Synthesis of Stable Analogues of Thiamine Di- and Triphosphate as Tools for Probing a New Phosphorylation Pathway

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Abstract: Thiamine (vitamin B1) is an essential nutritional factor metabolized inside the body in its mono-, di-, and triphosphate forms. Although the action of thiamine and thiamine diphosphate have been intensely investigated, many questions remain unanswered and the role of thiamine triphosphate is still especially unknown. To probe recent hypotheses on the implication of thiamine triphosphate in a new phosphorylation pathway involving synaptic proteins, we synthesized a series of thiamine di- and triphosphate analogues that are resistant to both enzymatic and chemical hydrolyses. The key step in the preparation of the title compounds is the coupling of thiamine propyl disulfide with adequately protected methylenebisphosphonic acid, the corresponding triphosphate analogue, and difluoromethylenebisphosphonic acid.

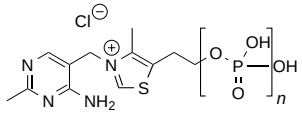
Keywords: difluoromethylenephosphonate · phosphorylation · triphosphates · vitamins

Introduction

Thiamine (**1**) was first isolated in rice bran in 1926[1] and its structure elucidated in 1936.[2] In 1937, thiamine diphosphate (ThDP) **3** was identified as cocarboxylase, the essential cofactor of a number of key enzymes.[3] Thiamine is a vitamin (vitamin B1) required by the human body. The recommended daily intake is approximately 1.5 mg and about 30 mg are stored in the body with 80 % as the diphosphate **3**, 10 % as triphosphate (ThTP) **4**, and the rest as thiamine and its monophosphate (ThMP) **2**. Thiamine is produced on an industrial scale (around 4000 tons annually) and is routinely added to bread in the western world to prevent deficiency diseases.

Since the early times, investigations on thiamine and thiamine derivatives have continued (≈200 papers in year 2001) and the implications of the presence of these compounds in various medical conditions (beri beri, Wernicke's encephalopathy, Korsakoff psychosis, Leigh syndrome, megaloblastic anaemia, and others) have been documented.[4] However, these studies mainly focused on thiamine and its pyrophosphate (90–95 % of the results published in the literature), and very few investigations were directed toward ThTP. Very recently the involvement of ThTP in the specific phosphorylation of *Torpedo* 43 K Rapsyn has been reported.[5, 6] 43 K Rapsyn is a peripheral protein specifically associated with the nicotinic acetylcholine receptor (nAChR) present in the post-synaptic membrane of the neuromuscular junction (NMJ) and of the electrocyte.[7] 43 K Rapsyn is essential for a functional NMJ.[8] The protein phosphorylation results from transfer of the γ-phosphate from ThTP onto a histidine residue by endogenous kinases that have not yet been identified.[5] The use of a phosphate donor (ThTP) belonging to the thiamine family represents a novel phosphorylation pathway possibly important for synaptic proteins and cell signaling.

To investigate the scope of that ThTP-dependent phosphorylation, analogues of the phosphate donor and its



1: $n = 0$
2: $n = 1$
3: $n = 2$
4: $n = 3$

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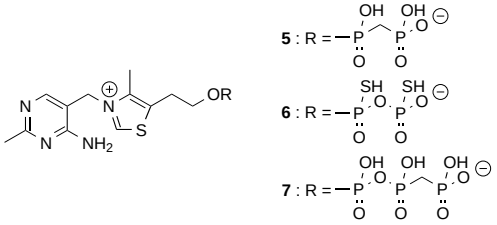
metabolites are required. Thus, we became interested in the design of enzymatically and chemically non-hydrolyzable analogues of thiamine di- and triphosphate. Herein we describe the synthesis of a series of ThDP and ThTP analogues.

Results and Discussion

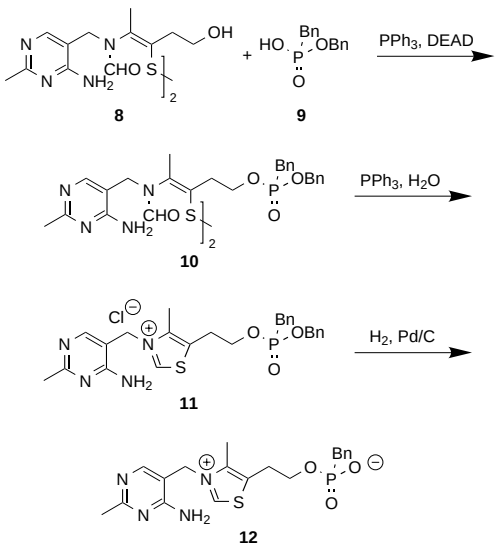
Though many thiamine and thiamine polyphosphate analogues have been described in the literature (for a review see[9]), only a very small number incorporate modifications of the polyphosphate chain (see below). To the best of our knowledge, three compounds are documented: thiamine α:β-methylenediphosphate (**5**),[10] thiamine α,β-dithiodiphosphate (**6**),[11] and thiamine β:γ-methylenetriphosphate (**7**).[12] Whereas the



dithio derivative and triphosphate analogue do not fulfill the stability requirements toward hydrolytic conditions, compound **5** appears to be a good candidate for probing part of the biological activity of the ThTP-dependent kinases. The original synthesis of that compound was achieved starting from thiamine disulfide and methylenebisphosphonic acid, but very few experimental details if any were reported.

During the past few years we have been involved in a program focused on the synthesis of stable analogues of nucleotides[13–18] and dinucleoside polyphosphates.[19] Our strategy consists of preparing a polyphosphonate building block that can be regioselectively functionalized with adequately protected nucleosides in organic solvents and in high yields. Due to the lipophilicity of the compounds, separation problems are largely overcome and purification can be performed by flash chromatography over silica gel. The final compounds are obtained after a quasi-quantitative polydeprotection of both phosphonyl and nucleoside moieties.

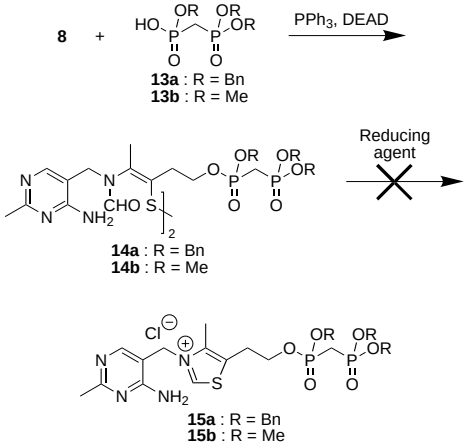
In our first attempt to prepare compound **5** we tried to use the same strategy. Thiamine chloride hydrochloride being insoluble in organic solvents, we used thiamine disulfide **8** as starting material (Scheme 1). First, we set up the reaction sequence with benzylphosphonic monobenzyl ester **9**[20] as a model phosphonate. Phosphonylation of the dimeric precursor of thiamine was carried out under the Mitsunobu conditions.[21] Bis-phosphonylated compound **10** was obtained in 81% yield. The well-documented reduction of the disulfide bridge with rapid cyclization and thiazolium formation[22] was realized in 97% yield using triphenylphosphine in the presence of water. The resulting mixed benzyl thiamine phosphonate **11** was debenzylated by catalytic hydrogenolysis to afford thiamine phosphonate **12** in 78% yield. The presence of a benzyliminium moiety and of a cyclic sulfide



Scheme 1. Synthesis of ThMP analogue **12**.

in the molecule did not cause any problem in that last transformation.

