Synthetic methodology towards ADP-ribosylation related molecular tools

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2 Phosphanylmethylphosphonates as Reagents for the Synthesis of Terminal Methylene Bisphosphates

Introduction

Pyrophosphates are present in numerous natural products that are involved in a wide variety of fundamental physiological processes, including cell metabolism, immunity and genome maintenance.[1–5] Consequently, pyrophosphates are important components of molecular tools or probes that aim to study and influence these processes.[6–8] Pyrophosphates are inherently susceptible to hydrolysis, trans-esterification and enzymatic cleavage.[9,10] If a higher stability is desired, methylene bisphosphonates are attractive pyrophosphate bioisosteres that are less prone to undergo hydrolysis both during synthesis and in physiological surroundings.[11] Methylene bisphosphonate isosteres of sugar nucleotides and nucleoside di- and triphoshates have been applied in studies that aim to elucidate the function of various enzymes.[8,12,13] Terminal methylene bisphosphonates monoesters have been synthesized in the past using either methylene bisphosphonic dichloride or partially protected monochloridite derivatives as the phosphonylating agent[14,15]. (Un)symmetric methylene bisphosphonates diesters can be accessed using Mitsunobu chemistry or by condensing a hydroxyl of a target (bio)molecule with an independently prepared terminal methylene bisphosphonate.[16,17] The published methods use unselective reagents and limit the regioselectivity of substitution reactions at the bisphosphonate core. Moreover, no generic strategy exists that readily allows for the introduction of methylene bisphosphonate moieties in structurally diverse pyrophosphate-containing (bio)molecules. This chapter describes the development of such a strategy and its application in the synthesis of terminal methylene bisphosphates.

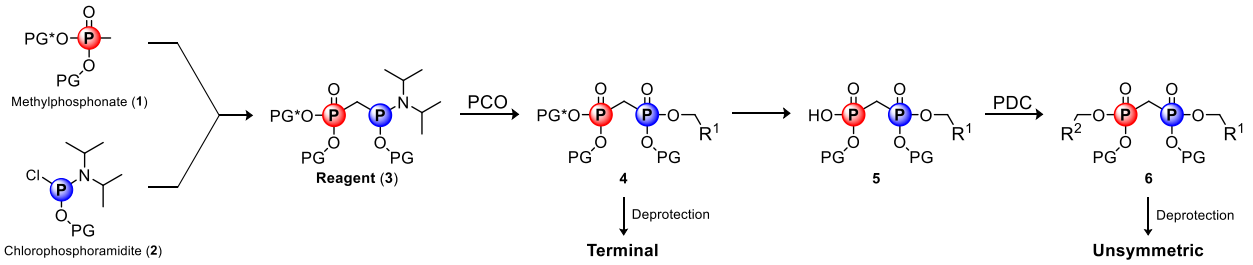
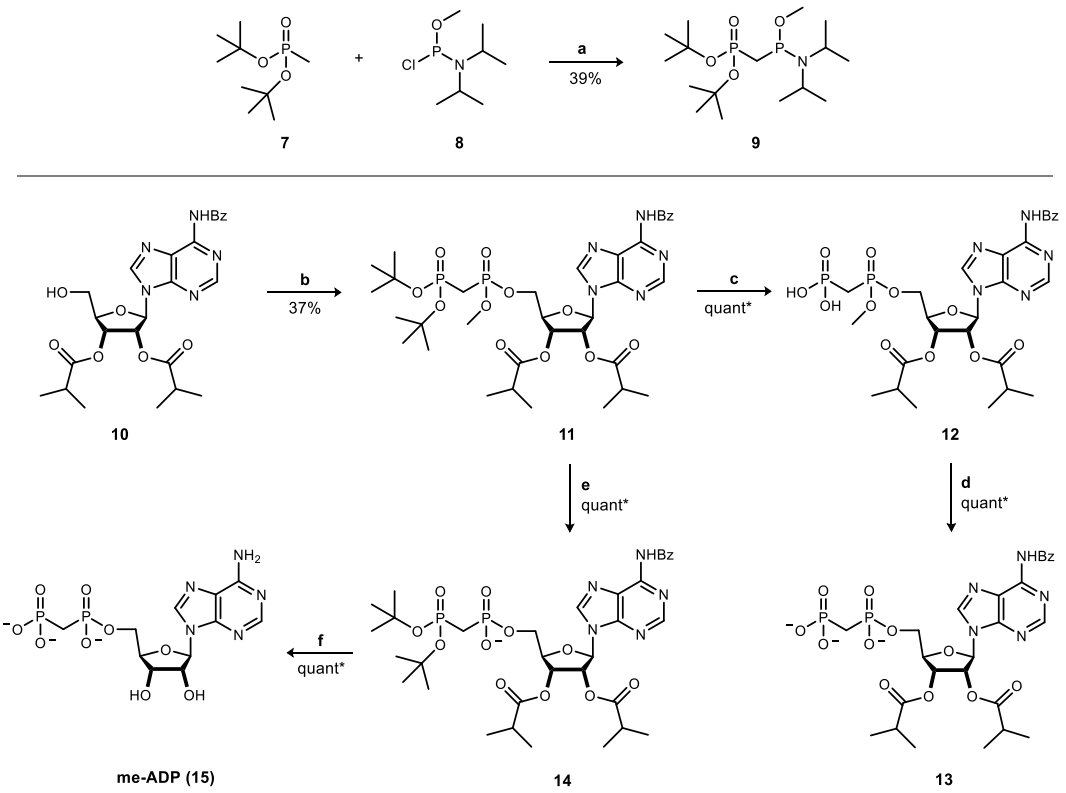


Figure 1: Methodology for the synthesis of terminal or unsymmetric methylene bisphosphates. Reagent **3** features orthogonal removable protecting groups PG and PG* and can be prepared from methylphosphonate **1** and chlorophosphoramidite **2**. The reagent is coupled to an alcohol containing substrate (R^1OH) via a (P^{III}) phosphoramidite coupling-oxidation sequence (PCO), to give protected intermediate **4**, a precursor for terminal methylene bisphosphonates. Alternatively, after selective PG*-deprotection (**5**), a second alcohol (R^2OH) can be introduced through phosphordiester condensation (PDC), leading to protected intermediate **6**, precursor of unsymmetric methylene bisphosphates.

Results and Discussion

It was envisioned that universal reagents capable of providing access to both terminal and unsymmetric methylene bisphosphonates had to be orthogonally protected to allow two sequential condensations under conditions compatible with common biomolecules. These requirements could be met through the application of phosphanylmethylphosphonate reagents (**3**, Figure 1). Azole-mediated condensation between an alcohol and **3**, followed by *in situ* oxidation, would give fully protected methylene bisphosphonate tetraester **4**. Global deprotection of **4** would provide terminal methylene bisphosphonate functionalized molecules. Alternatively, selective deprotection of **4** to bisphosphonotriester **5**, consecutive P^V-condensation with a second alcohol would give bisphosphonotetraester **6** and removal of the protecting groups in **6** would provide unsymmetric methylene bisphosphonates. This chapter describes the development and optimization of the syntheses of several phosphanylmethylphosphonate reagents and their application in the preparation of terminal methylene bisphosphonates, both in solution and on solid-support.



Scheme 1: Exploratory study towards the synthetic accessibility and application of phosphanylmethylphosphonates. Reagents and conditions: [**a**] *n*-BuLi, THF, -78 ˚C, 0.5h » add **16**, 15 min. [**b**] i: N^6-Benzoyl-2,3-*O*-di-*iso*-butyryladenosine (**9**), tetrazole, MeCN, 10 min. ii: ^{t}BuOOH, 15 min. [**c**] 10% TFA in DCM, 0.5h. [**d**] PhSH/Et_3N/dioxane (1:2:2), 48h. [**e**] PhSH/Et_3N/dioxane (1:2:2), 16h. [**f**] i: 10% TFA in DCM, 0.5h. ii: NH_4OH, 16h. [*] Yields based on ^{31}P NMR and LCMS analysis.

To evaluate the proposed methodology, phosphanylmethylphosphonate **9** was chosen as a reagent suitable for the introduction of terminal methylene bisphosphonate monoesters (Scheme 1). Lithiation of di-*tert*-butyl methylphosphonate (**7**) with 1 equivalent of *n*-BuLi, followed by the addition of

phosphoramidite **8**, provided the desired phosphanylmethylphosphonate **9** in 39% yield.[18] The phosphorylating properties of reagent **9** were investigated by exploring the synthesis of known adenosine-5'-methylene bisphosphonates (**13**, Scheme 1).[19] Condensation of phosphoramidite **9** with partially protected adenosine **10**[20] was accomplished using 1*H*-tetrazole as activator. Subsequent *in situ* *t*BuOOH-mediated oxidation of the phosphonite-phosphonate intermediate afforded methylene bisphosphonates of adenosine **11** in 37% yield. Next, the deprotection of the *tert*-butyl groups was investigated. ^{31}P-NMR spectroscopy was used to screen the reaction progression at varying concentrations of trifluoroacetic acid (TFA) in DCM. Optimal conditions were found at 10% TFA; providing clean conversion within 30 minutes. Successive demethylation of **12** with thiophenol in the presence of TEA proceeded sluggishly, taking 48

