First Synthesis of Fully Deprotected Diimidotriphosphoric Acid and Derivatives Designed for the Synthesis of "PNPNP" Nucleotides and Dinucleotides

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The first synthesis of fully deprotected diimidotriphosphoric acid is described. The PNPNP sequence is obtained from an all protected cyclic precursor that can be regioselectively mono- or bisfunctionalized with alcohols and amines in classical organic solvents. All protective groups can be removed at the end of the synthesis by catalytic hydrogenation to afford the corresponding PNPNP derivatives as a salt. The strategy developed allows a straightforward access to a new class of nucleotide and dinucleotide analogs as well as other triphosphorylated compounds of biological relevance.

Introduction

Since the early 1960s, chemists have paid much attention to the synthesis of nucleotide analogs and especially phosphate-modified analogs. Advances in synthetic chemistry of nucleotides have led to a series of analogs which have already allowed certain key questions to be answered concerning the role of nucleotides in many biological processes. The first phosphate-modified nucleotide to be prepared was adenylyl methylene diphosphonate (AMPPCP) in which a methylene group replaced the β,γ-bridge oxygen in the triphosphate chain.[1] Then a number of other phosphonate analogs were synthesized.[2] The advantage of these analogs is the extreme stability of the P–C–P bonds which preclude any enzymatic (or accidental) cleavage. However the structural characteristics of the P–C–P group are substantially different from that of the original pyrophosphate they replace so that often these analogs are ineffective as substrates, effectors, or inhibitors. Consequently, other structural modifications of the triphosphate sequence aimed to better mimic the steric, electronic, and ionization characteristics of the naturally occurring pyrophosphate chain. They led to the preparation of thiophosphate analogs[3] and β,γ-imido analogs of nucleosides triphosphate.[4] In these last compounds (Nucleoside-PPNP) the oxygen atom in between P_β and P_γ is replaced with the isosteric and isoelectronic NH group. These modified nucleotides showed good enzymatic stability and proved to be in many cases fairly good inhibitors of various nucleotidase activities. Some enzymes exhibited even better affinity for PPNP nucleosides than for natural ones.[5] As far as geometry of the P–N–P link is concerned, it was found close to that of pyrophosphate.[6,7] The P–X bond length increases from P–O (1.61 Å) to P–N (1.68 Å) and the P–O–P and P–N–P bond angles are very similar, 130° and 127°, respectively.

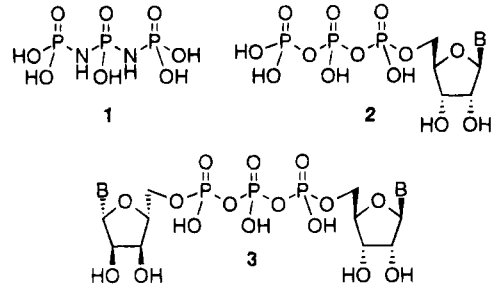

Figure 1.

In the course of our work on the synthesis of analogs of biological phosphates and pyrophosphates and following the considerations described above, we developed a strategy toward the synthesis of compound **1** and some of its esters as analogs of the triphosphate sequence in which the oxygen atom of the two pyrophosphate links were replaced with NH groups in order to give an access to triphosphate analogs of nucleotides **2** and dinucleotides **3** and to allow a study of their biological properties (Figure 1).[8]

To our knowledge the synthesis of the nonhydrolyzable $\alpha,\beta;\beta,\gamma$-diimido analogs of **2** and **3** has never been reported in the literature. Herein we wish to describe an original synthetic pathway for the unequivocal preparation of fully protected, monodeprotected, or wholly deprotected linear PNPNP sequences whose generic structure **4** is given in (Figure 2).

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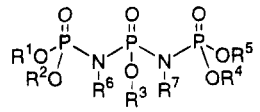

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Figure 2.