That same reaction sequence starting from methylenebisphosphonic tribenzyl ester **13a**[20] turned out differently (Scheme 2). Whereas the double phosphonylation of thiamine



Scheme 2. Tentative preparation of protected ThDP analogues starting from thiamine disulfide **8**.

disulfide could be carried out in 62% yield to give **14a**, further reduction of the disulfide bridge proved ineffective. Whatever the experimental conditions (PPh_3/H_2O, PBu_3/H_2O, $PBu_3/H_2O/HBF_4$, β-mercaptoethanol, *n*BuSH, *i*PrSH, *t*BuSH) we invariably failed in forming the reduced compound, and the starting material remained unchanged. A plausible explanation for this inert behavior toward reduction could be some compact folding of the dimer, possibly due to cumulative π-stacking interactions between the six phenyl and two pyrimidine rings in the molecule. To test the hypothesis we conducted parallel experiments in the methyl ester series. Phosphonylation of **8** with methylenebisphosphonic trimethyl ester **13b** yielded dimeric compound **14b**, which, however, proved equally resistant to disulfide reduction. Additional

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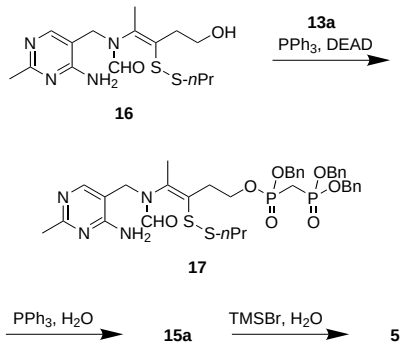
experiments were carried out in which **14a** was first submitted to hydrogenolysis before attempting disulfide reduction. We could neither identify nor detect the expected products in the crude reaction mixtures.

These negative results prompted us to revise our strategy. If the problem indeed resulted from a tight fit of the disulfide bridge within the dimer, breaking the symmetry of the molecule should restore disulfide accessibility toward reducing agents. Thus, phosphonylation of thiamine propyl disulfide **16**[23] with **13a** under the Mitsunobu conditions afforded **17** in 63% yield (Scheme 3). The latter compound was then treated

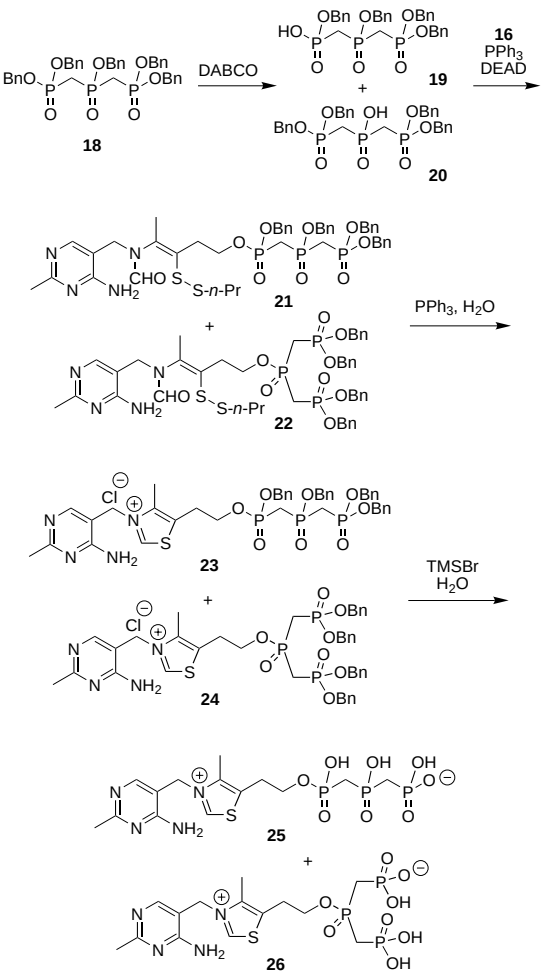


Scheme 3. Synthesis of ThDP analogue **5**.

with triphenylphosphine in aqueous 1,2-dimethoxyethane (DME) and thiamine phosphonate **15a** was obtained in 96% yield. This validates hypothesis of a compact conformation of dimers **14a** and **14b** and preventing access of any reducing agent close to the disulfide bridge. Hydrogenolysis of **15a**, however, proved troublesome. Deprotection of compound **11** could be achieved within 30 minutes without formation of byproducts, whereas the full debenzylation of **15a** required several hours. This is not compatible with the preservation of the thiazolium and benzyliminium moieties and by-products rapidly become the major products. According to previous results obtained with AZT derivatives by some of us[16, 18] debenzylation of **15a** could be achieved in a very clean manner using bromotrimethylsilane, and **5** was obtained in 96% yield.

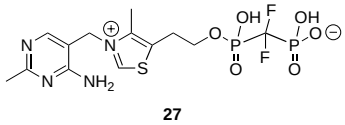
We then focused our attention on the synthesis of the thiamine triphosphate analogue **25** (Scheme 4). According to the previous strategy, disulfide **16** was condensed with phosphonic acid **19** which results from the monodeprotection of pentabenzyl ester **18**.[20, 24]

Phosphonic acid **19** was obtained as a mixture with phosphinic acid **20** (**19**:**20** 8:2). As it could not be purified by flash chromatography over silica gel, the mixture was directly used in the coupling reaction with **16**. The two isomers **21** and **22** were obtained in 42% yield (**21**:**22** 8:2). Compound **21** was present in the crude mixture as two diastereomers and was not separated from **22**. Reduction of the mixture with triphenylphosphine in the presence of water afforded compounds **23** and **24** that were directly involved in the debenzylation step using bromotrimethylsilane. Compounds **25** and **26** were finally separated by reversed-phase HPLC.



Scheme 4. Synthesis of ThTP analogues **25** and **26**.

To obtain a ThDP analogue with pK_a values closer to those of ThDP than compound **5**, we studied fluorinated compound **27**.

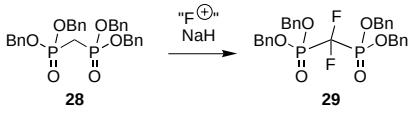


All the difluoromethylenebisphosphonic acid monoesters described so far in the literature result from nucleophilic displacement of tosylates by a quaternary ammonium salt of difluoromethylenebisphosphonic acid (Scheme 5).[25–28]

Scheme 5. General scheme for the preparation of difluoromethylenebisphosphonic monoesters described in the literature.[25–28]

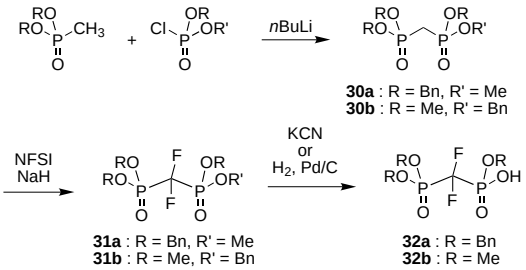
Due to the high electronegativity of the fluorine atom, difluoromethylenebisphosphonic acid is a very poor nucleophile. Consequently tosylate substitution is very slow and yields remain low. To adapt our overall strategy to the

synthesis of the difluoromethylene analogue of ThDP **27**, we prepared difluoromethylenebisphosphonic tetrabenzyl ester by electrophilic fluorination[29] of **28** (Scheme 6).



