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Synthesis of phosphinate analogues of the phospholipid anti-tumour agent hexadecylphosphocholine (miltefosine)

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ABSTRACT

Efficient synthesis of phosphinate analogues (in six steps and 68–69% overall yields) of the anti-tumour agent miltefosine are reported, which involve a radical hydrophosphinylation addition reaction followed by conversion to the P(III) silyloxy intermediate and Michael-type addition as the key steps.

Alkylphosphocholine (APC) analogues (prototype: miltefosine **1**, Fig. 1) of natural phospholipids are a family of anti-cancer drugs with a wide range of pharmacological behaviour.[1] There is a growing interest in synthetic anti-cancer phospholipids owing to their selectivity against tumours, which has resulted in a much lower toxicity as compared with some other classical anti-cancer chemotherapeutic agents.[1c]

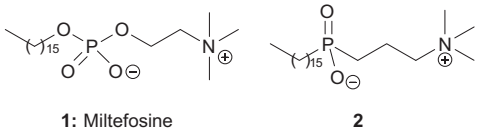
Many conventional anti-tumour agents run the risk of damaging healthy fast-proliferating tissues via a direct effect on cellular DNA. Alkylphosphocholines display two important advantages in contrast to common anti-tumour agents: (a) they target the plasma membrane rather than directly interacting with cellular DNA, and (b) they reveal a strong apoptosis-inducing ability.[1b,2] A remarkable feature of APCs lies in the fact that, whereas malignant cells are highly sensitive to their lethal action, normal cells remain relatively unaffected, illustrating the potentially selective anti-tumour properties of this class of compounds.[1c]

However, due to the presence of the phosphate diester functionality, alkylphosphocholines are prone to biodegradation by phospholipid-metabolising enzymes such as phospholipases C and D.[1b,3] In order to address this problem, replacement of both of the O–P bonds of the phosphate diester in miltefosine **1** with two C–P bonds would result in a sterically similar phosphinate–phospholipid analogue **2**, which should be resistant to hydrolysis by phospholipid-metabolising enzymes (Fig. 1).

It is a characteristic of phosphinic acids that the carbon–phosphorus bond is strongly resistant to hydrolytic cleavage. In addition, their wide range of activity has been attributed to the physical and structural similarity with biologically important phosphate esters and peptides.[4]

Carbon–phosphorus bonds have traditionally been constructed by addition reactions of a phosphorus-centred radical to an sp^2 carbon,[5] or through interaction between a trivalent phosphorus atom and an electrophilic centre (e.g., the Arbuzov and Pudovik reactions).[6] Our previous work in this latter field[7] suggested the possibility of preparing phosphinate analogue **2** from hypophosphorous acid **5** by Michael-type addition to acrylonitrile **6**, followed by Arbuzov-type alkylation of the resulting 2-cyanoethylphosphinic acid **4** with hexadecyl iodide **3** (Scheme 1, path A).

A second retrosynthetic analysis on the target molecule **2** (Scheme 1, path B) revealed the possibility of a potentially shorter approach, by the sequential radical addition reaction of hypophos-



1: Miltefosine **2**

Figure 1. Structures of miltefosine **1** and phosphinate analogue **2**.