Results and Discussion

Compound **4** was originally designed for the preparation of biological triphosphate analogs. Consequently it would require regioselective introduction of an aglycon moiety on a phosphate analog followed by selective removal of all other phosphorus ester protecting groups. This has to be accomplished respecting the structural integrity of the PNPNP sequence. Following this idea, we attempted to take advantage of benzyl esters that can be removed under mild hydrogenolysis conditions and so proved very useful in organic synthesis.[9]

A retrosynthetic analysis of compound **5** allows the consideration of different pathways to the target structure (Scheme 1). We developed each one of these potential accesses, bringing to light their limitations and advantages. The analysis of the results obtained allowed us to work out an efficient and successful strategy for the preparation of diimidotriphosphoric acid and its derivatives.

In our first attempt, we tried to prepare compound **1** using two successive coupling reactions between dibenzyl chlorophosphate (**6**) and di(*N*-benzyl)phosphoramidic acid benzyl ester **7** (Scheme 1, route A).

Dibenzyl chlorophosphate (**6**) was readily obtained from dibenzyl phosphite using sulfuryl chloride as chlorinating reagent.[10] Phosphoramide **7** was obtained in a one-pot procedure by successive addition of benzyl alcohol and benzylamine to phosphorus oxychloride in the presence of triethylamine. Extrapolating on a patented procedure describing a coupling between a phosphoramide and a chlorophosphate,[11] sodium hydride was added to a mixture of reagents **6** and **7** in xylene. Though hydrogen evolved, no reaction took place at room temperature as confirmed by the recovery of all starting phosphoramide **7**. When running the experiment at higher temperature, tribenzyl phosphate as well as polar products were obtained. This could be explained by the decomposition of **7** according to the following scheme (Scheme 2).

Due both to the poor reactivity of the phosphoramides and to the relative fragility of the phosphorus benzyl esters when compared to alkyl or phenyl esters, this synthetic route was not suitable for the preparation of compound **5**. We tried to mitigate the lack of reactivity of **7**, replacing dibenzyl chlorophosphate **6** with phosphorus oxychloride ($POCl_3$), dibenzyl chlorophosphite ($(BnO)_2PCl$), or phosphorus trichloride (PCl_3). In these three cases, we could only isolate benzyl chloride and unidentified polar products. This could account for a possible intramolecular attack of chloride on a benzylic position following the phosphorylation step (Scheme 3).

Reversing the reactivity of the reagents, we therefore tried to couple benzyl dichlorophosphate or phosphorus oxychloride with 2 equiv of phosphoramide **8** (Scheme 1, route B). In both experiments and whatever the base employed (NaH, Et_3N, C_5H_5N, DMAP) no reaction occurred at room temperature. Increasing the temperature resulted in the formation of benzyl chloride and polar products revealing the same type of reactivity as already described (cf. Scheme 3).

The results of these experiments indicate that dichlorophosphate as well as phosphorus oxychloride or trichloride cannot be directly used to achieve the synthesis of **5**. In order to avoid the side reaction yielding benzyl chloride and leading rapidly to the total degradation of the product, we tried to couple compound **10**, prepared from the corresponding acid **9**[12] using oxalyl chloride, with the phosphoramide **8** (Scheme 1, route C). All attempts to effect this coupling reaction failed. The use of triethylamine in the reaction mixture led to the formation of benzyl chloride. Alternatively, when NaH was used, phosphoramide **11** was obtained in fairly good yield resulting from the attack by the anion of **8** on benzyl chloride resulting from decomposition of **10** following a similar mechanism as described in Scheme 3 (Scheme 4).

We tried to avoid this side reaction by reversing the reactivity of the reagents (Scheme 1, route D). We prepared compound **12** from **10** and despite our efforts, phosphoramide **12** was totally unreactive toward dibenzyl chlorophosphate (**6**) (Scheme 5). The use of sodium hydride as a base led to the formation of tribenzyl phosphate whereas triethylamine afforded benzyl chloride.