Scheme 6. Electrophilic fluorination of **28**.

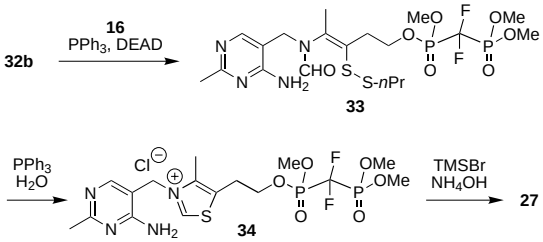
Different fluorinating agents have been tested (*N*-fluoro-*N*-methyl-*p*-toluenesulfonamide, Selectfluor, *N*-fluorobenzenesulfonimide (NFSI)); NFSI gave the best results and afforded **29** in 68% yield. The latter compound, however, proved labile. Whereas selective monodebenzylation of **28** can be achieved in refluxing toluene with 1 equiv DABCO to afford **13a**,[20] compound **29** led to a non-separable complex mixture of acids, even under milder experimental conditions. Consequently we prepared the mixed ester **30a** from dibenzyl methanephosphonate and benzyl methyl chlorophosphate (Scheme 7).



Scheme 7. Synthesis of difluoromethylenebisphosphonic acid triesters **32a** and **32b**.

Electrophilic fluorination of compound **30a** was achieved with 72% yield and removal of methyl ester in **31a** using 1 equiv KCN in hot DMF[30] was quantitative. Our attempts to perform the condensation of thiamine propyl disulfide **16** with **32a** under the Mitsunobu conditions however failed, presumably due to a rapid displacement of some benzyl groups by *N*,*N*′-dicarboxyethyl hydrazine or its deprotonated precursor. Considering the high sensitivity of benzylic positions toward nucleophiles, we transposed the reaction sequence in the methyl series assuming that methyl esters are more resistant and should prevent degradation. Trimethyl benzyl ester **30b** was fluorinated under the same conditions as for **30a**. The resulting monobenzyl ester **31b** was hydrogenolyzed to yield phosphonic acid **32b** quantitatively.

Esterification of the latter compound with **16** afforded **33** in 66% yield (Scheme 8). Although more stable than in the benzyl series, the compound slowly decomposes after a few hours, even at $-20\,^{\circ}C$. Consequently the reduction of the disulfide bridge was carried out immediately after purification. Compound **34** was purified by dissolving the crude reaction mixture in water and washing it with diethyl ether. It was obtained along with some compounds resulting from the partial deprotection of methyl esters. That mixture was not further purified butdirectly treated with bromotrimethylsilane to convert the remaining methyl esters to their corresponding phosphonic acids. Finally, compound **27** was then obtained in 38% yield after purification by reversed-phase HPLC.



Scheme 8. Synthesis of fluorinated ThDP analogue **27**.

Conclusion

Four different analogues of polyphosphorylated thiamine species (compounds **5**, **25**, **26**, and **27**) have been prepared. Whereas compounds **5** and **27** are clearly ThDP analogues, and **25** a ThTP analogue, **26** can be considered either as a ThTP analogue or as a "supercharged" ThDP analogue as earlier defined in the nucleotide series by Blackburn.[31] Compounds **5**, **25**, **26**, and **27** are currently under biological evaluation. Extensive description of the biological properties of the compounds will be reported elsewhere.

Experimental Section

General: ^{1}H, ^{13}C, ^{31}P, and ^{19}F NMR chemical shifts δ are reported in ppm relative to their standard reference (^{1}H: $CHCl_3$ at 7.27 ppm, HDO at 4.63 ppm, CD_2HOD at 3.31 ppm; ^{13}C: $CDCl_3$ at 77.0 ppm, CD_3OD at 49.0 ppm; ^{31}P: H_3PO_4 external at 0.00 ppm; ^{19}F: $CFCl_3$ external at 0.00 ppm). IR spectra were recorded in wavenumbers (cm^{-1}). Mass spectra (MS) were recorded as chemical ionization (CI) or in the electrospray (ES) mode. Mass data are reported in mass units (m/z). Analytical HPLC studies were carried out in the isocratic mode using a reversed-phase column (Zorbax SB C_{18}, 250 × 4.6 mm, 5 μm; flow rate 1 mL min^{-1} at 25 °C) and a photodiode array detector (LKB 2410, detection at 230 nm). Preparative HPLC was carried out in the isocratic mode (Zorbax SB C_{18}, 250 × 21.2 mm, 7 μm; flow rate 5 mL min^{-1} for 3 min then 20 mL min^{-1} at 25 °C). Aqueous triethylamine was acidified using CO_2. Abbreviations: s, singlet; d, doublet; t, triplet; q, quadruplet; m, multiplet; br, broad.

Thiamine α;β-methylenediphosphate (5): Bromotrimethylsilane (250 μL, 1.93 mmol) was added to a suspension of compound **15a** (199 mg, 260 μmol) in anhydrous methylene chloride (25 mL) at room temperature. The reaction mixture was stirred for 6 h before the solvent was removed under vacuum and water (5 mL) added to the crude residue. The aqueous solution was washed with AcOEt (2 × 5 mL) and methylene chloride (2 × 5 mL), and was lyophylized to yield **5** (105 mg, 96%) as a white hygroscopic powder. Analytical HPLC (aqueous Et_3N 62 mM, pH 7.3/CH_3CN 99.9:0.1): $t_R = 30.3$ min; ^{1}H NMR (D_2O, 300 MHz): $\delta = 9.67$ (s, 1H), 7.95 (s, 1H), 5.55 (s, 2H), 4.19 (dt, $J = 6.0$, 5.3 Hz, 2H), 3.22 (t, $J = 5.3$ Hz, 2H), 2.61 (s, 3H), 2.54 (s, 3H), 2.36 (dd, $J = 20.2$, 20.3 Hz, 2H); ^{13}C NMR (D_2O, 75 MHz): $\delta =$ 163.8, 163.6 (m), 155.6, 144.9, 144.0, 136.4, 107.0, 64.3 (d, $J = 5.8$ Hz), 50.5, 28.1 (d, $J = 7.2$ Hz), 26.8 (t, $J = 127.1$ Hz), 21.6, 11.8; ^{31}P NMR (D_2O, 121 MHz): $\delta = 24.46$ (d, $J = 8.4$ Hz, 1P), 20.96 (d, $J = 8.4$ Hz, 1P); IR (KBr): $\tilde{\nu} = 3332$ (b), 1644, 1221, 1009; MS (ES): m/z (%): 422 $[M]^+$ (100), 444 $[M - H + Na]^+$ (4).