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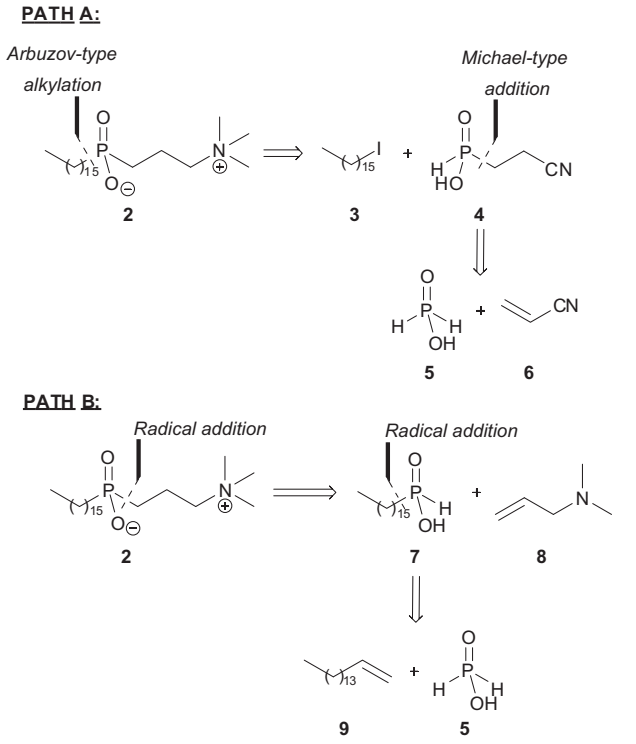


Scheme 1. Retrosynthetic analyses of **2**.

phorous acid **5** to hexadecene **9** and *N,N*-dimethylallylamine **8** (in either order). The involvement of hypophosphorous acid **5** in the formation of phosphinates through radical chain reactions has been reported by Nifant'ev.[5] Strategically, both routes could offer useful flexibility over the order of assembly of the hydrophilic polar head group and the hydrophobic tail of the phosphinate analogue **2**.

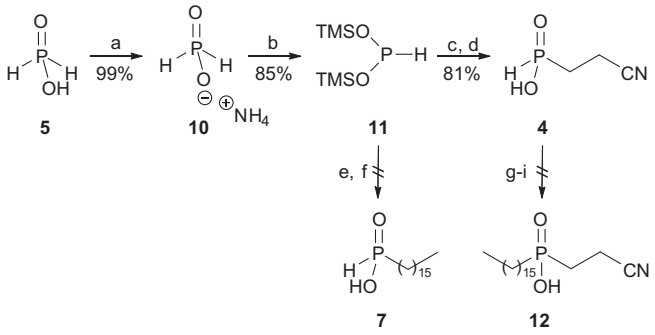
We describe herein an efficient synthesis of phosphinate analogue **2**, exploiting a combination of our Michael-type addition protocol using silyl phosphonites together with a radical addition step, in order to form the two key C–P bonds. This has also enabled us to prepare further analogues for biological testing.

Ammonium hypophosphite **10** was conveniently prepared in quantitative yield by neutralisation of 50% aqueous hypophosphorous acid **5** with ammonium hydroxide solution (Scheme 2). Double silylation of the salt **10** using hexamethyldisilazane (HMDS) afforded, after careful fractional distillation of the reaction mixture, the highly nucleophilic bis(trimethylsilyl) phosphonite (BTSP) **11**.[7] Care should be taken in handling bis(trimethylsilyl) phosphonite **11**, owing to its pyrophoric nature exhibited on exposure to air or moisture. Michael-type addition of the silyl phosphonite **11** to acrylonitrile **6**, followed by acidic hydrolysis of the silyl groups, afforded the intermediate 2-cyanoethylphosphinic acid **4** in 81% yield. Attempts to react the mono-substituted phosphinic acid **4** with hexadecyl iodide **3** using our silyl phosphonite alkylation methodology[7b] proved to be unsuccessful. Owing to the disappointing results in obtaining **12** via the Arbuzov-type alkylation of **4**, it was then decided to change the order of the reactions. However, once again, attempts to alkylate **11** with hexadecyl iodide **3** to give the mono-substituted phosphinic acid **7** were not successful.

