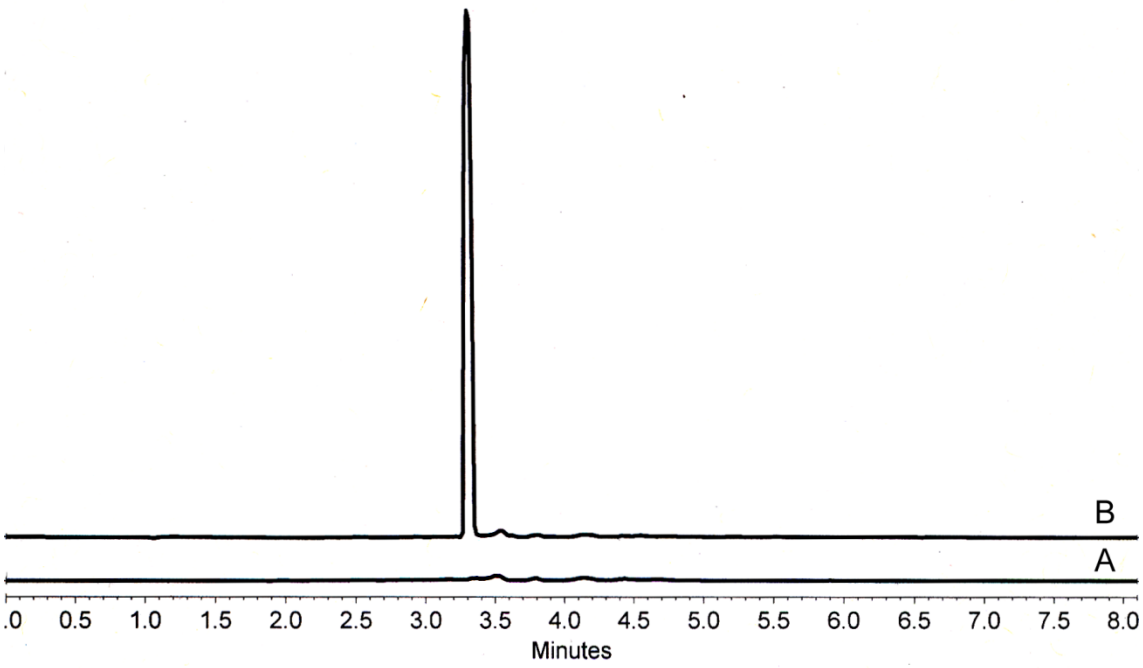


**Figure S3.** Purity of bisphosphonate prodrug **14** is > 95% as determined by reversed-phase HPLC, using a $C_{18}$ Rocket$^{TM}$ column. Method: 100% water to 100% acetonitrile, over 3 min, then 5 min at 100% acetonitrile. Retention time = 3.3 min. Detected at $\lambda_{max}$ of 270 nm. Minor peaks observed in the chromatogram of **14** (B) are artifacts detected in the background chromatogram (A).

