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Synthesis and Biological Evaluation of 1-Alkylaminomethyl-1,1-Bisphosphonic Acids against *Trypanosoma cruzi* and *Toxoplasma gondii*

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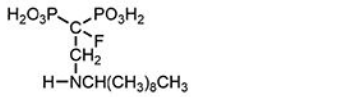
Abstract

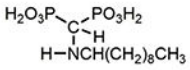
As an extension of our project aimed at the search for new chemotherapeutic agents against Chagas disease and toxoplasmosis, several 1,1-bisphosphonates were designed, synthesized and biologically evaluated against *Trypanosoma cruzi* and *Toxoplasma gondii*, the etiologic agents of these diseases, respectively. In particular, and based on the antiparasitic activity exhibited by 2-alkylaminoethyl-1,1-bisphosphonates targeting farnesyl diphosphate synthase, a series of linear 2-alkylaminomethyl-1,1-bisphosphonic acids (compounds **21–33**), that is, the position of the amino group was one carbon closer to the gem-phosphonate moiety, were evaluated as growth inhibitors against the clinically more relevant dividing form (amastigotes) of *T. cruzi*. Although all of these compounds resulted to be devoid of antiparasitic activity, these results were valuable for a rigorous SAR study. In addition, unexpectedly, the synthetic designed 2-cycloalkylaminoethyl-1,1-bisphosphonic acids **47–49** were free of antiparasitic activity. Moreover, long chain sulfur-containing 1,1-bisphosphonic acids, such as compounds **54–56**, **59**, turned out to be nanomolar growth inhibitors of tachyzoites of *T. gondii*. As many bisphosphonate-containing molecules are FDA-approved drugs for the treatment of bone resorption disorders, their potential nontoxicity makes them good candidates to control American trypanosomiasis and toxoplasmosis.

Graphical Abstract

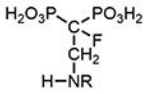
Supplementary Data: Copies of the ^{1}H NMR, ^{13}C NMR and ^{31}P NMR spectra of the target molecules and the corresponding intermediates are included as supporting information.



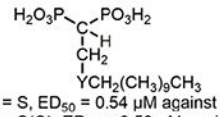
20f, ED_{50} > 10.0 μM against *T. cruzi*
ED_{50} = 4.0 μM against *T. gondii*
IC_{50} = 0.085 μM against *Tc*FPPS
IC_{50} = 0.079 μM against *Tg*FPPS



28, ED_{50} = 5.3 μM against *T. cruzi*



47, R = cypentyl,
ED_{50} > 10.0 μM against *T. cruzi*
ED_{50} > 20.0 μM against *T. gondii*
49, R = cycloheptyl,
ED_{50} > 10.0 μM against *T. cruzi*
ED_{50} > 20.0 μM against *T. gondii*



54, Y = S, ED_{50} = 0.54 μM against *T. gondii*
55, Y = S(O), ED_{50} = 0.53 μM against *T. gondii*
56, Y = $S(O_2)$, ED_{50} = 0.38 μM against *T. gondii*

Introduction

American Trypanosomiasis (Chagas disease) is a chronic zoonosis produced by the kinetoplastid parasite *Trypanosoma cruzi*, is the major parasitic disease burden of the American continent, and can be considered as one of the most important diseases in the world together with malaria and schistosomiasis.[1] The commonly affected organ is the heart with myocarditis and the central nervous system is also commonly attacked.[2–4] In addition, the second most common symptoms are the so-called megavisceras; therefore, the most commonly affected system is the digestive tract. The current chemotherapy is still deficient and based on two empirically-discovered drugs: nifurtimox (Lampit®, Bayer - El Salvador), which is available under a CDC investigational protocols[5] and benznidazole (Abarax®, Elea - Argentina), which was a recently FDA-approved drug but for pediatric use only.[5] (https://www.fda.gov/newsevents/newsroom/pressannouncements/ucm573942.htm). In most of endemic countries benznidazole is a drug of choice to treat the acute phase of the infection and is indicated to treat the chronic phase even with low efficiency.[6] As both of these drugs are associated to long term treatment and severe side effects there is a serious necessity to develop new safe drugs based on the knowledge of the biochemistry and physiology of these microorganisms. In AIDS-infected patients, chronic suppressive therapy is required and this is associated with neurotoxicity due to the drugs.

On the other hand, *T. gondii* is an opportunistic protozoan parasite that is responsible for toxoplasmosis.[7] *T. gondii* is able to infect a wide range of hosts, particularly humans and warm-blooded animals.[8] Toxoplasmosis is positively one of the most prevalent parasitic diseases affecting close to one billion people worldwide.[9] Particularly, this parasite can cause mortality among immunocompromised individuals such as AIDS patients, organ transplant recipients, as well as congenitally infected children. Toxoplasmosis may lead to severe central nervous system disease. As occurs with Chagas disease treatment, the current chemotherapy for toxoplasmosis is still deficient as well.[10]

Several enzymes of the isoprenoid pathway, involved in the synthesis of sterols and farnesyl diphosphate, have been reported to be excellent drug targets against trypanosomatids.[11,12] Regardless of their different structures and functional diversity, all isoprenoids have a common precursor: isopentenyl diphosphate, and its isomer, dimethylallyl diphosphate. In *T.*

cruzi, isopentenyl diphosphate is biosynthesized exclusively *via* the so-called mevalonate pathway, which has the 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase as the fundamental directing enzyme. Although *T. cruzi* takes up sterols from its mammalian host (largely cholesterol), it has an essential requirement for de novo sterol biosynthesis in all stages of its life cycle and is extremely susceptible to sterol biosynthesis inhibitors. On the other hand, *T. gondii* lacks the mevalonate pathway and employs an apicoplast localized biosynthesis, prokaryotic-type, 1-deoxy-D-xylulose-5-phosphate (DOXP) pathway, instead.[13,14] The DOXP pathway is not present in the host. In addition, as *T. gondii* does not synthesize cholesterol, which is taken from the host indicating that inhibitors of the host isoprenoid biosynthesis would be putative growth inhibitors of *T. gondii*[15] Certainly, mevalonate pathway inhibitors are able to control proliferation of a number of Apicomplexan parasites such as *Babesia divergens*,[16] *Plasmodium falciparum*,[17] Cryptosporidium parvum,[18] and *T. gondii*,[19] indicating that parasites lacking the mevalonate pathway are reliant on host precursors of isoprenoid biosynthesis. Interestingly, the synergistic effect of host and parasite isoprenoid pathway inhibitors has been reported.[20] For example, the used of two commonly used drugs, zoledronic acid and atorvastatin, exhibited a strong synergism in modulating *T. gondii* multiplication.[20] It would be possible to take advantage the fact of *T. cruzi* and *T. gondii* proliferate intracellularly to be used as a model for two major groups of parasites: trypanosomatids (*T. cruzi*, African trypanosomes, *Leishmania* spp) and Apicomplexan (*P. falciparum, C. parvum*, etc.). *T. gondii* main proliferative stage in the mammalian host is the rapidly dividing or tachyzoite stage, which is the form used in this study. Sometimes they are described as cells because these are unicellular parasites. We now use tachyzoites all the time to avoid confusion.[9] Scheme 1 illustrates ergosterol biosynthesis for trypanosomatids. Farnesyl diphosphate synthase (FPPS) is a key enzyme of the isoprenoid biosynthesis that catalyzes the consecutive condensation of isopentenyl diphosphate with dimethylallyl diphosphate and with geranyl diphosphate to form farnesyl diphosphate, which is the common substrate for enzymes to produce sterols, ubiquinones, dolichols, heme a, and prenylated proteins. Farnesyl diphosphate is able to be condensed with an additional molecule of isopentenyl diphosphate by the geranylgeranyl diphosphate synthase (GGPPS) to form the 20-carbon isoprenoid geranylgeranyl diphosphate.[21]

On the other hand, Scheme 2 shows the alternate pathway found in Apicomplexan parasites to biosynthesize isopentenyl and dimethylallyl diphosphate. It is well-documented that FPPS is essential for trypanosomatids.[22] TgFPPS can be considered a key enzyme only under certain circumstances, to be precise, when the isoprenoid biosynthesis of the host is inhibited, process known as genetic validation.[20] The essentiality of FPPS was demonstrated in *T. brucei* rather than *T. cruzi* because of the availability of better genetic tools for work with *T. brucei*22

The recent report of the crystal structure of some complexes of lineal 1,1-bisphosphonates, developed in our laboratory such as 2-alkylaminoethyl-1,1-bisphosphonates, with the target enzyme *T. cruzi* farnesyl diphosphate synthase (*Tc*FPPS) provided conclusive data in determining the precise mechanism of action of these antiparasitic agents.[23] These results, together with previous findings by other groups[24,25] indicated that these compounds behave as competitive inhibitors of the substrate by binding to the allylic site of the enzyme, with

the phosphonic moieties coordinating three magnesium ions at the active site.[23] It is worth mentioning that predictions of the inhibitors optimal chain length is not straightforward bearing in mind that small changes in the number of carbons of the alkyl chain brings about a dramatic influence on biological action.[26] In addition, FPPS of *T. gondii* is a bifunctional enzyme that catalyzes the condensation of isopentenyl diphosphate with three allylic substrates: dimethylallyl diphosphate, geranyl diphosphate, and farnesyl disphosphate.[27] Interestingly, *T. gondii* FPPS has less than 50% identity of *Tc*FPPS; therefore, it will be possible to access extremely selective inhibitors for both enzymes.[9]

We were able to develop effective inhibitors against either *Tc*FPPS or *Tg*FPPS.[21] Until now all the FPPSs are homodimeric enzymes that involve Mg^{2+} or Mn^{2+} for activity.[28] *Tc*FPPS is localized to the cytosol.[29]

Bisphosphonates (**2**) are diphosphate (**1**) analogues in which a substituted methylene group replaces the oxygen atom bridge between the two phosphorus atoms of the pyrophosphate moiety giving rise to a large family of compounds.[30] Several bisphosphonates are potent inhibitors of bone resorption and are in clinical use for the treatment and prevention of osteoporosis, Paget's disease, hypercalcemia, tumor bone metastases, and other bone diseases.[30–32] Aminobisphosphonates became putative antiparasitic drugs when these molecules were first found to be effective inhibitors *T. cruzi* proliferation in *in vitro* and *in vivo* assays without toxicity to the host cells.[33] In addition, this behavior was not limited exclusively to *T. cruzi* but was also observed in other trypanosomatids (*T. brucei rhodesiense* and *Leishmania donovani)* and Apicomplexan parasites, such as *T. gondii* and *P. falciparum*[34–37], indicating that bisphosphonates are promising molecules to control parasitic infections. Selective action on bone is based on the binding of the bisphosphonate moiety to the bone mineral.[30] Interestingly, the acidocalcisomes are equivalent in composition to the bone mineral and that accumulation of bisphosphonates in these organelles, as they do in bone mineral, aids their antiparasitic activity.[4,38–41] The isoprenoid pathway is the target of bisphosphonates through the inhibition of the enzymatic activity of FPPS.[21,42]