Thiamine disulfide bis(benzyl benzylphosphonate) (10): Triphenylphosphine (629 mg, 2.4 mmol) in anhydrous THF (3 mL) was added dropwise to

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a mixture of thiamine disulfide (225 mg, 0.4 mmol), phosphonic acid monobenzyl ester **9**[20] (214 mg, 0.8 mmol), and DEAD (0.4 mL, 2.6 mmol) in THF (15 mL) at room temperature. The initially heterogeneous reaction mixture rapidly became clear and was stirred for 30 min before the solvent was removed under vacuum. The oily residue was purified by flash chromatography over silica gel (EtOAc/MeOH 10:0 to 6:4) and **10** (340 mg, 81%) was obtained as a slightly yellow solid (mixture of two diastereomers). TLC: $R_f = 0.35$ (EtOAc/MeOH 6:4); ^{1}H NMR ($CDCl_3$, 200 MHz): $\delta = 7.76$ (s, 2H), 7.65 (s, 2H), 7.39–7.25 (m, 20H), 6.25 (s, 4H), 5.02 (A part of ABX syst., $J = -11.7$, 8.7 Hz, 2H), 4.94 (B part of ABX syst., $J = -11.7$, 9.2 Hz, 2H), 3.92–3.70 (m, 4H), 3.15 (d, $J = 21.6$ Hz, 4H), 2.47 (s, 6H), 2.44–2.32 (m, 4H), 1.75 (s, 6H); ^{13}C NMR ($CDCl_3$, 50 MHz): $\delta = 168.0$, 163.1, 162.1, 156.2, 136.1 (d, $J = 5.8$ Hz), 130.9 (d, $J = 8.7$ Hz), 129.8, 129.6, 128.7, 128.6, 128.1, 127.0 (m), 107.8, 68.2 (d, $J = 5.8$ Hz), 62.6 (d, $J = 7.2$ Hz), 40.0, 33.7 (d, $J = 135.7$ Hz), 31.0 (d, $J = 5.8$ Hz), 25.6, 18.2; ^{31}P NMR ($CDCl_3$, 121 MHz): $\delta = 28.89$; IR (film): $\tilde{\nu} = 3366$, 1660, 1458, 1008 cm^{-1}; MS (CI/NH_3): m/z (%): 1052 $[M+H]^+$ (100).

Thiamine chloride (benzyl benzylphosphonate) (11): Compound **10** (42 mg, 40 μmol) and triphenylphosphine (21 mg, 80 μmol) were dissolved in DME/water (4:1, 2 mL) and the pH brought to 1 with aqueous HCl. The reaction mixture was stirred at room temperature for 30 min and the solvent removed under vacuum. Water (5 mL) was added to the crude residue and the resulting solution washed with methylene chloride (3 × 5 mL) and diethyl ether (5 mL). The aqueous layer was evaporated to yield compound **11** (45 mg, 97%) as a slightly yellow solid that was used without further purification. TLC: $R_f = 0.40$ ($CHCl_3$/MeOH/H_2O 20:8:1); ^{1}H NMR (CD_3OD, 200 MHz): $\delta = 9.85$ (s, 1H), 8.32 (s, 1H), 7.51–7.26 (m, 10H), 5.58 (s, 2H), 5.07 (A part of ABX syst., $J = -11.5$, 8.0 Hz, 1H), 5.04 (B part of ABX syst., $J = -11.5$, 9.4 Hz, 1H), 4.19 (dt, $J = 6.9$, 5.5 Hz, 2H), 3.35 (d, $J = 21.2$ Hz, 2H), 3.24 (t, $J = 5.5$ Hz, 2H), 2.66 (s, 3H), 2.56 (s, 3H).

Thiamine benzylphosphonic acid (12): Compound **11** (49 mg, 84 μmol) and Pd/C 10% (45 mg) in MeOH/water (1:1, 3 mL) were vigorously stirred under a hydrogen atmosphere (1 bar) at room temperature for 30 min. The mixture was filtered over a Celite pad and the filtrate lyophylized to yield **12** (30 mg, 78%) as a white hygroscopic powder. TLC: $R_f = 0.10$ ($CHCl_3$/MeOH/H_2O 10:6:1); ^{1}H NMR (CD_3OD/D_2O 1:1, 200 MHz): $\delta = 9.83$ (s, 1H), 9.28 (s, 1H), 7.37–7.28 (m, 5H), 5.59 (s, 2H), 4.25–4.16 (m, 2H), 3.29 (t, $J = 4.1$ Hz, 2H), 3.23 (d, $J = 21.6$ Hz, 2H), 2.65 (s, 3H), 2.57 (s, 3H).

Methylenebisphosphonic acid trimethyl ester 13b: Compound **30b** (560 mg, 1.82 mmol) and Pd/C 10% (56 mg) in MeOH (15 mL) were vigorously stirred under a hydrogen atmosphere (1 bar) at room temperature for 15 min. The mixture was filtered over a Celite pad and the solvent removed under vacuum to yield **13b** (396 mg, 99%) as a colorless oil that was used without further purification. ^{1}H NMR ($CDCl_3$, 200 MHz): $\delta = 3.64$ (d, $J = 11.3$ Hz, 6H), 3.61 (d, $J = 11.3$ Hz, 3H), 2.40 (t, $J = 21.2$ Hz, 2H); ^{13}C NMR ($CDCl_3$, 50 MHz): $\delta = 53.2$ (d, $J = 6.4$ Hz), 52.4 (d, $J = 6.4$ Hz), 23.8 (t, $J = 135.2$ Hz); ^{31}P NMR ($CDCl_3$, 121 MHz): $\delta = 24.97$ (s, 1P), 19.43 (s, 1P); IR (film): $\tilde{\nu} = 3412$ (b), 2961, 1462, 1236, 1039 cm^{-1}; MS (CI/NH_3): m/z (%): 219 $[M+H]^+$ (100).

Thiamine disulfide bis[benzyl (dibenzylphosphonomethane)phosphonate] (14a): DEAD (1.04 mL, 6.6 mmol) was added dropwise to triphenylphosphine (1.73 g, 6.6 mmol) in anhydrous THF (5 mL) at 0 °C. The mixture was stirred for 15 min before being added to thiamine disulfide **8** (0.50 g, 0.89 mmol) and phosphonic acid **13a** (0.84 g, 1.88 mmol) in THF (5 mL). The resulting solution was stirred at room temperature for 1 h and the solvent removed under vacuum. The crude residue was chromatographed over silica gel (EtOAc/EtOH 10:0 to 7:3) to yield compound **14a** (0.78 g, 62%) as a yellow solid (mixture of two diastereomers). TLC: $R_f = 0.30$ (EtOAc/EtOH 7:3); ^{1}H NMR (CD_3OD, 200 MHz): $\delta = 8.18$ (s, 2H), 7.86 (s, 2H), 7.45–7.11 (m, 30H), 5.52–4.87 (m, 12H), 4.63–4.06 (m, 8H), 3.11–2.93 (m, 4H), 2.33 (s, 3H), 2.29 (s, 3H), 1.98 (s, 6H), 2.09–1.90 (m, 4H); IR (KBr): $\tilde{\nu} = 3030$, 1660, 1466, 1420, 1012 cm^{-1}; MS (CI/NH_3): m/z (%): 1421 $[M+H]^+$ (100), 1438 $[M+NH_4]^+$ (17).