These results led us to the conclusion that phosphoramide benzyl ester cannot be phosphorylated under mild conditions unless using highly activated phosphorus species. Benzyl dichlorophosphate and dichlorophosphite are activated phosphorus species but unfortunately induced rapid decomposition of the reaction products. To overcome this difficulty, it seemed reasonable to us to lower the activation energy of the coupling reactions between the phosphorylating species and the phosphoramide moiety in order to be able to perform the transformation in experimental conditions mild enough to preserve the integrity of the benzyl esters present in the molecule. An efficient way to reduce the activation energy of the system is to proceed in such a manner that the reaction goes through an unimolecular mechanism instead of a bimolecular one. So we imagined a strategy in which the coupling reaction between the phosphorylating agent and the amides results in the formation of a cyclic compound (Scheme 6).

Developing on this idea, we designed the following synthetic route to compound **1** (Scheme 7).

In the above scheme, a bis-phosphoramide compound reacts with a highly reactive benzyl dichlorophosphate (X = O) at one phosphoramide moiety. Now there is a competition between the degradation reaction leading to the formation of benzyl chloride and a cyclization reaction yielding the PNPNP compound. The lowering of the activation energy level due to the ring closure should act

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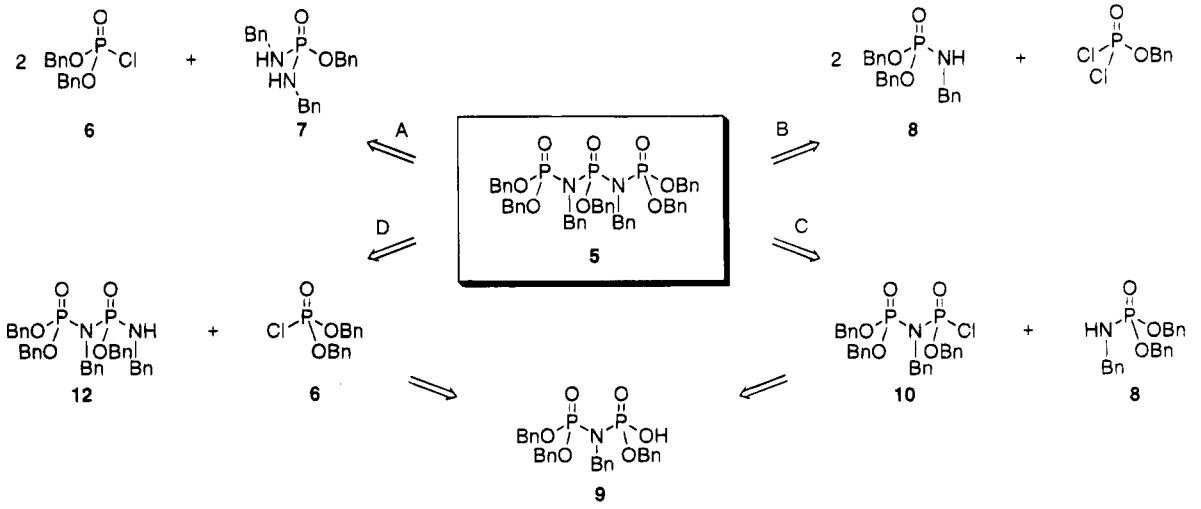
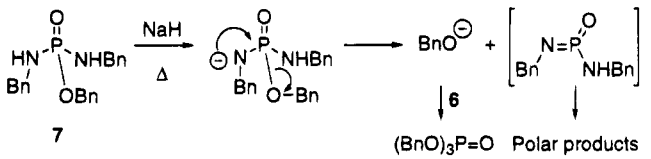
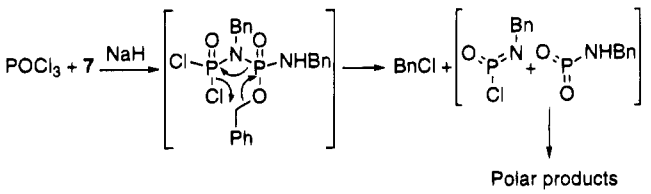
Scheme 1