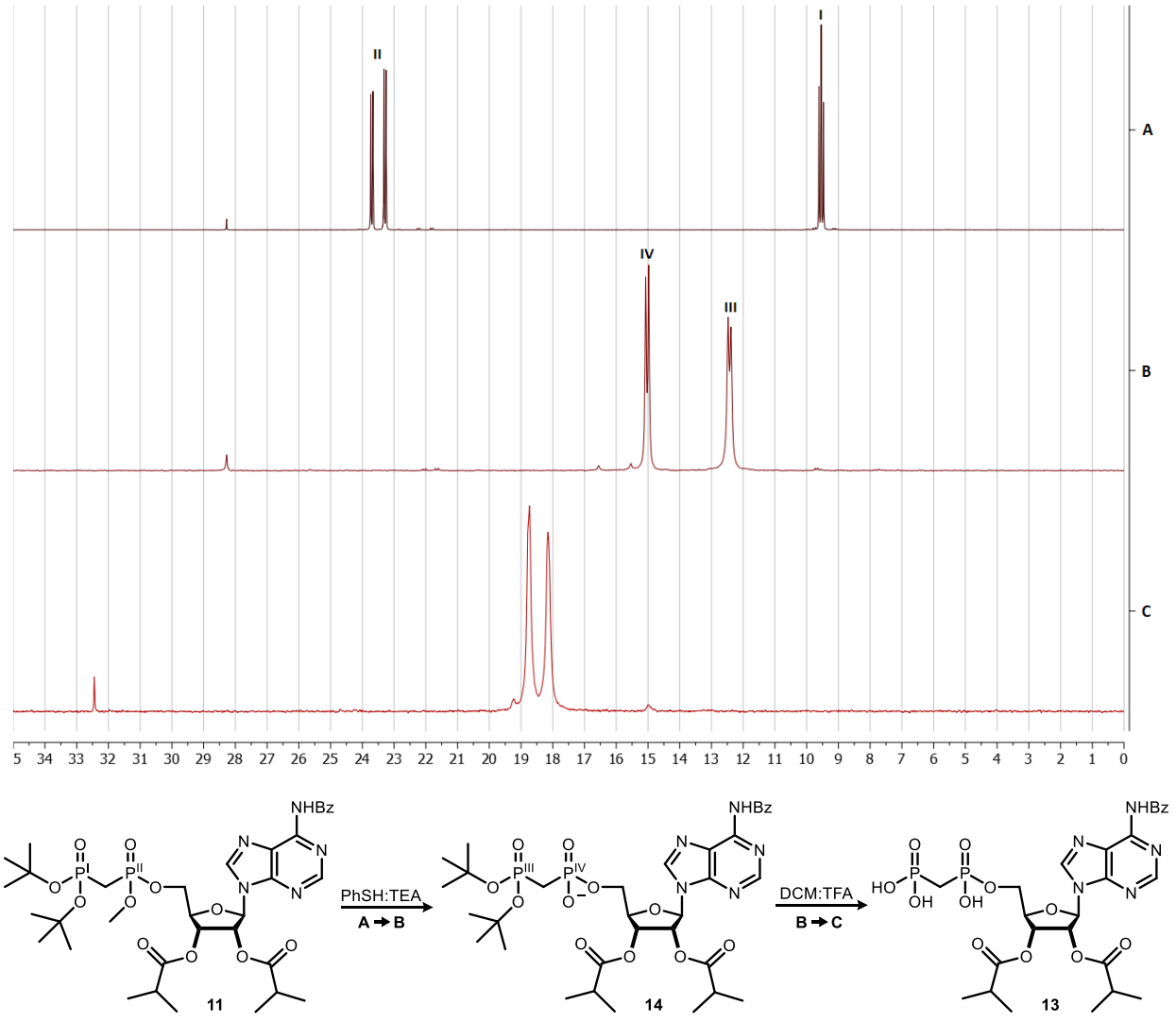
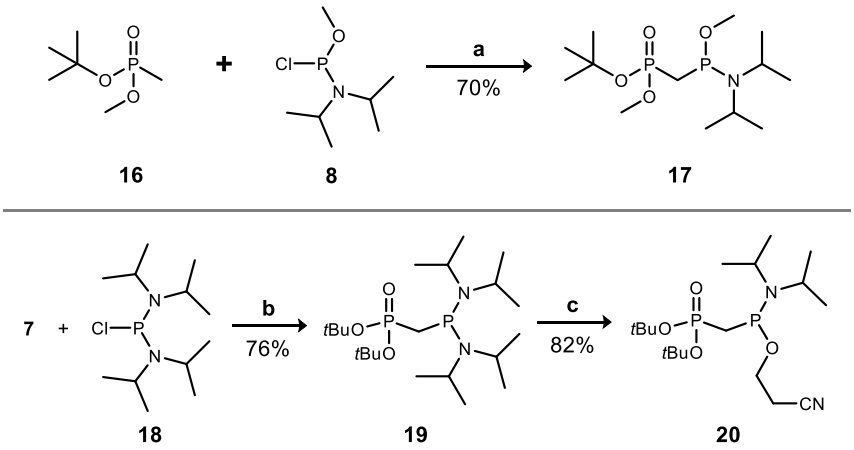


Figure 2: Exploratory sequential deprotection of compound **11**, monitored by ^{31}P NMR spectrometry (162 MHz). A NMR tube was charged with an aliquot of the reaction mixture and fitted with an acetone-d_6 capillary (required for locking). **A:** Fully protected **11**, before addition of PhSH:TEA. **B:** The reaction mixture after 16h treatment with PhSH:TEA:dioxane (1:2:2); showing complete conversion into demethylated intermediate **14**. **C:** After extractive work-up (removal of PhSH and TEA) and concentration, the residue was treated with 10% TFA in DCM for 30 minutes, resulting in cleavage of the *tert*-butyl groups (intermediate **13**).

hours to complete. It was reasoned that the terminal phosphate becomes deprotonated under basic demethylation conditions. The resulting increased electron density surrounding the phosphor lowers the leaving group capacity of the methyl phosphate. Indeed, demethylation proceeded significantly smoother after reversing the order of deprotection (Figure 2, **11** » **14**).). ^{31}P-NMR spectrometry and LCMS analysis showed clean peak-to-peak conversion of **11** (Figure 2, A) to **14** (Figure 2, B) within 16 hours. After extractive work-up, crude methylene diphosphate **14** was deprotected using the previously determined TFA conditions, providing protonated bisphosphonate monoester **13** (Figure 2). Finally, ammonia mediated deacylation provided me-ADP (Scheme 1, **15**) in quantitative yield (based on ^{31}P-NMR spectrometry and LCMS).

With the proposed methodology validated, efforts were directed towards the preparation of protected phosphanyl methylphosphonates **17** and **20**. Reagent **17** is orthogonally protected with a *tert*-butyl group at the terminal phosphate. After the coupling-oxidation sequence of reagent **17** with a specific alcohol, selective deprotection of the *tert*-butyl group in the acquired intermediate would allow for a P^V-condensation with second relevant alcohol, leading to unsymmetric methylene bisphosphonate diesters. The application of reagent **17** will be discussed in Chapter 3. Reagent **20** contains the base labile 2-cyanoethanol protecting group to accommodate the synthesis of terminal methylene bisphosphonates monoesters both in solution and on solid-support.



Scheme 2: Synthesis of phosphanyl methylphosphonate **17** and **20**. Reagents and conditions: [**a**] i: *n*-BuLi, THF, -78 °C, 0.5 h. ii: **8**, -78 °C, 15 min [**b**] i: LDA, THF, -78 ˚C, 0.5h. ii: **18**, -78 ˚C » RT, 16h. [**c**] DCI, 2-cyanoethanol, DCM, 3.5h.

Phosphanylmethylphosphonate reagent **17** is prepared according to a similar procedure as described for the synthesis of otherwise protected reagent **9** (Scheme 2). Lithiation of methylphosphonate **16** with 1 equivalent of *n*-BuLi was followed by the addition of chlorophosphine **8**, providing target phosphoramidite **17** in 32% yield. During refinement of this reaction, it was found that the relatively low yield could be attributed to two unwanted modes of quenching and partial P^{III}-oxidation during column purification. First of all, the diminished yield was partially caused by proton-exchange between emerging bisphosphonate **17** and lithiated methylphosphonate **16**, neutralizing the reactant. This problem was circumvented by switching to 2.1 equivalents LDA, increasing the yield to 45%. In addition, it was found that commercially

available **8** was contaminated with diisopropylamine hydrochloride (DIPA·HCl), another species capable of quenching the lithiated methylphosphonate **16**. Removal of DIPA·HCl, by precipitation in *n*-hexane prior to addition and applying the solution through a filter, further increased the yield of the reaction. During the purification of **17**, partial oxidation of the P^{III} (up to 30%) would sporadically occur during column purification. It was empirically determined that this could be attributed to silica gel impurities (potentially trace metals). The oxidation was prevented when high-purity grade silica was used to purify **17**. After these refinements were implemented the synthesis of **17** proceeded cleanly, as determined by ^{31}P NMR spectroscopy (Figure 3), increasing product yield to 70%. The monitoring of the synthesis of **17** is shown in Figure 3. Addition of lithiated **16** onto chlorophosphoramidite **8** gave rise to wide variety of phosphor species. The lithiation of formed **17** by excess LDA introduces an additional stereocenter, providing four additional (distinguishable) diastereoisomers (Figure 3, A). Subsequent quenching with aqueous sodium

