Organic & Biomolecular Chemistry

Dynamic Article Links

Cite this: *Org. Biomol. Chem.*, 2012, **10**, 1424

www.rsc.org/obc

PAPER

1-(Fluoroalkylidene)-1,1-bisphosphonic acids are potent and selective inhibitors of the enzymatic activity of *Toxoplasma gondii* farnesyl pyrophosphate synthase†

Sergio H. Szajnman,[a] Valeria S. Rosso,[a,b] Leena Malayil,[b] Alyssa Smith,[b] Silvia N. J. Moreno,[b] Roberto Docampo[b] and Juan B. Rodriguez*[a]

Received 19th September 2011, Accepted 8th November 2011
DOI: 10.1039/c1ob06602a

α-Fluorinated-1,1-bisphosphonic acids derived from fatty acids were designed, synthesized and biologically evaluated against *Trypanosoma cruzi*, the etiologic agent of Chagas disease, and against *Toxoplasma gondii*, the agent responsible for toxoplasmosis, and also towards the target parasitic enzymes farnesyl pyrophosphate synthase of *T. cruzi* (*Tc*FPPS) and *T. gondii* (*Tg*FPPS). Interestingly, 1-fluorononylidene-1,1-bisphosphonic acid (compound **43**) proved to be an extremely potent inhibitor of the enzymatic activity of *Tg*FPPS at the low nanomolar range, exhibiting an IC_{50} of 30 nM. This compound was two-fold more potent than risedronate (IC_{50} = 74 nM) that was taken as a positive control. This enzymatic activity was associated with a strong cell growth inhibition against tachyzoites of *T. gondii*, with an IC_{50} value of 2.7 μM.

Introduction

Bisphosphonates of general formula **1** are metabolically stable pyrophosphate (**2**) analogues, in which a methylene group replaces the oxygen atom bridge between the two phosphorus atoms of the pyrophosphate moiety. Substitution at the carbon atom with different side chains has generated a large family of compounds.[1] Several bisphosphonates, such as pamidronate (**3**), alendronate (**4**), risedronate (**5**) and ibandronate (**6**), are in clinical use for the treatment and prevention of osteoclast-mediated bone resorption associated with osteoporosis, Paget's disease, hypercalcemia, tumor bone metastases and other bone diseases (Chart1).[2–4] Bisphosphonates became compounds of pharmacological importance since calcification studies were done more than 40 years ago.[5–7]

Selective action on bone is based on the binding of the bisphosphonate moiety to the bone mineral.[8] It has been postulated that the acidocalcisomes are equivalent in composition to bone mineral and that accumulation of bisphosphonates in these organelles, as occurs in bone mineral, assists their antiparasitic action.[8] Bisphosphonates act by a mechanism that leads to osteoclast apoptosis.[9] The site of action of aminobisphosphonates has been narrowed down to the isoprenoid pathway and, more specifically, to an inhibition of protein prenylation.[10] Farnesyl pyrophosphate synthase (FPPS) constitutes the principal target of bisphosphonates.[11–15] This enzyme catalyzes the two mandatory biosynthetic steps to form farnesyl pyrophosphate, as indicated briefly in Scheme 1. Inhibition of the enzymatic activity of FPPS blocks farnesyl pyrophosphate and geranylgeranyl pyrophosphate formation, which are required for the post-translational prenylation of small GTP-binding proteins, which are also GTPases, such as Rab, Rho and Rac, within osteoclasts.[16]

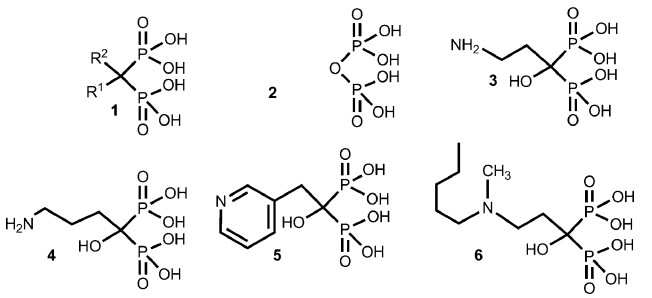
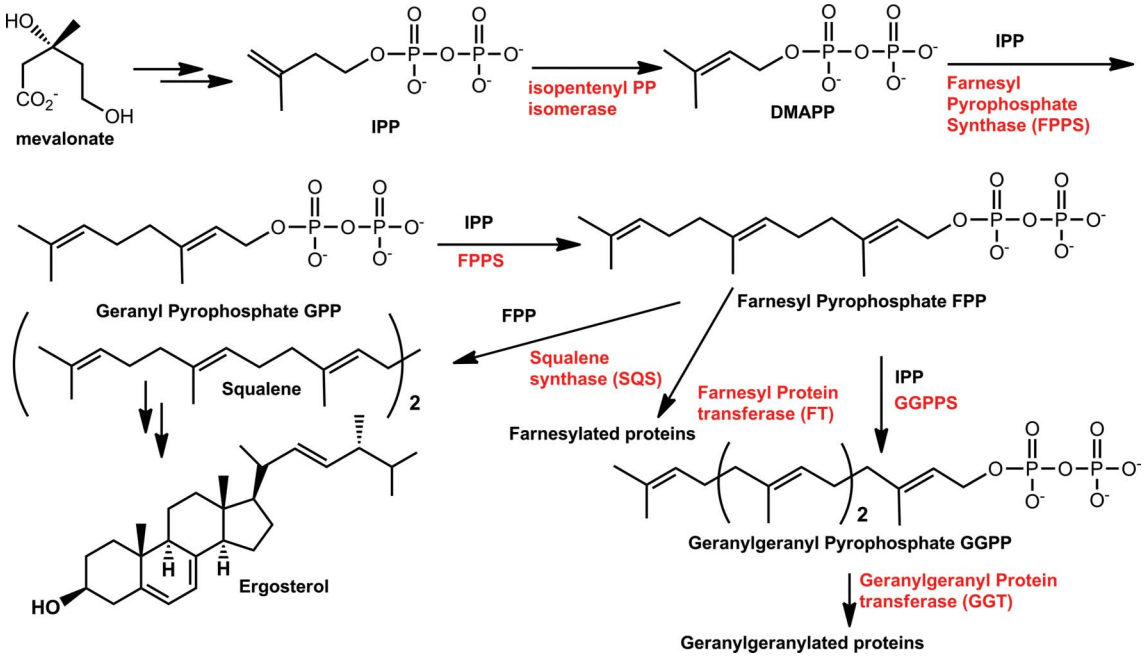


Chart 1 The general formula and chemical structure of representative FDA-approved bisphosphonates clinically employed for different bone disorders.

Besides their use in long-term treatments for different bone disorders, bisphosphonates additionally exhibit a wide range of biological actions, such as stimulation of γδ T cells of the immune system,[17] antibacterial action,[18] herbicidal properties,[19] anticancer action,[20–23] as potent and selective inhibitors of the enzymatic activity of acid sphingomyelinase[24] and, particularly, as antiparasitic agents.[25–29] Certainly, at the beginning, aminobisphosphonates

[a]*Departamento de Química Orgánica and UMYMFOR (CONICET–FCEyN), Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Pabellón 2, Ciudad Universitaria, C1428EHA, Buenos Aires, Argentina. E-mail: jbr@qo.fcen.ar; Tel: +54 11 4576-3385*
[b]*Center for Tropical and Emerging Global Diseases and Department of Cellular Biology, University of Georgia, Athens, Georgia, 30602, USA*
† Electronic supplementary information (ESI) available: See DOI: 10.1039/c1ob06602a



Scheme 1 Isoprenoid biosynthesis in trypanosomatids and apicomplexan parasites.

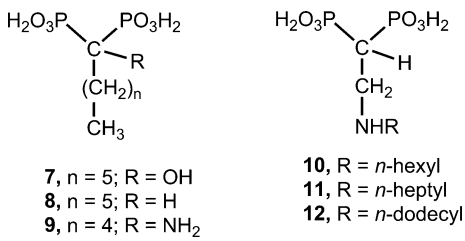


Chart 2 The chemical structure of representative members of bisphosphonic acids derived from fatty acids.

have proven to be effective growth inhibitors of *T. cruzi* in *in vitro* and *in vivo* assays without toxicity to the host cells.[8] Inspired by this work, different bisphosphonates were found to be potent inhibitors of the proliferation of pathogenic trypanosomatids other than *T. cruzi*, such as *T. brucei rhodesiense*, *Leishmania donovani* and *L. mexicana*, and apicomplexan parasites, such as *Toxoplasma gondii* and *Plasmodium falciparum*.[27,28,30–36] *In vivo* assays of bisphosphonates have shown that risedronate can significantly increase the survival of *T. cruzi*-infected mice.[37] In view of the above results, it is possible to assume that bisphosphonates are potential candidates for chemotherapy of neglected infectious diseases. In addition, bisphosphonates have the advantage that their synthesis is straightforward and relatively inexpensive. It is reasonable to assume a low toxicity for bisphosphonate-containing drugs, bearing in mind that many bisphosphonates are FDA-approved drugs for the long-term treatment of different bone disorders.[38]

Bisphosphonates derived from fatty acids have become interesting putative antiparasitic agents, especially 2-alkylaminoethyl derivatives, which were shown to be potent growth inhibitors against the clinically more relevant form of *T. cruzi* possessing IC_{50} values at the nanomolar range against the target enzyme.[31,32] Compounds **10–12** arise as representative members of this type of bisphosphonates, which have proven to be by far more efficient than their parent drugs 1-hydroxy-, 1-alkyl-, and 1-amino-bisphosphonates as growth inhibitors of trypanosomatids (Chart 2).[33–35]

T. cruzi, which is the etiologic agent of American trypanosomiasis, has a complex life cycle involving blood-sucking Reduviid insects and mammals.[39,40] It multiplies in the insect gut as an epimastigote form and is spread as a non-dividing metacyclic trypomastigote from the insect feces by contamination of intact mucosa or wounds produced by the blood-sucking activity of the vector. In the mammalian host, the parasite proliferates intracellularly in the amastigote form and is subsequently released into the blood stream as a non-dividing trypomastigote.[40] On the other hand, the opportunistic pathogen *T. gondii* causes a broad spectrum of disease, but most infections are asymptomatic.[41] This apicomplexan parasite has adopted an essential intracellular life style. The parasite actively penetrates host cells, sets up a privileged compartment in which it replicates and finally kills the cell.[42] There are two asexual forms that can cause disease in humans. The tachyzoite form can invade all types of cells and proliferate leading to host cell death. The bradyzoite form divides slowly and forms cysts in muscle and brain. The sexual cycle occurs in the superficial epithelium of the small intestine of members of the cat family. Oocysts, which are shed in feces of recently infected cats, remain in the upper soil horizon, where they may contaminate skin and may be ingested by hand-to-mouth transmission or on natural vegetables. Oocysts require at least 12 h in order to complete sporulation and afterwards they are infectious by mouth.[43–46] Chemotherapy for these neglected diseases is still deficient and based on old and empirically discovered drugs.[47,48] Therefore, there is a critical need to develop new safe drugs based on the knowledge of the biochemistry and physiology of these microorganisms.