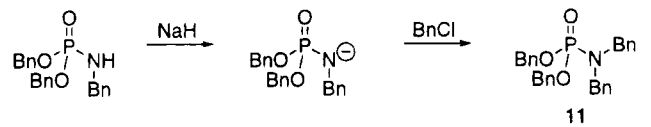
Scheme 2



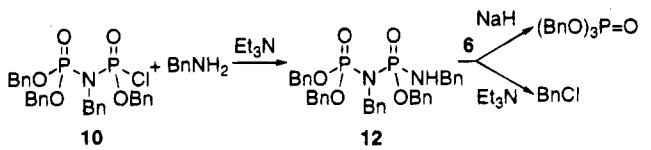
Scheme 3



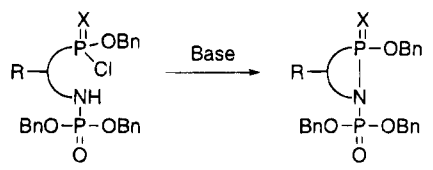
Scheme 4



Scheme 5



Scheme 6

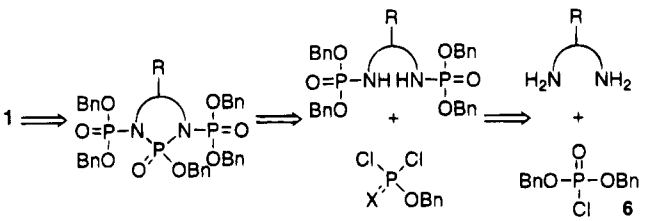


in favor of the formation of the cyclic compound, and degradation should be at least partly avoided.

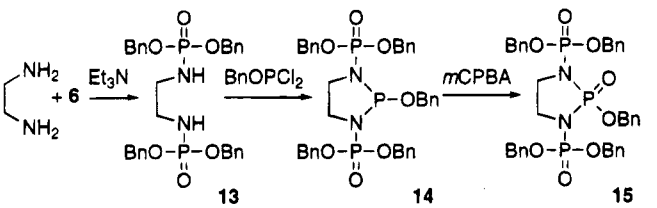
Firstly we tried to validate our hypothesis on a model system. Starting from ethylenediamine and dibenzyl chlorophosphate (**6**), we prepared bis-phosphoramide **13** which afforded the stable five-membered ring compounds **14** and **15** (Scheme 8).

Compound **13** was obtained from ethylenediamine, dibenzyl chlorophosphate (**6**) and triethylamine. Cyclization could be realized in 65% yield using benzyl dichlorophosphite.[13] Resulting phosphoramidite **14** was then quantitatively oxidized to **15** using *m*-chloroperbenzoic acid.[14] Benzyl dichlorophosphate failed to cyclize with **13** to form directly cyclic compound **15**.

Scheme 7



Scheme 8



These results attested the validity of our hypothesis. However compound **1** cannot be generated directly from **15**. In order to manage with a total removal of alkyl substituents on nitrogen by hydrogenolysis, we attempted to introduce two phenyl rings on the ethylenediamine bridge. Unfortunately we were not able to prepare *N,N'*-bis-phosphoramide from 1,2-diphenylethylenediamine[15] and dibenzyl chlorophosphate (**6**) or benzyl dichlorophosphite. This is probably due to steric hindrance introduced by the two aromatic rings at the two amino groups. In order to avoid this problem, we used *o*-xylenediamine **16** which displays two nonsubstituted benzylic positions.[16] This diamine could be conveniently bis-phosphorylated with dibenzyl chlorophosphate **6** to yield the correspond-