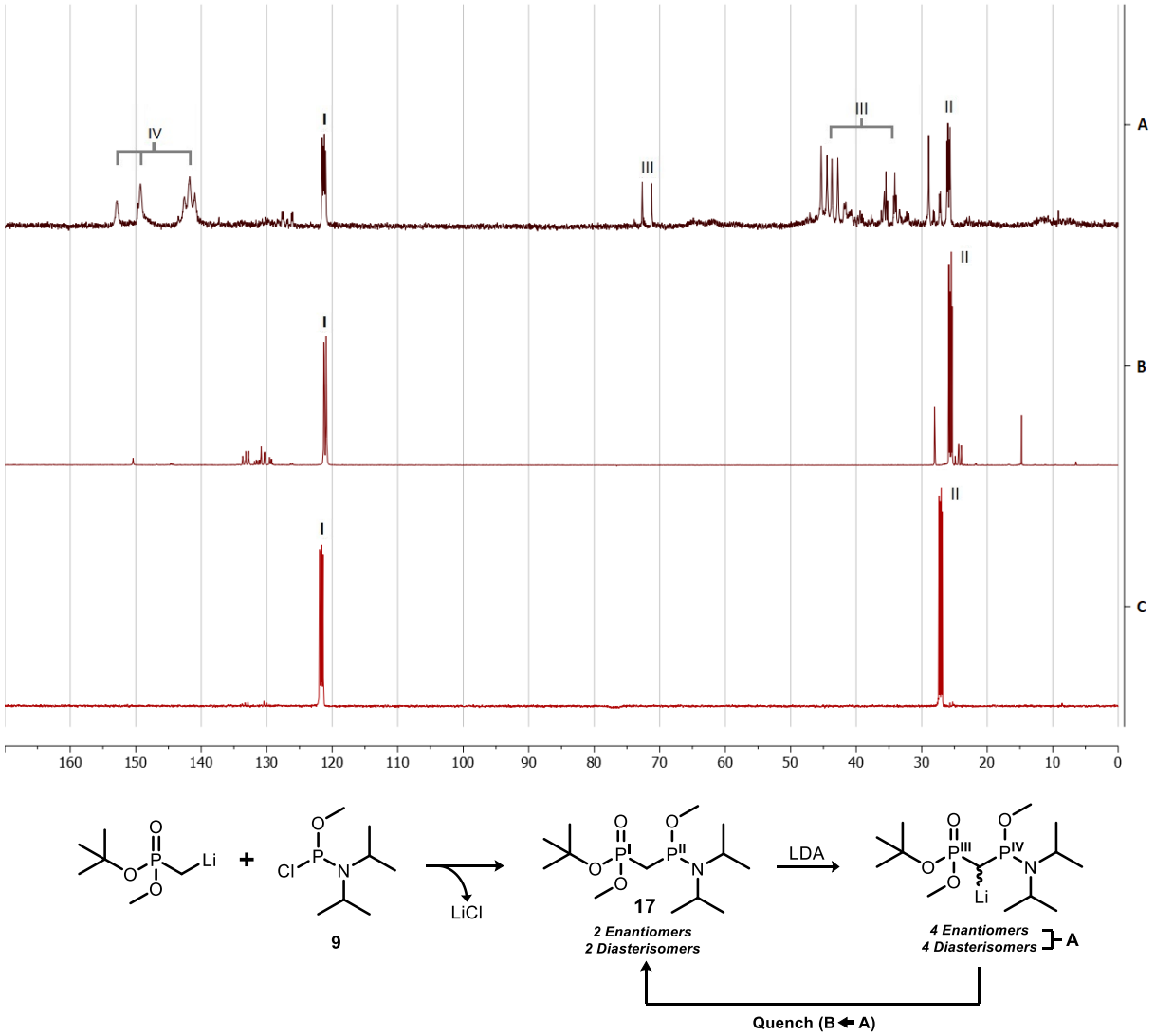
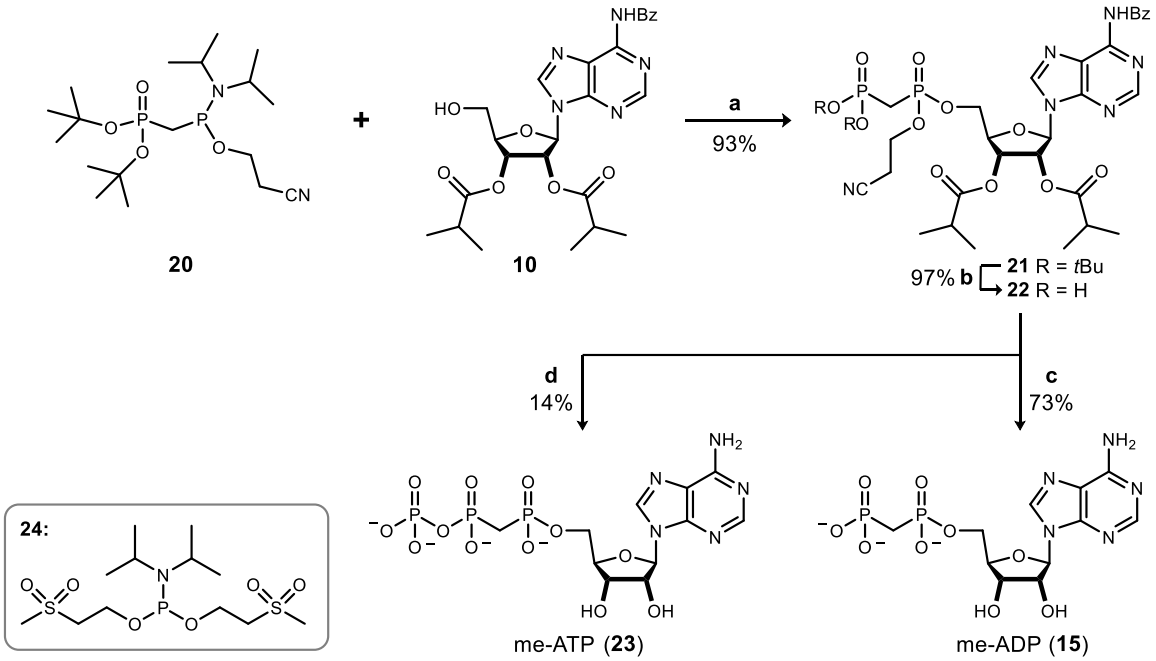


Figure 3: Synthesis of phosphanyl methylphosphonate reagent **17**, monitored by ^{31}P NMR spectrometry (162 MHz). A NMR tube was charged with an aliquot of the reaction mixture and fitted with an acetone-d6 capillary (required for locking). **A:** The reaction mixture after complete addition of the chlorophosphoramidite. **B:** The crude reaction mixture after quenching with aqueous sodium bicarbonate. **C:** The product after column purification using high purity grade silica.

bicarbonate protonates the lithium species, converting it into the desired bisphosphonate (Figure 3, B) which was isolated cleanly using high-purity grade silica (Figure 3, C).

Reagent **20**, fitted with the more labile cyanoethyl protecting group, was prepared via adjustment of the above described protocol (Scheme 2). *tert*-Butyl methylphosphonate **7** was deprotonated by LDA and subsequent reaction with bis(diisopropylamino)chlorophosphoramidite **18** afforded bis-amidite **19** in 76% yield. Selective substitution of one of the diisopropylamine groups with 2-cyanoethanol, under the agency of 0.6 eq. 4,5-dicyanoimidazole (DCI), provided reagent **20** in 82% yield.

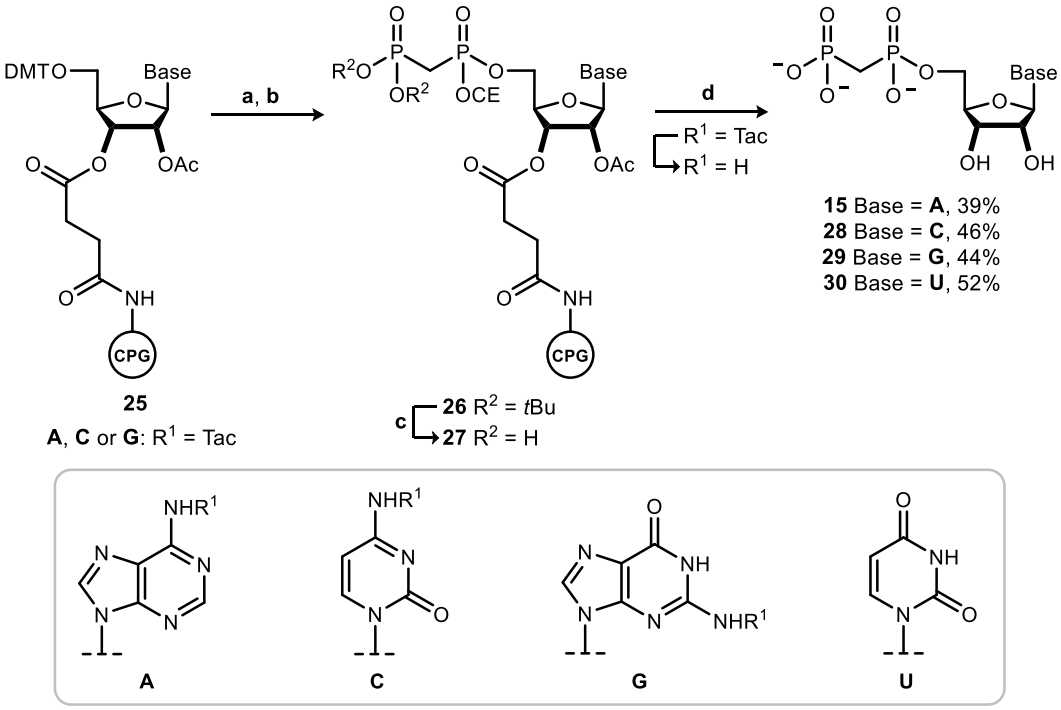


Scheme 3: Solution-phase synthesis of me-ADP **15** and methylene analogue of ATP **23** Reagents and conditions: [**a**] i: **20**, DCI, MeCN, 15 min. ii: *t*BuOOH, 15 min. [**b**] HCl (1.2 eq, 0.5M), HFIP, 4h. [**c**] Ammonia, 16h. [**d**] i: **23**, ETT, MeCN, 15 min. ii: *t*BuOOH, 15 min. iii: DBU, 0.5h. iv: ammonia, 16h.

Having the reagents in hand, the synthesis of the methylene bisphosphonate analogues of adenosine di- and triphosphate[21] (me-ADP **15** and me-ATP **23**, Scheme 3) with reagent **20** was undertaken. Coupling of **20** with adenosine derivative **10** under the influence of DCI and subsequent oxidation of the P^{III}-P^{V} intermediate gave protected me-ADP **21** in 93% isolated yield. Removal of the terminal *tert*-butyl protecting groups was carried out with HCl in hexafluoroisopropanol (HFIP), to obtain partially protected ADP analogue **22** in 97% yield. Global deprotection of **22** by treatment with aqueous ammonia provided adenosine methylene bisphosphonate (**15**) in 73% yield. The syntheses of adenosine methylene triphosphate **23** was effectuated in a one-pot procedure. Methylene diphosphate **22** was first phosphorylated with bis(methylsulfonyl)ethyl diisopropylphosphoramidite (**24**)[22] in the presence of 5-ethylthiotetrazole (ETT). Ensuing *in situ* oxidation of the phosphite-phosphate anhydride intermediate by *t*BuOOH gave the desired methylene triphosphate intermediate, as was determined by ^{31}P-NMR spectroscopy. The cyanoethyl and methylsulfonyl)ethyl protective groups were simultaneously cleaved

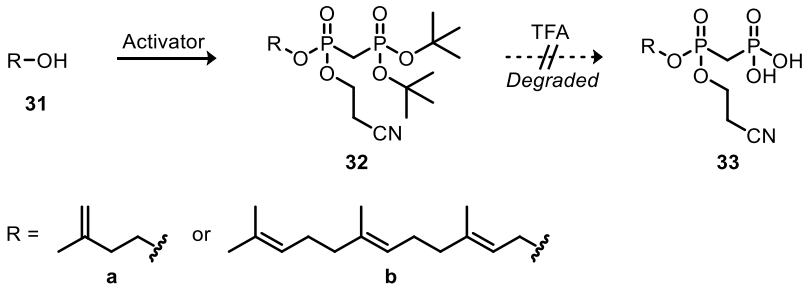
by DBU, followed by global deacylation with ammonia. Crude me-ATP **23** was purified by size-exclusion and anion-exchange chromatography.