Thiamine disulfide bis[methyl (dimethylphosphonomethane)phosphonate] (14b): Compound **14b** (0.19 g, 56%) was obtained as a yellow hygroscopic solid (mixture of two diastereomers) starting from compounds **8** and **13b** by following the same procedure as described for **14a**. TLC: $R_f = 0.30$ (EtOAc/MeOH 7:3); ^{1}H NMR ($CDCl_3$/CD_3OD 1:1, 200 MHz): $\delta = 8.13$ (s, 2H), 7.80 (s, 2H), 4.42–4.10 (m, 4H), 3.89–3.70 (m, 18H), 3.65–3.43 (m, 4H), 3.08–2.91 (m, 4H), 2.40 (s, 6H), 2.00 (s, 6H), 1.90 (t, $J = 20.1$ Hz, 4H); MS (CI/NH_3): m/z (%): 964 $[M+H]^+$ (100).

Thiamine α:β-methylenediphosphate tribenzyl ester (15a): Compound **15a** (293 mg, 97%) was obtained from **17** as a yellow solid by following the same procedure as described for **11**. TLC: $R_f = 0.20$ ($CHCl_3$/MeOH/H_2O 10:6:1); m.p. 155–156 °C; ^{1}H NMR (CD_3OD, 200 MHz): $\delta = 9.85$ (s, 1H), 8.31 (s, 1H), 7.43–7.28 (m, 15H), 5.55 (s, 2H), 5.18–5.00 (m, 6H), 4.39–4.15 (m, 2H), 3.27 (t, $J = 5.5$ Hz, 2H), 2.97 (t, $J = 21.2$ Hz, 2H), 2.71 (s, 3H), 2.64 (s, 3H); ^{13}C NMR (CD_3OD, 50 MHz): $\delta = 165.3$, 164.0 (m), 156.2, 147.4, 145.5, 137.2 (d, $J = 4.3$ Hz), 136.0, 129.7, 129.4, 129.2 (m), 106.5, 70.0 (d, $J = 5.8$ Hz), 69.7 (d, $J = 5.8$ Hz), 69.6 (d, $J = 5.8$ Hz), 66.5 (d, $J = 5.8$ Hz), 51.8, 28.7 (d, $J = 7.2$ Hz), 25.1 (t, $J = 135.7$ Hz), 21.9, 12.3; ^{31}P NMR (CD_3OD, 121 MHz): $\delta = 22.28$ (d, $J = 6.7$ Hz, 1P), 21.69 (d, $J = 6.7$ Hz, 1P); IR (film): $\tilde{\nu} = 3376$, 3064, 1653, 1244, 1016 cm^{-1}.

Thiamine propyl disulfide [benzyl (dibenzylphosphonomethane)phosphonate] (17): Compound **17** (650 mg, 63%) was obtained as a yellow hygroscopic solid starting from thiamine propyl disulfide **16**[23] and phosphonic acid **13a**[20], and following the same procedure as described for **14a**. TLC: $R_f = 0.40$ (EtOAc/MeOH 9:1); ^{1}H NMR ($CDCl_3$, 200 MHz): $\delta = 7.89$ (s, 1H), 7.80 (s, 1H), 7.36–7.28 (m, 15H), 6.02 (br s, 2H), 5.14–5.02 (m, 6H), 4.75, 4.15 (2 br s, 2H), 4.12–3.97 (m, 2H), 2.85 (t, $J = 6.4$ Hz, 2H), 2.53 (t, $J = 21.2$ Hz, 2H), 2.43 (s, 3H), 2.28 (t, $J = 6.9$ Hz, 2H), 1.90 (s, 3H), 1.45 (tq, $J = 6.9$, 7.3 Hz, 2H), 0.90 (t, $J = 7.3$ Hz, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): $\delta = 168.0$, 163.7, 162.1, 156.4, 135.9 (d, $J = 5.8$ Hz), 134.0, 133.2, 128.6–128.1 (m), 108.2, 68.4, 68.1, 68.0 (3 d, $J = 5.8$ Hz), 63.7 (d, $J = 6.5$ Hz), 41.5, 40.1, 30.5 (d, $J = 6.5$ Hz), 26.0 (t, $J = 135.8$ Hz), 25.6, 22.0, 18.9, 13.0; ^{31}P NMR ($CDCl_3$, 121 MHz): $\delta = 20.55$ (d, $J = 5.9$ Hz, 1P), 20.36 (d, $J = 5.9$ Hz, 1P); IR (film): $\tilde{\nu} = 3331$, 2961, 1660, 1258, 998 cm^{-1}; MS (CI/NH_3): m/z (%): 786 $[M+H]^+$ (100), 803 $[M+NH_4]^+$ (22).

Compounds 21 and 22: These two isomers are obtained as a mixture (140 mg, 42%; **21** (two diastereomers):**22** 78:22) starting from thiamine propyl disulfide **16** and a mixture of phosphonic and phosphinic acids **19** and **20**, and by following the same procedure as described for **14a**. TLC: $R_f = 0.45$ ($CHCl_3$/EtOH 9:1); ^{1}H NMR ($CDCl_3$, 300 MHz): $\delta = 7.96$, 7.92, 7.89 (3 s, 1H), 7.82, 7.79 (2 s, 1H), 7.37–7.25 (m, 20H), 6.08 (br s, 2H), 5.20–4.93 (m, 8H), 4.17–3.94 (m, 2H), 3.05–2.70 (m, 6H), 2.43 (s, 3H), 2.29, 2.28 (2 t, $J = 6.8$ Hz, 2H), 2.04, 1.94, 1.88 (3 s, 3H), 1.52–1.40 (m, 2H), 0.90, 0.89 (2 t, $J = 7.2$ Hz, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): $\delta = 167.7$, 163.8, 163.7, 162.2, 156.1, 136.0–135.7 (m), 134.0, 133.9, 133.4, 133.3, 128.6–127.9 (m), 108.4, 108.3, 68.6–67.0 (m), 63.8, 63.5 (2 d, $J = 6.3$, 6.8 Hz), 41.5, 40.0, 30.5, 28.6 (d, $J = 88.3$ Hz), 28.5 (d, $J = 132.1$ Hz), 25.3, 21.9, 19.0, 18.9 (2 s), 12.9; ^{31}P NMR ($CDCl_3$, 81 MHz): $\delta = 38.83$ (dd, $J = 2.5$, 4.5 Hz, 0.4P), 38.69 (dd, $J = 3.5$, 5.0 Hz, 0.4P), 38.48 (t, $J = 4.0$ Hz, 0.2P), 21.07 (d, $J = 2.5$ Hz, 0.4P), 20.97 (d, $J = 3.5$ Hz, 0.4P), 20.91 (d, $J = 4.5$ Hz, 0.4P), 20.83 (d, $J = 5.0$ Hz, 0.4P), 20.68 (d, $J = 4.0$ Hz, 0.4P); IR (film): $\tilde{\nu} = 3336$ (b), 2962, 1655, 1248, 1012; MS (CI/NH_3): m/z (%): 954 $[M+H]^+$ (100), 971 $[M+NH_4]^+$ (43).