A relevant achievement of our laboratory was the finding that linear 1,1-bisphosphonates turned out to be efficient antiparasitic agents[43–45] where, in most of them, the hydroxyl group at the C-1 position, usually found in those bisphosphonates currently employed in the treatment of bone disorders, was absent.[42] Compounds **6–9** were the first examples of linear bisphosphonates that exhibited antiparasitic activity against trypanosomatids and Apicomplexan parasites targeting parasitic FPPS.[27,43–46] For example, **6** was a moderate growth inhibitor of intracellular *T. cruzi*43 and also against tachyzoites of *T. gondii*[27,46] whereas **7** was effective against *P. falciparum*.[27] **8** was practically devoid of activity,[44] whereas **9** showed similar cellular activity against *T. cruzi*.[45] In addition, α-fluoro-1,1-bisphosphonates such as **10** and **11** were neither effective against amastigotes of *T. cruzi* nor the target enzyme *Tc*FPPS; nevertheless, they were extremely effective inhibitors of the enzymatic activity of *Tg*FPPS.[47] Of paramount concern were the 2-alkyl(amino)ethyl-1,1-bisphosphonate derivatives, which were potent inhibitors of *T. cruzi* proliferation targeting *Tc*FPPS with IC_{50} values in the low nanomolar concentrations.[48,49] Unquestionable, compounds **12–14** emerge as pertinent members of this type of bisphosphonates. For

example, **12** was significantly more potent than the well-known antiparasitic agent **WC-9** against *T. cruzi* (amastigotes),[48] under the same assays conditions;[50] while **13** was a potent inhibitor of the enzymatic activities of *Tc*FPPS.[48] Moreover, **14**, the bisphosphonate bearing a long aliphatic chain, was an effective growth inhibitor of *T. cruzi*.[49] **15** was a motivating example of a linear bisphosphonate that was designed and synthesized in order to optimize structures **12–14**. **15** had been designed based on the fact that the presence of electron withdrawing group (HO-) at C-1 would enhance the ability to coordinate Mg^{2+}, would increase pK_a and also by the fact that most bisphosphonates clinically in use have this functionality at C-1.[51] Unfortunately, **15** is devoid of activity against *T. cruzi* growth and *Tc*FPPS, but exhibited a potent and selective inhibition of the enzymatic activity towards *Tg*FPPS.[51] Linear sulfur-containing bisphosphonates are interesting examples of selective anti-*Toxoplasma* agents as it is the case of **16** and **17**.[52] Certainly, **16** is a potent inhibitor of *T. gondii* proliferation. This cellular activity was associated with a potent action against the target enzyme *Tg*FPPS,[52] whereas **17** is an unusually potent inhibitor towards *Tg*FPPS.[52] A strong synergistic effect is observed when the sulfur-containing 1,1-bisphosphonate **16** was used in combination with statins against the hypervirulent RH strain of *T. gondii* in *in vivo* assays.[53] Statins, which block the mevalonate pathway within the mammalian cells, had exhibited a rather modest inhibitory effect against *T. gondii* cells when tested alone.[53] Moreover, the sulfone-containing derivative **18** exhibited a very potent *in vitro* activity against *T. gondii* (ED_{50} = 0.1 μM).[54] This compound also showed high activity in vivo (ED_{50} of 0.02 mg/kg) in a toxoplasmosis mouse model employing the hypervirulent RH strain of *T. gondii*.[54] It is worth mentioning that **18** did not behave as an effective inhibitor of the enzymatic activity of *Tg*FPPS (IC_{50} = 0.27 μM) indicating that *Tg*FPPS was not the primary target of this molecule.[55] The methylsulfonium derivative **19** has proven to be a moderate growth inhibitor against both *T. cruzi* and *T. gondii* cells, but a very potent inhibitor of the enzymatic activity towards the target enzymes *Tc*FPPS and *Tg*FPPS.[52] Finally, α-fluoro-2-alkyl(amino)ethyl derivatives such as **20a–20h** were unpredictably free of cellular activity.[55] The structures of these representative compounds are illustrated in Figure 2, whereas relevant already published antiparasitic data are listed in Table 1.

Rationale

The lack of antiparasitic activity found in the α-fluoro-2-alkyl(amino)ethyl derivatives was rather unexpected. In fact we further evaluated compounds **20a–20h** as inhibitors of the enzymatic activity against the target enzymes *Tc*FPPS and *Tg*FPPS, respectively.

On the other hand, in order to study the influence of the position of the nitrogen atom in our linear 1,1-bisphosphonates, 2-alkylaminomethyl-1,1-bisphosphonates such as **21–33** could be considered as very exciting structural variations taking into account the selective and potent antiparasitic activity exhibited by 2-alkylaminoethyl-1,1-bisphosphonates such as **12–14** towards both *T. cruzi* cells and *Tc*FPPS.[23,48,49] Although some of the title compounds **21–33** had been previously described as growth inhibitors of *Entamoeba histolytica* and *Plasmodium* species in in vitro and in vivo studies, there were not spectroscopic data available for these compounds.[56] Therefore, the influence of the position of the nitrogen atom (C-3 versus C-2) seemed to be relevant from the pharmacological point of view.

Taking into account that the 2-alkylaminoethyl-1,1-bisphosphonates of type **12–14** were extremely effective growth inhibitors either of *T. cruzi* cells or of the enzymatic activity of the target enzyme *Tc*FPPS,[23,48,49] based on the biological activity exhibited by the cyclohexylamine-containing bisphosphonate,[48] which showed an IC_{50} value of 0.013 μM against *Tc*FPPS but limited biological activity against intracellular *T. cruzi*, it was decided to prepare some 1,1-bisphosphonate derivatives bearing a cycloalkylamino group in their structure such as **47–49**.

Results and discussion

Preparation of the respective 2-alkylaminomethyl-1,1-bisphosphonic acids **21–33** was successfully carried out according to already published methods.[57,58] Thus, following a slightly modified published protocol,[59] a suitable alkyl or cycloalkyl amine treated with triethyl orthoformate and diethyl phosphite at 135 °C for 2 hours gave rise to the corresponding tetraethyl ester derivatives **34–46** in a relatively modest but reproducible yields. Then, on treatment with concentrated hydrochloric acid at 100 °C overnight the esters were converted into the title molecules **21–33** in very good yields as shown in Scheme 3.

The 2-cycloalkylaminoethyl-1,1-bisphosphonates **47–49** were easily synthesized starting from the corresponding cycloalkylamine and the well-known Michael-type acceptor **50**.[60–62] Then, conjugate addition of the cycloalkylamine to form the respective tetraethyl esters **47–49** with excellent yields followed by hydrolysis by treatment with concentrated hydrochlroric acid yielded the desired molecules **47–49** as presented in Scheme 4.

Long chain sulfur-containing 1,1-bisphosphonates such as **54–59** were synthesized according to our previous results.[52] Therefore, the Michael-type acceptor **50** was treated with undecylmercaptan and dodecylmercaptan to produce the respective tetraethyl esters **60** and **61**, respectively. On treatment with bromotrimethylsilane in methylane chloride, which is the protocol developed by McKenna,[63,64] followed by digestion with methanol, **60** and **61** were converted into the free bisphosphonic acids **54** and **57**. Sulfur-containing 1,1-bisphosphonate esters are quite sensitive to hydrolysis by treatment with concentrated hydrochlroric acid.[52] Each compound, in independent experiments, was oxidized by treatment with hydrogen peroxide (one equivalent) to yield the corresponding sulfoxides **55** and **58**, respectively or with two equivalents of hydrogen peroxide to produce the respective sulfones **56** and **59**, respectively as shown in Scheme 5.

Biological data are shown in Table 2 and 3. As previously mentioned, the α-fluoro-2-alkyl(amino)ethyl derivatives **20a–20h** resulted to be almost free of antiparasitic activity. In spite of not having a significant cellular activity compounds **20a–20h** were further evaluated against the main target of the linear bisphosphonates, FPPS. All of these fluorine-containing-1,1-bisphosphonates exhibited a potent inhibitory action towards the enzymatic activity of either *Tc*FPPS or of *Tg*FPPS. Certainly, most of these compounds showed to be nanomolar inhibitors of these key enzymes. Long chain derivatives such as **20f** and **20g** were representative examples of potent inhibitors showing IC_{50} values of 0.085 μM and 0.079 μM against *Tc*FPPS and *Tg*FPPS, respectively for **20f** and 0.034 μM and 0.051 μM, respectively

for **20g**. Unfortunately, it is difficult to rationalize the lack of cellular activity of this family of compounds. Evidently, α-fluoro-2-alkyl(amino)ethyl-1,1-bisphosphonates do not have the appropriate physicochemical parameters to cross two cell membranes (the mammalian one and the parasite cell membrane) to reach the target enzyme. Moreover, the long chain α-fluoro-2-alkyl(amino)ethyl-1,1-bisphosphonates **20f–20h** were cytotoxic molecules making these derivatives of low interest in Medicinal Chemistry. This decrease in the antiparasitic activity by making small structural variations on nitrogen-containing bisphosphonates such as such **12–14**[48,49] to give **15**[51] has also been observed in other bisphosphonates acting on bone mineral affinity targeting the mevalonate pathway between α-hydroxy- and α-fluorobisphosphonates.[65]

Biological evaluation of 1-alkylaminomethyl-1,1-bisphosphonic acids **21–29** indicated that this structural variation was not beneficial for the biological activity. With the exception of compound **28**, which resulted to be a fairly modest growth inhibitor of intracellular *T. cruzi* (ED_{50} 5.3 μM), all the title compounds were devoid of cellular activity and, for that reasons, they were not further analyzed.

Unexpectedly, the designed 2-cycloalkyl(amino)ethyl-1,1-bisphosphonates **47–49** were devoid of activity against *T. cruzi* cells and *T. gondii* cells. Interestingly, the long chain sulfur-containing-1,1-bisphosphonates were effective inhibitors of tachyzoites of *T. gondii* as anticipated according to our previous data in closely related sulfur-containing analogues.[52–54] All of them **54–56**, **59** were nanomolar inhibitors of tachyzoites of *T. gondii* being **56** the most effective growth inhibitor with an ED_{50} value of 0.53 μM. In this regard, it is important to note that the reference structure **18** (Figure 2), which behaved as a potent inhibitor of tachyzoites of *T. gondii* proliferation (ED_{50} = 0.1 μM), did not have *Tg*FPPS as a primary target exhibiting a modest a fairly modest inhibitory action towards this target enzyme (IC_{50} value of 0.8 μM).[54] Indeed, these long chain sulfur-containing -1,1-bisphosphonates will require further efforts to investigate their precise mode of action.

In order to account for the lack of activity of compounds bearing the amino group at position 2, Molecular Dynamics (MD) and Quantum Mechanical (QM) optimizations were carried out. The protein ligand complex for the compound with the amino group at position 2 (compound **21**) was generated from the crystal structure for compound **62** (pdb id 4DXJ). Although no specific interactions between the amino groups and the receptor were observed in the 10 ns MD simulations, the side chain is fixed in one orientation, directed towards the unsymmetrical magnesium ion (Figure 3). A plot for the dihedral angle P-C1-C2-N for **21** and P-C1-N-C3 for **62** (Figure 4) shows that this angle remains constant throughout the simulation.

QM optimizations at the B3LYP/6-311+G(d,p) level were carried out for the main conformers of each model compound (**63** and **64**), simulating the aqueous solvent with the polarizable continuum method (PCM). Consistent with previous studies,[51] the phosphate groups were considered to be doubly protonated, as it is expected for a physiological pH of 6.5. We have considered both protonation states for the amino group, yielding equivalent conclusions. The molecules were completed by the addition of one magnesium atom. The lowest energy conformations (Figure 5) show a strong intramolecular hydrogen bond

between the phosphate groups for both compounds. However, compound **64** shows a weak H-bond between the amino and phosphate groups (bond length 2.32 Å and N-H…O angle 120°) while the equivalent H-bond is relatively strong for compound **63** (1.77 Å, 151°). The formation of this strong H-bond is expected to decrease the conformational entropy in solution, therefore decreasing the entropy penalty upon complex formation, and yielding a higher inhibition activity. It is worth mentioning that, consistently with our MD and QM studies, ITC measurements[23] found that bisphosphonate compounds bind to the active site due to a combination of a favorable entropic driving force and an unfavorable enthalpic energy.

In conclusion, the lack of antiparasitic activity shown by the regioisomers of the nitrogen-containing bisphosphonates **12–14**, that is, amino derivatives **21–29** provided significant insights concerning chemical structure-biological activity relationship. The simple moving of the nitrogen atom in representative compounds **12–14** had a marked outcome on the biological activity giving rise to inactive compounds. Efforts in optimizing linear bisphosphonates are 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 dry argon. Unless otherwise noted, chemicals were commercially available and used without further purification. Solvents were distilled before use. Dichloromethane was distilled from phosphorus pentoxide. Nuclear magnetic resonance spectra were performed with a Bruker AVANCE NEO 500 or with a Bruker Fourier 300 spectrometers. The ^{1}H NMR spectra are referenced with respect to the residual $CHCl_3$ proton of the solvent $CDCl_3$ at $\delta = 7.26$ ppm. Coupling constants are reported in Hz. ^{13}C NMR spectra were fully decoupled and are referenced to the middle peak of the solvent $CDCl_3$ at $\delta = 77.0$ ppm. ^{31}P NMR spectra are referenced with respect to the peak of 85% H_3PO_4 as external reference. For comparative purposes, all NMR spectra acquired in D_2O for free bisphosphonic acids were recorded at the same conditions. Splitting patterns are designated as s, singlet; d, doublet; t, triplet; q, quadruplet; dd, double doublet, etc. Melting points were determined with a Fisher–Johns apparatus and are uncorrected. IR spectra were recorded with a Nicolet Magna 550 spectrometer. Elemental analyses were performed with an Exeter CE-440 Elemental Analyzer. Analytical TLC was performed on commercial 0.2 mm aluminum-coated silica gel plates (F_{254}) and visualized by 254 nm UV or immersion in an aqueous solution of $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$ (0.04 M), $Ce(SO_4)_2$ (0.003 M) in concentrated H_2SO_4 (10%).

As judged from the homogeneity of the ^{1}H, ^{13}C, ^{31}P NMR spectra and HPLC analyses of the title compounds employing a Beckmann Ultrasphere ODS-2 column 5 μM, 250 × 10 mm eluting with water-acetonitrile (9:1) at 3.00 mL/min with a refractive index detector indicated a purity >97%.