Next, the applicability of newly developed methodology for automated solid-phase synthesis was investigated. For this purpose, the methylene bisphosphonates analogues of all nucleosides (me-ADP **15**, me-CDP **28**, me-GDP **29** and me-UDP **30**, Scheme 4) were selected as target molecules. Commercially available controlled pore glass (CPG), pre-loaded with the respective suitably protected nucleoside through a succinyl linker (**25**)[23], was loaded into an automated solid-phase oligonucleotide synthesizer. The four-step cycle procedure started with coupling of the immobilized nucleoside with phosphanyl methylphosphonate reagent **20**, using ETT as the activating agent. Subsequent oxidation with (1S)-(+)-(10-camphorsulfonyl)-oxaziridine (CSO) yielded protected resin-bound methylene bisphosphonate nucleotide **26**. The terminal *tert*-butyl groups in intermediate **26** were cleaved with HCl in HFIP (**27**), followed by DBU mediated removal of the 2-cyanoethyl group. Final ammonolysis of the acyl protecting groups with concomitant release from the solid support was effected by treatment with aqueous ammonia. The crude products were purified by gel filtration, providing methylene bisphosphonate nucleotides **15** (39%), **28** (46%), **29** (44%) and **30** (52%).



Scheme 4: Automated solid-phase synthesis of methylene bisphosphonate nucleotides. Reagents and conditions: [**a**] 4 x HCl (50 mM), HFIP, 1 min. [**b**] i: 2 x ETT and **20**, MeCN, 5 min. ii: 2 x CSO, MeCN, 5 min. [**c**] i: 4 x HCl (50 mM), HFIP, 1 min. [**d**] i: 2 x DBU, DMF, 2 min. ii: NH_4OH (35%), 1h. CE = 2-cyanoethanol

The necessity of strong basic conditions during the preparation of the phosphanylmethylphosphonate reagents impedes the use of base-labile protecting groups at the P^{V}-position. The use of bis(diisopropylamino)-chlorophosphoramidite circumvents this issue at the P^{III}-position, as was demonstrated for compound **20**. The reliance on base-stable protecting groups, such as the *tert*-butyl, limits the scope to acid stable substrates. This restriction became evident while attempting to synthesize the acid labile mono-substituted methylene bisphosphonate analogue of farnesyl pyrophosphate[24] and isopentenyl pyrophosphate[25]. Although the key phosphoramidite coupling proceeded efficiently (**32a** in 71%, **32b** in 65%), the acid sensitive aliphatic tails decomposed during *tert*-butyl removal. It can be concluded that the method, in its present form, is limited to substrates stable to at least diluted TFA. In Chapter 7 (Future Prospects) strategies are discussed that enable the installation of base-labile protecting groups.



Scheme 6: Attempted **s**ynthesis of isopentenyl- (**a**) and farnesyl (**b**) methylene bisphosphonate phosphonate analogues.

Conclusion

A new class of orthogonally protected phosphanylmethylphosphonate reagents has been developed for the synthesis of methylene bisphosphonate esters. Relevant examples are **17**, **19** and **20**, which are provided with selectively removable *tert*-butyl groups. These reagents are readily accessible through a one-pot procedure by reacting a lithiated methylphosphonate diester with the corresponding chlorophosphoramidite. Terminal methylene bisphosphonates could be prepared by phosphitylation of an alcohol with phosphanyl-methylphosphonate **20**, followed by *in situ* oxidation using *t*BuOOH, as has been demonstrated in the solution-phase synthesis of analogues of adenosine di- and triphosphate (me-ADP **15** and me-ATP **23**). In addition, the method has proven to be suitable for solid-phase synthesis, as shown for nucleotides methylene bisphosphonates (**15**, **28**-**30**). Phosphanylmethyl bis(amidite) **19** enables potential installation of other relevant protecting groups. Reagent **17** was designed and prepared while its application for the synthesis of unsymmetric methylene bisphosphates is described in the next Chapter.

Experimental Section

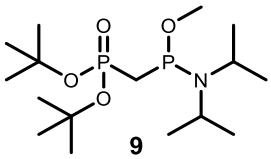
General: All solvents used under anhydrous conditions were stored over 4Å molecular sieves except for methanol which was stored over 3Å molecular sieves. Solvents used for workup and column chromatography were of technical grade from Sigma Aldrich and used directly. Unless stated otherwise, solvents were removed by rotary evaporation under reduced pressure at 40 °C. Reactions were monitored by TLC-analysis using Merck aluminum DC Silicagel 60 F_{254} with detection by spraying with 20% H_2SO_4 in EtOH, $(NH_4)_6Mo_7O_{24}{\cdot}4(H_2O)$ (25 g/L) and $(NH_4)_4Ce(SO_4)_4{\cdot}2(H_2O)$ (10 g/L) in 10% sulfuric acid or by spraying with a solution of ninhydrin (3 g/L) in EtOH / AcOH (20/1 v/v), followed by charring at approx. 250°C. Column chromatography was performed on Fluka silicagel (0.04 – 0.063 mm) or for methylene bis(phosphorus) compounds on high-purity grade silica (Sigma-Aldrich, Davisil Grade, 633).

NMR: ^{1}H-, ^{13}C- and ^{31}P-NMR Experiments were carried out on a Brüker AV-400 (400 MHz) or a Brüker AV-500 (500 MHz) and AVIII-Brüker DMX-600 (600 MHz). Chemical shifts are given in ppm (δ) and directly referenced to TMS (0.00 ppm) in $CDCl_3$ or D_2O via the solvent residual signal. $CDCl_3$ used in the characterization of phosphoramidite containing compounds was neutralized before use by filtering over aluminum oxide (Type WB-5: Basic). ^{31}P Chemical shifts are indirectly referenced to H_3PO_4 (0.00 ppm) according to the IUPAC method. ^{31}P NMR spectra measured to monitor reactions were made by charging a NMR tube with an aliquot of the reaction mixture and fitting the tube with an acetone-d_6 capillary.

LC-MS: Analysis were carried out on a JASCO HPLC system (detection simultaneously at 214 and 254 nm) coupled to a PE/SCIEX API 165 single quadruple mass spectrometer (Perkin-Elmer) using an analytical Gemini C18 column (Phenomex, 50 x 4.60 mm, 3 micron) in combination eluents A: H_2O; B: MeCN and C: 0.1 M aq. NH_4OAc or a Thermo Finnigan LCQ Advantage MAX ion-trap mass spectrometer with an electrospray ion source coupled to Surveyor HPLC system (Thermo Finnegan) using an analytical Gemini C18 column (Phenomex, 50 x 4.60 mm, 3 micron) in combination with eluents A: H_2O; B: MeCN and C: 1% aq. TFA as the solvent system. High resolution mass spectra were recorded by direct injection (2 µL of a 2 µM solution in water/acetonitrile; 50/50; v/v and 0.1% formic acid) on a mass spectrometer (Thermo Finnigan LTQ Orbitrap) equipped with an electrospray ion source in positive mode (source voltage 3.5 kV, sheath gas flow 10, capillary temperature 250 °C) with resolution R = 60000 at m/z 400 (mass range m/z = 150-2000) and dioctylpthalate (m/z = 391.2842) as a “lock mass”.

Trace Metal Chelation: High purity grade silica should be used when purifying methylene mono-phosphonic acids by silica gel column chromatography. Methylene mono-phosphonic acids (**8**) could be purified using silica gel column chromatography. However, it was found that after column purification the characteristic ^{31}P NMR peaks showed extreme signal broadening, to such an extent that the signals became difficult to detect. This broadening effect could be reversed by passing the purified methylene mono-phosphonic acid over an EDTA functionalized resin (a resin used to capture metal ions). This indicates that signal broadening resulted from metal ions bound to the phosphate, which could only have occurred during column purification.

Preparation of Phosphoramidite Reagents:



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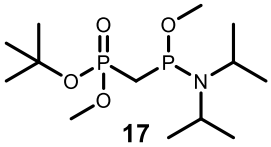
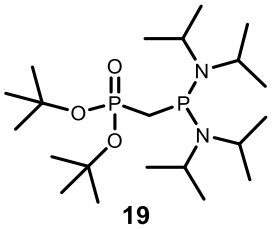
Phosphanylmethylphosphonate 9: Reagent was prepared according to the procedure described for **17**, using the following amounts: 2.5M solution of *n*-BuLi in *n*-hexane (4.2 ml, 10.5 mmol, 2.1 eq), diisopropylamine (1.6 ml, 11.2 mmol, 2.2 eq), di-*tert*-butyl methylphosphonate[26] (1.0 g, 5.0 mmol, 1.0 eq) in THF (5 mL) and chloro(diisopropylamino) methoxyphosphine (1.17 ml, 6.0 mmol, 1.2 eq) in *n*-hexane (5 mL). Purification by silica gel column chromatography (12.5% » 15% EtOAc in PE + 1% TEA) provided the product as a colorless oil (1.0 g, 2.7 mmol, 54%). **^{1}H NMR:** (400 MHz, $CDCl_3$) δ 3.53 – 3.36 (m, 2H), 3.45 (d, *J* = 14.0, 3H), 2.23 (ddd, *J* = 18.8, 14.4, 1.5 Hz, 1H), 1.86 (ddd, *J* = 20.2, 14.5, 1.0 Hz, 1H), 1.51 (s, 18H), 1.20 (d, *J* = 6.7 Hz, 6H), 1.13 (d, *J* = 6.8 Hz, 6H). **^{13}C NMR:** (101 MHz, $CDCl_3$) δ 81.62, 81.54, 81.47, 54.18, 53.97, 44.63, 44.52, 36.55, 36.23, 35.17, 34.85, 30.41, 30.37, 30.30, 24.36, 24.25, 24.17. **^{31}P NMR:** (122 MHz, $CDCl_3$) δ 122.04 (d, *J* = 57.1 Hz), 19.41 (d, *J* = 57.1 Hz). **HRMS:** Calculated for $C_{16}H_{38}NO_4P_2$ 370.22761 $[M+H]^+$; found 370.22703