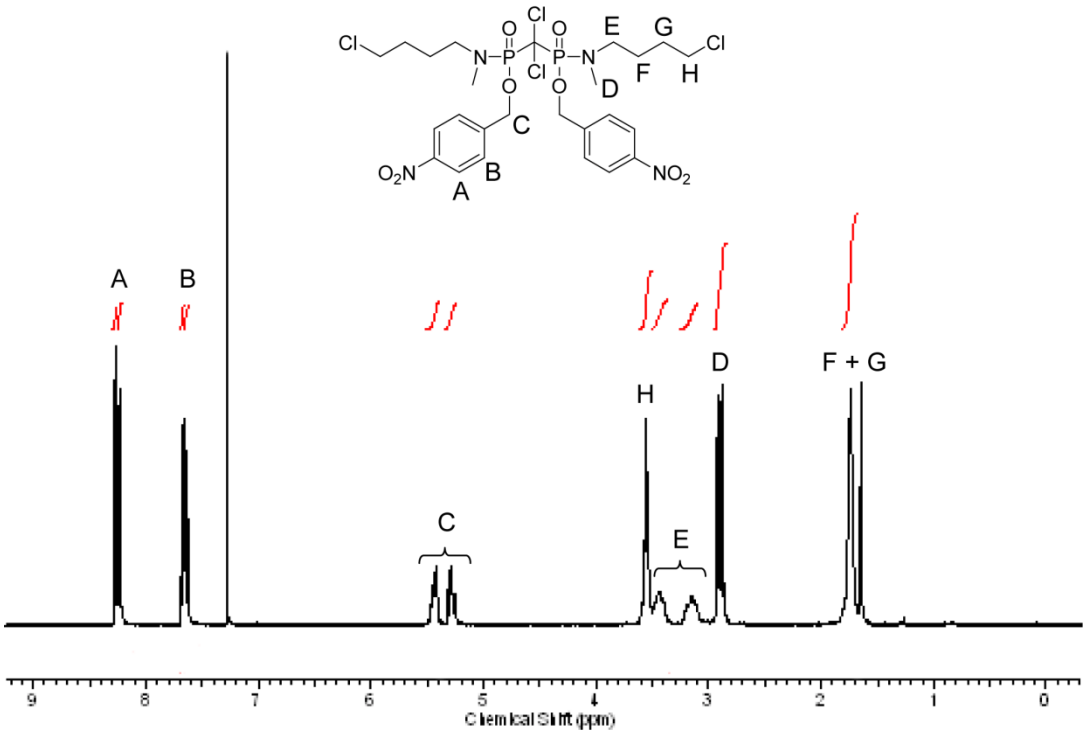


**Figure S4**. $^{1}H$ NMR of clodronate prodrug **15** acquired in $CDCl_3$ on a 400 MHz Bruker NMR. Chemical shifts are reported in the accompanying publication (Experimental Procedures).

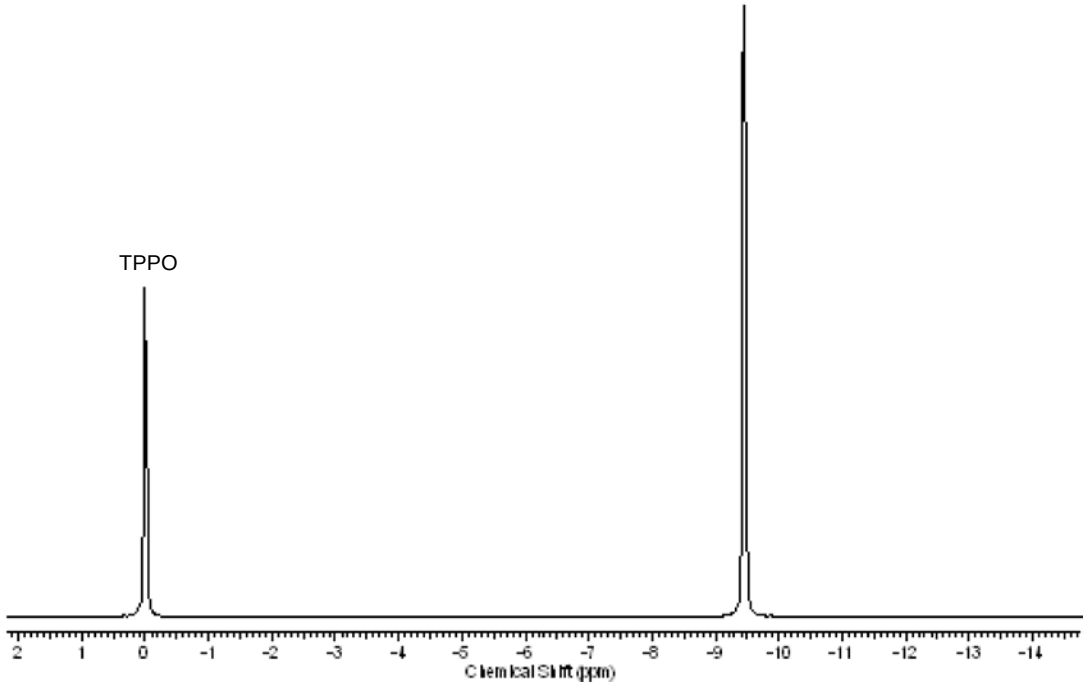


**Figure S5**. $^{31}P$ NMR of clodronate prodrug **15** acquired in $CDCl_3$ on a 400 MHz Bruker NMR, using TPPO as an external standard. Chemical shift is reported in the accompanying publication (Experimental Procedures).