Rationale

The precise mechanism of action by which bisphophonates inhibit the enzymatic activity of the target enzyme remains unsolved. The main members of the 2-alkylaminoethyl bisphosphonates family were originally designed in order to maintain the ability to coordinate Mg^{2+} in a tridentate manner as 1-hydroxy- and 1-amino- derivatives do.[32] However, preliminary studies on the interaction of inhibitor **11** (IC_{50} = 58 nM) with *Tc*FPPS based on the X-ray crystallographic structure of **11**–*Tc*FPPS have indicated that the nitrogen atom did not coordinate[49] to the Mg^{2+} present at the active site of the target enzyme.[50,51] The tridentate coordination structure is circumvented to the hydroxyl groups bonded to the phosphorus atoms either for 2-alkylaminoethyl- or 1-hydroxy-1,1-bisphosphonates.[52,53] In addition, the X-ray structure of the complex risedronate–*Tc*FPPS indicated that the residue Asp250 forms a hydrogen bond with the hydroxyl group at the C-1 position of risedronate; an event not possible with the 2-aminoalkyl derivatives.[31,32,54] In connection with the above ideas, it has been postulated that the presence of an electron withdrawing group at C-1 would enhance the ability to coordinate Ca^{2+} or Mg^{2+} in a tridentate manner.[55–57] Derivatives where the hydroxyl group is absent, that is, replaced by a hydrogen atom would coordinate in a bidentate manner. In fact, we have found that these analogues exhibited less potency as inhibitors towards the target enzyme than 1-hydroxy,[34] or 1-amino derivatives.[35] In addition, the influence of the hydroxyl group at C-1 on biological action in many bisphosphonates remains unclear; as a matter of fact there exists strong evidence available showing that the hydroxyl group at C-1 does not interact with Mg^{2+} at the active site of FPPS, suggesting that this group would influence the capacity of the adjacent bisphosphonic moiety to chelate Mg^{2+} and to increase the pK_a of the *gem*-phopshonate functionality as well.

The introduction of a fluorine atom at the C-1 position of an 1-alkyl-1,1-bisphosphonate derivative, such as **8**, would seem of interest for a number of reasons: (a) isosteric replacement of hydroxyl groups or hydrogen atoms by fluorine atoms is usually employed in drug design based on the stability of the carbon–fluorine bond and the small atomic size that is beneficial for molecular recognition;[58] (b) the presence of a fluorine atom would increase metabolic stability; (c) 1-fluoro-1,1-bisphosphonic acids would mimic pK_a values of inorganic pyrophophoric acid quite well;[59–62] (d) these fluorine-containing bisphosphonic acid derivatives would increase acidity by at least one order of magnitude compared to conventional ones;[63] (e) it has been recently depicted that some fluorine-containing bisphosphonate with an aromatic ring at the side chain have proven to be inhibitors of the enzymatic activity of human FPPS.[64]

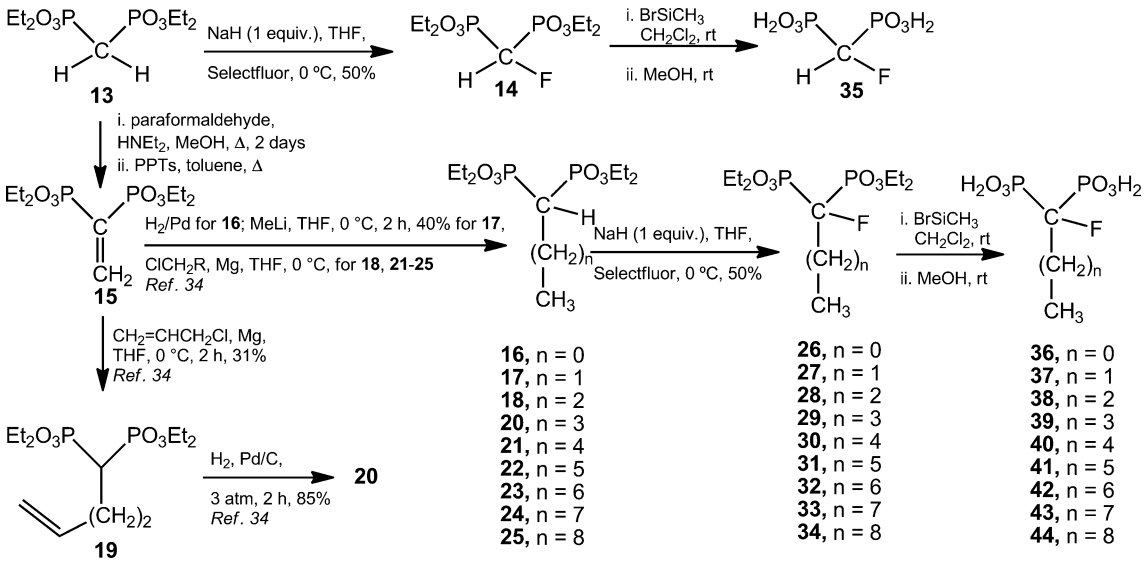
Results and discussion

The formation of a carbon–fluorine bond can be envisioned through a reaction between an appropriate carbanion and an electrophilic fluorine donor, such as Selectfluor™ (1-chloromethyl-4-fluoro-1,4-diazobicyclo[2.2.2]octane bis(tetrafluoroborate), F-TEDABF4).[65,66] Sodium hydride has proven to be a good base to form a carbanion at the carbon bridge of a *gem*-phosphonate unit to react with Selectfluor™ to afford the desired monofluoro bisphosphonate derivatives.[59,67,68] There were two synthetic approaches to follow in order to access our title compounds (α-fluorinated bisphosphonates derived from fatty acids) taking **7–12** as reference structures (Chart 2): (a) to employ the already depicted tetraethyl 1-(fluoromethylidene)-1,1-bisphosphonate (compound **14**) as a common synthetic intermediate.[67] Alkylation of the respective enolate-type carbanion of **14**[67] by treatment with different alkyl halides followed by hydrolysis of the alkylated α-fluoro-tetraethyl esters would yield the title compounds; (b) fluorination of the already depicted tetraethyl 1-alkyl-1,1-bisphosphonates[34] followed by hydrolysis of the resulting products would be the latter approach. It would be possible to obtain the corresponding precursors of the title compounds from the simplest fluorine-containing intermediate (compound **14**) *via* an alkylation reaction, as recently described.[68] However, the first route resulted to be impractical from the synthetic point of view and, in particular, this transformation was always been associated with low reaction yields. The presence of a fluorine atom would increase the acidity of the hydrogen atom bonded at C-1, resulting in a weak and less reactive conjugate base. We succeeded in preparing the title drugs using the second approach. Fluorination of compound **13** gave rise to **14** in 50% yield according to the literature.[67] We were able to fluorinate all of the tetraethyl 1-alkyl-1,1-bisphosphonates in moderate, but reproducible yields. Precursors (**16–25**) were obtained *via* either a hydrogenation reaction or a Michael addition on the already depicted tetraethyl ethenylidene bisphosphonate **15**.[69] Hydrolysis by treatment with bromotrimethylsilane in anhydrous methylene chloride followed by digestion with methanol afforded the desired 1-fluoro-1,1-bisphosphonic acids **35–44** (Scheme 2).

It is worth mentioning the difficulty of monitoring the course of the reaction by t.l.c. These reactions, which involved compounds without chromophore groups and without sensitivity for universal revelators, should be followed by NMR.

Biological evaluation of the title compounds **35–44** was very surprising. All of these compounds were almost devoid of antiparasitic activity against the amastigote form of *T. cruzi*. Only compound **43** exhibited a marginal activity against intracellular amastigotes, with 13% of growth inhibition at a concentration of 10 μM. In addition, these results were consistent with inhibition studies towards the enzymatic activity of FPPS. Then, compounds **35–44** were devoid of activity against *T. cruzi* FPPS. These data were quite unexpected bearing in mind the inhibition action exhibited by lineal structurally related bisphosphonates.[31–36] On the other hand, these fluorine-containing bisphosphonic acids exhibited an extremely potent activity as inhibitors of the enzymatic activity of *T. gondii* FPPS.[70] In fact, relatively aliphatic long chain derivatives, such as **40–44**, showed an inhibitory action at the nanomolar level. Compounds **43** and **44** arose as the main members of these drugs exhibiting an unusual potency against *Tg*FPPS, possessing IC_{50} values of 35 nM and 60 nM, respectively, and were more effective than risedronate (IC_{50} = 74 nM) taken as the positive control. This high selectively towards *Tg*FPPS *versus* *Tc*FPPS might be understood by a comparison of the amino acid sequence between both of these enzymes with less than 50% identity.[71] Compound **43** was able to control the growth of tachyzoites of *T. gondii*, exhibiting an IC_{50} value of 2.67 μM (Table 1).

In summary, lineal α-fluoro-1,1-bisphosphonic acids represent an interesting new family of bisphosphonates that was able to efficiently and selectively inhibit the enzymatic activity of



Scheme 2 Reagents and conditions for the preparation of the title compounds.

Table 1 Biological activity of 1-fluoro-1,1-bisphosphonates against *Tc*FPPS, *Tg*FPPS, *T. cruzi* (amastigotes), and tachyzoites of *T. gondii*

	IC_{50} (μM)	IC_{50} (μM)	IC_{50} (μM)	IC_{50} (μM)
Comp	*Tc*FPPS	*Tg*FPPS	*T. cruzi* amastigotes	*T. gondii* tachyzoites
35	>1	>10	>10	>10
36	>1	>10	>10	>10
37	>1	>10	>10	>10
38	>1	>10	>10	>10
39	>1	3.42 ± 1.67	>10	>10
40	>1	0.160 ± 0.078	>10	>10
41	>1	0.241 ± 0.120	>10	>10
42	>1	0.616 ± 0.259	>10	>10
43	>1	0.035 ± 0.019	13% at 10 μM	2.67
44	>1	0.060 ± 0.006	>10	>10
Risedronate	0.027 ± 0.003	0.074 ± 0.017	—	
Benznidazole	—		2.768 ± 0.488	

T. gondii farnesyl pyrophosphate synthase. Particularly, compound **43**, which contains nine carbon atoms in the aliphatic chain, exhibited superior activity against *T. gondii* FPPS. As a result of this enzymatic action, this compound was able to impair tachyzoites of *T. gondii* growth. Structural variation in the aliphatic chain that includes the addition of conformational restriction tools will be considered in the near future in order to optimize its structure. Work aimed at exploiting the potential usefulness of these drugs is currently being pursued in our laboratory.