17

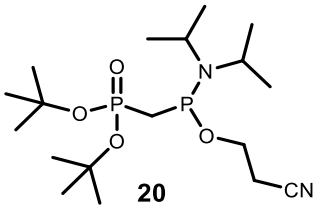
Phosphanylmethylphosphonate 17: A 2.5 M solution of *n*-BuLi in *n*-hexane (3.4 ml, 8.4 mmol, 2.1 eq) was carefully added to a cooled solution of (-78 ˚C) diisopropylamine (1.2 ml, 8.4 mmol, 2.1 eq) in dry THF (4 mL), under argon atmosphere. The reaction mixture was stirred for 30 minutes. Next, a solution of *tert*-butyl methyl methylphosphonate[27] (0.63ml, 4.0 mmol, 1 eq) in THF (4 mL) was added dropwise over 5 minutes, and the reaction was stirred for an additional 30 minutes. Lastly, commercially acquired chloro(diisopropylamino)methoxyphosphine (0.93 ml, 4.8 mmol, 1.2 eq) was purified by dissolving in *n*-hexane (4 mL), to precipitating DIPA·HCl contaminates. The resulting solution was added dropwise (±5 minutes) through a syringe fitted with silica filter and small layer of molecular sieves (±1 cm, 4Å). The cold reaction mixture was stirred for 30 minutes, before it was quenched with ethanol (2 mL) and poured into a two-layer system of DCM and saturated aqueous sodium bicarbonate. After extraction, the organic layer was dried over sodium sulfate, filtered and concentrated *in vacuo*. The concentrate was purified by silica gel column chromatography (30% EtOAc in PE + 1% TEA), providing **17** as a colorless oil (0.91 g, 2.8 mmol, 70%). **^{1}H NMR:** (400 MHz, $CDCl_3$) δ 3.72 (d, *J* = 11.2 Hz, 3H), 3.44 (dd, *J* = 14.2, 1.2 Hz, 3H), 2.32 (dddd, *J* = 19.8, 14.8, 11.8, 2.9 Hz, 1H), 1.89 (dddd, *J* = 20.9, 17.5, 14.5, 2.9 Hz, 1H), 1.52 (d, *J* = 2.2 Hz, 9H), 1.21 (d, *J* = 6.7 Hz, 6H), 1.13 (d, *J* = 6.8 Hz, 6H). **^{13}C NMR:** (101 MHz, $CDCl_3$) δ 82.26, 82.18, 82.13, 82.05, 54.18, 53.97, 51.73, 44.52, 44.41, 33.32, 33.20, 33.01, 32.89, 31.96, 31.84, 31.65, 31.53, 30.27, 30.23, 30.22, 30.21, 30.17, 24.32, 24.27, 24.10, 24.02. **^{31}P NMR:** (162 MHz, $CDCl_3$) δ = 121.03 (d, *J* = 56.7 Hz), 120.85 (d, *J* = 55.1

Hz), 26.20 (d, *J* = 56.7 Hz), 26.03 (d, *J* = 55.1 Hz). **HRMS:** Calculated for $C_{13}H_{31}NO_4P_2$ 328.18011 $[M+H]^+$; found 328.17960.



19

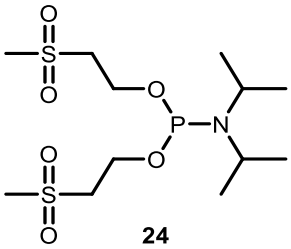
Phosphanylmethylphosphonate 19: A 2.5M solution of *n*-BuLi in *n*-hexane (8.4 ml, 21 mmol, 2.1 eq) was carefully added to a cooled solution (-78 ˚C) of diisopropylamine (3.14 ml, 21 mmol, 2.1 eq) in dry THF (10 mL), under argon atmosphere. The reaction mixture was stirred for 30 minutes. Next, a solution of di-*tert*-butyl methylphosphonate (2.08 g, 10 mmol) in THF (10 mL) was added dropwise over 5 minutes, and the reaction mixture was stirred for an additional 30 minutes. Lastly, a solution of bis(diisopropylamino)chlorophosphoamidite (3.2 g, 12 mmol) in *n*-hexane:THF (2:1 v:v, 15 mL) was prepared and added dropwise (±5 minutes) through a syringe fitted with a silica filter. After addition, the cooling bath was removed and the solution was stirred overnight at room temperature. The reaction mixture was poured into saturated aqueous sodium bicarbonate and was extracted with EtOAc. The organic layer was dried over sodium sulfate, filtered and concentrated *in vacuo*. Purification by silica gel column chromatography (5% » 10% EtOAc in PE + 2% TEA) yielded the product as a colorless oil (3.33 g, 7.6 mmol, 76%), that crystalized to a white solid. **^{1}H NMR:** (400 MHz, $CDCl_3$) δ 3.38 (dq, *J* = 18.0, 6.6 Hz, 4H), 2.12 (dd, *J* = 20.7, 1.6 Hz, 2H), 1.52 (s, 18H), 1.19 (t, *J* = 7.5 Hz, 24H). **^{13}C NMR:** (101 MHz, $CDCl_3$) δ 81.15, 81.06, 46.98, 46.86, 32.31, 32.00, 30.93, 30.63, 30.46, 30.43, 24.16, 24.10, 23.89, 23.81. **^{31}P NMR:** (162 MHz, $CDCl_3$) δ 39.63 (d, *J* = 89.1 Hz), 21.78 (d, *J* = 89.1 Hz). **HRMS:** Calculated for $C_{21}H_{51}NO_4P_2$ 457.33186 $[M+H+H_2O]^+$; found 457.33178.



20

Phosphanylmethylphosphonate 20: DCI (0.538 g, 4.56 mmol, 0.6 eq) was added to a dry solution of di-*tert*-butyl ((bis(diisopropylamino)phosphaneyl)methyl)phosphonate (3.33 g, 7.59 mmol) and 2-cyanoethanol (0.519 ml, 7.59 mmol) in DCM (50 mL). The reaction progression was followed by ^{31}P NMR, which indicated completion after 3.5 hours. The solution was concentrated *in vacuo* and purified by silica gel column chromatography (30% » 40% EtOAc + 1% TEA), providing phosphanyl methylphosphonate **20** (2.54 g, 6.22 mmol, 82%) as a colorless oil. **^{1}H NMR:** (500 MHz, $CDCl_3$) δ 3.92 – 3.74 (m, 2H), 3.58 – 3.40 (m, 2H), 2.65 (q, *J* = 6.3, 5.9 Hz, 2H), 2.30 (ddd, *J* = 19.0, 14.4, 1.1 Hz, 1H), 1.89 (ddd, *J* = 20.3, 14.5, 1.5 Hz, 1H), 1.58 – 1.45 (m, 18H), 1.21 (d, *J* = 6.7 Hz, 6H), 1.14 (d, *J* = 6.8 Hz, 6H). **^{13}C NMR:** (126 MHz, $CDCl_3$) δ = 117.57, 81.62, 81.58, 81.55, 81.51, 61.58, 61.38, 44.80, 35.99, 35.74, 34.89, 34.64, 30.22, 30.19, 23.95,

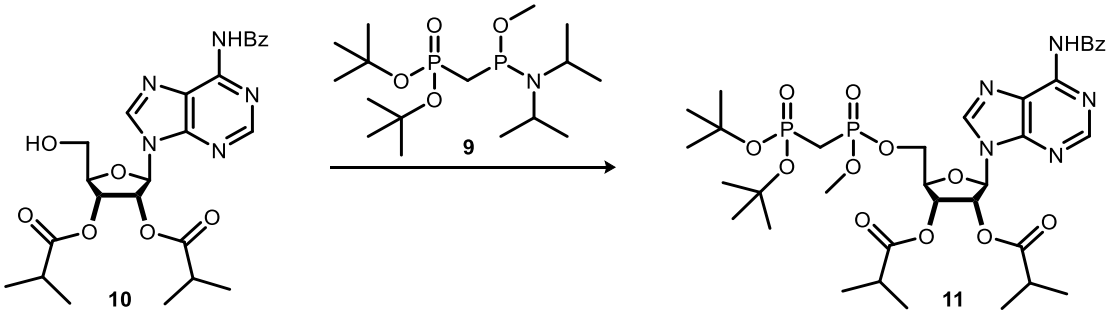
20.08, 20.02. **^{31}P NMR:** (202 MHz, $CDCl_3$) δ = 117.95 (d, *J* = 60.6 Hz), 15.25 (d, J = 60.6 Hz). Calculated for $C_{18}H_{39}N_2O_4P_2$ 409.23796 $[M+H]^+$; found 409.23792.



24

Bis(methylsulfonylethanol) Phosphoramidite 24: Diisopropylamine (1.63 ml, 11.46 mmol, 2 eq) was added to a cooled (0 °C) solution of DIPEA (2,0 ml, 11,46 mmol) and trichlorophosphane (0.50 ml, 5.73 mmol, 1 eq) in THF (20 mL). After 10 minutes, a solution of methylsulfonylethanol (1.42 g, 11.46 mmol, 2 eq) in THF (10 mL) was carefully added. The cooling bath was removed and the reaction mixture was stirred for 3 hours, after which ^{31}P NMR: indicated completion of the reaction. The mixture was filtered, rinsed and diluted with EtOAc. The filtrate was washed with saturated aqueous sodium bicarbonate and brine. The organic layer was isolated, dried over magnesium sulfate and concentrated *in vacuo*. The solid residue was recrystallized by cooling a supersaturated solution of the impure product in acetone : *n*-hexane (1:2 v:v, 5 mL), affording phosphoramidite **24** (1.15 g, 3.05 mmol, 53%) as a white crystalline substance.[22] Alternatively, the residue can be purified using silica gel column chromatography (20% » 30% acetone in PE + 1% TEA). **^{1}H NMR:** (400 MHz, $CDCl_3$) δ = 4.19 – 3.98 (m, 2H), 3.58 (dh, *J*=10.4, 6.8, 1H), 3.38 – 3.20 (m, 2H), 3.01 (d, *J*=0.9, 3H), 1.20 (d, *J*=6.9, 6H). **^{13}C NMR:** (101 MHz, $CDCl_3$) δ = 57.51, 57.31, 56.00, 55.92, 43.17, 43.04, 42.67, 24.49, 24.42. **^{31}P NMR:** (162 MHz, $CDCl_3$) δ = 148.27.