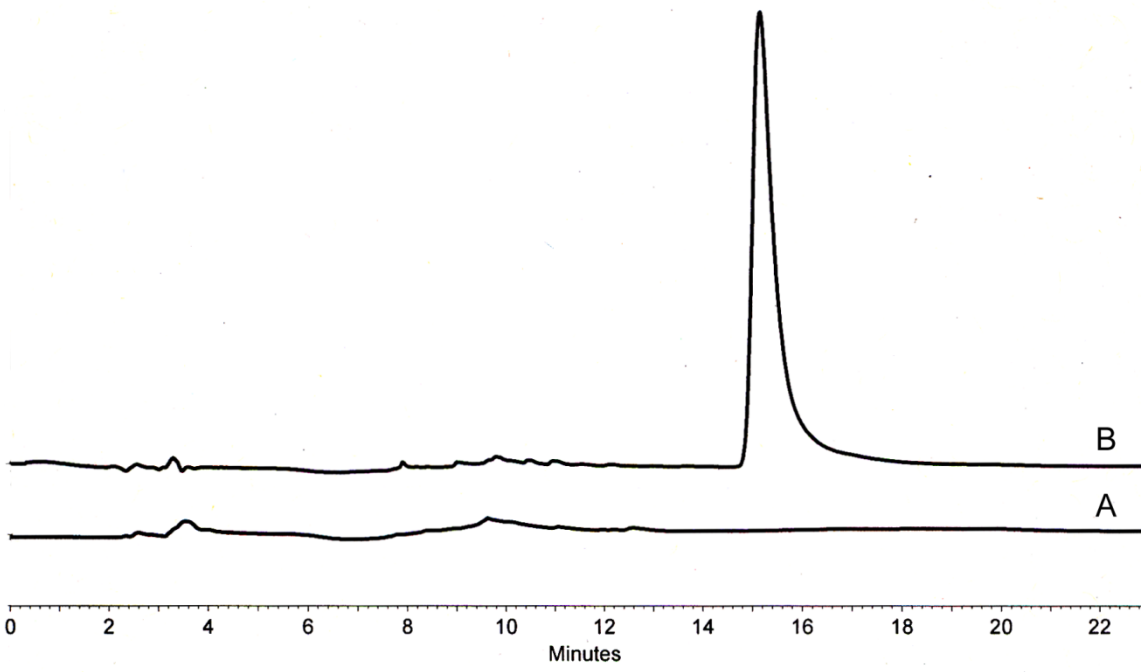


**Figure S6.** Purity of bisphosphonate prodrug **15** is > 95% as determined by reversed-phase HPLC (Altima $C_{18}$ column, 250 mm × 4.6 mm I.D.). Method*:* 5:95 water/acetonitrile to 75% acetonitrile over 5 min, 75% acetonitrile for 5 min, 75-100% acetonitrile over 5 min, then 100% acetonitrile for 5 min. Retention time = 15.2 min. Detected at $\lambda_{max}$ of 270 nm. Minor peaks observed in the chromatogram of **15** (B) are artifacts detected in the background chromatogram (A).