Compounds 23 and 24: These compounds were obtained as a mixture of three non-separated isomers (106 mg, 90%) starting from the previous mixture of compounds **21** and **22**, and following the same procedure as described for **11**. M.p. 170–171 °C; ^{1}H NMR (CD_3OD, 300 MHz): $\delta = 9.86$, 9.85, 9.84 (3 s, 1H), 8.27 (s, 1H), 7.41–7.32 (m, 20H), 5.48 (s, 2H), 5.20–4.75 (m, 8H), 4.43–4.15 (m, 2H), 3.39–3.22 (m, 2H), 3.21–2.60 (m, 4H), 2.62, 2.60 (2 s, 3H), 2.56, 2.55 (2 s, 3H); ^{13}C NMR (CD_3OD, 50 MHz): $\delta = 165.0$, 164.0, 156.4, 156.3, 147.3, 147.1, 147.0, 145.7, 145.5, 138.0–137.0 (m), 136.4, 136.0, 129.7–128.2 (m), 106.7, 106.6, 106.5, 69.9–68.4 (m), 66.6 (d, $J = 4.8$ Hz), 51.7, 29.5 (dd, $J = 85.9$, 115.9 Hz), 28.6 (dd, $J = 85.9$, 115.9 Hz), 28.8, 28.5 (2 d, $J = 7.7$ Hz), 21.8, 12.3; ^{31}P NMR (CD_3OD, 121 MHz): $\delta = 42.9$–32.9 (m, 1P), 23.4–21.5 (m, 1P), 20.0–18.1 (m, 1P); IR (film): $\tilde{\nu} = 3040$ (b), 1652, 1245, 999 cm^{-1}.

Thiamine α:β,β:γ-bismethylenetriphosphate (25): Compound **25** (41 mg, 57%) was obtained as a white, very hygroscopic powder from the previous mixture of compounds **23** and **24** by following the same procedure as described for **5**. Purification was achieved by preparative reversed-phase HPLC and compound **25** was obtained as its triethyl ammonium salt. Analytical HPLC (aqueous Et_3N 62 mM, pH 7.67/CH_3CN 99.9:0.1): $t_R = 31.8$ min; ^{1}H NMR (D_2O, 300 MHz): $\delta = 9.41$ (s, 1H), 7.82 (s, 1H), 5.77 (s, 2H), 4.02 (dd, $J = 2.9$, 4.5 Hz, 2H), 3.19 (t, $J = 2.9$ Hz, 2H), 3.10–2.85 (m, 6H), 2.41 (s, 6H), 2.22–1.97 (m, 4H), 1.18–1.08 (m, 9H); ^{13}C NMR (D_2O, 75 MHz): $\delta = 163.8$, 163.5, 155.6, 145.0, 144.0, 136.2, 106.8, 64.4 (d, $J =$

5.8 Hz), 50.6, 30.9 (t, $J = 127.1$ Hz), 30.0 (dd, $J = 51;3$, 74.4 Hz), 28.4 (d, $J =$ 6.5 Hz), 21.6, 11.9; ^{31}P NMR (D_2O, 121 MHz): $\delta = 28.76$ (brs, 1P), 19.09 (brs, 1P), 15.39 (brs, 1P); IR (KBr): $\tilde{\nu} = 3412$ (b), 2935, 2672, 1662, 1622, 1192, 1035 cm^{-1}; MS (ES): m/z (%): 500 $[M]^+$ (100), 522 $[M - H+Na]^+$ (36).

Compound 26: Compound **26** (15 mg, 21%) was isolated as a white, very hygroscopic powder during the HPLC purification of **25**. Analytical HPLC (aqueous Et_3N 62 mM, pH 7.67/CH_3CN 99.9:0.1): $t_R = 26.2$ min; ^{1}H NMR (D_2O, 300 MHz): $\delta = 7.85$ (s, 1H), 5.26 (s, 2H), 4.20–4.09 (m, 2H), 3.20 (t, $J = 5.7$ Hz, 2H), 2.40 (s, 3H), 2.32 (s, 3H), 2.32 (t, $J = 19.1$ Hz, 4H); ^{13}C NMR (D_2O, 75 MHz): $\delta = 163.8$, 163.5, 155.7, 145.1, 144.4, 135.7, 106.8, 65.1 (d, $J = 6.5$ Hz), 50.7, 30.7 (dd, $J = 72.2$, 125.6 Hz), 28.1 (d, $J = 8.7$ Hz), 21.6, 11.8; ^{31}P NMR (D_2O, 121 MHz): $\delta = 52.78$ (brs, 1P), 10.56 (d, $J =$ 6.7 Hz, 2P); IR (KBr): $\tilde{\nu} = 3417$ (b), 2968, 1661, 1183, 1043 cm^{-1}; MS (ES): m/z (%): 500 $[M]^+$ (100), 522 $[M - H+Na]^+$ (42).

Thiamine α:β-difluoromethylenediphosphate (27): A suspension of compound **34** (91 mg, 158 μmol) and bromotrimethylsilane (1 mL, 7.5 mmol) in anhydrous chloroform (7 mL) was sonicated for 4 h at room temperature. Solvent and excess reagent were removed under vacuum. The crude residue was decomposed by the addition of water (5 mL) and the mixture purified by preparative HPLC to yield the triethylammonium salt of **27** (25 mg, 38%) as a white hygroscopic solid. Analytical HPLC (aqueous Et_3N 62 mM, pH 7.67/CH_3CN 99.9:0.1): $t_R = 59.3$ min; ^{1}H NMR (D_2O, 300 MHz): $\delta = 7.89$ (s, 1H), 5.38 (s, 2H), 4.20 (dd, $J = 6.0$, 6.4 Hz, 2H), 3.23 (t, $J = 6.4$ Hz, 2H), 3.10 (q, $J = 7.1$ Hz, 6H), 2.49 (s, 3H), 2.43 (s, 3H), 1.21 (t, $J = 7.1$ Hz, 9H); ^{13}C NMR (D_2O, 75 MHz): $\delta = 164.1$, 162.6, 162.5, 155.1, 145.7, 143.5, 136.0, 106.7, 65.4, 50.1, 46.8, 28.1, 21.6, 11.2, 8.3; ^{31}P NMR (D_2O, 121 MHz): $\delta = 6.02 - 3.09$ (m, 2P); ^{19}F NMR (D_2O, 188 MHz): $\delta = 120.55$ (t, $J = 82.1$ Hz); MS (ES): m/z (%): 458 $[M]^+$ (100), 480 $[M - H+Na]^+$ (59).

Methylenebisphosphonic acid tetrabenzyl ester 28: Dibenzyl methanephosphonate (7.82 g, 28.3 mmol) in anhydrous THF (125 mL) was treated dropwise with *n*-butyllithium (1.6 M in hexane, 17.7 mL, 28.3 mmol) at $-78\,^\circ$C. The mixture was stirred for 30 min and added in one portion to dibenzyl chlorophosphate (4.20 g, 14.2 mmol) in THF (20 mL) at $-78\,^\circ$C. The reaction mixture was stirred for 1 h at that temperature before being decomposed by the addition of aqueous ammonium chloride. The resulting solution was allowed to warm to room temperature and extracted with EtOAc. The organic layer was dried over $MgSO_4$, reduced under vacuum and purified over silica gel (EtOAc/nC_6H_{14} 4:6) to yield **28** (5.2 g, 68%) as a white solid. TLC: $R_f = 0.50$ (EtOAc/nC_6H_{14} 8:2); ^{1}H NMR ($CDCl_3$, 200 MHz): $\delta = 7.37 - 7.28$ (m, 20H), 5.06 (A part of ABX syst., $J = -11.7$, 8.9 Hz, 4H), 5.03 (B part of ABX syst., $J = -11.7$, 8.1 Hz, 4H), 2.54 (t, $J =$ 21.1 Hz, 2H); ^{13}C NMR ($CDCl_3$, 50 MHz): $\delta = 135.8$ (d, $J = 5.8$ Hz), 128.5, 128.4, 128.0, 67.9 (d, $J = 5.8$ Hz), 26.1 (t, $J = 136.1$ Hz); ^{31}P NMR ($CDCl_3$, 121 MHz): $\delta = 20.53$; IR (film): $\tilde{\nu} = 3070$, 3033, 2953, 1468, 1250, 998 cm^{-1}; MS (CI/NH_3): m/z (%): 537 $[M+H]^+$ (100), 554 $[M+NH_4]^+$ (29).