General procedure for the preparation of tetraethyl 1-*n*-alkylaminomethyl-1,1-bisphosphonates

A mixture of the corresponding alkyl or cycloalkyl amine (30 mmol, 4.14 g), triethyl orthoformate (30 mmol, 5.0 mL) and diethyl phosphite (60 mmol, 7.7 mL) was heated at 135 °C for 2 h. Then, volatile components were evaporated and the respective residues were purified by column chromatography (silica gel) eluting with mixtures of hexane–acetone giving rise to the desired tetraethyl esters.

General procedure for hydrolysis of tetraethyl 1-*n*-alkylaminomethyl-1,1-bisphosphonates

The solvent was evaporated and the residue was treated with a concentrated aqueous solution of hydrochloric acid (2 mL). The resulting mixture was heated to reflux for 24 h. The solvent was evaporated and the residue was crystallized from ethanol–water.

Synthesis of tetraethyl 2-[(alkylthio)ethyl] 1,1-bisphosphonates. General procedure

To a solution of tetraethyl ethenylidenbisphosphonate (**50**; 300 mg, 1.0 mmol) in anhydrous dichloromethane (10 mL) was added triethylamine (1 mmol) and the corresponding alkylmercaptan (1 mmol). The reaction mixture was stirred at room temperature for 1 h. Water (20 mL) was added, and the mixture was extracted with dichloromethane (3 × 10 mL). The combined organic layers were washed with brine (20 mL), dried ($MgSO_4$), and the solvent was evaporated.

Synthesis of 2-(alkylthio)ethyl-1,1-bisphosphonic acids. General procedure.— A solution of the corresponding tetraethyl 2-[(alkylthio)ethyl] 1,1-biphosphonate (1 mmol) in anhydrous methylene chloride (10 mL) was treated with trimethylsilyl bromide (10 equiv.) under an argon atmosphere. The reaction mixture was stirred at room temperature for 48 h. Then, methanol (1.0 mL) was added and the solvent was evaporated. The residue was dissolved in methanol (8 mL) and the mixture was stirred at room temperature for 24 h. The solvent was evaporated and the residue redissolved/evaporated in methanol four times, to complete the hydrolysis of remaining trimethylsilyl bromide and eliminate the hydrobromic acid created. The residue was purified by column chromatography on reverse phase with a mixture of water–methanol as eluent and the pure compound was obtained after lyophilization.

Tetraethyl 1-*n*-propylaminomethyl-1,1-bisphosphonate (34).—Colorless oil; 16% yield; ^{1}H NMR (500.13 MHz, $CDCl_3$) δ 0.92 (t, *J* = 7.4 Hz, 3H, H-5), 1.35 (dt, *J* = 7.1, 0.9 Hz, 12H, CH_2CH_3), 1.49 (sext, *J* = 7.2 Hz, 2H, H-4), 2.80 (t, *J* = 7.2 Hz, 2H, H-3), 3.25 (t, *J* = 21.7 Hz, 1H, H-1), 4.22 (m, 8H, CH_2CH_3); ^{13}C NMR (125.77 MHz, $CDCl_3$) δ 11.5 (C-5), 16.39 (t, *J* = 3.1 Hz, CH_2CH_3), 16.44 (t, *J* = 3.1 Hz, CH_2CH_3), 23.1 (C-4), 52.4 (t, *J* = 6.0 Hz, C-3), 54.3 (t, *J* = 145.5 Hz, C-1), 62.8 (t, *J* = 3.3 Hz, CH_2CH_3), 63.2 (t, *J* = 3.3 Hz, CH_2CH_3); ^{31}P NMR (202.46 MHz, $CDCl_3$) δ 19.74 ppm.

Tetraethyl 1-*n*-butylaminomethyl-1,1-bisphosphonate (35).—Colorless oil; 15% yield; ^{1}H NMR (500.13 MHz, $CDCl_3$) δ 0.90 (t, *J* = 7.3 Hz, 3H, H-6), 1.35 (dt, *J* = 7.1, 1.0 Hz, 12H, CH_2CH_3), 1.37 (m, *J* = 7.2 Hz, 2H, H-4), 1.45 (m, *J* = 7.2 Hz, 2H, H-5), 2.83 (t, *J* = 7.1 Hz, 2H, H-3), 3.24 (t, *J* = 21.7 Hz, 1H, H-1), 4.21 (m, 8H, CH_2CH_3); ^{13}C NMR

(125.77 MHz, $CDCl_3$) δ 13.9 (C-6), 16.4 (t, J = 3.0 Hz, CH_2CH_3), 16.5 (t, J = 3.1 Hz, CH_2CH_3), 20.1 (C-5), 32.1 (C-4), 50.3 (t, J = 6.1 Hz, C-3), 54.4 (t, J = 97.4 Hz, C-1), 62.8 (t, J = 3.4 Hz, CH_2CH_3), 63.3 (t, J = 3.2 Hz, CH_2CH_3); ^{31}P NMR (202.46 MHz, $CDCl_3$) δ 19.77 ppm.

Tetraethyl 1-*n*-pentylaminomethyl-1,1-bisphosphonate (36).—Colorless oil; 11% yield; ^{1}H NMR (500.13 MHz, $CDCl_3$) δ 0.88 (t, J = 7.0 Hz, 3H, H-7), 1.30 (m, 4H, H-5, H-6), 1.35 (dt, J = 7.1, 1.0 Hz, 12H, CH_2CH_3), 1.47 (m, 2H, H-5), 2.82 (t, J = 7.2 Hz, 2H, H-3), 3.25 (t, J = 21.8 Hz, 1H, H-1), 4.22 (m, 8H, CH_2CH_3); ^{13}C NMR (125.77 MHz, $CDCl_3$) δ 14.0 (C-7), 16.39 (t, J = 3.1 Hz, CH_2CH_3), 16.44 (t, J = 3.1 Hz, CH_2CH_3), 22.5 (C-6), 29.1 (C-5), 29.7 (C-4), 50.6 (t, J = 6.0 Hz, C-3), 54.3 (t, J = 145.4 Hz, C-1), 62.8 (t, J = 3.3 Hz, CH_2CH_3), 63.2 (t, J = 3.2 Hz, CH_2CH_3); ^{31}P NMR (202.46 MHz, $CDCl_3$) δ 19.74 ppm.

Tetraethyl 1-*n*-hexylaminomethyl-1,1-bisphosphonate (37).—Colorless oil; 27% yield; ^{1}H NMR (500.13 MHz, $CDCl_3$) δ 0.88 (t, J = 7.0 Hz, 3H, H-8), 1.30 (m, 6H, H-5, H-6, H-7), 1.35 (dt, J = 7.1, 1.1 Hz, 12H, CH_2CH_3), 1.46 (p, J = 7.3 Hz, 2H, H-4), 2.82 (t, J = 7.2 Hz, 2H, H-3), 3.25 (t, J = 21.7 Hz, 1H, H-1), 4.22 (m, 8H, CH_2CH_3); ^{13}C NMR (125.77 MHz, $CDCl_3$) δ 14.0 (C-8), 16.42 (t, J = 3.1 Hz, CH_2CH_3), 16.46 (t, J = 3.0 Hz, CH_2CH_3), 22.6 (C-7), 26.7 (C-5), 30.0 (C-4), 31.5 (C-6), 50.6 (t, J = 6.0 Hz, C-3), 54.3 (t, J = 145.4 Hz, C-1), 62.8 (t, J = 3.3 Hz, CH_2CH_3), 63.2 (t, J = 3.0 Hz, CH_2CH_3); ^{31}P NMR (202.46 MHz, $CDCl_3$) δ 19.76 ppm.

Tetraethyl 1-*n*-hepylaminomethyl-1,1-bisphosphonate (38).—Colorless oil; 11% yield; ^{1}H NMR (500.13 MHz, $CDCl_3$) δ 0.86 (t, J = 6.8 Hz, 3H, H-9), 1.26 (m, 8H, H-5, H-6, H-7, H-8), 1.33 (t, J = 7.1 Hz, 12H, CH_2CH_3), 1.44 (p, J = 7.1 Hz, 2H, H-4), 2.80 (t, J = 7.1 Hz, 2H, H-3), 3.23 (t, J = 21.7 Hz, 1H, H-1), 4.22 (m, 8H, CH_2CH_3); ^{13}C NMR (125.77 MHz, $CDCl_3$) δ 14.0 (C-9), 16.40 (t, J = 3.1 Hz, CH_2CH_3), 16.44 (t, J = 3.0 Hz, CH_2CH_3), 22.6 (C-8), 27.0 (C-5), 29.1 (C-6), 30.0 (C-4), 31.8 (C-7), 50.6 (t, J = 6.0 Hz, C-3), 54.3 (t, J = 145.4 Hz, C-1), 62.8 (t, J = 3.2 Hz, CH_2CH_3), 63.2 (t, J = 3.2 Hz, CH_2CH_3); ^{31}P NMR (202.46 MHz, $CDCl_3$) δ 19.74 ppm.

Tetraethyl 1-*n*-octylaminomethyl-1,1-bisphosphonate (39).—Colorless oil; 23% yield; ^{1}H NMR (500.13 MHz, $CDCl_3$) δ 0.88 (t, J = 7.0 Hz, 3H, H-10), 1.26 (m, 10H, -CH_2-), 1.35 (t, J = 7.1 Hz, 12H, CH_2CH_3), 1.46 (p, J = 7.21 Hz, 2H, H-4), 2.82 (dt, J = 7.1, 1.1 Hz, 2H, H-3), 3.24 (t, J = 21.7 Hz, 1H, H-1), 4.21 (m, 8H, CH_2CH_3); ^{13}C NMR (125.77 MHz, $CDCl_3$) δ 14.1 (C-10), 16.4 (t, J = 3.1 Hz, CH_2CH_3), 16.5 (t, J = 3.0 Hz, CH_2CH_3), 22.6 (C-9), 27.0 (C-5), 29.2 (C-6), 29.4 (C-7), 30.0 (C-4), 31.8 (C-8), 50.6 (t, J = 6.0 Hz, C-3), 54.4 (t, J = 145.4 Hz, C-1), 62.8 (t, J = 3.3 Hz, CH_2CH_3), 63.2 (t, J = 3.2 Hz, CH_2CH_3); ^{31}P NMR (202.46 MHz, $CDCl_3$) δ 19.76 ppm.

Tetraethyl 1-*n*-nonylaminomethyl-1,1-bisphosphonate (40).—Colorless oil; 22% yield; ^{1}H NMR (500.13 MHz, $CDCl_3$) δ 0.88 (t, J = 7.0 Hz, 3H, H-11), 1.25 (m, 12H, -CH_2-), 1.35 (dt, J = 7.0, 1.0 Hz, 12H, CH_2CH_3), 1.45 (p, J = 7.2 Hz, 2H, H-4), 2.81 (t, J = 7.2, 1.1 Hz, 2H, H-3), 3.25 (t, J = 21.7 Hz, 1H, H-1), 4.20 (m, 8H, CH_2CH_3); ^{13}C NMR (125.77 MHz, $CDCl_3$) δ 14.0 (C-11), 16.37 (t, J = 3.1 Hz, CH_2CH_3), 16.42 (t, J = 3.1 Hz,

$CH_2C'H_3$), 22.6 (C-10), 27.0 (C-5), 29.2 (C-6), 29.4 (C-7), 29.4 (C-8), 30.0 (C-4), 31.8 (C-9), 50.8 (t, J = 5.7 Hz, C-3), 54.3 (t, J = 145.4 Hz, C-1), 62.8 (t, J = 3.3 Hz, CH_2CH_3), 63.2 (t, J = 2.9 Hz, $C'H_2CH_3$); ^{31}P NMR (202.46 MHz, $CDCl_3$) δ 19.73 ppm.

Tetraethyl 1-*n*-decylaminomethyl-1,1-bisphosphonate (41).—Colorless oil; 26% yield; ^{1}H NMR (500.13 MHz, $CDCl_3$) δ 0.86 (t, J = 6.8 Hz, 3H, H-12), 1.26 (m, 14H, -CH_2-), 1.33 (t, J = 6.7 Hz, 12H, CH_2CH_3), 1.43 (p, J = 7.2 Hz, 2H, H-4), 2.79 (t, J = 7.0, 1.1 Hz, 2H, H-3), 3.23 (t, J = 21.7 Hz, 1H, H-1), 4.19 (m, 8H, CH_2CH_3); ^{13}C NMR (125.77 MHz, $CDCl_3$) δ 14.1 (C-12), 16.39 (t, J = 3.0 Hz, CH_2CH_3), 16.44 (t, J = 3.1 Hz, $CH_2C'H_3$), 22.6 (C-11), 27.0 (C-5), 29.3 (C-6), 29.4 (C-7), 29.5 (C-8), 29.6 (C-9), 30.0 (C-4), 31.8 (C-10), 50.6 (t, J = 6.0 Hz, C-3), 54.3 (t, J = 145.4 Hz, C-1), 62.8 (t, J = 3.3 Hz, CH_2CH_3), 63.2 (t, J = 3.0 Hz, $C'H_2CH_3$); ^{31}P NMR (202.46 MHz, $CDCl_3$) δ 19.74 ppm.