Experimental section

The glassware used in air and/or moisture sensitive reactions was flame-dried and reactions were carried out under a dry argon atmosphere. Unless otherwise noted, chemicals were commercially available and used without further purification. Solvents were distilled before use. Tetrahydrofuran and ethyl ether were distilled from sodium/benzophenone ketyl. Anhydrous *N*,*N*-dimethylformamide was used as supplied from Aldrich. Tetraethyl methylenebisphosphonate and Selectfluor™ were purchased from Aldrich.

Nuclear magnetic resonance spectra were recorded using a Bruker AM-500 MHz or a Bruker AC-200 MHz spectrometers. Chemical shifts are reported in parts per million (δ) relative to tetramethylsilane. Coupling constants are reported in Hertz. ^{13}C-NMR spectra were fully decoupled. ^{31}P-NMR spectra were fully decoupled. Chemical shifts are reported in parts per million relative to phosphoric acid. Splitting patterns are designated as s, singlet; d, doublet; t, triplet; q, quartet.

High resolution mass spectra were conducted using a Bruker micrOTOF-Q II spectrometer, which is a hybrid quadrupole time of flight mass spectrometer with MS/MS capability. Low-resolution mass spectra were obtained on a VG TRIO 2 or VG ZAB2-SEQ instruments in electron impact mode at 70 eV (direct inlet).

Melting points were determined using a Fisher–Johns apparatus and are uncorrected. IR spectra were recorded using a Nicolet Magna 550 spectrometer.

Column chromatography was performed with E. Merck silica gel (Kieselgel 60, 230–400 mesh). Analytical thin layer chromatography was performed employing 0.2 mm coated commercial silica gel plates (E. Merck, DC-Aluminum sheets, Kieselgel 60 F_{254}).

Elemental analyses were performed by Atlantic Microlab, Norcross, Georgia, USA.

As judged from the homogeneity of the ^{1}H, ^{13}C, ^{31}P NMR spectra of the title compounds **35–44** and HPLC analyses of the committed intermediates **14**, **16–18**, **20–25**, **26–34**, employing a Beckmann Ultrasphere ODS-2 column 5 μM, 250 × 10 mm eluting with acetonitrile–water (1 : 1) at 3.00 mL min^{-1} with a refractive index detector indicated a purity >97%.

Tetraethyl ethylidene-1,1-bisphosphonate (16)

A solution of **15** (200 mg, 0.66 mmol) in ethyl acetate (10 mL) in the presence of 10% palladium on charcoal (30 mg) was treated with hydrogen under 3 atm at room temperature for 4 h. Then the reaction mixture was filtered and the solvent was evaporated to afford 190 mg (95% yield) of compound **16** as a colorless oil: R_f 0.20 (EtOAc); IR (film, cm^{-1}) 2985, 1645, 1251, 1022, 968, 819; ^{1}H NMR (200.13 MHz, $CDCl_3$) δ 1.34 (t, J = 7.1 Hz, 12H, H-2′), 1.46 (dt, J = 17.4, 7.3 Hz, 3H, H-2), 2.39 (tq, J = 30.8, 7.2 Hz, 1H, H-1), 4.18 (m, 8H, H-1′); ^{13}C NMR (50.33 MHz, $CDCl_3$) δ 10.1 (t, J = 6.1 Hz, C-2), 16.3 (d, J = 5.4 Hz, C-2′), 31.0 (t, J = 136.3 Hz, C-1), 62.46 (d, J = 6.8, C-1′); ^{31}P NMR (202.45 MHz, $CDCl_3$) δ 21.47; MS (m/z, relative intensity) 302 (M^+, 1), 275 (5), 247 (6), 191 (13), 165 (100).

Tetraethyl *n*-propylidene-1,1-bisphosphonate (17)

A solution of **15** (200 mg, 0.66 mmol) in anhydrous tetrahydrofuran (10 mL) cooled at −78 °C under argon atmosphere was treated with 1.6 M of methyllithium in diethyl ether (0.4 mL). The reaction mixture was stirred at 0 °C for 2 h. The reaction was worked up by addition of an aqueous saturated solution of ammonium chloride (10 mL). The aqueous phase was extracted with chloroform (3 × 10 mL). The combined organic layers were dried ($MgSO_4$) and the solvent was evaporated. The residue was purified by column chromatography (silica gel) eluting with hexane–EtOAct (9 : 1) as eluent to give 83.2 mg (40% yield) of **17** as a colorless oil: R_f 0.67 (CH_2Cl_2–^{i}PrOH, 19 : 1); IR (film, cm^{-1}) 3664, 2983, 1246, 1024, 962; ^{1}H NMR (200.13 MHz, $CDCl_3$) δ 1.15 (t, J = 7.3 Hz, 3H, H-3), 1.33 (t, J = 7.1 Hz, 12H, H-2′), 1.94 (m, 2H, H-2), 2.20 (tt, J = 23.9, 5.8 Hz, 1H, H-1), 4.17 (m, 8H, H-1′); ^{13}C NMR (125.77 MHz, $CDCl_3$) δ 13.9 (dd, J = 6.3, 3.6 Hz, C-2), 16.4 (dd, J = 6.8, 3.4 Hz, C-2′), 19.1 (t, J = 5.5 Hz, C-3), 38.2 (t, J = 133.5 Hz, C-1), 62.7 (dd, J = 15.4, 6.4 Hz, C-1′); ^{31}P NMR (202.45 MHz, $CDCl_3$) 23.84; MS (m/z, relative intensity) 316 (M^+, 1), 301 (11), 288 (17), 261 (14), 233 (15), 179 (100), 123 (45).

Tetraethyl *n*-butylidene-1,1-bisphosphonate (18)

A solution of ethyl iodide (1.0 mL, 8.3 mmol) in anhydrous ethyl ether (10 mL) was added to magnesium turnings (204.8 mg, 8.3 mmol) in the presence of iodine (30 mg) under an argon atmosphere. The reaction mixture was stirred at room temperature for 2 h. The resulting dark gray mixture was added slowly *via* a cannula to a solution of **15** (500 mg, 1.6 mmol) in ethyl ether (10 mL) cooled at 0 °C. The mixture was stirred for 2 h and the reaction was quenched, as described for the preparation of **17**. The residue was purified by column chromatography (silica gel), eluting with hexane–EtOAc (4 : 1) to afford 450 mg (85% yield) of **18** as a colorless oil: R_f 0.72 (AcOEt–^{i}PrOH–H_2O, 6 : 3 : 1); IR (film, cm^{-1}) 2980, 1394, 1245, 1165, 1033, 969; ^{1}H NMR (200.13 MHz, $CDCl_3$) δ 0.89 (t, J = 7.3 Hz, 3H, H-4), 1.31 (t, J = 6.9 Hz, 12H, H-2′), 1.55 (m, 2H, H-3), 1.86 (m, 2H, H-2), 2.25 (tt, J = 24.1, 5.8 Hz, 1H, H-1), 4.14 (m, 8H, H-1′); ^{13}C NMR (50.33 MHz, $CDCl_3$) δ 13.7 (C-4), 16.3 (d, J = 6.8 Hz, C-2′), 26.3 (t, J = 6.8 Hz, C-2), 27.5 (t, J = 4.7 Hz, C-3), 36.5 (t, J = 133.6 Hz, C-1), 62.4 (d, J = 7.5 Hz, C-1′); ^{31}P NMR (202.45 MHz, $CDCl_3$) δ 24.15; MS (m/z, relative intensity) 331 ([M + 1]$^+$, 11), 301 (34), 288 (69), 261 (38), 193 (100), 165 (53), 152 (67), 137 (81). Anal. calcd for $C_{12}H_{28}O_6P_2 \cdot H_2O$: C 41.38, H 8.68; found C 41.64, H 8.78.

Tetraethyl *n*-pent-4-enylidene-1,1-bisphosphonate (19)

A solution of allyl chloride (0.4 mL, 5.0 mmol) in anhydrous tetrahydrofuran (10 mL) was added to magnesium turnings (121.5 mg, 5.0 mmol) in the presence of iodine (20 mg) under an argon atmosphere. The mixture was stirred at room temperature for 2 h. Then the mixture was cooled at 0 °C and a solution of **15** (415.7 mg, 1.38 mmol) in tetrahydrofuran (5 mL) was added. The reaction mixture was treated as described for the preparation of **18**. The residue was purified by column chromatography (silica gel), eluting with hexane–EtOAc (1 : 1) to afford 150 mg (31% yield) of **19** as a colorless oil: R_f 0.75 (AcOEt–^{i}PrOH–H_2O, 6 : 3 : 1); IR (film, cm^{-1}) 2983, 2933, 2910, 1641, 1456, 1392, 1247, 1165, 1024, 972; ^{1}H NMR (200.13 MHz, $CDCl_3$) δ 1.35 (t, J = 7.1 Hz, 12H, H-2′), 2.19 (m, 5H, H-1, H-2, H-3); 4.19 (m, 8H, H-1′), 5.07 (m, 2H, H-5), 5.77 (ddt, J = 17.1, 10.2, 6.7 Hz, 1H, H-4); ^{13}C NMR (50.33 MHz, $CDCl_3$) δ 16.3 (t, J = 6.8 Hz, C-2′), 24.6 (t, J = 4.7 Hz, C-2), 32.7 (t, J = 6.1 Hz, C-3), 35.6 (t, J = 133.6, C-1), 62.5 (t, J = 6.8 Hz, C-1′), 116.0 (C-5), 137.1 (C-4); ^{31}P NMR δ (202.45 MHz, $CDCl_3$) 24.13; MS (m/z, relative intensity) 343 ([M + 1]$^+$, 5), 288 (70), 261 (51), 233 (45), 205 (100), 152 (92), 109 (50).

Tetraethyl *n*-pentylidene-1,1-bisphosphonate (20)

A solution of **19** (100 mg, 0.29 mmol) in ethyl acetate (10 mL) in the presence of 10% palladium on charcoal (30 mg) was treated as described for the preparation of compound **16** to give 85.1 mg (85% yield) of **20** as a colorless oil: IR (film, cm^{-1}) 2960, 1662, 1471, 1247, 1024, 970; ^{1}H NMR (500.13 MHz, $CDCl_3$) δ 0.91 (t, J = 7.1 Hz, 3H, H-5), 1.35 (t, J = 7.1, 12H, H-2′), 1.73 (m, 6H, -CH_2-), 2.28 (tt, J = 24.1, 6.0 Hz, 1H, H-1) 4.17 (m, 8H, H-1′); ^{13}C NMR (125.77 MHz, $CDCl_3$) δ 13.7 (C-5), 16.3 (dd, J = 6.4, 2.3 Hz, C-2′), 22.4 (C-4), 25.2 (t, J = 5.0 Hz, C-3), 31.2 (t, J = 6.4 Hz, C-2), 36.7 (t, J = 133.4 Hz, C-1), 62.4 (dd, J = 18.5, 6.7 Hz, C-1′); ^{31}P NMR (202.45 MHz, $CDCl_3$) δ 24.14; MS (m/z, relative intensity) 344 (M^+, 5), 301 (12), 288 (28), 207 (21), 152 (27), 57 (46), 41 (100).