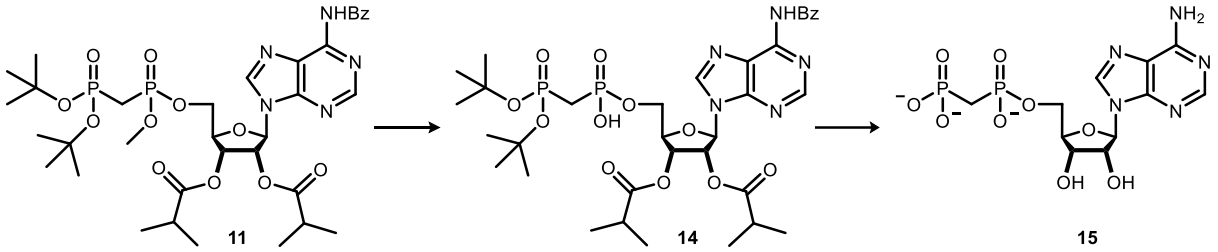
Coupling Reactions in Solution:



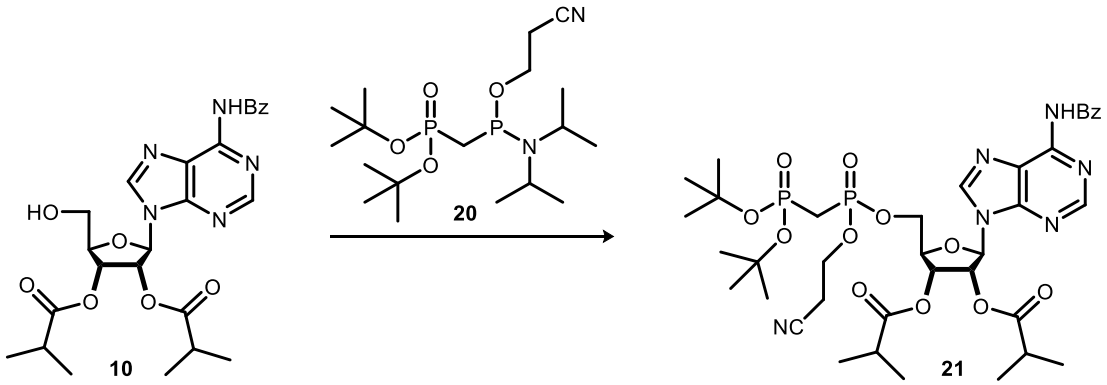
10 **9** **11**

Protected me-ADP 11: N^6-Benzoyl-2,3-*O*-di-*iso*-butyryladenosine (0.213 g, 0.416 mmol) was co-evaporated with pyridine (10 mL) and MeCN (10 mL) and put under argon atmosphere. Phosphanyl methylphosphonate **9** (122 mg, 0.330 mmol) was added as a solution (1 mL; 15% pyridine in MeCN) and the mixture was reduced in volume. The concentrated mixture was co-evaporated once more with MeCN, redissolved in MeCN (4 mL) and put under argon atmosphere. Tetrazole (1.1 mL, 0.495 mmol) in MeCN (0.45M) was added. ^{31}P NMR indicated full conversion within 10 minutes. *tert*-butyl hydroperoxide (180 µl, 0.991 mmol) was added, and the reaction mixture was stirred for an additional 15 minutes. The reaction was concentrated *in vacuo* and redissolved in EtOAc. The solution was washed with aqueous

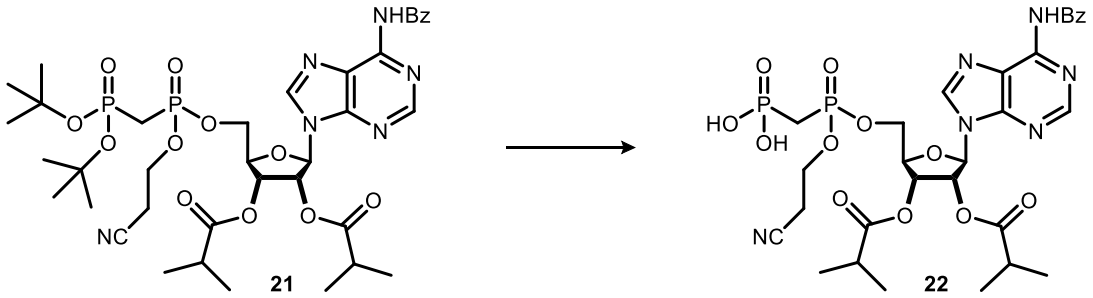
sodium bicarbonate and brine. The organic layer was dried over magnesium sulfate and concentrated *in vacuo*. Purification by silica gel column chromatography (100% EtOAc » 1% MeOH in EtOAc » 3% MeOH) yielded methylene bisphosphonate **11** as a colorless oil (86 mg, 0.108 mmol, 33%). **^{1}H NMR:** (400 MHz, $CDCl_3$) δ 9.48 (d, J = 10.0 Hz, 1H), 8.82 (s, 1H), 8.53 (d, J = 20.4 Hz, 1H), 8.10 – 7.99 (m, 2H), 7.63 – 7.55 (m, 1H), 7.51 (dd, J = 8.2, 6.7 Hz, 2H), 6.34 (t, J = 6.3 Hz, 1H), 5.89 (dt, J = 18.0, 5.9 Hz, 1H), 5.70 (ddd, J = 15.6, 5.7, 3.3 Hz, 1H), 4.46 (d, J = 6.4 Hz, 2H), 3.85 – 3.64 (m, 4H), 2.65 (dt, J = 14.0, 6.9 Hz, 1H), 2.58 – 2.31 (m, 3H), 1.52 (dd, J = 3.7, 1.4 Hz, 18H), 1.23 (d, J = 7.0 Hz, 6H), 1.11 (dd, J = 14.6, 7.0 Hz, 6H). **^{13}C NMR:** (101 MHz, $CDCl_3$) δ 175.61, 175.55, 175.21, 164.72, 152.82, 151.70, 151.66, 149.70, 141.59, 133.55, 132.63, 128.67, 127.88, 122.96, 85.58, 83.88, 83.82, 83.80, 83.74, 83.71, 81.93, 81.86, 81.79, 81.72, 73.21, 73.11, 70.54, 70.45, 65.06, 64.99, 64.93, 53.27, 53.21, 53.11, 33.68, 33.49, 30.20, 30.16, 30.13, 18.82, 18.80, 18.71, 18.64, 18.54. **^{31}P NMR:** (162 MHz, CDCl3) δ 23.73, 23.66, 23.32, 23.25, 9.61, 9.54, 9.47. **LCMS:** Calculated for $C_{35}H_{52}N_5O_{12}P_2$ 796.31 $[M+H]^+$; found 796.00



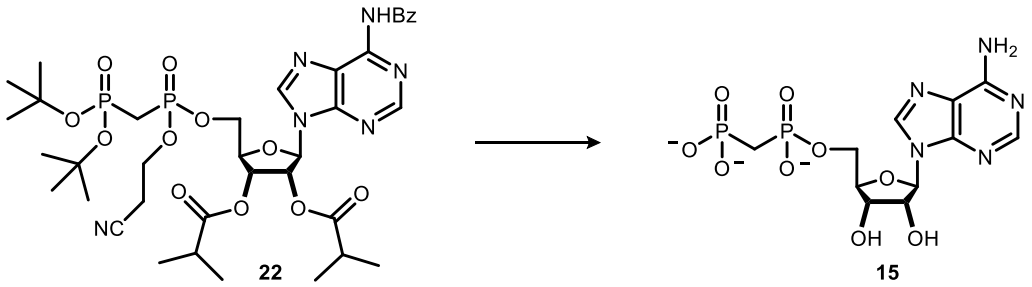
me-ADP 15: Methylene bisphosphonate **11** (37 mg, 46 µmol, 1 eq) was dissolved in a mixture of thiophenol:dioxane: TEA 1:2:2 (1.94 M, 0.5 mL) and the reaction was stirred overnight. ^{31}P NMR and LCMS analysis indicated complete conversion into intermediate **14** (see page S16). The reaction mixture was poured into a separation funnel containing water, acidified with acetic acid and washed with Et_2O (3x, to remove thiophenol). The water layer was isolated and concentrated *in vacuo*. The residue was dissolved in 10% TFA in DCM (0.5 mL). After 30 minutes, the reaction mixture was analyzed by ^{31}P NMR spectrometry, showing complete conversion. The reaction mixture co-evaporated with dioxane and chloroform. Ammonia (30%, 1 mL) was added and solution was stirred overnight. The final reaction mixture was analyzed by LCMS analysis. This was an exploratory experiment to determine the effectiveness of the deprotection conditions, therefore the product was not isolated.