Tetraethyl 1-*n*-Undecylaminomethyl-1,1-bisphosphonate (42).—Colorless oil; 19% yield; ^{1}H NMR (500.13 MHz, $CDCl_3$) δ 0.86 (t, J = 6.8 Hz, 3H, H-13), 1.23 (m, 16H, -CH_2-), 1.33 (t, J = 6.7 Hz, 12H, CH_2CH_3), 1.43 (p, J = 7.2 Hz, 2H, H-4), 2.79 (t, J = 7.0 Hz, 2H, H-3), 3.22 (t, J = 21.7 Hz, 1H, H-1), 4.19 (m, 8H, CH_2CH_3); ^{13}C NMR (125.77 MHz, $CDCl_3$) δ 14.1 (C-13), 16.39 (t, J = 3.0 Hz, CH_2CH_3), 16.44 (t, J = 3.1 Hz, $CH_2C'H_3$), 22.6 (C-12), 27.0 (C-5), 29.3 (C-6), 29.47 (C-7), 29.57 (C-8), 29.59 (C-9, C-10), 30.0 (C-4), 31.9 (C-11), 50.6 (t, J = 6.1 Hz, C-3), 54.3 (t, J = 145.6 Hz, C-1), 62.8 (t, J = 3.3 Hz, CH_2CH_3), 63.3 (t, J = 3.2 Hz, $C'H_2CH_3$); ^{31}P NMR (202.46 MHz, $CDCl_3$) δ 19.75 ppm.

Tetraethyl (Cyclopentylamino)methyl-1,1-bisphosphonate (43).—Colorless oil; 28% yield; ^{1}H NMR (500.13 MHz, $CDCl_3$) δ 1.35 (dt, J = 7.1, 1.6 Hz, 12H, CH_2CH_3), 1.52 (m, 4H, -CH_2-), 1.72 (m, 4H, -CH_2-), 3.32 (t, J = 22.0 Hz, 1H, H-1), 3.48 (t, J = 5.8 Hz, 2H, H-3), 4.19 (m, 8H, CH_2CH_3); ^{13}C NMR (125.77 MHz, $CDCl_3$) δ 16.4 (t, J = 3.1 Hz, CH_2CH_3), 16.5 (t, J = 3.1 Hz, $CH_2C'H_3$), 23.9 (C-5), 32.8 (C-4), 52.6 (t, J = 145.4 Hz, C-1), 59.2 (t, J = 5.9 Hz, C-3), 62.8 (t, J = 3.4 Hz, CH_2CH_3), 63.4 (t, J = 3.4 Hz, $C'H_2CH_3$); ^{31}P NMR (202.46 MHz, $CDCl_3$) δ 19.93 ppm.

Tetraethyl (Cyclohexylamino)methyl-1,1-bisphosphonate (44).—Colorless oil; 11% yield; ^{1}H NMR (500.13 MHz, $CDCl_3$) δ 1.06–1.25 (m, 5H, -CH_2-), 1.35 (dt, J = 7.1, 1.3 Hz, 12H, CH_2CH_3), 1.49 (m, 1H, -CH_2-), 1.59 (m, 1H, -CH_2-), 1.72 (m, 2H, -CH_2-), 1.83 (m, 3H, -CH_2-), 2.76 (tt, J = 9.9, 3.7 Hz, 2H, H-3), 3.42 (t, J = 22.0 Hz, 1H, H-1), 4.19 (m, 8H, CH_2CH_3); ^{13}C NMR (125.77 MHz, $CDCl_3$) δ 16.4 (t, J = 3.1 Hz, CH_2CH_3), 16.5 (t, J = 2.9 Hz, $CH_2C'H_3$), 24.7 (C-5), 26.0 (C-6), 33.1 (C-4), 51.0 (t, J = 145.4 Hz, C-1), 56.2 (t, J = 5.8 Hz, C-3), 62.8 (t, J = 3.3 Hz, CH_2CH_3), 63.3 (t, J = 3.2 Hz, $C'H_2CH_3$); ^{31}P NMR (202.46 MHz, $CDCl_3$) δ 19.94 ppm.

Tetraethyl (Cycloheptylamino)methyl-1,1-bisphosphonate (45).—Colorless oil; 15% yield; ^{1}H NMR (500.13 MHz, $CDCl_3$) δ 1.34 (dt, J = 7.1, 1.2 Hz, 12H, CH_2CH_3), 1.39 (m, 2H, -CH_2-), 1.52 (m, 4H, -CH_2-), 1.64 (m, 2H, -CH_2-), 1.78 (m, 4H, -CH_2-), 2.98 (p, J = 4.1 Hz, 2H, H-3), 3.36 (t, J = 22.1 Hz, 1H, H-1), 4.21 (m, 8H, CH_2CH_3); ^{13}C NMR (125.77 MHz, $CDCl_3$) δ 16.4 (t, J = 2.9 Hz, CH_2CH_3), 16.5 (t, J = 3.3 Hz, $CH_2C'H_3$), 24.0 (C-5), 28.4 (C-6), 34.3 (C-4), 51.7 (t, J = 145.3 Hz, C-1), 58.4 (t, J = 5.7 Hz, C-3), 62.7 (t, J = 3.3

Hz, CH_2CH_3), 63.4 (t, J = 3.2 Hz, CH_2CH_3); ^{31}P NMR (202.46 MHz, $CDCl_3$) δ 19.99 ppm.

Tetraethyl (Piperidin-1-yl)methyl-1,1-bisphosphonate (46).—Colorless oil; 37% yield; ^{1}H NMR (500.13 MHz, $CDCl_3$) δ 1.35 (dt, J = 7.1, 1.2 Hz, 12H, CH_2CH_3), 1.42 (m, 2H, -CH_2-), 1.54 (p, J = 5.4 Hz, 4H, -CH_2-), 1.64 (m, 2H, -CH_2-), 1.78 (m, 4H, -CH_2-), 2.96 (m, 4H, H-3), 3.34 (t, J = 25.0 Hz, 1H, H-1), 4.20 (m, 8H, CH_2CH_3); ^{13}C NMR (125.77 MHz, $CDCl_3$) δ 16.4 (t, J = 2.9 Hz, CH_2CH_3), 16.5 (t, J = 3.1 Hz, CH_2CH_3), 23.9 (C-5), 26.8 (C-4), 53.0 (t, J = 4.5 Hz, C-3), 62.4 (t, J = 140.8 Hz, C-1), 62.7 (t, J = 3.3 Hz, CH_2CH_3), 63.4 (t, J = 3.2 Hz, CH_2CH_3); ^{31}P NMR (202.46 MHz, $CDCl_3$) δ 18.56 ppm.

Tetraethyl 1-[(Cyclopentylamino)ethyl] 1,1-bisphosphonate (51).—Colorless oil; 95% yield; ^{1}H NMR (500.13 MHz, $CDCl_3$) δ 1.32 (t, J = 7.1 Hz, 12H, CH_2CH_3), 1.36 (m, 1H, -CH_2-), 1.51 (m, 2H, -CH_2-), 1.66 (m, 2H, -CH_2-), 1.80 (m, 2H, -CH_2-), 2.70 (tt, J = 23.5, 5.7 Hz, 1H, H-1), 3.12 (dt, J = 16.7, 6.0 Hz, 2H, H-2), 3.06 (q, J = 6.9 Hz, 1H, H-4), 4.16 (m, 8H, CH_2CH_3); ^{13}C NMR (125.77 MHz, $CDCl_3$) δ 16.3 (t, J = 3.2 Hz, CH_2CH_3), 16.4 (t, J = 3.1 Hz, CH_2CH_3), 24.0 (C-5), 32.6 (C-4), 37.2 (t, J = 132.9 Hz, C-1), 44.2 (t, J = 4.2 Hz, C-2), 58.9 (C-4), 62.5 (t, J = 6.8 Hz, CH_2CH_3), 62.8 (t, J = 6.8 Hz, CH_2CH_3); ^{31}P NMR (202.46 MHz, $CDCl_3$) δ 22.60 ppm.

Tetraethyl 1-[(Cycloheptylamino)ethyl] 1,1-bisphosphonate (52).—Colorless oil; 99% yield; ^{1}H NMR (500.13 MHz, $CDCl_3$) δ 1.35 (t, J = 7.1 Hz, 12H, CH_2CH_3), 2.63 (tt, J = 23.6, 5.8 Hz, 1H, H-1), 3.15 (dt, J = 16.7, 5.9 Hz, 2H, H-2), 4.18 (m, 8H, CH_2CH_3); ^{13}C NMR (125.77 MHz, $CDCl_3$) δ 16.39 (t, J = 2.9 Hz, CH_2CH_3), 16.43 (t, J = 3.1 Hz, CH_2CH_3), 24.3 (C-6), 28.3 (C-7), 34.5 (C-5), 37.4 (t, J = 132.2 Hz, C-1), 43.2 (t, J = 4.3 Hz, C-2), 57.9 (C-4), 62.5 (t, J = 6.6 Hz, CH_2CH_3), 62.8 (t, J = 6.8 Hz, CH_2CH_3); ^{31}P NMR (202.46 MHz, $CDCl_3$) δ 22.87 ppm.

Tetraethyl 1-[(4-methylcyclohexylamino)ethyl] 1,1-bisphosphonic acid (53).—Colorless oil, 94% yield; ^{1}H NMR (500.13 MHz, $CDCl_3$) δ 0.87, 0.90 (d, J = 6.5 Hz, 3H CH_3), 1.342, 1.347 (t, J = 7.0 Hz, 12H, CH_2CH_3), 2.63 (tt, J = 22.9, 5.6 Hz, 1H, H-1), 3.15, 3.19 (dt, J = 16.5, 5.6 Hz, 2H, H-2), 4.19 (m, 8H, CH_2CH_3); ^{31}P NMR (202.46 MHz, $CDCl_3$) δ 22.78, 22.90 ppm.

1-*n*-Propylaminomethyl-1,1-bisphosphonic acid (21).—White solid, 73% yield; mp 207 °C; ^{1}H NMR (500.13 MHz, D_2O) δ 0.91 (t, J = 7.5 Hz, 3H, H-5), 1.64 (sext, J = 7.5 Hz, 2H, H-4), 2.90 (t, J = 16.4 Hz, 1H, H-1), 3.22 (t, J = 7.5 Hz, 2H, H-3); ^{13}C NMR (125.77 MHz, D_2O) δ 10.1 (C-5), 19.8 (C-4), 51.1 (t, J = 2.8 Hz, C-3), 58.3 (t, J = 115.8 Hz, C-1); ^{31}P NMR (202.46 MHz, D_2O) δ 8.40 ppm. HRMS (ESI) calcd. for ($C_4H_{13}O_6NP_2Na$) [M +Na]$^+$ 256.0110; found 256.0110. *Anal.* calcd. For ($C_4H_{13}O_6NP_2.½H_2O$): C, 19.84; H, 5.83; N, 5.79. Found C, 19.34; H, 6.08; N, 5.62.

1-*n*-Butylaminomethyl-1,1-bisphosphonic acid (22).—White solid; 100% yield; mp 215 °C; ^{1}H NMR (500.13 MHz, D_2O) δ 0.85 (t, J = 7.4 Hz, 3H, H-6), 1.32 (sext, J = 7.5 Hz, 2H, H-5), 1.61 (q, J = 7.6 Hz, 2H, H-4), 2.90 (t, J = 16.4 Hz, 1H, H-1), 3.25 (t, J = 7.5 Hz, 2H, H-3); ^{13}C NMR (125.77 MHz, D_2O) δ 12.9 (C-6), 19.0 (C-5), 28.3 (C-4), 49.5 (t, J =

2.9 Hz, C-3), 58.4 (t, *J* = 115.2 Hz, C-1); ^{31}P NMR (202.46 MHz, D_2O) δ 8.39 ppm. HRMS (ESI) calcd. for ($C_5H_{15}O_6NP_2Na$) $[M + Na]^+$ 270.0267; found *210.0262. Anal*, calcd. for ($C_5H_{15}O_6NP_2$): C, 24.30; H, 6.12; N, 5.67. Found C, 24.69; H, 6.60; N, 5.39.