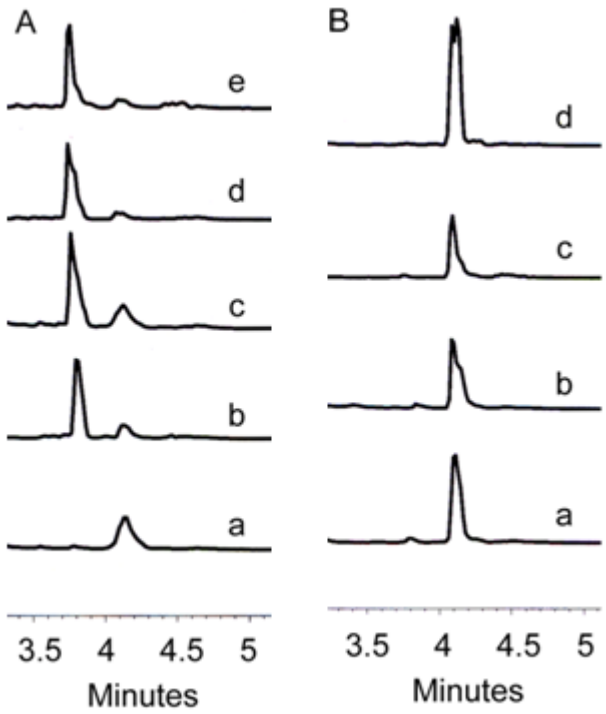


**Figure S7.** Prodrugs **14** and **15** are stable in 1640 RPMI media over 72 at 37ºC. Stability was monitored by reversed-phase HPLC, using a $C_{18}$ Rocket$^{TM}$ column (monitored at $\lambda_{max}$ of 270 nm). Method: 5-100% acetonitrile over 3 min, 100% acetonitrile for 5 min. Retention time of **14** = 3.7 min. Retention time of **15** = 4.1 min. **Figure 4A):** Stability of prodrug **14** in media over 72 hours. a) media only at 24 h; b) **14** in media, 0 h; c) **14** in media, 24 h; d) **14** in media, 48 h; e) **14** in media, 72h. **Figure 4B):** Stability of prodrug **15** in media over 72 hours. A small decrease in the peak area of **15** (~ 20%) was observed by 48 hours, but without concomitant appearance of a new species in the chromatogram. A thin film accumulating on the vial from these samples was confirmed to be pure prodrug **15**, indicating **15** is stable over several days in media at 37ºC. a) **15** in media, 0 h; b) **15** in media, 48 h; c) **15** in media, 72 h; d) film dissolved in DMSO and re-subjected to HPLC analysis.

**Chemical conversion of prodrug 14 to derivatized bisphosphonate 19.** Tetra-silyl bisphosphonate **18** was detected in A549 cells treated with prodrug **14**. Here the possibility that the prodrug itself is converted to **18** under the derivatization conditions was considered. In order to rule this out as a possible explanation for the observed derivatized bisphosphonate **18** in prodrug-treated cells, A549 cell lysate (prepared as described in Experimental Protocols) was spiked with prodrug **14** (1920 ng/mL), and the lysate was immediately subjected to derivatization conditions. Under these conditions, minimal conversion to **18** was observed, confirming that **18** observed in prodrug-treated cells is largely a result of intracellular prodrug activation and release of bisphosphonate **1**.

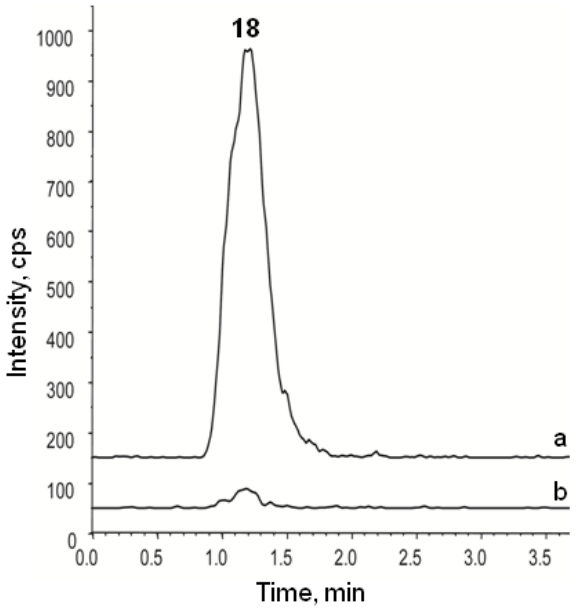


**Figure S8.** Prodrug **14** undergoes minimal conversion to derivatized bisphosphonate **18** in the presence of MTBSTFA. a) tetra-silyl bisphosphonate **18** detected in A549 cells treated with prodrug **14**. The prodrug concentration in this sample was determined to be 474 ng/mL (169.3 ng/$10^4$ cells, Figure 4A); b) tetra-silyl bisphosphonate **18** detected in A549 cell lysate spiked with prodrug **14** immediately prior to derivatization with MTBSTFA. The prodrug concentration in this sample was 1920 ng/mL.