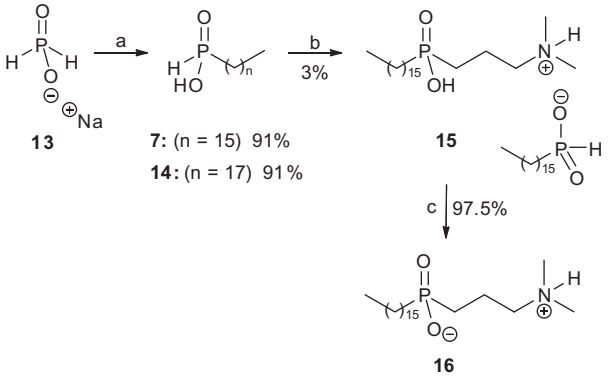
Attention was then turned to free radical reactions (Scheme 3). Mono-substituted hexadecyl- and octadecyl-phosphinic acids **7** and **14** were conveniently prepared under free radical conditions from sodium phosphinate **13** and the appropriate terminal alkenes in high yields.[5] However, subsequent hydrophosphinylation of *N,N*-dimethylallylamine **8** with hexadecylphosphinic acid **7**, under free radical conditions, afforded only a 3% yield of the desired addition product, which was isolated as a phosphinate salt **15** (formed with the starting phosphinic acid **7**). Extensive experimentation in efforts to improve this free radical step, in order to obtain synthetically useful yields, proved to be unsuccessful. Attempts included the use of different radical initiators such as AIBN or Et_3B/O_2,[8] and also the use of acrylonitrile as the substrate.

The phosphinate salt **15** was then dissolved in hot ethyl acetate and treated with concentrated hydrochloric acid to give the zwitterionic neutral form **16**. The ^{31}P (proton-decoupled mode) NMR spectrum of **16** displayed a signal at δ 53.59 for P–$O^{(-)}$, which moved downfield to δ 56.69 for P–OH when acidified with hydrochloric acid.

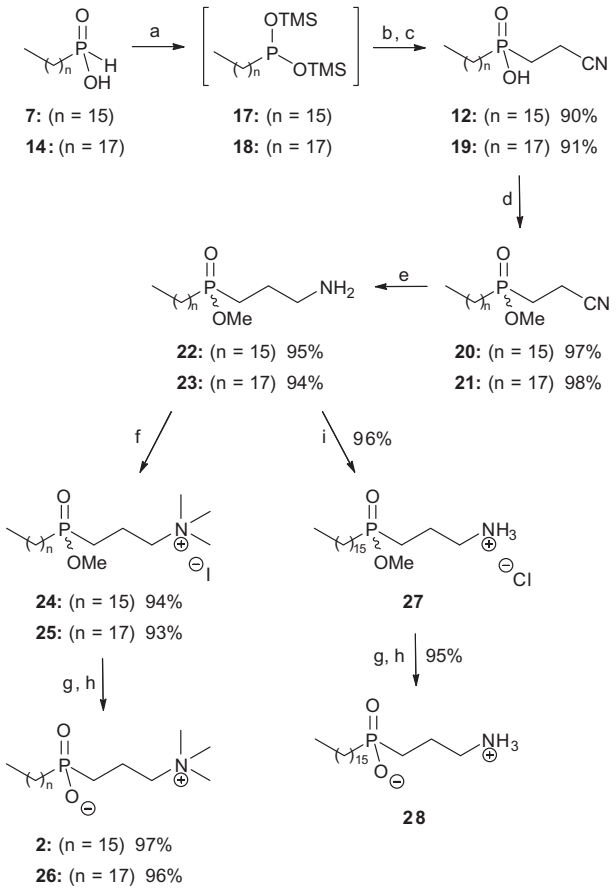
Efficient syntheses of phosphinate analogues of miltefosine **1** were finally realised as outlined in Scheme 4, by combining the high-yielding radical-mediated preparation of mono-alkyl phosphinic acids **7** and **14** with a silyl phosphonite mediated addition as the second step. Following a similar procedure to that described for the preparation of 2-cyanoethylphosphinic acid **4**, the desired di-substituted phosphinic acids **12** and **19** were successfully formed in high yields. Initial attempts at esterification of **12** and **19** included the use of Cs_2CO_3 or Et_3N together with MeI, Et_3N with ethyl chloroformate, and reaction with an alcohol with azeotropic removal of water.[9a] However, all these attempts proved to be low yielding (<20%). The best results were achieved upon refluxing phosphinic acids **12** and **19** in excess trimethyl orthoformate.[9b] Hydrogenation of the nitriles **20** and **21** at atmospheric pressure in the presence of Raney-Nickel then afforded the amines **22** and **23** in 95% and 94% yields, respectively. Other reducing conditions,