1-*n*-Pentylaminomethyl-1,1-bisphosphonic acid (23).—white solid; 70% yield; mp = 201 °C; ^{1}H NMR (500.13 MHz, D_2O) δ 0.79 (t, *J* = 7.1 Hz, 3H, H-7), 1.25 (m, 4H, H-5, H-6), 1.62 (q, *J* = 7.5 Hz, 2H, H-4), 2.88 (t, *J* = 16.3 Hz, 1H, H-1), 3.24 (t, *J* = 7.8 Hz, 2H, H-3); ^{13}C NMR (125.77 MHz, D_2O) δ 13.1 (C-7), 21.6 (C-6), 26.0 (C-5), 27.7 (C-4), 49.8 (t, *J* = 2.6 Hz, C-3), 58.5 (t, *J* = 115.4 Hz, C-1); ^{31}P NMR (202.46 MHz, D_2O) δ 8.45 ppm. HRMS (ESI) calcd. for ($C_6H_{18}O_6NP_2$) $[M+H]^+$ 262.0604; found 262.0606.

1-*n*-Hexylaminomethyl-1,1-bisphosphonic acid (24).—White solid, 81% yield; mp = 210 °C; ^{1}H NMR (500.13 MHz, D_2O) δ 0.77 (t, *J* = 7.1 Hz, 3H, H-8), 1.23 (m, 4H, H-6, H-7), 1.31 (m, 2H, H-5), 1.61 (q, *J* = 7.6 Hz, 2H, H-4), 2.87 (t, *J* = 16.3 Hz, 1H, H-1), 3.23 (t, *J* = 7.6 Hz, 2H, H-3); ^{13}C NMR (125.77 MHz, D_2O) δ 13.3 (C-8), 21.7 (C-7), 25.2 (C-5), 26.3 (C-4), 30.3 (C-6), 49.8 (t, *J* = 3.2 Hz, C-3), 58.5 (t, *J* = 116.3 Hz, C-1); ^{31}P NMR (202.46 MHz, D_2O) δ 8.46 ppm. HRMS (ESI) calcd. for ($C_7H_{20}O_6NP_2$) $[M + H]^+$ 276.0760; found 277.0767. *Anal*, calcd. for ($C_7H_{19}O_6NP_2$): C, 30.55; H, 6.96; N, 5.09. Found C, 30.17; H, 7.06; N, 4.97.

1-*n*-Heptylaminomethyl-1,1-bisphosphonic acid (25).—White solid; mp 198 °C. ^{1}H NMR (500.13 MHz, D_2O) δ 0.78 (t, *J* = 6.9 Hz, 3H, H-9), 1.21 (m, 4H, H-7, H-8), 1.29 (m, 2H, H-5, H-6), 1.63 (p, *J* = 7.7 Hz, 2H, H-4), 2.90 (t, *J* = 16.4 Hz, 1H, H-1), 3.25 (t, *J* = 7.6 Hz, 2H, H-3); ^{13}C NMR (125.77 MHz, D_2O) δ 13.3 (C-9), 21.9 (C-8), 25.5 (C-5), 26.3 (C-4), 28.0 (C-6), 30.8 (C-7), 49.8 (t, *J* = 3.1 Hz, C-3), 58.4 (t, *J* = 116.0 Hz, C-1); ^{31}P NMR (202.46 MHz, D_2O) δ 8.36 ppm. HRMS (ESI) calcd. for ($C_8H_{21}O_6NP_2Na$) $[M + Na]^+$ 312.0736; found 312.0735. *Anal*, calcd. for ($C_8H_{21}O_6NP_2$): C, 33.22; H, 7.32; N, 4.84. Found C, 33.65; H, 7.01; N, 5.11.

1-*n*-Octylaminomethyl-1,1-bisphosphonic acid (26).—White solid; 97% yield; mp = 201 °C. ^{1}H NMR (500.13 MHz, D_2O) δ 0.75 (m, 3H, H-10), 1.17 (m, 6H, -C*H*$_2$-), 1.28 (m, 4H, -C*H*$_2$-), 1.61 (m, 2H, H-4), 2.87 (t, *J* = 16.2 Hz, 1H, H-1), 3.22 (t, *J* = 7.0 Hz, 2H, H-3); ^{13}C NMR (125.77 MHz, D_2O) δ 13.4 (C-10), 22.0 (C-9), 25.6 (C-5), 26.3 (C-4), 28.1 (C-6), 28.2 (C-8), 31.0 (C-7), 49.8 (t, *J* = 2.8 Hz, C-3), 58.5 (t, *J* = 115.5 Hz, C-1); ^{31}P NMR (202.46 MHz, D_2O) δ 8.51 ppm. HRMS (ESI) calcd. for ($C_9H_{23}O_6NP_2Na$) $[M+Na]^+$ 326.0893; found 326.0850. *Anal*, calcd. for ($C_9H_{23}O_6NP_2$): C, 35.65; H, 7.65; N, 4.62. Found C, 35.48; H, 8.01; N, 3.86.

1-*n*-Nonylaminomethyl-1,1-bisphosphonic acid (27).—White solid; 82% yield; mp = 195 °C. ^{1}H NMR (500.13 MHz, D_2O) δ 0.77 (t, *J* = 6.8 Hz, 3H, H-11), 1.20 (m, 8H, -C*H*$_2$-), 1.28 (m, 4H, -C*H*$_2$-), 1.64 (p, *J* = 7.5 Hz, 2H, H-4), 3.00 (t, *J* = 16.3 Hz, 1H, H-1), 3.25 (t, *J* = 7.6 Hz, 2H, H-3); ^{13}C NMR (125.77 MHz, D_2O) δ 13.4 (C-11), 22.0 (C-10), 25.6 (C-5), 26.2 (C-4), 28.26 (C-6), 28.31 (C-9), 28.4 (C-8), 31.1 (C-7), 49.7 (t, *J* = 3.1 Hz, C-3), 57.0 (t, *J* = 113.3 Hz, C-1); ^{31}P NMR (202.46 MHz, D_2O) δ 8.34 ppm. HRMS (ESI) calcd. for ($C_{10}H_{26}O_6NP_2$) $[M+H]^+$ 318.1230; found 318.1220. *Anal.* calcd. for ($C_{10}H_{25}O_6NP_2$): C, 37.85; H, 7.94; N, 4.41. Found C, 37.82; H, 8.07; N, 4.44.

1-*n*-Decylaminomethyl-1,1-bisphosphonic acid (28).—White solid; 100% yield; mp = 197 °C; ^{1}H NMR (500.13 MHz, D_2O) δ 0.76 (t, *J* = 6.9 Hz, 3H, H-12), 1.18 (m, 10H, -C*H*$_2$-), 1.28 (m, 4H, -C*H*$_2$-), 1.62 (p, *J* = 7.4 Hz, 2H, H-4), 2.88 (t, *J* = 16.8 Hz, 1H, H-1), 3.23 (t, *J* = 7.6 Hz, 2H, H-3); ^{13}C NMR (125.77 MHz, D_2O) δ 13.4 (C-12), 22.0 (C-11), 25.6 (C-5), 26.4 (C-4), 28.26 (C-6), 28.41 (C-10), 28.45 (C-9), 28.6 (C-8), 31.1 (C-7), 49.9 (t, *J* = 2.9 Hz, C-3), 58.5 (t, *J* = 115.6 Hz, C-1); ^{31}P NMR (202.46 MHz, D_2O) δ 8.44 ppm. HRMS (ESI) calcd. for ($C_{11}H_{27}O_6NP_2Na$) $[M+Na]^+$ 354.1206; found 354.1208. *Anal.* calcd. for ($C_{11}H_{27}O_6NP_2$): C, 39.88; H, 8.21; N, 4.23. Found C, 39.51; H, 8.01; N, 3.98.

1-*n*-Undecylaminomethyl-1,1-bisphosphonic acid (29).—White solid; 100% yield; mp = 203 °C; ^{1}H NMR (500.13 MHz, D_2O) δ 0.77 (m, 3H, H-13), 1.19 (m, 16H, -C*H*$_2$-), 1.64 (m, 2H, H-4), 3.11 (t, *J* = 15.7 Hz, 1H, H-1), 3.25 (m, 2H, H-3); ^{13}C NMR (125.77 MHz, D_2O) δ 13.4 (C-13), 22.0 (C-12), 25.6 (C-5), 26.1 (C-4), 28.25 (C-6), 28.46 (C-10, C-11), 28.6 (C-9), 28.7 (C-8), 31.2 (C-7), 49.5 (t, *J* = 3.3 Hz, C-3), 55.5 (t, *J* = 111.8 Hz, C-1); ^{31}P NMR (202.46 MHz, D_2O) δ 8.33 ppm. HRMS (ESI) calcd. for ($C_{12}H_{29}O_6NP_2Na$) $[M+Na]^+$ 368.1362; found 368.1361. *Anal.* calcd. for ($C_{12}H_{29}O_6NP_2$): C, 41.74; H, 8.46; N, 4.06. Found C, 41.21; H, 8.32; N, 3.93.

(Cyclopentylamino)methyl-1,1-bisphosphonic acid (30).—White solid; 99% yield; mp 210 °C; ^{1}H NMR (500.13 MHz, $CDCl_3$) δ 1.62 (m, 6H, H4a, H-5), 2.05 (m, 2H, H-4b), 3.43 (t, *J* = 18.1 Hz, 1H, H-1), 3.98 (p, *J* = 7.2 Hz, 1H, H-3); ^{13}C NMR (125.77 MHz, $CDCl_3$) δ 23.6 (C-5), 29.2 (C-4), 54.0 (t, *J* = 124.3 Hz, C-1), 61.3 (t, *J* = 3.2 Hz, C-3); ^{31}P NMR (202.46 MHz, D_2O) δ 8.02 ppm. HRMS (ESI) calcd. for $C_6H_{16}NO_6P_2$ [M + H] $^+$ 260.0447; found 260.0454. *Anal.* calcd. for ($C_6H_{15}O_6NP_2$): C, 27.81; H, 5.83; N, 5.41. Found C, 28.17; H, 5.72; N, 5.08.

(Cyclohexylamino)methyl-1,1-bisphosphonic acid (31).—white solid; 94% yield; mp 213 °C; ^{1}H NMR (500.13 MHz, D_2O) δ 1.14 (m, 2H, H-6$_{ax}$), 1.28 (sext, *J* = 11.5 Hz, 4H, H-5), 1.54 (m, 2H, H-6$_{eq}$), 1.71 (m, 2H, H-4$_{ax}$), 2.04 (m, 2H, H-4$_{eq}$), 3.06 (t, *J* = 16.2 Hz, 1H, H-1), 3.46 (m, 1H, H-3); ^{13}C NMR (125.77 MHz, $CDCl_3$) δ 23.7 (C-6), 24.6 (C-5), 29.5 (C-4), 54.9 (t, *J* = 124.3 Hz, C-1), 57.9 (C-3); ^{31}P NMR (202.46 MHz, D_2O) δ 8.72 ppm. HRMS (ESI) calcd. for $C_7H_{18}NO_6P_2$ $[M + H]^+$ 274.0604; found 274.0606. *Anal.* calcd. for ($C_7H_{17}O_6NP_2$): C, 30.78; H, 6.27; N, 5.13. Found C, 30.43; H, 6.18; N, 4.99.

(Cycloheptylamino)methyl-1,1-bisphosphonic acid (32).—White solid; 83% yield; mp 225 °C; ^{1}H NMR (500.13 MHz, D_2O) δ 1.47 (m, 6H, -C*H*$_2$-), 1.62 (m, 6H, -C*H*$_2$-), 2.04 (p, *J* = 6.5 Hz, 2H, H-5$_b$), 3.12 (t, *J* = 16.3 Hz, 1H, H-1), 3.62 (sept, *J* = 4.4 Hz, 1H, H-3); ^{13}C NMR (125.77 MHz, D_2O) δ 23.1 (C-6), 27.4 (C-5), 30.8 (C-4), 54.4 (t, *J* = 112.4 Hz, C-1), 61.0 (t, *J* = 3.1 Hz, C-3); ^{31}P NMR (202.46 MHz, D_2O) δ 8.74 ppm. HRMS (ESI) calcd. for $C_8H_{20}NO_6P_2$ $[M+H]^+$ 288.0760; found 288.0758. *Anal.* calcd. for ($C_8H_{19}O_6NP_2$): C, 33.46; H, 6.67; N, 4.88. Found C, 33.70; H, 6.58; N, 4.65.

(Piperidin-1-yl)methyl-1,1-bisphosphonic acid (33).—White solid; 82% yield; mp 238 °C; ^{1}H NMR (500.13 MHz, D_2O) δ 1.69 (m, 2H, H-5), 1.87 (m, 4H, H-4), 3.15 (t, *J* = 17.6 Hz, 1H, H-1), 3.52 (m, 4H, H-3); ^{13}C NMR (125.77 MHz, D_2O) δ 21.0 (C-5), 24.3 (C-4), 53.8 (C-3), 63.8 (t, *J* = 106.8 Hz, C-1); ^{31}P NMR (202.46 MHz, D_2O) δ 6.77 ppm.