Tetraethyl *n*-hexylidene-1,1-bisphosphonate (21)

A solution of butyl chloride (0.42 mL, 4.3 mmol) in anhydrous tetrahydrofuran (10 mL) was treated with magnesium turnings (100 mg, 4.3 mmol), as described for the preparation of **19**. Purification by column chromatography (silica gel), eluting with hexane–EtOAc (4 : 1) afforded 48 mg (33% yield) of **21** as a colorless oil: R_f 0.20 (AcOEt–^{i}PrOH, 19 : 1); IR (film, cm^{-1}) 2983, 1662, 1458, 1394, 1247, 1165, 1026, 972, 804; ^{1}H NMR (200.13 MHz, $CDCl_3$) δ 0.88 (t, J = 6.6 Hz, 3H, H-6), 1.33 (t, J = 7.1 Hz, 12H, H-2′), 1,25–1,4 (m, 4H, H-5, H-4) 1.59 (m, 2H, H-3), 1.91

(m, 2H, H-2), 2.26 (tt, J = 24.1, 5.8 Hz, 1H, H-1), 4.17 (m, 8H, H-1′); ^{13}C NMR (50.33 MHz, $CDCl_3$) δ 13.9 (C-6), 16.3 (d, J = 5.4 Hz, C-2′), 22.2 (C-5), 25.5 (t, J = 4.8 Hz, C-3), 28.8 (t, J = 6.1 Hz, C-2), 31.5 (C-4), 36.8 (t, J = 132.9 Hz, C-1), 62.4 (t, J = 6.8 Hz, C-1′); ^{31}P NMR (202.45 MHz, $CDCl_3$) δ 24.16; MS (m/z, relative intensity) 359 ([M + 1]$^+$, 2), 301 (39), 288 (97), 261 (47), 233 (32), 221 (100), 165 (55), 152 (88). Anal. calcd for $C_{14}H_{32}O_6P_2 \cdot H_2O$: C 44.68, H 9.11; found C 44.97, H 8.71.

Tetraethyl *n*-heptylidene-1,1-bisphosphonate (22)

A solution of *n*-pentyl bromide (0.62 mL, 5,0 mmol) in anhydrous tetrahydrofuran (10 mL) was treated with magnesium turnings (121.5 mg, 5.0 mmol), as described for the preparation of **19**. The residue was purified by column chromatography (silica gel), eluting with hexane–EtOAc (4 : 1) to yield 63.2 mg (17% yield) of **22** as a colorless oil: R_f 0.23 (AcOEt–^{i}PrOH, 19 : 1); IR (film, cm^{-1}) 2981, 2929, 2860, 1647, 1471, 1394, 1249, 1165, 1026, 970, 839, 792; ^{1}H NMR (500.13 MHz, $CDCl_3$) δ 0.88 (t, J = 6.9 Hz, 3H, H-7), 1.34 (t, J = 7.1 Hz, 12H, H-2′), 1.30 (m, 6H, –CH_2–), 1.55 (p, J = 7.4 Hz, 2H, H-3), 1.91 (m, 2H, H-2), 2.27 (tt, J = 24.2, 6.0 Hz, 1H, H-1), 4.17 (m, 8H, H-1′); ^{13}C NMR (125.77 MHz, $CDCl_3$) δ 14.0 (C-7), 16.4 (dd, J = 6.6, 3.0 Hz, C-2′), 22.5 (C-6), 25.5 (t, J = 5.5 Hz, C-3), 28.9 (C-4), 29.1 (t, J = 6.4 Hz, C-2), 31.4 (C-5), 36.7 (t, J = 133.2 Hz, C-1), 62.4 (dd, J = 18.5, 6.8 Hz, C-1′); ^{31}P NMR (202.45 MHz, $CDCl_3$) δ 24.16; MS (m/z, relative intensity) 373 ([M + 1]$^+$, 10), 301 (39), 288 (90), 261 (40), 235 (100), 207 (24), 189 (15), 179 (42), 165 (24), 152 (72), 137 (27), 125 (21). Anal. calcd for $C_{15}H_{34}O_6P_2$: C 46.92, H 9.00; found C 47.41, H 9.13.

Tetraethyl *n*-octylidene-1,1-bisphosphonate (23)

A solution of *n*-hexyl bromide (0.70 mL, 5.0 mmol) in anhydrous tetrahydrofuran (10 mL) was treated with magnesium turnings (121.5 mg, 5.0 mmol), as described for the preparation of **19**. The product was purified by column chromatography (silica gel), eluting with hexane–EtOAc (2 : 3) to afford 112.6 mg (30% yield) of **23** as a colorless oil: R_f 0.22 (AcOEt–^{i}PrOH, 19 : 1); IR (film, cm^{-1}) 2927, 2856, 1652, 1465, 1394, 1247, 1165, 1026, 970, 856, 800; ^{1}H NMR (500.13 MHz, $CDCl_3$) δ 0.87 (t, J = 6.8 Hz, 3H, H-8), 1.32 (m, 20H, –CH_2–, H-2′), 1.55 (m, 2H, H-3), 1.92 (m, 2H, H-2), 2.27 (tt, J = 24.2, 6.1 Hz, 1H, H-1), 4.17 (m, 8H, H-1′); ^{13}C NMR (125.77 MHz, $CDCl_3$) δ 14.1 (C-8), 16.4 (dd, J = 5.9, 2.9 Hz, C-2′), 22.6 (C-7), 25.5 (t, J = 5.4 Hz, C-3), 28.9 (C-4), 29.1 (t, J = 6.3 Hz, C-2), 29.3 (C-5), 31.7 (C-6), 36.8 (t, J = 133.5 Hz, C-1) 62.4 (dd, J = 18.8, 6.7 Hz, C-1′); ^{31}P NMR (202.45 MHz, $CDCl_3$) δ 24.15; MS (m/z, relative intensity) 387 ([M + 1]$^+$, 3), 301 (42), 288 (100), 261 (37), 249 (96), 193 (30), 152 (67). Anal. calcd for $C_{16}H_{36}O_6P_2 \cdot 0.2H_2O$: C 49.27, H 9.41; found C 49.21, H 9.61.

Tetraethyl *n*-nonylidene-1,1-bisphosphonate (24)

A solution of *n*-heptyl chloride (0.76 mL, 5.0 mmol) in anhydrous tetrahydrofuran (10 mL) was treated with magnesium turnings (121.5 mg, 5.0 mmol), as described for the preparation of **19**. Column chromatography purification, eluting with hexane–EtOAc (9 : 1) afforded 160 mg (40% yield) of **24** as a colorless oil: R_f 0.28 (AcOEt–^{i}PrOH, 19 : 1); IR (film, cm^{-1}) 2927, 2856, 1647, 1467, 1444, 1392, 1251, 1165, 1024, 970, 835, 794; ^{1}H NMR (200.13 MHz, $CDCl_3$) δ 0.87 (t, J = 6.4 Hz, 3H, H-9), 1.32 (m, 10H, -CH_2-), 1.34 (t, J = 7.1 Hz, 12H, H-2′), 1.56 (m, 2H, H-3), 1.90 (m, 2H, H-2), 2.26 (tt, J = 23.9, 6.0 Hz, 1H, H-1), 4.17 (m, 8H, H-1′); ^{13}C NMR (50.33 MHz, $CDCl_3$) δ 14.0 (C-9), 16.4 (d, J = 6.8 Hz, C-2′), 22.6 (C-8), 25.5 (t, J = 5.4 Hz, C-3), 25.7 (C-4), 29.1 (t, J = 6.5 Hz, C-2), 29.2 (C-5),* 29.3 (C-6),* 31.8 (C-7), 36.7 (t, J = 133.6 Hz, C-1), 62.4 (t, J = 7.5 Hz, C-1′); ^{31}P NMR (202.45 MHz, $CDCl_3$) δ 24.15; MS (m/z, relative intensity) 401 ([M + 1]$^+$, 28), 301 (38), 288 (100), 263 (90), 261 (35), 152 (58). Anal. calcd for $C_{17}H_{38}O_6P_2 \cdot H_2O$: C 48.80, H 9.64; found C 48.48, H 9.57.

Tetraethyl *n*-decylidene-1,1-bisphosphonate (25)

A solution of *n*-octyl chloride (0.85 mL, 5.0 mmol) in anhydrous tetrahydrofuran (10 mL) was treated with magnesium turnings (121.5 mg, 5.0 mmol), as described for the preparation of **19**. The product was purified by column chromatography (silica gel), eluting with hexane–EtOAc (19 : 1) to afford 186.3 mg (45% yield) of **25** as a colorless oil: R_f 0.12 (hexane–EtOAc, 4 : 1); IR (film, cm^{-1}): 2979, 2929, 2873, 1458, 1392, 1251, 1165, 1026, 968, 813; ^{1}H NMR (200.13 MHz, $CDCl_3$) δ 0.89 (t, J = 6.7 Hz, 3H, H-10), 1.28 (m, 12H, –CH_2–), 1.34 (t, J = 6.7 Hz, 12H, H-2′), 1.51 (m, 2H, H-3), 1.89 (m, 2H, H-2), 2.22 (tt, J = 24.3, 5.8 Hz, 1H, H-1), 4.17 (m, 8H, H-1′); ^{13}C NMR (50.33 MHz, $CDCl_3$) δ 14.0 (C-10), 16.3 (d, J = 6.8 Hz, C-2′), 23.0 (C-9), 21.7 (t, J = 5.4 Hz, C-3), 29.1 (t, J = 6.2 Hz, C-2), 29.5 (C-4, C-7), 28,7 (C-5, C-6), 32.4 (C-8), 37.0 (t, J = 133.6 Hz, C-1), 62.3 (t, J = 6.1 Hz, C-1′); ^{31}P NMR (202.45 MHz, $CDCl_3$) δ 24.15; MS (m/z, relative intensity) 415 ([M+1]$^+$, 20), 385 (5), 371 (5), 357 (6), 315 (10), 301 (28), 288 (100), 277 (67), 261 (32), 165 (23), 152 (49). Anal. calcd for $C_{18}H_{40}O_6P_2 \cdot H_2O$: C 49.99, H 9.79; found C 50.39, H 9.76.