Scheme 2. Reagents and conditions: (a) concd NH_4OH (aq), 0 °C, 2.5 h, toluene azeotrope; (b) HMDS, 120–130 °C, 2 h; (c) acrylonitrile, CH_2Cl_2, 0 °C, 2 h, then rt, 12 h; (d) THF–H_3O^+, 0 °C to rt, 2 h; (e) hexadecyl iodide, CH_2Cl_2, 0 °C to reflux, 2 days; (f) THF–H_3O^+, 0 °C to rt, 2 h; (g) TMSCl, Et_3N, CH_2Cl_2, 0 °C to rt, 2 h; (h) hexadecyl iodide, rt to reflux, 5 days; (i) THF–H_3O^+, 0 °C to rt, 2 h.



Scheme 3. Reagents and conditions: (a) terminal alkene, concd H_2SO_4, AIBN, EtOH, reflux, 1 day; (b) *N,N*-dimethylallylamine **8**, 1,1′-azobis(cyclohexanecarbonitrile), EtOH, reflux, 5 days; (c) concd HCl, hot EtOAc, rt, 1 h.



Scheme 4. Reagents and conditions: (a) TMSCl, Et_3N, CH_2Cl_2, 0 °C to rt, 2–3 h; (b) acrylonitrile, 0 °C to rt, overnight; (c) 1 M HCl, 0 °C to rt, 1 h; (d) trimethyl orthoformate, reflux, 3.5 days; (e) H_2 (g), Raney-Ni (cat.), concd NH_4OH, MeOH, 55 °C, 1 atm, 2 h; (f) methyl iodide, anhydrous K_2CO_3, MeOH–$CHCl_3$, reflux, 4 days; (g) TMSI, CH_2Cl_2, rt, overnight; (h) MeOH, rt, 30 min; (i) concd HCl, EtOAc, rt, 10 min.

including $CoCl_2/NaBH_4$,[10a] Ra-Ni/$NaBH_4$,[10b] or H_2–Pd/C[10c], proved to be less efficient (40–50% yields). The primary amines **22** and **23** were then quaternised with excess MeI in the presence of anhydrous K_2CO_3. In addition, the primary amine **22** was converted to the hydrochloride salt **27** by treatment with concentrated HCl. This was in order to provide—after de-esterification—a phosphinate analogue **28** with modified hydrophilic polar head group, for biological testing.

Finally, de-esterification of methyl phosphinate esters **24**, **25**, and **27** was achieved with iodotrimethylsilane (TMSI)[11] followed by methanolysis, to afford the ammonium phosphinate inner salts **2**, **26**, and **28** in high yields.

In summary, an efficient and flexible synthetic strategy has been developed for the synthesis of phosphinate analogues of the anti-tumour agent hexadecylphosphocholine (miltefosine) **1**, making use of a radical hydrophosphinylation addition reaction of terminal olefins to introduce the hydrophobic tail, in combination with a Michael-type addition protocol using silyl phosphonites to attach the hydrophilic polar head group. Overall, the synthesis of phosphinate analogues **2** (C16, $^+NMe_3$), **26** (C18, $^+NMe_3$) and **28** (C16, $^+NH_3$) proceeded in six steps and 68–69% overall yields. By suitable editing of the hydrophobic tail and the hydrophilic polar head group, further nonhydrolysable analogues may be designed in order to explore biological structure–activity relationships.

Acknowledgments

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Supplementary data

Supplementary data (experimental procedures and characterisation data for all new compounds along with copies of ^1H, ^{13}C and ^{31}P NMR spectra) associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2011.03.107.

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