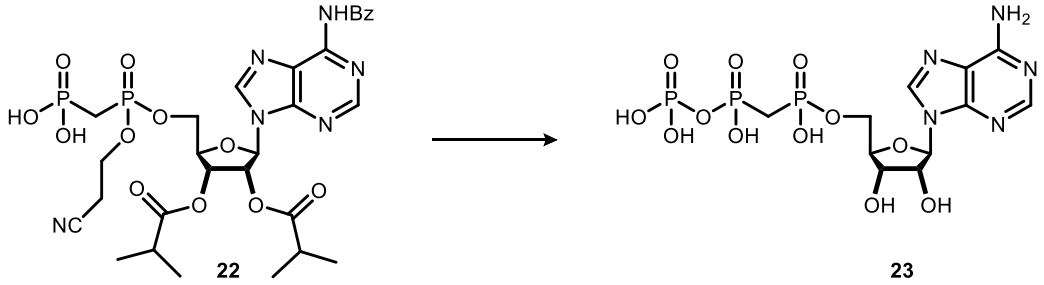
Protected me-ADP 21: Adenosine **10** (0.465 g, 0.909 mmol, 1.1 eq) and phosphanyl methylphosphonate **20** (0.408 g, 1.00 mmol, 1 eq) were co-evaporated with MeCN (8 mL), redissolved in MeCN (4 mL) and put under argon atmosphere. The reaction was stirred until all solids were dissolved and DCI (0.161 g, 1.36 mmol, 1.5 eq) was added. ^{31}P-NMR indicated full conversion within 15 minutes. Oxidation was initiated the addition of *tert*-butyl hydroperoxide (0.331 ml, 1.82 mmol, 2 eq) in decane (5.5 M). The reaction mixture was stirred for an additional 15 minutes. The solution was poured into a separation funnel containing water and EtOAc, washed with aqueous sodium bicarbonate (to remove DCI) and brine. The organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. Purification by silica gel column chromatography (25% » 50% acetone in DCM) yielded compound **21** (705 mg, 0.845 mmol, 93%) as white foam. **^{1}H NMR:** (500 MHz, $CDCl_3$) δ 9.31 (s, 1H), 8.76 (d, J = 2.9 Hz, 1H), 8.40 (d, J = 44.2 Hz, 1H), 8.06 – 7.93 (m, 2H), 7.60 – 7.52 (m, 1H), 7.47 (dd, J = 8.4, 7.0 Hz, 2H), 6.27 (dd, J = 21.0, 5.9 Hz, 1H), 5.88 (dt, J = 38.9, 5.8 Hz, 1H), 5.68 (ddd, J = 23.2, 5.6, 3.7 Hz, 1H), 4.51 – 4.38 (m, 3H), 4.35 – 4.23 (m, 2H), 2.74 (dp, J = 7.6, 6.2 Hz, 2H), 2.60 (h, J = 7.0 Hz, 1H), 2.55 – 2.39 (m, 3H), 1.53 – 1.42 (m, 18H), 1.19 (d, J = 7.0 Hz, 6H), 1.12 – 1.03 (m, 7H). **^{13}C NMR:** (126 MHz, $CDCl_3$) δ 175.77, 175.66, 175.43, 175.40, 164.86, 152.94, 151.87, 151.82, 149.85, 133.63, 132.83, 128.86, 128.01, 123.60, 123.48, 116.95, 116.86, 86.24, 85.77, 84.25, 84.23, 84.18, 84.17, 84.16, 84.09, 84.07, 84.01, 81.88, 81.82, 81.61, 81.55, 73.19, 73.08, 70.48, 70.39, 65.33, 64.98, 61.28, 61.24, 33.82, 33.63, 30.73, 30.55, 30.37, 30.34, 30.31, 30.28, 29.65, 29.61, 29.48, 29.43, 28.53, 28.36, 19.90, 19.85, 18.98, 18.96, 18.86, 18.82, 18.81, 18.70. **^{31}P NMR:** (202 MHz, $CDCl_3$) δ 22.86 (d, *J* = 6.1 Hz), 22.53 (d, *J* = 6.1 Hz), 8.73, 8.64. **HRMS:** Calculated for $C_{37}H_{53}N_6O_{12}P_2$ 835.31912 $[M+H]^+$; found 835.31958



Protected me-ADP 22: A 6M HCl in dioxane : water (1:1 v:v, 84 µl, 0.505 mmol, 1.2 eq) was added to a solution of adenosine methylene bisphosphonate **21** (351 mg, 0.420 mmol, 1 eq) in HFIP (1 mL). The reaction was shortly sonicated until the HCl solution was dissolved in the HFIP. The reaction was monitored by ^{31}P NMR, which indicated full conversion after 4 hours. The reaction was diluted with dioxane : toluene (1:1 v:v, 20 mL) and co-evaporated, this was repeated thrice to remove all traces of HFIP, providing adenosine methylenephosphonic acid **22** as a colorless oil (295 mg, 0.408 mmol, 97%). The product was used without further purification. **^{31}P NMR:** (162 MHz, Acetone-D_6) δ = 22.08 (d, *J* = 8.1 Hz), 21.68 (d, *J* = 8.1 Hz), 16.55 (d, *J* = 8.1 Hz), 16.46 (d, *J* = 8.1 Hz).

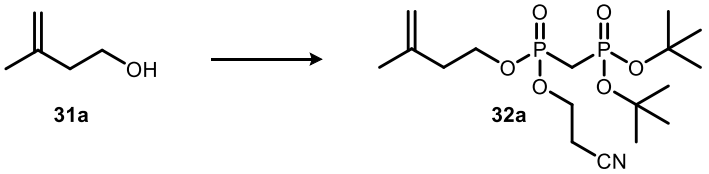


me-ADP 15: A 6M HCl in dioxane : water (1:1 v:v, 39 µL, 0.236 mmol, 1.2 eq) was added to a solution of adenosine methylene bisphosphonate **22** (164 mg, 0.196 mmol) in HFIP (1 mL). The reaction was monitored by ^{31}P NMR spectrometry, which indicated full conversion after 4 hours. The reaction was quenched with ammonium hydroxide (0.1 mL) and concentrated *in vacuo*. The concentrate was redissolved in ammonium hydroxide (1 mL, 17.5 mmol) and the reaction was stirred overnight. The excess ammonia was purged by stirring under vacuum. The crude material was purified using size-exclusion (HW40-column, 0.15 M aqueous NH_4OAc) chromatography. Lyophilization provided me-ADP **15** (61 mg, 0.143 mmol, 73%) as a white powder. **^{1}H NMR:** (600 MHz, D_2O) δ 8.25 (s, 1H), 7.87 (s, 1H), 5.87 (d, J = 5.3 Hz, 1H), 4.57 (t, J = 5.2 Hz, 1H), 4.37 (t, J = 4.7 Hz, 1H), 4.22 (d, J = 3.8 Hz, 1H), 4.03 (m, J = 4.9, 4.3 Hz, 2H), 2.05 (t, J = 19.9, 2.0 Hz, 2H). **^{13}C NMR:** (151 MHz, D_2O) δ 155.95, 153.36, 149.39, 140.58, 119.08, 88.00, 84.58, 75.15, 71.10, 64.47, 29.33, 28.51, 27.68. **^{31}P NMR:** (202 MHz, D_2O) δ = 18.20 (d, *J* = 8.1 Hz), 21.68 (d, *J* = 8.1 Hz), 16.55 (d, *J* = 8.1 Hz), 16.46 (d, *J* = 8.1 Hz).

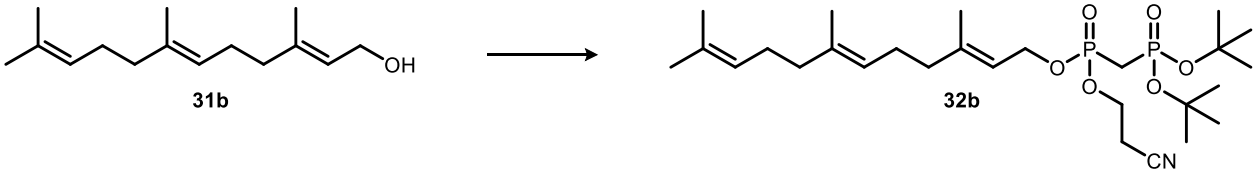


me-ATP 23: Crude adenosine methylene bisphosphonate **22** (295 mg, 0.408 mmol, 1 eq) and bis(2-(methylsulfonyl)ethyl) diisopropylphosphoramidite **24** (185 mg, 0.490 mmol, 1.2 eq) were co-evaporated with MeCN (8 mL), redissolved in dry MeCN (4 mL) and put under argon atmosphere. The reaction was stirred until all solids were dissolved, after which ETT (80 mg, 0.612 mmol, 1.5 eq) was added. 31P-NMR indicated full conversion within 15 minutes. Oxidation was initiated by the addition of *tert*-butyl hydroperoxide (111 µl, 0.612 mmol, 1.5 eq) in decane (5.5 M). After 15 minutes, DBU (431 µl, 2.86 mmol, 7 eq) was added and the reaction mixture was stirred for an additional 30 minutes. Ammonia (30%, 5 mL) was added and the reaction mixture was stirred overnight. The excess ammonia was purged by stirring under vacuum. The crude material was purified using size-exclusion (HW40-column, 0.15 M aqueous NH_4OAc) and anion-exchange chromatography (Source 15Q, 10 mM » 1 M, aqueous NH_4OAc). Lyophilization provided me-ATP **23** (14.4 mg, 0.029 mmol, 14%) as a white powder. **^{1}H NMR:** (500 MHz, D_2O) δ 8.55 (s, 1H), 8.20 (s, 1H), 6.06 (d, *J* = 5.4 Hz, 1H), 4.96 – 4.92 (m, 1H), 4.72 (t, *J* = 5.2 Hz, 1H), 4.53 (t, *J* = 4.5 Hz, 1H), 4.36 (t, *J* = 3.4 Hz, 1H), 4.18 (q, *J* = 4.2 Hz, 2H), 2.37 (t, *J* = 20.3 Hz, 2H). **^{13}C NMR:** (126 MHz,

D_2O) δ 153.86, 150.58, 148.66, 118.37, 87.16, 84.03, 83.97, 74.36, 70.16, 63.61, 63.57, 28.63, 27.62, 26.58. **^{31}P NMR** (202 MHz, D_2O) δ 18.25 (d, *J* = 9.9 Hz), 7.72 (dd, *J* = 25.3, 9.2 Hz), -9.65 (d, *J* = 24.6 Hz). **HRMS:** Calculated for $C_{11}H_{19}N_5O_{12}P_3$ 506.02431 $[M+H]^+$; found 506.02347.



Protected me-Isopentenyl Diphosphate 32a: Isoprenol (41.8 µL, 0.414 mmol) and di-*tert*-butyl (((2-cyanoethoxy) (diisopropylamino) phosphanyl)methyl)phosphonate (203 mg, 0,497 mmol, 1.2 eq) were co-evaporated with MeCN (4 mL), redissolved in MeCN (2 mL) and put under argon atmosphere. The reaction was stirred until all solids were dissolved and DCI (73.4 mg, 0.621 mmol, 1.5 eq) was added. 31P-NMR indicated full conversion within 15 minutes. Oxidation was initiated the addition of *tert*-butyl hydroperoxide (151 µl, 0.828 mmol, 2 eq) in decane (5.5 M). The reaction mixture was stirred for an additional 15 minutes. The solution was poured into a separation funnel containing water and EtOAc, washed with sodium bicarbonate. The organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. Purification by silica gel column chromatography (25% » 50% acetone in DCM) yielded the title compound (121 mg, 0.296 mmol, 71%) as colorless oil. **^{1}H NMR:** (400 MHz, $CDCl_3$) δ 4.81 (d, *J* = 23.3 Hz, 2H), 4.39 – 4.28 (m, 2H), 4.24 (q, *J* = 7.2 Hz, 2H), 2.86 – 2.70 (m, 2H), 2.50 – 2.34 (m, 4H), 1.77 (s, 3H), 1.56 – 1.51 (m, 18H). **^{13}C NMR:** (101 MHz, $CDCl_3$) δ 140.96, 116.69, 112.71, 64.45, 60.75, 38.40, 30.90, 30.28, 29.54, 28.14, 22.41, 19.90. **^{31}P NMR:** (162 MHz, $CDCl_3$) δ 21.00, 8.80.