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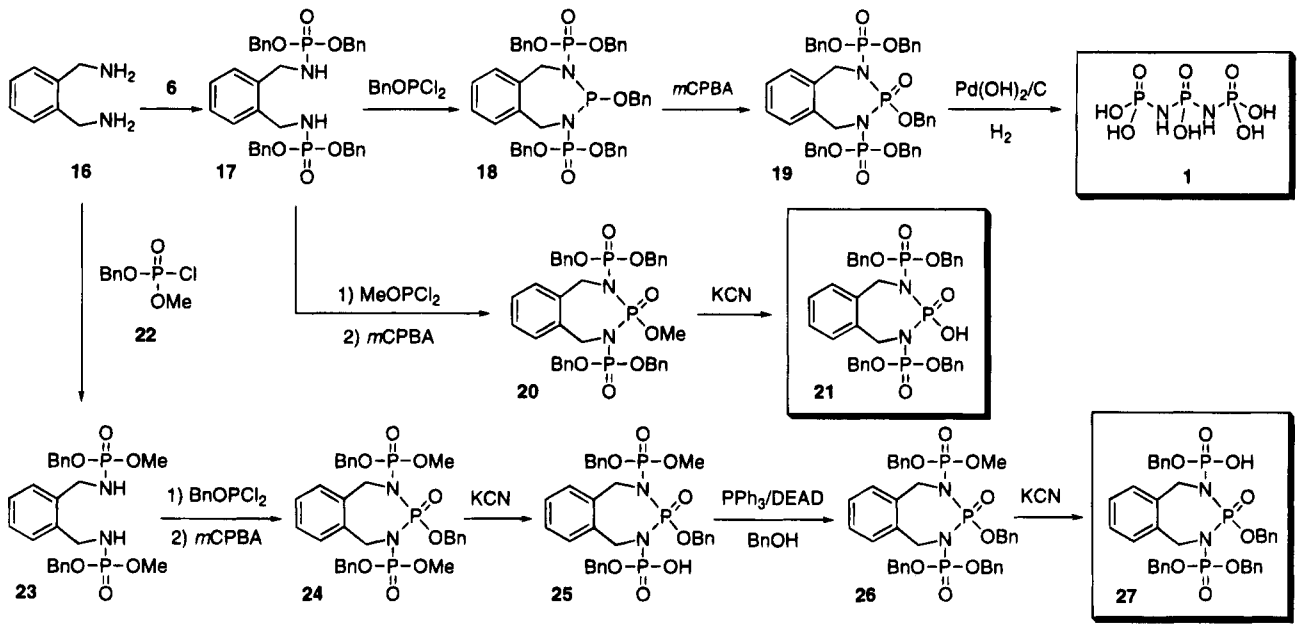
Scheme 9



ing bis-phosphoramide **17** (Scheme 9). Cyclization and oxidation were performed as with compound **13** using benzyl dichlorophosphite.

The conversion of **16** into **19** could be realized very efficiently without isolating the intermediates **17** and **18**, with an overall yield of 62%. All the substituents on the PNPNP sequence are now of the benzyl type and can be quantitatively removed by hydrogenolysis without formation of any byproduct. We used the Pearlman catalyst in a water/*tert*-butyl alcohol mixture, and the reaction was conducted under hydrogen pressure.[17] It is noteworthy that when hydrogenolysis was performed in a water/methanol mixture partial displacement of P–N bonds by methanol occurred as revealed by ^{1}H-, ^{13}C-, and $_{31}$P-NMR analyses.

As earlier established by Robins concerning imidodiphosphoric acids, triethylammonium salts are the more stable form of such species.[18] Consequently diimidotriphosphoric acid **1** was lyophilized in a triethylammonium bicarbonate aqueous solution (TEAB) prior to storage. Moreover hydrogenolysis could be performed directly in a *t*-BuOH/TEAB mixture.

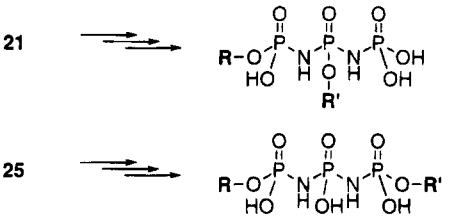
The synthetic pathway we have developed gives a straightforward access to several differently functionalized PNPNP sequences on which regioselective monodeprotection can be performed. To demonstrate the versatility of our approach we have worked out two examples.

In the first one, bis-phosphoramide **17** was cyclized with methyl dichlorophosphite and the resulting phosphoramidite was oxidized using *m*CPBA to give **20** in 40% yield (Scheme 9). The methyl ester was then selectively removed using KCN[19] in DMF to yield quantitatively the acid **21**. This compound allows the introduction of any alcohol (or amine) of interest at the central phosphorus atom by common esterification methods.