Fluorination reaction: general procedure

Sodium hydride (10.5 mmol, 60% in mineral oil) was washed with anhydrous hexane under an argon atmosphere and then anhydrous tetrahydrofuran (190 mL) was added. The suspension was cooled to 0 °C and, in independent experiments, each tetraethyl 1-alkyl-1,1-bisphosphonate, **14**, **16–18**, **20–25** (10.0 mmol), in anhydrous tetrahydrofuran (10 mL) was added. The solution was stirred at 0 °C for 15 min, then the mixture was allowed to reach room temperature and was stirred for 60 min. The mixture was cooled to 0 °C and Selectfluor™ (12.5 mmol) was added in one portion followed by the addition of anhydrous *N*,*N*-dimethylformamide (3.5 mL). The reaction mixture was allowed to reach room temperature and was stirred for 4 h. The mixture was partitioned between methylene chloride (30 mL) and an aqueous saturated solution of ammonium chloride (30 mL). The aqueous phase was extracted with methylene chloride (2 × 30 mL). The combined organic layers were washed with water (2 × 30 mL), dried (MgSO4) and the solvent was evaporated. The residue was purified by column chromatography, eluting with mixtures of (EtOAc–CH_3OH) in a ratio indicated in each case to afford the corresponding 1-fluoro tetraethyl ester derivatives (compounds **14**, **26–34**).

Tetraethyl 1-(fluoromethylidene)-1,1-bisphosphonate (14). 50% yield; colorless oil; IR (film, cm^{-1}) 2987, 2935, 2914, 1479, 1444, 1394, 1371, 1261, 1164, 1028, 981, 869, 794; ^{1}H NMR (500.13 MHz, $CDCl_3$) δ 1.38 (dt, J = 7.1, 1.4 Hz, 12H, H-2′), 4.29 (m, 8H, H-1′), 5.01 (dt, J = 45.9, 13.9 Hz, H-1); ^{13}C NMR (50.3 MHz,

$CDCl_3$) δ 15.8 (C-2′), 63.9 (dt, J = 17.7, 2.7 Hz, Hz, C-1′), 82.78 (dt, J = 192.0, 157.1 Hz, C-1), ^{31}P NMR (202.46 MHz, $CDCl_3$) δ 11.08 (d, J = 74.6 Hz); HRMS (ESI) calcd for ($C_9H_{21}O_6P_2F$) [M + Na]$^+$: 329.0695; found 329.0697.

Tetraethyl 1-(fluoroethylidene)-1,1-bisphosphonate (26). 43% yield; colorless oil; IR (film, cm^{-1}) 2984, 2935, 2874, 1445, 1393, 1369, 1256, 1165, 1024, 970, 845, 816, 793, 536; ^{1}H NMR (500.13 MHz, $CDCl_3$) δ 1.37 (d, J = 7.1 Hz, 12H, H-2′), 1.83 (dt, J = 25.7, 15.4 Hz, 3H, H-2), 4.28 (m, 8H, H-1′);^{13}C NMR (125 MHz, $CDCl_3$) δ 16.4 (dd, J = 7.1, 2.9 Hz, C-2), 19.1 (d, J = 20.9 Hz, C-2′), 64.0 (dt, J = 28.0, 3.2 Hz, C-1′), 93.0 (dt, J = 183.9, 160.5 Hz, C-1); ^{31}P NMR (202.46 MHz, $CDCl_3$) δ 14.92 (d, J = 72.1 Hz). HRMS (ESI) calcd for ($C_{10}H_{24}O_6P_2F$) [M + H]$^+$: 321.1032; found 321.1022.

Tetraethyl 1-(fluoro-*n*-propylidene)-1,1-bisphosphonate (27). 36% yield; colorless oil; IR (film, cm^{-1}) 2981, 2933, 1444, 1394, 1369, 1263, 1164, 1022, 972, 784, 769; ^{1}H NMR (500.13 MHz, $CDCl_3$) δ 1.17 (t, J = Hz, 3H, H-3), 1.37 (t, J = Hz, 12H, H-2′), 2.24 (m, 2H, H-2), 4.29 (m, 8H, H-1′); ^{13}C NMR (125.77 MHz, $CDCl_3$) δ 7.8 (dd, J = 12.7, 4.6 Hz), 16.4 (dt, J = 5.9, 3.0 Hz), 26.5 (d, J = 20.9 Hz, C-2′), 63.8 (dt, J = 28.2, 3.2 Hz, C-1′); ^{31}P NMR (202.46 MHz, $CDCl_3$) δ 15.00 (d, J = 74.6 Hz). HRMS (ESI) calcd for ($C_{11}H_{25}FO_6P_2Na$) [M + Na]$^+$: 357.1008; found 357.1013.

Tetraethyl 1-(fluoro-*n*-butylidene)-1,1-bisphosphonate (28). 47% Yield; Colorless oil; IR (film, cm^{-1}) 2981, 2935, 2875, 1649, 1444, 1392, 1369, 1261, 1164, 1022, 972, 794, 746; ^{1}H NMR (500.13 MHz, $CDCl_3$) δ 0.97 (t, J = 7.3 Hz, 3H, H-4), 1.38 (t, J = 7.0 Hz, 12H, H-2′), 1.68 (m, 2H, H-3), 2.14 (dtt, J = 47.0, 15.1, 8.6 Hz, 2H, H-2), 4.25 (m, 8H, H-1′); ^{13}C NMR (125.77 MHz, $CDCl_3$) δ 14.52 (C-4), 16.43 (p, J = 2.9 Hz, C-2′), 16.54 (q, J = 5.4 Hz), 35.29 (d, J = 20.0 Hz), 63.87 (dt, J = 27.4, 2.4 Hz, C-1′), 95.86 (dt, J = 187.3, 156.2 Hz, C-1); ^{31}P NMR (202.46 MHz, $CDCl_3$) δ 14.52 (d, J = 74.6 Hz). HRMS (ESI) calcd for ($C_{12}H_{28}FO_6P_2$) [M + H]$^+$: 349.1345; found: 349.1348.

Tetraethyl 1-(fluoro-*n*-pentylidene)-1,1-bisphosphonate (29). 49% yield; colorless oil; IR (film, cm^{-1}) 2964, 2934, 2874, 1445, 1393, 1261, 1165, 1024, 975, 797, 540; ^{1}H NMR (500.13 MHz, $CDCl_3$) δ 0.93 (t, J = 7.4 Hz, 3H, H-5), 1.37 (t, J = 7.1 Hz, 12H, H-2′), 1.70 (br s, 2H), 2.17 (m, 2H, H-2), 4.27 (m, 8H, H-1′); ^{13}C NMR (125.77 MHz, $CDCl_3$) δ 13.76, 14.43 (q, J = 2.9 Hz, C-2′), 23.09, 25.04 (t, J = 5.5. Hz), 32.99 (d, J = 20.1 Hz), 63.86 (dt, J = 27.2, 3.2 Hz, C-1′); ^{31}P NMR (202.46 MHz, $CDCl_3$) δ 15.79 (d, J = 74.6 Hz). HRMS (ESI) calcd for ($C_{13}H_{30}O_6P_2F$) [M + H]$^+$: 363.1502; found 363.1508.

Tetraethyl 1-(fluoro-*n*-hexylidene)-1,1-bisphosphonate (30). 57% yield; colorless oil; ^{1}H NMR (500.13 MHz, $CDCl_3$) δ 0.90 (t, J = 7.1 Hz, 3H, H-5), 1.30 (m, 8H, H-4, H-5, H-6, H-7), 1.37 (t, J = 7.1 Hz, 12H, H-2′),1.64 (p, J = 7.2 Hz, 2H, H-3), 2.15 (dt, J = 22.3, 15.3 Hz, H-2), 4.28 (m, 8H, H-1′); ^{13}C NMR (125.77 MHz, $CDCl_3$) δ 13.88 (C-6), 16.39 (p, J = 3.0 Hz, C-2′), 22.23 (C-5), 22.57 (q, J = 5.4 Hz, C-3), 32.09 (C-4), 33.17 (d, J = 20.0 Hz, C-2), 63.81 (dt, J = 27.2, 3.2 Hz, C-1′), 95.85 (dt, J = 187.1, 156.2 Hz, C-1); ^{31}P NMR (202.46 MHz, $CDCl_3$) δ 15.02 (d, J = 74.6 Hz).

Tetraethyl 1-(fluoro-*n*-heptylidene)-1,1-bisphosphonate (31). 35% yield; colorless oil; IR (film, cm^{-1}) 2984, 2961, 2932, 2872, 2858, 1456, 1393, 1369, 1261, 1164, 1022, 976, 794, 576; ^{1}H NMR (500.13 MHz, $CDCl_3$) δ 0.88 (t, J = 6.9 Hz, 3H, H-7), 1.30 (m, 6H, H-4, H-5, H-6), 1.36 (t, J = 7.1 Hz, 12H, H-2′), 1.64 (m, 2H, H-3), 2.15 (m, 2H, H-2), 4.26 (m, 8H, H-1′); ^{13}C NMR (125.77 MHz, $CDCl_3$) δ 13.98 (C-7), 16.38 (p, J = 2.7 Hz, C-2′), 22.45 (C-6), 22.84 (q, J = 5.4 Hz, C-3), 29.59 (C-5), 31.38 (C-4), 33.21 (d, J = 20.1 Hz, C-2), 63.78 (dt, J = 27.2, 3.2 Hz, C-1′), 95.84 (dt, J = 188.0, 156.2 Hz, C-1); ^{31}P NMR (202.46 MHz, $CDCl_3$) δ 14.76 (d, J = 75.1 Hz). Calcd for ($C_{15}H_{34}FO_6P_2$) [M + Na]$^+$: 413.1634; found: 413.1635.

Tetraethyl 1-(fluoro-*n*-octylidene)-1,1-bisphosphonate (32). 48% yield; colorless oil; IR (film, cm^{-1}) 2982, 2959, 2930, 2858, 2358, 1452, 1393, 1258, 1165, 1026, 970, 797, 580, 538; ^{1}H NMR (500.13 MHz, $CDCl_3$) δ 0.90 (t, J = 7.0 Hz, 3H, H-8), 1.30 (t, J = 7.1 Hz, 12H, H-2′), 2.15 (dtt, J = 23.8, 15.3, 8.4 Hz, 2H, H-2), 4.27 (m, 8H, H-1′); ^{13}C NMR (125.77 MHz, $CDCl_3$) δ 14.06 (C-8), 16.46 (p, J = 3.1 Hz, C-2′), 22.59 (C-7), 22.96 (q, J = 5.3 Hz, C-3), 28.90 (C-6), 29.93 (C-5), 31.70 (C-4), 33.27 (d, J = 19.8 Hz, C-2), 63.87 (dt, J = 27.3, 2.8 Hz, C-1′), 95.84 (dt, J = 188.0, 156.2 Hz, C-1); ^{31}P NMR (202.46 MHz, $CDCl_3$) δ 14.58 (d, J = 74.6 Hz). (F8OEt) HRMS (ESI) calcd for ($C_{16}H_{36}O_6P_2F$) [M + H]$^+$: 405.1971; found 405.1977.