Difluoromethylenebisphosphonic acid tetrabenzyl ester 29: Sodium hydride (60% in oil, 166 mg, 4.15 mmol) was added to compound **28** (1.11 g, 2.07 mmol) in anhydrous THF (40 mL) at $-10\,^\circ$C and the resulting mixture allowed to warm to room temperature for 10 min. It was then cooled to $-10\,^\circ$C again and *N*-fluorobenzenesulfonimide (1.31 g, 4.15 mmol) in THF (15 mL) was added dropwise. The temperature was maintained at -10–$0\,^\circ$C and a white precipitate appeared after a few minutes. The reaction mixture was decomposed with aqueous NH_4Cl, extracted with diethyl ether, dried over magnesium sulfate, and reduced under vacuum. The residue was chromatographed over silica gel (EtOAc/nC_6H_{14} 2:8) to yield **29** (0.81 g, 68%) as a white solid. TLC: $R_f = 0.35$ (EtOAc/nC_6H_{14} 3:7); m.p. 69–70 °C; ^{1}H NMR ($CDCl_3$, 200 MHz): $\delta = 7.38 - 7.30$ (m, 20H), 5.26 (A part of ABX syst., $J = -11.7$, 7.3 Hz, 4H), 5.19 (B part of ABX syst., $J =$ -11.7, 9.1 Hz, 4H); ^{13}C NMR ($CDCl_3$, 75 MHz): $\delta = 134.6$ (d, $J = 8.9$ Hz), 128.3, 128.1, 127.7, 115.6 (tt, $J = 188.0$, 280.2 Hz,), 69.9 (d, $J = 2.9$ Hz); ^{31}P NMR ($CDCl_3$, 121 MHz): $\delta = 4.86$ (t, $J = 87.6$ Hz); ^{19}F NMR ($CDCl_3$, 188 MHz): $\delta = -121.01$ (t, $J = 87.6$ Hz); IR (film): $\tilde{\nu} = 3066$, 1457, 1281, 1054, 1010, 998 cm^{-1}; MS (CI/NH_3): m/z (%): 573 $[M+H]^+$ (9), 590 $[M+NH_4]^+$ (100).

Methylenebisphosphonic acid tribenzyl methyl ester (30a): Compound **30a** (2.49 g, 78%) was obtained as a slightly yellow oil starting from benzyl methyl methanephosphonate and dibenzyl chlorophosphate and by following the same procedure as described for **28**. TLC: $R_f = 0.40$ (EtOAc); ^{1}H NMR ($CDCl_3$, 300 MHz): $\delta = 7.41 - 7.33$ (m, 15H), 5.17–4.99 (m, 6H), 3.68 (d, $J = 11.3$ Hz, 3H), 2.50 (t, $J = 21.1$ Hz, 2H); ^{13}C NMR ($CDCl_3$, 50 MHz): $\delta = 136.1$ (d, $J = 6.4$ Hz), 128.6, 128.5, 128.1, 68.2 (d, $J = 5.5$ Hz), 68.1 (d, $J = 6.6$ Hz), 53.0 (d, $J = 6.6$ Hz), 25.8 (t, $J = 136.1$ Hz); ^{31}P NMR ($CDCl_3$, 121 MHz): $\delta = 22.23$ (d, $J = 6.7$ Hz, 1P), 21.38 (d, $J = 6.7$ Hz, 1P); IR (film): $\tilde{\nu} = 3090$, 3030, 2955, 1465, 1250, 1010 cm^{-1}; MS (CI/NH_3): m/z (%): 461 $[M+H]^+$ (19), 478 $[M+NH_4]^+$ (100).

Methylenebisphosphonic acid benzyl trimethyl ester (30b): Compound **30b** (2.52 g, 60%) was obtained as a colorless oil starting from dimethyl methanephosphonate and benzyl methyl chlorophosphate by following the same procedure as described for **28**. TLC: $R_f = 0.40$ (EtOAc/MeOH 9:1); ^{1}H NMR ($CDCl_3$, 200 MHz): $\delta = 7.41 - 7.32$ (m, 5H), 5.14 (d, $J = 8.8$ Hz, 2H), 3.79 (d, $J = 12.1$ Hz, 3H), 3.77 (d, $J = 11.3$ Hz, 3H), 3.73 (d, $J =$ 11.7 Hz, 3H), 2.46 (t, $J = 21.2$ Hz, 2H); ^{13}C NMR ($CDCl_3$, 50 MHz): $\delta =$ 135.5 (d, $J = 5.8$ Hz), 128.1, 128.0, 127.6, 67.7 (d, $J = 5.8$ Hz), 52.7 (d, $J =$ 5.8 Hz), 52.5 (d, $J = 5.8$ Hz), 23.8 (t, $J = 136.4$ Hz); ^{31}P NMR ($CDCl_3$, 121 MHz): $\delta = 23.06$ (d, $J = 5.9$ Hz, 1P), 22.28 (d, $J = 5.9$ Hz, 1P); IR (film): $\tilde{\nu} = 3504$, 2957, 1457, 1259, 1037; MS (CI/NH_3): m/z (%): 309 $[M+H]^+$ (10), 326 $[M+NH_4]^+$ (100).

Difluoromethylenebisphosphonic acid tribenzyl methyl ester (31a): Compound **31a** (0.96 g, 72%) was obtained as a slightly yellow oil starting from compound **30a**, and by following the same procedure as described for **29**. TLC: $R_f = 0.50$ (EtOAc/nC_6H_{14} 5:5); ^{1}H NMR ($CDCl_3$, 200 MHz): $\delta =$ 7.39–7.32 (m, 15H), 5.37–5.12 (m, 6H), 3.87 (d, $J = 11.0$ Hz, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): $\delta = 135.0$ (d, $J = 4.3$ Hz), 128.7, 128.5, 128.1, 116.0 (tt, $J = 186.5$, 277.0 Hz), 70.3 (d, $J = 5.8$ Hz), 55.2 (d, $J = 5.8$ Hz); ^{31}P NMR ($CDCl_3$, 121 MHz): $\delta = 6.35$ (A part of ABX_2 syst., $J = -71.9$, 86.5 Hz, 1P), 5.21 (B part of ABX_2 syst., $J = -71.9$, 86.5 Hz, 1P); ^{19}F NMR ($CDCl_3$, 188 MHz): $\delta = -121.10$ (t, $J = 86.5$ Hz); IR (film): $\tilde{\nu} = 2962$, 1457, 1381, 1282, 1055, 1013; MS (CI/NH_3): m/z (%): 498 $[M+H]^+$ (16), 515 $[M+NH_4]^+$ (100).