HRMS (ESI) calcd. for $C_6H_{15}NO_6P_2Na$ $[M+Na]^+$ 282.0267; found 282.0258. *Anal.* calcd. for ($C_6H_{15}O_6NP_2$): C, 27.81; H, 5.83; N, 5.41. Found C, 27.44; H, 5.58; N, 5.06.

1-[(Cyclopentylamino)ethyl] 1,1-bisphosphonic acid (47).—White solid; 88% yield; mp 228 °C; ^{1}H NMR (500.13 MHz, D_2O) δ 1.62 (m, 6H, H-5_a, H-6), 1.95 (m, 2H, H-5_b), 2.39 (tt, *J* = 21.5, 7.3 Hz, 1H, H-1), 3.35 (dt, *J* = 14.3, 7.3 Hz, 2H, H-2), 3.55 (m. 1H, H-4); ^{13}C NMR (125.77 MHz, D_2O) δ 23.4 (C-6), 29.4 (C-5), 36.3 (t, *J* = 120.9 Hz, C-1), 43.6 (t, *J* = 2.4 Hz, C-2), 59.5 (C-4); ^{31}P NMR (202.46 MHz, D_2O) δ 15.58 ppm. HRMS (ESI) calcd. for $C_7H_{18}O_6NP_2$ $[M+H]^+$ 274.0604; found 274.0610.

1-[(Cycloheptylamino)ethyl] 1,1-bisphosphonic acid (48).—73% yield; white solid; mp 220 °C; ^{1}H NMR (500.13 MHz, D_2O) δ 1.45 (m, 6H, H-6_a, H-7), 1.60 (m, 4H, H-5_a, H-6_b), 1.97 (m, 2H, H-5_b), 2.37 (tt, *J* = 21.4, 7.3 Hz, 1H, H-1), 3.28 (hept, *J* = 4.4 Hz, 1H, H-4), 3.36 (dt, *J* = 14.3, 7.3 Hz, 2H, H-2); ^{13}C NMR (125.77 MHz, D_2O) δ 23.0 (C-7), 27.3 (C-6), 30.6 (C-5), 36.2 (t, *J* = 120.8 Hz, C-1), 42.3 (t, *J* = 2.3 Hz, C-2), 59.4 (C-4); ^{31}P NMR (202.46 MHz, D_2O) δ 15.59 ppm. HRMS (ESI) calcd. for $C_9H_{22}O_6NP_2$ $[M+H]^+$ 302.0917; found 302.0905.

1-[(4-methylcyclohexylamino)ethyl] 1,1-bisphosphonic acid (49).—76% yield; white solid; mp = 175 °C; ^{1}H NMR (125.77 MHz, D_2O) δ 0.80 (d, *J* = 6.6 Hz, 6H, $CH(CH_3)_2$), 0.84 (d, *J* = 6.6 Hz, 6H, $CH(CH'_3)_2$), 2.41 (tt, *J* = 21.4, 7.3 Hz, 1H, H-1), 3.25 (m. 1H, H-4), 3.36 (dt, 7= 14.0, 7.1 Hz, 2H, H-2); ^{13}C NMR (125.77 MHz, D_2O) δ 19.9 21.0 (CH_3), 25.4 (C-5), 28.4 28.9 (C-5), 30.9 32.2 (C-6), 36.1 (t, 7= 121.1 Hz, C-1), 36.2 (t, *J* = 120.8 Hz, C-1), 42.0 (t, *J* = 2.2 Hz, C-2), 42.5 (t, *J* = 2.6 Hz, C-2), 55.5 57.1 (C-4); ^{31}P NMR (202.46 MHz, D_2O) δ 15.59 ppm. HRMS (ESI) calcd. for $C_9H_{22}O_6NP_2$ $[M+H]^+$ 302.0917; found 302.0931.

Tetraethyl 1-[(*n*-Undec-1-ylthio)ethyl]-1,1-bisphosphonate (60).—74% yield, colorless oil; ^{1}H NMR (500.13 MHz, $CDCl_3$) δ 0.88 (t, *J* = 7.0 Hz, 3H, H-14), 1.26 (m,16H, -CH_2-), 1.35 (t, *J* = 7.1 Hz, 12H, CH_2CH_3), 1.59 (p, *J* = 7.3 Hz, 2H, H-5), 2.56 (t, *J* = 7.4 Hz, 2H, H-4), 2.59 (tt, *J* = 23.6, 6.0 Hz, 1H, H-1), 3.04 (dt, *J* = 16.3, 5.9 Hz, 2H, H-2), 4.21 (m, 8H, CH_2CH_3); ^{13}C NMR (125.77 MHz, $CDCl_3$) δ 14.1 (C-14), 16.3 (d, *J* = 6.4 Hz, CH_2CH_3), 22.6 (C-13), 27.7 (t, *J* = 5.2 Hz, C-2), 28.8 (C-12), 29.2 (C-11), 29.3 (C-10), 29.4 (C-9), 29.47 (C-8), 29.53 (C-6, C-7), 31.8 (C-5), 33.1 (C-4), 39.0 (t, *J* = 131.4 Hz, C-1), 62.8 (dd, *J* = 18.8, 6.8 Hz, CH_2CH_3); ^{31}P NMR (202.46 MHz, $CDCl_3$) δ 21.75.

Tetraethyl 1-[(n-Dodec-1-ylthio)ethyl]-1,1-bisphosphonate (61).—Colorless oil; 55% yield; ^{1}H NMR (500.13 MHz, $CDCl_3$) δ 0.88 (t, *J* = 7.0 Hz, 3H, H-15), 1.26 (m,16H, -CH_2-), 1.35 (t, *J* = 7.1 Hz, 12H, CH_2CH_3), 1.59 (p, *J* = 7.5 Hz, 2H, H-5), 2.56 (t, *J* = 7.5 Hz, 2H, H-4), 2.59 (tt, *J* = 23.9, 5.9 Hz, 1H, H-1), 3.04 (dt, *J* = 16.3, 5.9 Hz, 2H, H-2), 4.21 (m, 8H, CH_2CH_3); ^{13}C NMR (125.77 MHz, $CDCl_3$) δ 14.1 (C-15), 16.4 (d, *J* = 6.3 Hz, CH_2CH_3), 22.7 (C-14), 27.7 (t, *J* = 4.9 Hz, C-2), 28.9 (C-13), 29.2 (C-12), 29.3 C-11), 29.4 (C-10), 29.51 (C-9), 29.57 (C-8), 29.59 (C-7), 29.62 (C-6), 31.9 (C-5), 33.1 (C-4), 39.1 (t, *J* = 131.4 Hz, C-1), 62.8 (dd, *J* = 18.7, 6.6 Hz, CH_2CH_3); ^{31}P NMR (202.46 MHz, $CDCl_3$) δ 21.76.

1-[(*n*-Undecylthio)ethyl]-1,1-bisphosphonic acid (54).—45% yield; white solid; mp = 95 °C; ^{1}H NMR (500.13 MHz, DMSO-d_6) δ 0.84 (t, *J* = 6.9 Hz, 3H, H-14), 1.23 (m, 14H, -C*H*$_2$-), 1.31 (m, 2H, H-6), 1.49 (p, *J* = 7.3 Hz, 2H, H-5), 2.10 (tt, *J* = 22.5, 6.0 Hz, 1H, H-1), 2.84 (t, *J* = 15.5, 6.1 Hz, 2H, H-2); ^{31}P NMR (202.46 MHz, DMSO-d_6) δ 18.80 ppm. HRMS (ESI) Calcd. for $C_{13}H_{31}O_6P_2S$ $[M+H]^+$ 377.1311; found 377.1324.

1-[(*n*-Dodecylthio)ethyl]-1,1-bisphosphonic acid (57).—80% yield; white solid; mp = 110 °C; ^{1}H NMR (500.13 MHz, DMSO-d_6) δ 0.84 (t, *J* = 7.0 Hz, 3H, H-15), 1.23 (m, 16H, -C*H*$_2$-), 1.30 (p, *J* = 7.6 Hz, 2H, H-6), 1.49 (p, *J* = 7.3 Hz, 2H, H-5), 2.10 (tt, *J* = 22.5, 6.0 Hz, 1H, H-1), 2.84 (t, *J* = 15.5, 6.1 Hz, 2H, H-2); ^{31}P NMR (202.46 MHz, DMSO-d_6) δ 18.97 ppm. HRMS (ESI) Calcd. for $C_{14}H_{33}O_6P_2S$ $[M+H]^+$ 391.1468; found 391.1453

1-[(*n*-Undecylsulfmyl)ethyl]-1,1-bisphosphonic acid (55).—81% yield; white solid; mp = 97 °C; ^{1}H NMR (500.13 MHz, D_2O) δ 0.87 (t, *J* = 7.0 Hz, 3H, H-14), 1.20 (m, 12H, -C*H*$_2$-), 1.28 (p, *J* = 5.9 Hz, 2H, H-7), 1.39 (m, 2H, H-6), 1.69 (m, 2H, H-5), 2.39 (m, 1H, H-1), 2.76 (ddd, *J* = 13.9, 8.5, 6.0 Hz, 1H, H-4$_a$), 2.89 (ddd, *J* = 13.5, 8.4, 7.5 Hz, 1H, H-4$_b$), 3.15 (m, 2H, H-2); ^{31}P NMR (202.46 MHz, D_2O) δ 16.09 m_{AB}. HRMS (ESI) Calcd. for $C_{13}H_{30}O_7P_2SNa$ $[M+Na]^+$ 415.1080; found 415.0797.

1-[(*n*-Dodecylsulfinyl)ethyl]-1,1-bisphosphonic acid (58).—White solid, 67%; mp = 94 °C; ^{1}H NMR (500.13 MHz, DMSO-d_6) δ 0.84 (t, *J* = 7.0 Hz, 3H, H-15), 1.23 (m, 14H, -C*H*$_2$-), 1.38 (p, *J* = 6.9 Hz, 2H, H-6), 1.61 (p, *J* = 7.4 Hz, 2H, H-5), 2.57 (dt, *J* = 13.7, 6.9 Hz, 1H, H-4$_a$), 2.81 (dt, *J* = 13.1, 8.0 Hz, 1H, H-4$_b$), 3.05 (m, 2H, H-2); ^{31}P NMR (202.46 MHz, DMSO-d_6) δ 17.31 m_{AB}.

1-[(*n*-Undecylsulfonyl)ethyl]-1,1-biphosphonic acid (56).—58% yield; white solid; mp = 128 °C; ^{1}H NMR (500.13 MHz, DMSO-d_6) δ 0.84 (t, *J* = 6.9 Hz, 3H, H-14), 1.22 (m, 14H, CH2), 1.33 (p, J = 7.3 Hz, 2H, H-6), 1.65 (p, *J* = 7.7 Hz, 2H, H-5), 3.26 (m, 2H, H-4), 3.37 (t, *J* = 16.3, 5.2 Hz, 2H, H-2); ^{31}P NMR (202.46 MHz, DMSO-d_6) δ 17.13 ppm. HRMS (ESI) Calcd. for $C_{13}H_{31}O_8P_2S$ $[M+H]^+$ 409.1209; found 409.1198.

1-[(*n*-Dodecylsulfonyl)ethyl]-1,1-biphosphonic acid (59).—65% yield; white solid; mp 125 °C; ^{1}H NMR (500.13 MHz, DMSO-d_6) δ 0.84 (t, *J* = 6.9 Hz, 3H, H-15), 1.23 (m, 14H, -C*H*$_2$-),1.33 (p, *J* = 7.0 Hz, 2H, H-6), 1.64 (p, *J* = 7.6 Hz, 2H, H-5), 3.21 (m, 2H, H-4) 3.38 (dt, *J* = 16.1, 4.7 Hz, 1H, H-2); ^{13}C NMR (125.77 MHz, DMSO-d_6) δ 14.0 (C-15), 21.5 (C-14), 22.1 (C-5), 27.8 (C-6), 28.6 (C-7), 28.76 (C-8), 28.84 (C-9), 29.0 (C-12), 29.06 (C-10), 29.08 (C-11), 31.3 (C-13), 34.1 (t, *J* = 122.3 Hz, C-1), 49.7 (t, *J* = 4.0 Hz, C-2), 52.5 (C-4); ^{31}P NMR (202.46 MHz, DMSO-d_6) δ 17.04 ppm. HRMS (ESI) Calcd. for $C_{14}H_{32}O_8P_2SNa$ $[M+Na]^+$ 445.1185; found 445.1201.

Drug Screening

T. cruzi amastigote assays

These experiments were done as reported using tdTomato labeled trypomastigotes with the modifications described by Recher et al., 2013.[52] ED_{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 using *T. gondii* tachyzoites expressing red fluorescent protein with the modifications described by Recher et al., 2013.[52] Plates were read with covered lids, and both excitation (544 nm) and emission (590 nm) were read from the bottom.