Tetraethyl 1-(fluoro-*n*-nonylidene)-1,1-bisphosphonate (33). 24% yield; colorless oil; IR (film, cm^{-1}) 2981, 2959, 2928, 2856, 1467, 1392, 1369, 1261, 1163, 1024, 974, 797, 663, 577, 540; ^{1}H NMR (500.13 MHz, D_2O) δ 0.88 (t, J = 7.0 Hz, 3H, H-9), 1.30 (m, 10H), 1.37 (t, J = 7.1 Hz, 12H, H-2′), 1.64 (p, J = 7.3 Hz, 2H, H-3), 2.15 (dtt, J = 47.3, 15.3, 8.3 Hz, 2H, H-2), 4.27 (m, 8H, H-1′); ^{13}C NMR (125.77 MHz, $CDCl_3$) δ 14.06 (C-9), 16.43 (p, J = 2.7 Hz, C-2≡), 22.62 (C-8), 22.93 (q, J = 5.4 Hz, C-3), 29.15 (C-7), 29.19 (C-6), 29.98 (C-5), 31.80 (C-4), 33.25 (d, J = 20.1 Hz, C-2), 63.83 (dt, J = 27.3, 2.7 Hz, C-1′); ^{31}P NMR (202.46 MHz, $CDCl_3$) δ 15.01 (d, J = 74.6 Hz). HRMS (ESI) calcd for ($C_{17}H_{38}O_6P_2F$) [M + H]$^+$: 419.2128; found 419.2131.

Tetraethyl 1-(fluoro-*n*-decylidene)-1,1-bisphosphonate (34). 33% yield; colorless oil; IR (film, cm^{-1}) 2982, 2961, 2926, 2856, 1468, 1450, 1393, 1369, 1261, 1165, 1024, 976, 797, 663, 540; ^{1}H NMR (500.13 MHz, $CDCl_3$) δ 0.90 (t, J = 6.9 Hz, 3H, H-10), 1.32 (m, 6H, CH_2), 1.39 (t, J = 7.1 Hz, 8H, H-2′), 1.63 (m, 2H, H-3), 2.12 (dtt, J = 47.4, 15.4, 8.4 Hz, 2H, H-2), 4.26 (m, 8H, H-1′);^{13}C NMR (125.77 MHz, $CDCl_3$) δ 14.1 (C-10), 16.5 (p, J = 2.9 Hz, C-2′), 22.6 (C-9), 23.0 (q, J = 5.5 Hz, C-3), 29.2 (C-6), 29.5 (C-7), 30.0 (C-5), 31.9 (C-4), 33.3 (d, J = 19.6 Hz, C-2), 63.9 (dt, J = 27.9, 3.2 Hz, C-1′); ^{31}P NMR (202.46 MHz, $CDCl_3$) δ 14.78 (d, J = 74.6 Hz). HRMS (ESI) calcd for ($C_{10}H_{39}O_6P_2FNa$) [M + Na]$^+$: 455.2104; found 455.2105.

Silylation/methanolysis: general procedure

To a solution of the resulting tetraethyl ester (**14**, **26–34**; 1 equivalent) in anhydrous methylene chloride was added dropwise trimethylsilyl bromide (10 equivalents) in an argon atmosphere. The reaction mixture was stirred at room temperature for 48 h. After cooling at 0 °C, anhydrous methanol (10 mL) was added and the resulting mixture was allowed to reach room temperature. The solution was then concentrated under reduced pressure. The residue was dissolved in dry methanol (10 mL) and subsequently concentrated twice under reduced pressure. The solvent was evaporated and the residue was purified by reverse phase column

chromatography or crystallized from ethanol–water to afford the title compounds **35–44**.

1-(Fluoromethylene)-1,1-bisphosphonic acid (35). 93% yield; syrup; ^{1}H NMR (500.13 MHz, D_2O) δ 4.78 (dt, J = 45.1, 12.7 Hz, H-1); ^{13}C NMR (50.3 MHz, DMSO-d_6) δ 86.2 (dt, J = 185.3, 148.4 Hz, C-1); ^{31}P NMR (202.46 MHz, D_2O) δ 10.06 (d, J = 64.7 Hz); HRMS (ESI) calcd for ($CH_5O_6P_2F$) [M + Na]$^+$: 216.9443; found 216.9438.

1-(Fluoroethylidene)-1,1-bisphosphonic acid (36). 96% yield; syrup; IR (KBr, cm^{-1}) 3406, 2985, 2929, 2866, 1731, 1681, 1400, 1220, 1024, 935, 825, 767, 628, 518; ^{1}H NMR (500.13 MHz, D_2O) δ 1.39 (dt, J = 27.3, 14.8 Hz, H-2); ^{13}C NMR (125 MHz, CD_3OD) δ 19.8 (d, J = 20.9 Hz, C-2), 94.21 (dt, J = 180.3, 152.8 Hz, C-1); ^{31}P NMR (202.46 MHz, D_2O) δ 12.24 (d, J = 69.7 Hz). HRMS (ESI) calcd for ($C_2H_8O_6P_2F$) [M + H]$^+$: 208.9780; found 208.9780.

1-(Fluoro-*n*-propylidene)-1,1-bisphosphophonic acid (37). 96% yield; syrup; IR (KBr, cm^{-1}) 3604, 2995, 2950, 2931, 2314, 1728, 1703, 1467, 1205, 1022, 956, 763, 626; ^{1}H NMR (500.13 MHz, D_2O) δ 1.01 (t, J = 7.5 Hz, 3H, H-3), 2.07 (dtt, J = 45.7, 15.0, 7.7 Hz, 2H, H-2); ^{13}C NMR (125.77 MHz, DMSO-d_6) δ 9.41 (d, J = 5.4 Hz, C-3), 27.18 (d, J = 20.9 Hz, C-2), 97.86 (dt, J = 180.7, 141.2 Hz, C-1); ^{31}P NMR (202.46 MHz, D_2O) δ 14.16 (d, J = 74.6 Hz). HRMS (ESI) calcd for ($C_3H_9FO_6P_2Na$) [M + Na]$^+$: 244.9756; found: 244.9748.

1-(Fluoro-*n*-butylidene)-1,1-bisphosphonic acid (38). 90% yield; syrup; ^{1}H NMR (500.13 MHz, $CDCl_3$) δ 0.85 (t, J = 7.3 Hz, 3H, H-4), 1.55 (m, 2H, H-3), 1.96 (m, 2H, H-2); ^{13}C NMR (125.77 MHz, $CDCl_3$) δ 14.63, 16.62 (q, J = 5.6 Hz), 34.51 (d, J = 19.5 Hz), 94.73 (dt, J = 184.1, 146.7 Hz, C-1); ^{31}P NMR (202.46 MHz, $CDCl_3$) δ 13.20 (d, J = 72.1 Hz); HRMS (ESI) calcd for ($C_4H_{11}O_6P_2F$) [M + Na]$^+$: 258.9913; found 258.9903.

1-(Fluoro-*n*-pentylidene)-1,1-bisphosphonic (39). 93% yield; syrup; (KBr, cm^{-1}) 3155, 2962, 2875, 1653, 1468, 1402, 1213, 1022, 943, 660, 520; ^{1}H NMR (500.13 MHz, D_2O) δ 0.94 (t, J = 7.3 Hz, 3H, H-5), 1.36 (1.35 (sext, J = 7.4 Hz, 2H, H-4), 1.68 (m, 2H, H-3), 2.17 (m, 2H, H-2); ^{13}C NMR (125.77 MHz, D_2O) δ 13.01 (C-5), 22.59 (C-4), 25.19 (q, J = 5.1 Hz, C-3), 32.13 (d, J = 19.1 Hz, C-2) HRMS (ESI) calcd for ($C_5H_{13}O_6P_2FNa$) [M + Na]$^+$: 273.0069; found 273.0069.

1-(Fluoro-*n*-hexylidene)-1,1-bisphosphonic acid (40). 95% yield; syrup; (KBr, cm^{-1}) 3612, 2961, 2924, 2872, 1685, 1462, 1198, 1177, 1140, 1028, 1020, 943, 659, 513; ^{1}H NMR (500.13 MHz, CD_3OD) δ 0.90 (t, J = 6.9 Hz, 3H, H-6), 1.34 (m, 4H, H-4, H-5), 1.68 (m, 2H, H-3), 2.15 (m, 2H, H-2); ^{13}C NMR (125.77 MHz, CD_3OD) δ 14.39 (C-6), 23.40 (C-5), 24.07 (q, J = 4.5 Hz, C-3), 33.49 (C-4), 33.91 (d, J = 20.0 Hz, C-2); ^{31}P NMR (202.46 MHz, CD_3OD) δ 14.55 (d, J = 74.6 Hz). HRMS (ESI) calcd for ($C_6H_{16}O_6P_2F$) [M + H]$^+$: 265.0406; found 265.0401.

1-(Fluoro-*n*-heptylidene)-1,1-bisphosphonic acid (41). 99% yield; syrup; IR (KBr, cm^{-1}) 3611, 2951, 2937, 2916, 2856, 2246, 1712, 1470, 1200, 1120, 1049, 955, 895, 878, 717, 667, 561, 460; ^{1}H NMR (500.13 MHz, D_2O) δ 0.77 (t, J = 7.1 Hz, 3H, H-7), 1.34 (m, 6H, H-4, H-5, H-6), 1.51 (p, J = 7.6 Hz, 2H, H-3), 2.15 (m, 2H, H-2); ^{13}C NMR (125.77 MHz, D_2O) δ 13.30 (C-7), 21.86.45 (C-6), 22.98 (q, J = 5.5 Hz, C-3), 29.00 (C-5), 30.73 (C-4), 33.42 (d, J = 19.7 Hz, C-2), 95.89 (dt, J = 181.4, 144.5 Hz, C-1); ^{31}P NMR (202.46 MHz, D_2O) δ 14.76 (d, J = 72.15 Hz).HRMS (ESI) calcd for ($C_7H_{17}O_6P_2FNa$) [M + Na]$^+$: 301.0382; found 301.0390.

1-(Fluoro-*n*-octylidene)-1,1-bisphosphonic acid (42). 97% yield; syrup; IR (KBr, cm^{-1}) 3570, 2959, 2928, 2858, 1695, 1468, 1211, 1016, 960, 662, 567, 513; ^{1}H NMR (500.13 MHz, D_2O) δ 0.76 (t, J = 6.9 Hz, 3H, H-8), 1.20 (m, 8H, H-4, H-5, H-6, H-7), 1.51 (p, J = 7.9 Hz, 2H, H-3), 2.01 (m, 2H, H-2);^{13}C NMR (125.77 MHz, D_2O) δ 13.35 (C-8), 21.95 (C-7), 23.07 (q, J = 5.3 Hz, C-3), 28.13 (C-6), 29.32 (C-5), 31.00 (C-4), 33.42 (d, J = 18.8 Hz, C-2); ^{31}P NMR (202.46 MHz, D_2O) δ 14.2 (d, J = 74.6 Hz). HRMS (ESI) calcd for ($C_8H_{20}O_6P_2F$) [M+H]$^+$: 293.0719; found 293.0720.