Difluoromethylenebisphosphonic acid benzyl trimethyl ester (31b): Compound **31b** (0.94 g, 78%) was obtained as a colorless oil starting from compound **30b**, and following the same procedure as described for **29**. TLC: $R_f = 0.70$ (EtOAc/MeOH 9:1); ^{1}H NMR ($CDCl_3$, 200 MHz): $\delta =$ 7.42–7.30 (m, 5H), 5.28 (A part of ABX syst., $J = -10.2$, 7.2 Hz, 1H), 5.21 (B part of ABX syst., $J = -10.2$, 8.5 Hz, 1H), 3.92 (d, $J = 9.9$ Hz, 3H), 3.89 (d, $J = 9.1$ Hz, 3H), 3.87 (d, $J = 9.1$ Hz, 3H); ^{13}C NMR ($CDCl_3$, 50 MHz): $\delta = 134.8$ (d, $J = 4.3$ Hz), 128.6, 128.4, 127.9, 116.0 (tt, $J = 187.0$, 278 Hz), 70.1 (d, $J = 5.1$ Hz), 55.1 (d, $J = 4.3$ Hz), 55.0 (d, $J = 4.3$ Hz); ^{31}P NMR ($CDCl_3$, 121 MHz): $\delta = 6.11$ (A part of ABX_2 syst., $J = -71.0$, 85.1 Hz, 1P), 5.05 (B part of ABX_2 syst., $J = -71.0$, 85.1 Hz, 1P); ^{19}F NMR ($CDCl_3$, 188 MHz): $\delta = -121.42$ (t, $J = 85.1$ Hz); IR (film): $\tilde{\nu} = 2967$, 1284, 1051 cm^{-1}; MS (CI/NH_3): m/z (%): 345 $[M+H]^+$ (13), 362 $[M+NH_4]^+$ (100).

Difluoromethylenebisphosphonic acid tribenzyl ester (32a): Compound **31a** (0.55 g, 1.11 mmol) and potassium cyanide (0.08 g, 1.11 mmol) were stirred in anhydrous DMF (7 mL) for 3 h at 80 °C. The solvent was removed under vacuum, the residue dissolved in aqueous 10% HCl (10 mL), and the solution extracted with EtOAc and methylene chloride. The organic layer was dried over magnesium sulfate and reduced under vacuum to yield **32a** (0.53 g, 99%) as a colorless oil that was used without further purification. ^{1}H NMR ($CDCl_3$/CD_3OD 1:1, 200 MHz): $\delta = 7.28 - 7.21$ (m, 15H), 5.12–4.98 (m, 6H); ^{13}C NMR ($CDCl_3$/CD_3OD 1:1, 50 MHz): $\delta = 135.9$ (d, $J =$ 6.5 Hz), 134.8 (d, $J = 5.1$ Hz), 128.6, 128.4, 128.2, 128.1, 127.9, 127.5 (m), 116.4 (tt, $J = 180.1$, 277.3 Hz), 70.2 (d, $J = 6.5$ Hz), 69.4 (d, $J = 6.5$ Hz); ^{31}P NMR ($CDCl_3$/CD_3OD 1:1, 121 MHz): $\delta = 5.64$ (dt, $J = 68.0$, 90.2 Hz, 1P), 1.76 (dt, $J = 68.0$, 90.2 Hz, 1P); ^{19}F NMR ($CDCl_3$/CD_3OD 1:1, 188 MHz): $\delta = -121.86$ (dd, $J = 84.0$, 90.2 Hz); IR (film): $\tilde{\nu} = 2924$ (b), 1456, 1261, 1052; MS (CI/NH_3): m/z (%): 483 $[M+H]^+$ (5), 500 $[M+NH_4]^+$ (100).

Difluoromethylenebisphosphonic acid trimethyl ester (32b): Compound **32b** (141 mg, 99%) was obtained as a colorless oil starting from **31b**, and by following the same procedure as described for **13b**. ^{1}H NMR ($CDCl_3$, 300 MHz): $\delta = 11.98$ (brs, 1H), 3.95 (d, $J = 10.9$ Hz, 6H), 3.89 (d, $J =$ 10.9 Hz, 3H); ^{13}C NMR ($CDCl_3$, 50 MHz): $\delta = 116.3$ (tt, $J = 187.7$, 275.8 Hz), 55.5 (d, $J = 6.5$ Hz), 55.2 (d, $J = 6.5$ Hz); ^{31}P NMR ($CDCl_3$:CD_3OD 1:1, 121 MHz): $\delta = 7.99$ (dt, $J = 66.2$, 89.8 Hz, 1P), 2.72 (dt, $J = 66.2$, 73.2 Hz, 1P); ^{19}F NMR ($CDCl_3$, 188 MHz): $\delta = -122.20$ (dd, $J = 88.9$, 83.9 Hz); IR (film): $\tilde{\nu} = 3966$ (b), 1454, 1256, 1048 cm^{-1}; MS (CI/NH_3): m/z (%): 255 $[M+H]^+$ (2), 272 $[M+NH_4]^+$ (100), 526 $[2M+NH_4]^+$ (6).

Thiamine propyl disulfide [methyl (dimethylphosphonodifluoromethane)-phosphonate] (33): Compound **33** (155 mg, 66%) was prepared starting

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from thiamine propyl disulfide **16** and phosphonic acid **32b** and by following the same procedure as described for **14a**. TLC: $R_f = 0.30$ ($CHCl_3$/EtOH 9:1); ^{1}H NMR ($CDCl_3$, 200 MHz): $\delta = 7.95$ (s, 1H), 7.82 (s, 1H), 6.13 (br s, 2H), 4.72 (br s, 1H), 4.10 (br s, 1H), 4.37 (dd, $J = 5.9$, 6.5 Hz, 2H), 3.99 (d, $J = 8.8$ Hz, 3H), 3.98 (d, $J = 8.4$ Hz, 3H), 3.97 (d, $J = 8.0$ Hz, 3H), 3.02 (t, $J = 5.9$ Hz, 2H), 2.44 (s, 3H), 2.30 (t, $J = 7.3$ Hz, 2H), 2.05 (s, 3H), 1.47 (tq, $J = 7.3$, 6.9 Hz, 2H), 0.91 (t, $J = 6.9$ Hz, 3H); ^{31}P NMR ($CDCl_3$, 121 MHz): $\delta = 6.72$ (A part of ABX syst., $J = -72.5$, 84.3 Hz, 1P), 5.84 (B part of ABX syst., $J = -72.5$, 84.7 Hz, 1P).

Thiamine α:β-difluoromethylenediphosphate trimethyl ester (34): Compound **34** (133 mg, 91%) was obtained as a sligthly yellow solid starting from **33** and by following the same procedure as described for **15a**. It slowly decomposed to give phosphonic acids. ^{1}H NMR (D_2O, 200 MHz): $\delta = 9.49$ (s, 1H), 7.72 (s, 1H), 5.36 (s, 2H), 4.37 (dt, $J = 5.8$, 5.5 Hz, 2H), 3.72 (d, $J = 10.6$ Hz, 6H), 3.66 (d, $J = 11.3$ Hz, 3H), 3.24 (t, $J = 5.5$ Hz, 2H), 2.40 (s, 3H), 2.33 (s, 3H).

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