Protected Farnesol me-Diphosphate (32b): Farnesol (125 µL, 0.5 mmol) and (((2-cyanoethoxy) (diisopropylamino)phosphanyl)methyl)phosphonate (245 mg, 0.600 mmol, 1.2 eq) were co-evaporated with MeCN (4 mL), redissolved in MeCN (2 mL), put under argon atmosphere and charged with 2,6-lutidine (116 µL, 1.00 mmol, 2 eq). 2,6-lutidine·HCl (108 mg, 0.750 mmol, 1.5 eq) was added. ^{31}P-NMR indicated full conversion within 15 minutes. Oxidation was initiated the addition of *tert*-butyl hydroperoxide (182 µL, 1.00 mmol, 2 eq) in decane (5.5 M). The reaction mixture was stirred for an additional 15 minutes. The solution was poured into a separation funnel containing water and EtOAc, washed with sodium bicarbonate (to remove DCI). The organic layer was dried over sodium carbonate and concentrated *in vacuo*. Purification by silica gel column chromatography (10% » 25% acetone in DCM) yielded the title compound (176 mg, 0.323 mmol, 65%) as colorless oil. **^{1}H NMR:** (400 MHz, $CDCl_3$) δ 5.47 – 5.35 (t, 1H), 5.16 – 5.03 (m, 2H), 4.65 (t, *J* = 8.0 Hz, 2H), 4.33 (dtd, *J* = 7.8, 6.4, 4.3 Hz, 2H), 2.79 (q, *J* = 6.3 Hz, 2H), 2.53 – 2.30 (m, 2H), 2.07 (ddd, *J* = 18.6, 10.6, 4.7 Hz, 7H), 1.97 (dd, *J* = 9.2, 6.2 Hz, 1H), 1.75 – 1.65

(m, 7H), 1.61 (d, *J* = 4.7 Hz, 5H), 1.54 (d, *J* = 2.4 Hz, 18H). **^{31}P NMR:** (162 MHz, $CDCl_3$) δ 21.20 (d, *J* = 10.7 Hz), 8.86 (d, *J* = 10.5 Hz). **HRMS:** Calculated for $C_{27}H_{49}NO_6P_2Na$ 568.29273 $[M+Na]^+$; found 568.29244.

Nucleotide Synthesis on Solid Phase

Methylene bisphosphonate functionalized nucleotides me-ADP, me-CDP, me-GDP and me-UDP were prepared from CPG resin pre-loaded with the respective nucleoside. The nucleosides were attached to the resin through a succinyl linker connected at the O^3-position. The O^5-positions were capped with a DMT and the exocyclic amine in **A**, **C** and **G** were protected with the 4-*tert*-butylphenoxyacetyl (Tac) moiety:

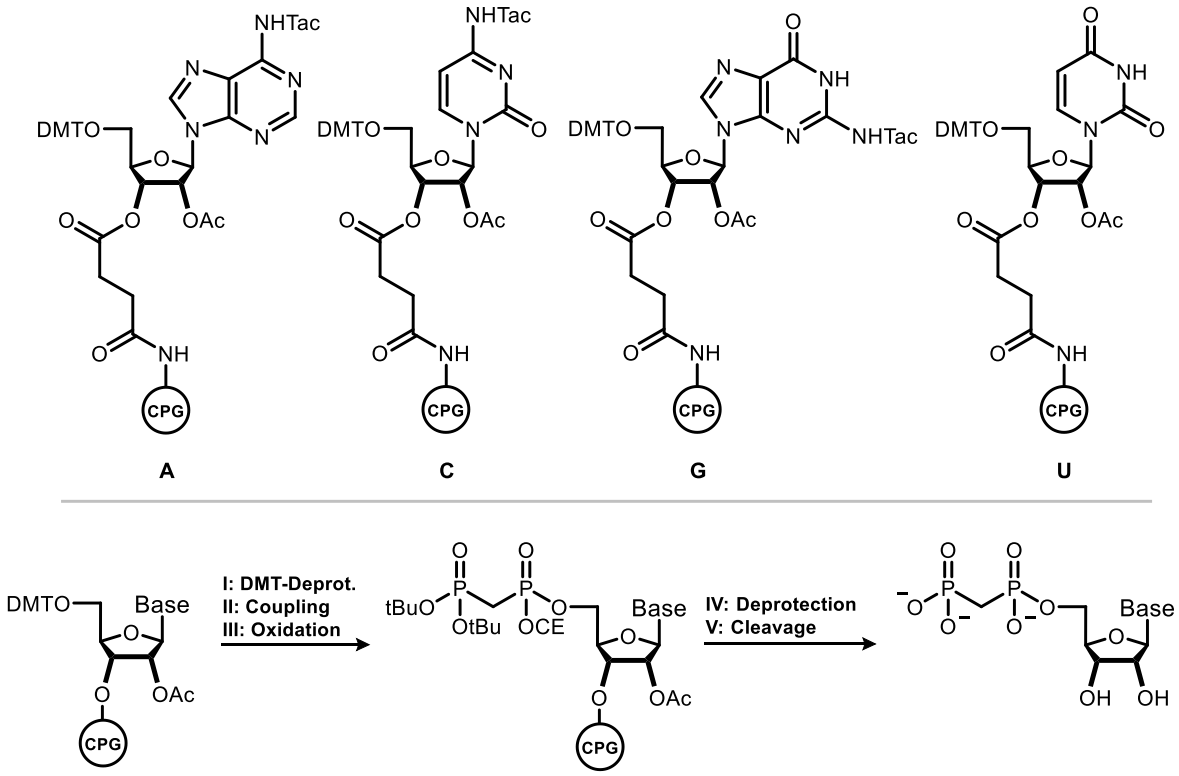


Figure S1: Overview of the solid-phase synthesis of the methylene bisphosphonate nucleotides me-ADP, me-CDP, me-GDP and me-UDP.

For each experiment 300 mg of pre-loaded GPC resin (±10 µmol loaded nucleoside) was loaded in a Mermade 6 oligonucleotide synthesizer. The synthesis was fully carried out under argon atmosphere. The nucleotides were purified using size-exclusion (HW40-column, 0.15 M aqueous NH_4OAc) chromatography.

I. DMT-Deprotection: The DMT was removed by treatment with HCl in hexafluoroisopropanol (50 mM, 1 mL) four times for 1 minute. The resin was washed with MeCN (3 x 2 mL).

II. **Coupling:** The reaction vessel was charged with phosphanyl methylphosphonate **20** (0.1 M in MeCN, 300 µL) and ETT (0.5 M in MeCN, 600 µL) and left to stand for 5 minutes. This cycle was repeated once, before the resin was rinsed with MeCN (3 x 2 mL).

III. **Oxidation:** The phosphinite-phosphonate intermediate was oxidized by treatment with (1S)-(+)-(10-camphorsulfonyl)oxaziridine (0.5 M in MeCN, 2 mL) twice for 5 minutes, and washed with MeCN (3 x 2 mL).

IV. **Deprotection:** The *tert*-butyl groups were removed by treating the resin with HCl in hexafluoroisopropanol (50 mM, 1 mL) four times for 1 minute. The resin was washed with MeCN (3 x 2 mL), before being treated with DBU (1 M in DMF, 1 mL) twice for 2 minutes, to eliminate the 2-cyanoethanol (CE). The resin was washed with MeCN (3 x 2 mL).

V. **Cleavage:** The resin was treated with NH_4OH (35%) for 1 hour, removing the remaining acyl-based protecting groups (Ac, TAC) and releasing the nucleotide from resin.

Yields and Data[28]**:**

me-ADP (15): 1.66 mg, 3.9 µmol, ±39%. For NMR data see solution phase synthesis for compound **15**.

me-CDP (28): 1.86 mg, 4.64 µmol, ±46%. **^{1}H NMR:** (500 MHz, D_2O) δ 8.17 (d, *J* = 7.8 Hz, 1H), 6.20 (d, J = 7.8 Hz, 1H), 5.91 (d, *J* = 2.9 Hz, 1H), 4.35 – 4.30 (m, 2H), 4.27 – 4.23 (m, 1H), 4.16 (m, *J* = 43.9, 11.9, 5.1, 2.4 Hz, 2H), 2.15 (td, *J* = 19.8, 1.5 Hz, 2H). **^{31}P NMR** (202 MHz, D_2O) δ 18.92 (d, *J* = 10.3 Hz), 15.29 (d, *J* = 10.1 Hz).

me-GDP (29): 1.92 mg, 4.35 µmol, ±44%. **^{1}H NMR**: (500 MHz, D_2O) δ 8.32 (s, 1H), 5.91 (d, *J* = 5.5 Hz, 1H), 4.73 (t, *J* = 5.4 Hz, 1H), 4.48 (t, *J* = 4.6 Hz, 1H), 4.30 (d, *J* = 3.8 Hz, 1H), 4.17 – 4.08 (m, 2H), 2.20 – 2.09 (t, *J* = 19.9, 2H). **^{31}P NMR:** (202 MHz, D_2O) δ 18.80, 15.78.

me-UDP (30): 2.07 mg, 5.25 µmol, ±52%. **^{1}H NMR** (500 MHz, D_2O) δ 7.87 (dt, *J* = 8.6, 1.3 Hz, 1H), 5.87 – 5.78 (m, 2H), 4.29 – 4.21 (m, 2H), 4.13 (d, *J* = 3.9 Hz, 1H), 4.10 – 3.93 (m, 2H), 2.05 (t, *J* = 19.7 Hz, 2H). **^{31}P NMR** (202 MHz, D_2O) δ 18.84 (d, *J* = 10.2 Hz), 15.50 (d, *J* = 9.7 Hz).

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