1-(Fluoro-*n*-nonylidene)-1,1-bisphosphonic acid (43). 96% yield; white solid; mp 119 °C; IR (KBr, cm^{-1}) 3608, 2950, 2916, 2852, 1469, 1201, 1126, 1053, 1028, 956, 667, 561, 462; ^{1}H NMR (500.13 MHz, D_2O) δ 0.74 (t, J = 6.9 Hz, 3H, H-9), 1.18 (m, 10H, H-4, H-5, H-6, H-7, H-8), 1.49 (m, 2H, H-3), 1.98 (m, 2H, H-2); ^{13}C NMR (125.77 MHz, D_2O) δ 13.4 (C-9), 22.0 (C-8), 28.4 (C-7), 28.5 (C-6), 29.5 (C-5), 31.1 (C-4), 32.4 (d, J = 20.4 Hz, C-3), 34.9 (C-2); ^{31}P NMR (202.46 MHz, D_2O) δ 13.95 (d, J = 69.8 Hz). HRMS (ESI) calcd for ($C_9H_{21}O_6P_2FNa$) [M + Na]$^+$: 329.0695; found 329.0686.

1-(Fluoro-*n*-decylidene)-1,1-bisphosphonic acid (44). 94% yield; white solid; mp 58–59 °C; IR (KBr, cm^{-1}) 3626, 2920, 28532, 1470, 1195, 1144, 1080, 1032, 947, 717, 665, 563, 514, 457;^{1}H NMR (500.13 MHz, D_2O) δ 0.70 (t, J = 7.0 Hz, 3H, H-10), 1.13 (m, 8H, CH_2), 1.17 (m, 4H, CH_2), 1.48 (p, J = 7.1 Hz, 2H, H-3), 2.01 (m, 2H, H-2); ^{13}C NMR (125.77 MHz, D_2O) δ 13.37 (C-10), 22.02 (C-9), 23.14 (q, J = 5.4 Hz, C-3), 28.42 (C-8), 28.46 (C-7), 28.66 (C-6), 29.39 (C-5), 31.13 (C-4), 33.41 (d, J = 20.0 Hz, C-2); ^{31}P NMR (202.46 MHz, D_2O) δ 14.22 (d, J = 71.6 Hz). HRMS (ESI) calcd for ($C_{10}H_{24}O_6P_2F$) [M + H]$^+$: 321.1032; found 321.1032.

Drug screening

***T. cruzi* amastigotes assays.** Gamma-irradiated (2,000 Rads) Vero cells (3.4×10^4 cells/well) were seeded in 96 well plates (black, clear bottom plates from Greiner Bio-One) in 100 µL RPMI media (Sigma) with 10% FBS. Plates were incubated overnight at 35 °C and 7% CO_2. After overnight incubation, Vero cells were challenged with 3.4×10^5 trypomastigotes/well (CL strain overexpressing a tdTomato red fluorescent protein) in 50 µL volume and incubated for 5 h at 35 °C and 7% CO_2. After infection, cells were washed once with Hanks solution (150 µL/well) to eliminate any extracellular parasites and compounds were added in serial dilutions in RPMI media in 150 µL volumes. Each dilution was tested in quadruplicate. Each plate also contained controls with host cells and no parasites (for background check), controls with two representative drug dilutions and no parasites (for cytotoxicity assays) and controls with parasites and no drugs (positive control). For each plate, benznidazole was also used as a positive control at 3.5 and 1.5 µM. After drug addition, plates were incubated at 35 °C and 7% CO_2. At day 3 post-infection, plates were assayed for fluorescence.[72] IC_{50} values were determined by non-linear regression analysis using SigmaPlot.

***T. gondii* tachyzoites assays.** Experiments on *T. gondii* tachyzoites were carried out as described previously[73] using *T.gondii* tachyzoites expressing red fluorescence protein.[74] Cells were routinely maintained in hTerT cells grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 1% fetal bovine serum, 2 mM glutamine and 1 mM pyruvate, at 37 °C in a humid 5% CO_2 atmosphere. Confluent monolayers grown in 96-well black plates with optical bottoms (Falcon/Becton-Dickinson, Franklin Lakes, NJ) were used and drugs dissolved in the same medium and serially diluted in the plates. Freshly isolated tachyzoites were filtered through a 3 μm filter and passed through a 22 gauge needle before use. The cultures were inoculated with 10^4 tachyzoites/well in the same media. The plates were incubated at 37 °C and read daily in a Molecular Devices fluorescence plate reader. To preserve sterility, the plates were read with covered lids and both excitation (510 nm) and emission (540 nm) were read from the bottom.[75] For the calculation of the IC_{50}, the percent of growth inhibition was plotted as a function of drug concentration by fitting the values to the function: $I = I_{max}\ C/(IC_{50} + C)$, where I is the percent inhibition, I_{max} = 100% inhibition, C is the concentration of the inhibitor and IC_{50} is the concentration for 50% growth inhibition.

***Tc*FPPS and *Tg*FPPS assays and product analysis.** For *Tc*FPPS,[76–78] 100 μL of assay buffer (10 mM Hepes, pH 7.4, 1 mM $MgCl_2$, 2 mM dithiothreitol, 4.7 μM [4-^{14}C]IPP (10 μCi/μmol)) and 55 μM DMAPP were prewarmed to 37 °C. The assay was initiated by the addition of recombinant protein (10–20 ng). The assay was allowed to proceed for 30 min at 37 °C and was quenched by the addition of 6 M HCl (10 μL). The reactions were made alkaline with 6.0 M NaOH (15 μL), diluted in water (0.7 mL) and extracted with hexane (1 mL). The hexane solution was washed with water and transferred to a scintillation vial for counting. One unit of enzyme activity was defined as the activity required to incorporate 1 nmol of [4-^{14}C]IPP into [14-^{14}C]FPP in 1 min.

Acknowledgements

This work was supported by grants from the National Research Council of Argentina (PIP 1888), ANPCyT (PICT 2008 #1690) and the Universidad de Buenos Aires (X-191) to J. B. R., and Bunge & Born Foundation to S. H. S., and the U. S. National Institutes of Health to R. D. (AI-082542) and S. N. J. M. (AI-068467). V. S. R. was supported in part by a training grant of the Ellison Medical Foundation to the Center for Tropical and Emerging Global Diseases.

References

1 A. J. Roelofs, K. Thompson, F. H. Ebetino, M. J. Rogers and F. P. Coxon, *Curr. Pharm. Des.*, 2010, **16**, 2950–2960.
2 A. A. Reszka and G. A. Rodan, *Mini-Rev Med. Chem.*, 2004, **4**, 711–717.
3 R. G. G. Russell and M. J. Rogers, *Bone*, 1999, **25**, 97–106.
4 A. A. Reszka and G. A. Rodan, *Curr. Osteoporosis Rep.*, 2003, **1**, 45–52.
5 H. Fleisch, R. G. G. Russell and F Straumann, *Nature*, 1966, **212**, 901–903.
6 H. Fleisch, R. G. G. Russell and M. D. Francis, *Science*, 1969, **165**, 1262–1264.
7 M. D. Francis, R. G. G. Russell and H. Fleisch, *Science*, 1969, **165**, 1264–1266.
8 J. A. Urbina, B. Moreno, S. Vierkotter, E. Oldfield, G. Payares, C. Sanoja, B. N. Bailey, W. Yan, D. A. Scott, S. N. J. Moreno and R. Docampo, *J. Biol. Chem.*, 1999, **274**, 33609–33615.
9 D. E. Hughes, K. R. Wright, H. L. Uy, A. Sasaki, T. Yoneda, G. D. Roodman, G. R. Mundy and B. F. Boyce, *J. Bone Miner. Res.*, 1995, **10**, 1478–1487.
10 M. J. Rogers, J. C. Frith, S. P. Luckman, F. P. Coxon, H. L. Benford, J. Mönkkönen, S. Auriola, K. M. Chilton and R. G. G. Russell, *Bone*, 1999, **24**, 73S–79S.
11 K. L. Kavanagh, K. Guo, J. E. Dunford, X. Wu, S. Knapp, F. H. Ebetino, M. J. Rogers, R. G. G. Russell and U. Oppermann, *Proc. Natl. Acad. Sci. U. S. A.*, 2006, **103**, 7829–7834.
12 D. J. Hosfield, Y. Zhang, D. R. Dougan, A. Broun, L. W. Tari, R. V. Swanson and J. Finn, *J. Biol. Chem.*, 2004, **279**, 8526–8529.
13 J.-M. Rondeau, F. Bitsch, E. Bourgier, M. Geiser, R. Hemmig, M. Kroemer, S. Lehmann, P. Ramage, S. Rieffel, A. Strauss, J. R. Green and W. Jahnke, *ChemMedChem*, 2006, **1**, 267–273.
14 F. Cheng and E. Oldfield, *J. Med. Chem.*, 2004, **47**, 5149–5158.
15 J. E. Dunford, K. Thompson, F. P. Coxon, S. P. Luckman, F. M. Hahn, C. D. Poulter, F. H. Ebetino and M. J. Rogers, *J. Pharmacol. Exp. Ther.*, 2001, **296**, 235–242.
16 F. P. Coxon, K. Thompson and M. J. Rogers, *Curr. Opin. Pharmacol.*, 2006, **6**, 307–312.
17 J. M. Sanders, S. Ghosh, J. M. W. Chan, G. Meints, H. Wang, A. M. Raker, Y. Song, A. Colantino, A. Burzynska, P. Kafarski, C. T. Morita and E. Oldfield, *J. Med. Chem.*, 2004, **47**, 375–384.
18 R. Reddy, E. Dietrich, Y. Lafontaine, T. J. Houghton, O. Belanger, A. Dubois, F. F. Arhin, I. Sarmiento, I. Fadhil, K. Laquerre, V. Ostiguy, D. Lehoux, G. Moeck, T. R. Parr Jr. and A. Rafai Far, *ChemMedChem*, 2008, **3**, 1863–1868.
19 G. Forlani, S. Giberti, Ł. Berlicki, D. Petrollino and P Kafarski, *J. Agric. Food Chem.*, 2007, **55**, 4340–4347.
20 P. Clézardin and M. Massaia, *Curr. Pharm. Des.*, 2010, **16**, 3007–3014.
21 K. Miller, R. Erez, E. Segal, D. Shabat and R. Satchi-Fainaro, *Angew. Chem., Int. Ed.*, 2009, **48**, 2949–2954.
22 Y. Zhang, R. Cao, F. Yin, M. P. Hudock, R.-T. Guo, K. Krysiak, S. Mukherjee, Y.-G. Gao, H. Robinson, Y. Song, J. H. No, K. Kyle Bergan, A. Leon, L. Cass, A. Goddard, T.-K. Chang, F.-Y. Lin, E. Van Beek, S. Papapoulos, A. H.-J. Wang, T. Kubo, M. Ochi, D. Mukkamala and E. Oldfield, *J. Am. Chem. Soc.*, 2009, **131**, 5153–5162.
23 R. E. Coleman, *Br. J. Cancer*, 2008, **98**, 1736–1740.
24 A. G. Roth, D. Drescher, Y. Yang, S. Redmer, S. Uhlig and C. Arenz, *Angew. Chem., Int. Ed.*, 2009, **48**, 7560–7563.
25 R. Docampo and S. N. J. Moreno, *Curr. Pharm. Des.*, 2008, **14**, 882–888.
26 E. Oldfield, *Acc. Chem. Res.*, 2010, **43**, 1216–1226.
27 S. Ghosh, J. M. W. Chan, C. R. Lea, G. A. Meints, J. C. Lewis, Z. S. Tovian, R. M. Flessner, T. C. Loftus, I. Bruchhaus, H. Kendrick, S. L. Croft, R. G. Kemp, S. Kobayashi, T. Nozaki and E. Oldfield, *J. Med. Chem.*, 2004, **47**, 175–187.
28 M. B. Martin, J. S. Grimley, J. C. Lewis, H. T. Heath III, B. N. Bailey, H. Kendrick, V. Yardley, A. Caldera, R. Lira, J. A. Urbina, S. N. J. Moreno, R. Docampo, S. L. Croft and E. Oldfield, *J. Med. Chem.*, 2001, **44**, 909–916.
29 V. Yardley, A. A. Khan, M. B. Martin, T. R. Slifer, G. Araujo Fausto, S. N. J. Moreno, R. Docampo, S. L. Croft and E. Oldfield, *Antimicrob. Agents Chemother.*, 2002, **46**, 929–931.
30 M. B. Martin, J. M. Sanders, H. Kendrick, K. De Luca-Fradley, J. C. Lewis, J. S. Grimley, E. M. Van Brussel, J. R. Olsen, G. A. Meints, A. Burzynska, P. Kafarski, S. L. Croft and E. Oldfield, *J. Med. Chem.*, 2002, **45**, 2904–2914.
31 V. S. Rosso, S. H. Szajnman, L. Malayil, M. Galizzi, S. N. J. Moreno, R. Docampo and J. B. Rodriguez, *Bioorg. Med. Chem.*, 2011, **19**, 2211–2217.
32 S. H. Szajnman, G. E. García Liñares, Z.-H. Li, M. Galizzi, C. Jiang, E. Bontempi, M. Ferella, S. N. J. Moreno, R. Docampo and J. B. Rodriguez, *Bioorg. Med. Chem.*, 2008, **16**, 3283–3290.
33 S. H. Szajnman, B. N. Bailey, R. Docampo and J. B. Rodriguez, *Bioorg. Med. Chem. Lett.*, 2001, **11**, 789–792.
34 S. H. Szajnman, A. Montalvetti, Y. Wang, R. Docampo and J. B. Rodriguez, *Bioorg. Med. Chem. Lett.*, 2003, **13**, 3231–3235.
35 S. H. Szajnman, E. L. Ravaschino, R. Docampo and J. B. Rodriguez, *Bioorg. Med. Chem. Lett.*, 2005, **15**, 4685–4690.
36 Y. Ling, G. Sahota, S. Odeh, J. M. W. Chan, F. G. Araujo, S. N. J. Moreno and E. Oldfield, *J. Med. Chem.*, 2005, **48**, 3130–3140.
37 B. Bouzahzah, L. A. Jelicks, S. A. Morris, L. M. Weiss and H. B. Tanowitz, *Parasitol. Res.*, 2005, **96**, 184–187.