Cytotoxicity for Vero cells.—The cytotoxicity was tested using the Alamar Blue™ assay as described by Recher et al., 2013.[52]

***Tc*FPPS and *Tg*FPPS assays and product analysis.—**The enzymatic activity of the target enzymes was performed according to our previous studies as described for Szajnman et al., 2008.[48]

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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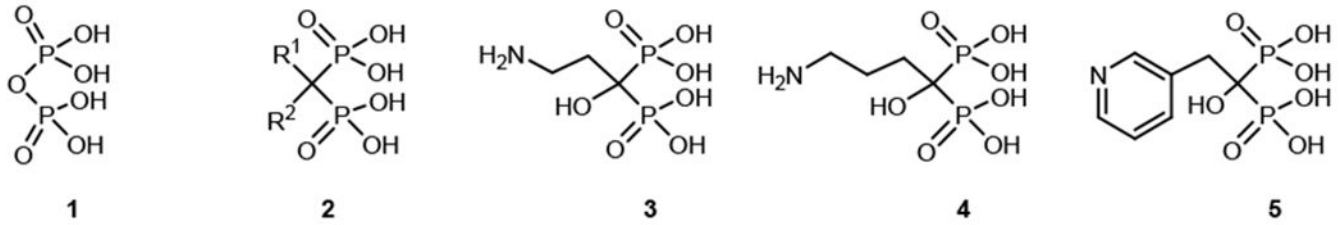
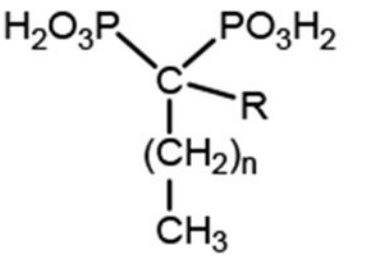
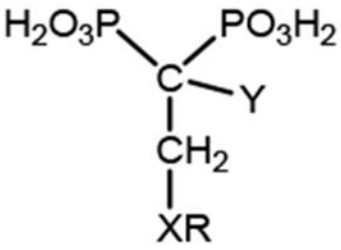


Figure 1.

Chemical structures of pyrophosphoric acid, general formula of bisphosphonic acids and representative FDA-approved bisphosphonates clinically employed for the treatment of different bone disorders.



6, n = 5; R = OH
7, n = 9; R = OH
8, n = 5; R = H
9, n = 4; R = NH_2
10, n = 7; R = F
11, n = 8; R = F



12, X = NH; Y = H; R = *n*-hexyl
13, X = NH; Y = H; R = *n*-heptyl
14, X = NH; Y = H; R = *n*-dodecyl
15, X = NH; Y = OH; R = *n*-octyl
16, X = S; Y = H; R = *n*-heptyl
17, X = S; Y = H; R = *n*-decyl
18, X = $S(O)_2$; Y = H; R = *n*-decyl
19, X = $S+(CH_3)$; Y = H; R = *n*-octyl
20, X = NH; Y = F; R = alkyl

20a, R = *n*-propyl; **20b,** R = *n*-butyl; **20c,** R = *n*-pentyl; **20d,** R = *n*-hexyl;
20e, R = *n*-heptyl; **20f,** R = *n*-octyl; **20g,** R = *n*-nonyl; **20h,** R = *n*-decyl;

Figure 2.
Chemical structures of representative lineal bisphosphonic acids developed in our laboratory as putative antiparasitic agents targeting FPPS.

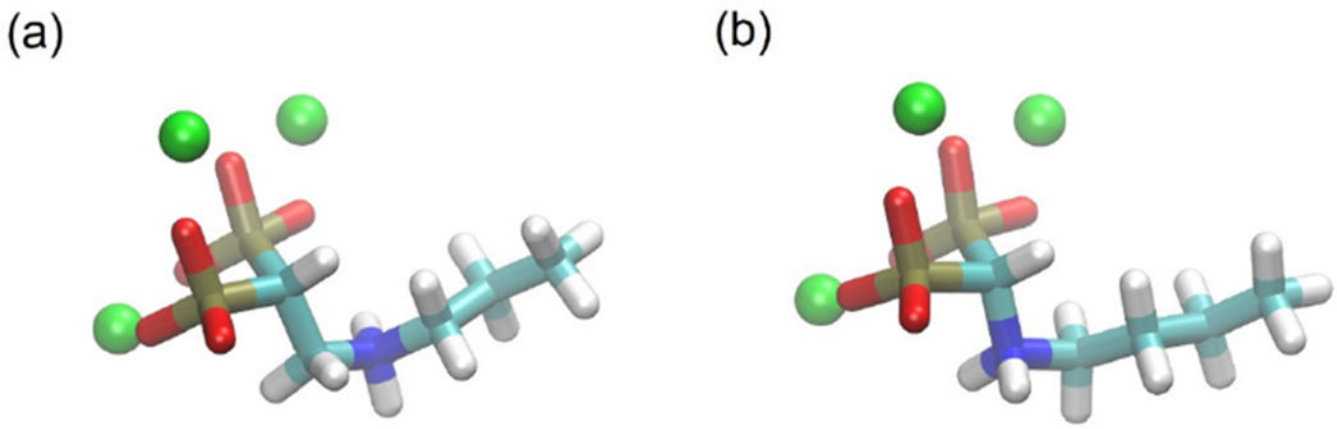


Figure 3.

The side chain is fixed in one orientation, directed towards the unsymmetrical magnesium ion present at the allylic site of the active site of *Tc*FPPS.

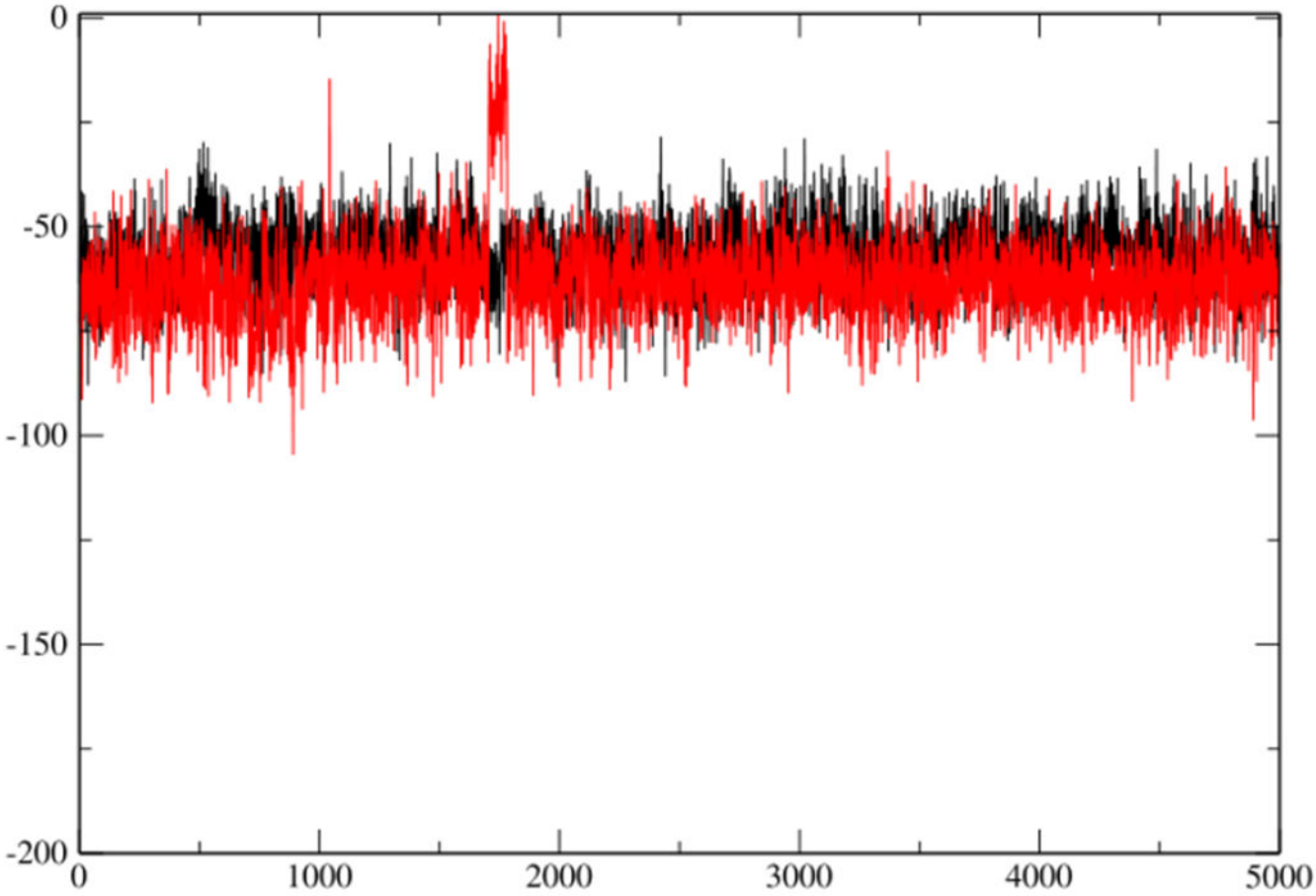


Figure 4.
Dihedral angle P-C1-C2-N for **21** (red) and P-C1-N-C3 for **62** (black) throughout the MD simulation

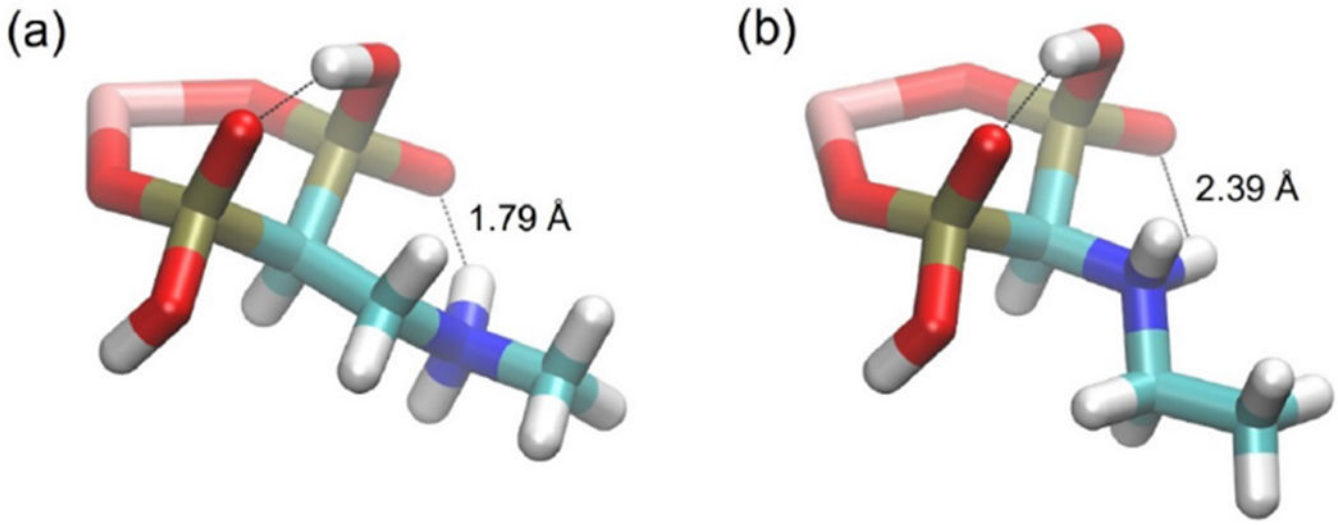
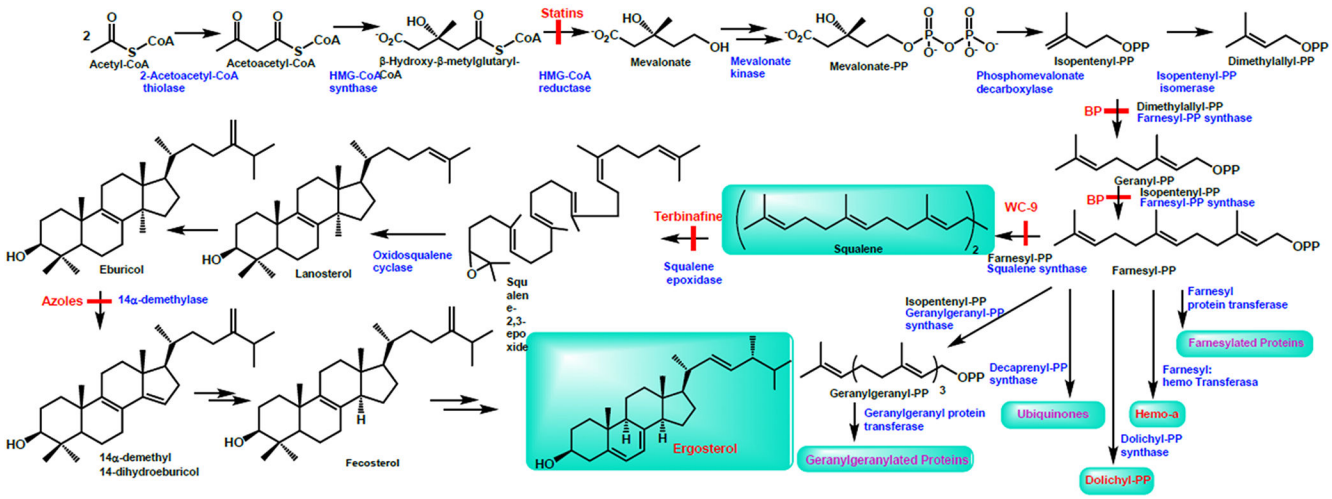
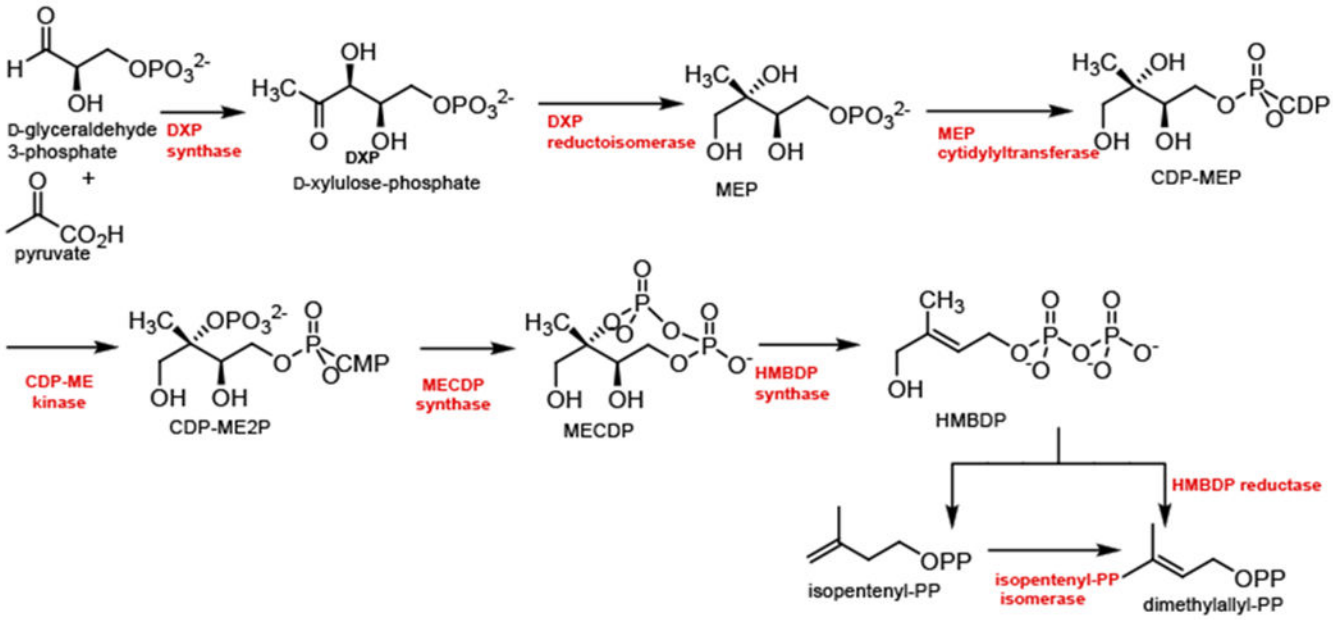