38 G. García Liñares, E. L. Ravaschino and J. B. Rodriguez, *Curr. Med. Chem.*, 2006, **13**, 335–360.
39 J. A. Urbina and R. Docampo, *Trends Parasitol.*, 2003, **19**, 495–501.
40 Z. Brener, *Annu. Rev. Microbiol.*, 1973, **27**, 347–382.
41 S. N. J. Moreno and Z.-H. Li, *Expert Opinion. Ther. Targets*, 2008, **12**, 253–263.
42 N. D. Levine, J. O. Corliss, F. E. G. Cox, G. Deroux, J. Grain, B. M. Honigberg, G. F. Leedale, A. R. Loeblich 3rd, J. Lom, D. Lynn, E. G. Merinfeld, F. C. Page, G. Poliansky, V. Sprague, J. Vavra and F. G. Wallace, *J. Protozool.*, 1980, **27**, 37–58.
43 V. Carruthers and J. C. Boothroyd, *Curr. Opin. Microbiol.*, 2007, **10**, 83–89.
44 M. E. Fichera and D. S. Roos, *Nature*, 1997, **390**, 407–409.
45 T. J. W. Stokkermans, J. D. Schwartzman, K. Keenan, N. S. Morrissette, L. G. Tilney and D. S. Roos, *Exp. Parasitol.*, 1996, **84**, 355–370.
46 F. Roberts, C. W. Roberts, J: J. Johnson, D. E. Kyle, T. Krell, J. R. Coggins, G. H. Coombs, W. K. Milhous, S. Tzipori, D. J. Ferguson, D. Chakrabarti and R. McLeod, *Nature*, 1998, **393**, 801–805.
47 J. A. Urbina, *Acta Tropica*, 2010, **115**, 55–68.
48 J. A. Urbina, *Drugs Fut*, 2010, **35**, 409–420.
49 L. M. Amzel, personal communication.
50 D. J. Hosfield, Y. Zhang, D. R. Dougan, A. Broun, L. W. Tari, R. Swanson and J. Finn, *J. Biol. Chem.*, 2003, **278**, 18401–18407.
51 L. C. Tarshis, P. J. Proteau, B. A. Kellogg, J. C. Sacchettini and C. D. Poulter, *Proc. Natl. Acad. Sci. USA*, 1996, **93**, 15018–15023.
52 C.-H. Huang, S. B. Gabelli, E. Oldfield and M. L. Amzel, *Proteins*, 2010, **78**, 888–899.
53 R. Cao, C. K.-M. Chen, R.-T. Guo, A. H. Wang and E. Oldfield, *Proteins*, 2008, **73**, 431–439 .
54 S. B. Gabelli, J. S. McLellan, A. Montalvetti, E. Oldfield, R. Docampo and M. L. Amzel, *Proteins*, 2006, **62**, 80–88.
55 E. Van Beek, C. Lowik, I. Que and S. Papapoulos, *J. Bone Miner. Res.*, 1996, **11**, 1492–1497.
56 F. H. Ebetino, M. D. Francis, M. J. Rogers and R. G. G. Russell, *Rev. Contemp. Pharmacother.*, 1998, **9**, 233–243.
57 A. Jung, S. Bisaz and H. Fleisch, *Calcif. Tissue Res.*, 1973, **11**, 269–280.
58 S. Purser, P. R. Moore, S. Swallow and V. Gouverneur, *Chem. Soc. Rev.*, 2008, **37**, 320–330.
59 M. S. Marma, Z. Xia, C. Stewart, F. Coxon, J. E. Dunford, R. Baron, B. A. Kashemirov, F. H. Ebetino, J. T. Triffit, R. G. G. Russell and C. E. McKenna, *J. Med. Chem.*, 2007, **50**, 5967–5975.
60 C. E. McKenna and P.-D. Shen, *J. Org. Chem.*, 1981, **46**, 4573–4576.
61 D. B. Berkowitz and M. Bose, *J. Fluorine Chem.*, 2001, **112**, 13–33.
62 G. M. Blackburn, D. A. England and F. Kolkmann, *Chem. Commun.*, 1981, 930–932.
63 V. D. Romanenko and V. P. Kukhar, *Chem. Rev.*, 2006, **106**, 3868–3935.
64 J. W. De Schutter, S. Zaretsky, S. Welbourn, A. Pause and Y. S. Tsantrizos, *Bioorg. Med. Chem. Lett.*, 2010, **20**, 5781–5786.
65 G. S. Lal, *J. Org. Chem.*, 1993, **57**, 4676–4683.
66 G. S. Lal, G. P. Pez and R. G. Syvret, *Chem. Rev.*, 1996, **96**, 1737–1755.
67 Y. Xu, Y. L. Qian and G. D. Prestwich, *Org. Lett.*, 2003, **5**, 2267–2270.
68 P. Beier, S. Opekar, M. Mikhail Zibinsky, I. Inessa Bychinskaya and G. K. S. Prakash, *Org. Biomol. Chem.*, 2011, **9**, 4035–4038.
69 C. R. Degenhardt and D. C. Burdsall, *J. Org. Chem.*, 1986, **51**, 3488–3490.
70 Y. Ling, Z.-H. Li, K. Miranda, E. Oldfield and S. N. J. Moreno, *J. Biol. Chem.*, 2007, **282**, 30804–30816.
71 The amino acid sequence of the proteins was obtained from the protein database from NCBI http://www.ncbi.nlm.nih.gov/protein (*T. cruzi*, 1YHK_A; *T. brucei*, gi:30522953; *L. donovani*, gi:112363472; *T. gondii*, gi:108745448; Human, 2F7M_F). Sequence alignments were created with ClustalX: F. Jeanmougin, J. D. Thompson, M. Gouy, D. G. Higgins and T. J. Gibson, *Trends Biochem. Sciences*, 1998, **23**, 403–405.
72 A. M. Canavaci, J. M. Bustamante, A. M. Padilla, C. M. Pereza Brandan, L. J. Simpson, D. Xu, C. L. Boehlke and R. L. Tarleton, *PLOS Negl. Trop. Dis.*, 2010, **4**, e740.
73 M. J. Gubbels, C. Li and B. Striepen, *Antimicrob. Agents Chemother.*, 2003, **43**, 309–316.
74 S. Agrawal, G. G. van Dooren, W. L. Beatty and B. Striepen, *J. Biol. Chem.*, 2009, **284**, 33683–33691.
75 E. L. Ravaschino, R. Docampo and J. B. Rodriguez, *J. Med. Chem.*, 2006, **49**, 426–435.
76 A. Montalvetti, B. N. Bailey, M. B. Martin, G. W. Severin, E. Oldfield and R. Docampo, *J. Biol. Chem.*, 2001, **276**, 33930–33937.
77 A. Montalvetti, A. Fernandez, J. M. Sanders, S. Ghosh, E. Van Brussel, E. Oldfield and R. Docampo, *J. Biol. Chem.*, 2003, **278**, 17075–17083.
78 K. Ogura, T. Nishino, T. Shinka and S. Seto, *Methods Enzymol.*, 1985, **110**, 167–171.