Figure 5.
A strong intramolecular hydrogen bond between the phosphate groups for both compounds.



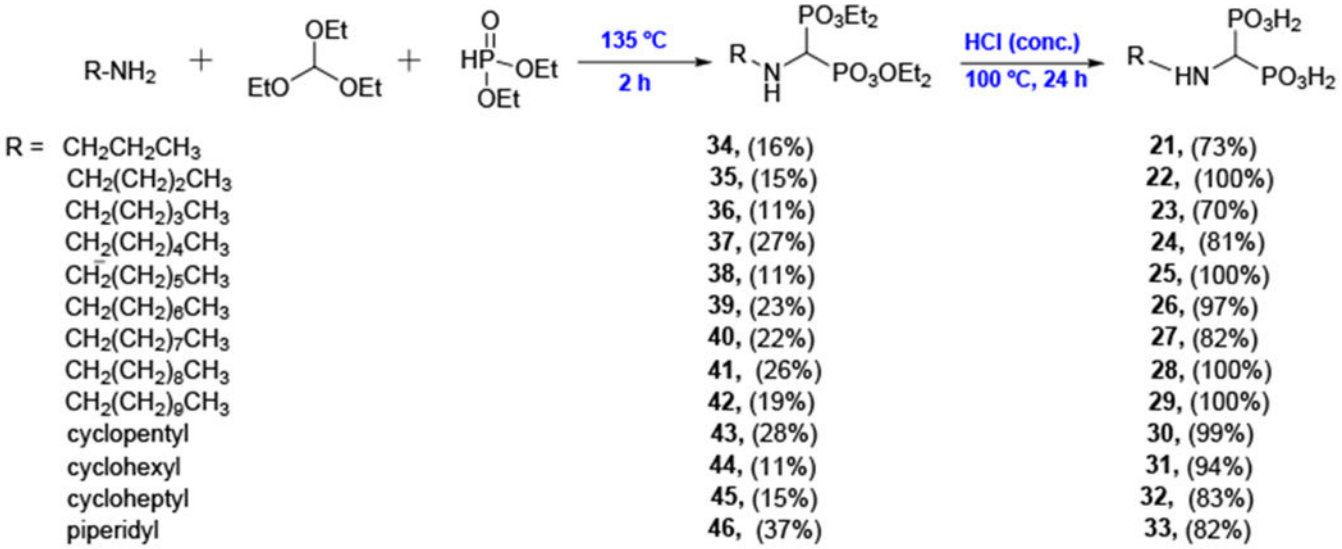
Scheme 1.

Ergosterol biosynthesis for trypanosomatids.



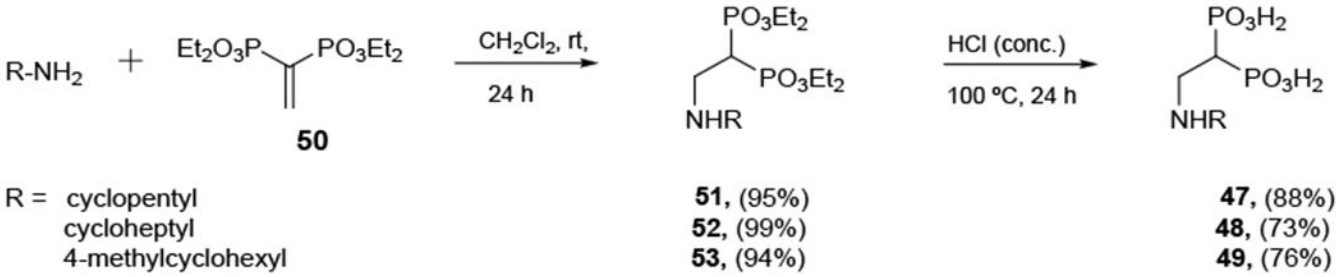
Scheme 2.

The 1-deoxy-D-xylulose-5-phosphate (DOXP) pathway to biosynthesize isopentenyl diphosphate and dimethylallyl diphosphate in Apicomplexan parasites.



Scheme 3.

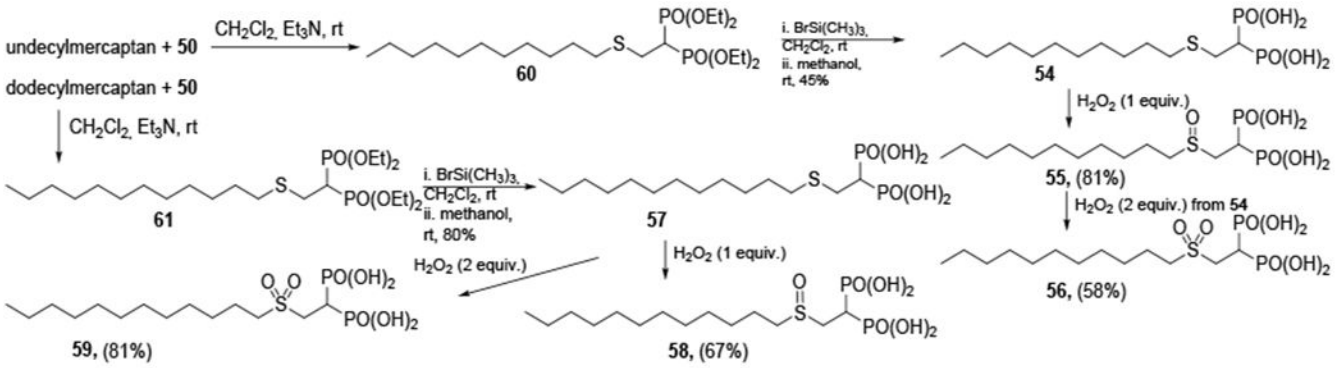
Synthetic approach for the preparation of 2-alkylaminomethyl-1,1-bisphosphonic acids.



R = cyclopentyl — **51**, (95%) — **47**, (88%)
cycloheptyl — **52**, (99%) — **48**, (73%)
4-methylcyclohexyl — **53**, (94%) — **49**, (76%)

Scheme 4.

Synthetic approach for the synthesis of cycloalkyl 2-aminoethyl-1,1-bisphosphonates.



Scheme 5.

Synthetic approach for the preparation of long chain sulfur-containing-1,1-bisphosphonates.

Table 1.

Biological activity of bisphosphonates previously prepared in our laboratory against *T. cruzi* (amastigotes), *T. gondii* (tachyzoites), *Tc*FPPS, and *Tg*FPPS.

Compound	*T. cruzi* growth ED_{50} (μM)	*Tc*FPPS IC_{50} (μM)	*T. gondii* growth ED_{50} (μM)	*Tg*FPPS IC_{50} (μM)
10	> 10.0	> 1.0	2.67	0.035
11	> 10.0	> 1.0	> 10.0	0.060
12	0.84	0.49	9.4	0.14
13	10.0	0.058	> 50.0	0.095
14	0.67	0.81	6.23	0.093
15	> 10.0	> 10.0	2.0	0.039
16	> 20.0	> 10.0	0.97	0.069
17	> 20.0	0.097	1.8	0.021
18	NT	NT	0.11	0.27
19	>20.0	0.040	7.0	0.013

Table 2.

Biological activity of bisphosphonates against *T. cruzi* (amastigotes), *T. gondii* (tachyzoites), *Tc*FPPS, *Tg*FPPS, and Vero cells

Compound	*T. cruzi* cells ED_{50} μM	*T. gondii* cells ED_{50} μM	*Tc*FPPS IC_{50} (μM)	*Tg*FPPS IC_{50} (μM)	Cytotoxicity ED_{50} (μM)
20a	> 10.0[55]		0.060 ± 0.03	0.245 ± 0.081	NT
20b	> 10.0[55]	> 10.0[55]	0.173 ± 0.04	0.286 ± 0.087	> 200[55]
20c	> 10.0[55]	> 10.0[55]	0.611 ± 0.37	0.299 ± 0.028	NT
20d	> 10.0[55]	>10.0[55]	0.915 ± 0.18	0.131 ± 0.017	NT
20e	> 10.0[55]	>10.0[55]	0.071 ± 0.02	0.088 ± 0.005	NT
20f	> 10.0[55]	4.008 ± 1.191[55]	0.085 ± 0.03	0.079 ± 0.013	161.7[55]
20g	cytotoxic[55]	2.24 ± 0.31	0.034 ± 0.023	0.051 ± 0.006	15.0
20h	cytotoxic[55]	2.26 ± 0.75	0.136 ± 0.047	0.080 ± 0.004	20.0

Table 3.

Biological activity of bisphosphonates against *T. cruzi* (amastigotes), *T. gondii* (tachyzoites), and Vero cells

Compound	*T. cruzi* cells ED_{50} μM	*T. gondii* cells ED_{50} μM	Cytotoxicity ED_{50} (μM)
21	> 10.0		
22	> 10.0		
23	> 10.0		
24	> 10.0		
25	> 10.0		
26	> 10.0		
27	> 10.0		
28	5.26 ± 0.45		
29	Cytotoxic[a]		10.0
30	> 10.0		
31	> 10.0		
32	> 10.0		
33	> 10.0		
47	> 10.0	> 20.0	
48	> 10.0	> 20.0	
49	> 10.0	> 20.0	
54		0.54 ± 0.10	
55		0.53 ± 0.096	
56		0.38 ± 0.082	
59		0.93 ± 0.24	
benznidazole	2.58 ± 0.50		

[a]cytotoxic at 10 μM, 5 μM, no inhibition at 2.5 μM