In the second example, benzyl methyl chlorophosphate (**22**) reacting with diamine **16** led to bis-phosphoramide **23** (Scheme 9). This compound was cyclized with benzyl dichlorophosphite and oxidized with *m*CPBA to yield **24**. Compound **24** was then specifically monodeprotected at one methyl ester to yield **25** and esterified with benzyl alcohol (or another alcohol of interest) to give **26**. The esterification was efficiently achieved *via* the Mitsunobu reaction. Removal of the second methyl ester was realized as for **24** to yield **27**. The overall yield of the transformation from **16** to **27** was 49%.

Compound **27** can be now condensed in the same manner as **25** with different aglycon moieties bearing a free alcohol or amine.

Scheme 10



Conclusion

We were able to synthesize molecules that are precursors of linear diimidotriphosphoric acid. The strategy we developed goes through the formation of cyclic structures that can be variously functionalized. Final hydrogenolysis allows the removal of all benzyl protective groups both at the phosphorus and nitrogen atoms in the PNPNP sequence. The regioselective work out of successive monodeprotections as well as the very mild reaction conditions necessary for the final removal of residual benzyl groups provides an access to various types of potentially interesting structures (Scheme 10).

For example, these compounds open perspectives on the preparation of a new class of nucleotides and dinucleotides analogs and other triphosphorylated species of biological relevance which should exhibit interesting properties. This is actually now under investigation.

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Experimental Section

General. Full general statements were described elsewhere.[20] When NMR spectra were recorded in a $CDCl_3/CD_3OD$ 1:1 mixture, chemical shifts were referenced to methanol. FAB-MS were recorded in negative ion mode in a 2-hydroxyethyl disulfide (HEDS) or triethanolamine (TEA) matrix. Analytical HPLC was performed on a column Superpac PEPS C2/C18 (250 × 4.6 mm) from Pharmacia Biotech with a system composed of a Shimadzu LC9 solvent delivery system and a LKB 2140 UV detector. Elutions were conducted at 35 °C with a solvent flow rate of 1.5 mL/min.

***N,N'*-Dibenzylphosphoramidic Acid Benzyl Ester (7).** Freshly distilled anhydrous triethylamine (11.98 mL, 85.99 mmol, 3.1 equiv) was added dropwise to phosphorus oxychloride (2.59 mL, 27.74 mmol, 1.0 equiv) in THF (50 mL) at −78 °C over 10 min. Benzyl alcohol (2.88 mL, 27.74 mmol, 1.0 equiv) in THF (20 mL) was then slowly added and the mixture was stirred for 3 h at −78 °C prior to the dropwise addition of benzylamine (6.06 mL, 55.48 mmol, 2.0 equiv) in THF (5 mL). The mixture was warmed at room temperature and stirred for 8 h. The precipitate was removed by filtration, and the filtrate was reduced *in vacuo*. The residue was chromatographed (AcOEt/hexane 80/20 to 100/0) to give **7** (9.15 g, 90%) as a white powder. TLC R_f 0.5 (AcOEt). Analytical HPLC ($MeOH/H_2O/Et_3N$ 65:35:0.1) t_R 9.1 min. Mp 89−91 °C, ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 7.34−7.21 (15 H, m); 5.01 (2 H, d, J = 7.9 Hz); 4.10 (4 H, AB part of ABX syst, J_{AB} = 7.1, J_{AX} = 2.3, J_{BX} = 2.2 Hz, υ_A = 4.12, υ_B = 4.07); 2.87 (2 H, td, J = 10.5, J = 6.0 Hz). ^{13}C-NMR ($CDCl_3$, 50 MHz) δ 139.92 (d, J = 6.6 Hz); 136.96 (d, J = 6.9 Hz); 128.51; 128.46; 128.79; 127.77; 127.36; 127.21; 66.99 (d, J = 4.7 Hz); 45.20. ^{31}P-NMR ($CDCl_3/H_3PO_4$, 81.015 MHz) δ 15.49 (s). IR (CH_2Cl_2): 3410; 3230; 3015; 1496; 1454; 1410; 1216; 1093; 1072; 1016.

Benzylphosphoramidic Acid Dibenzyl Ester (8). Compound **8** (13.6 g, 87%) was obtained as a white solid following the preceding procedure using 2 equiv of benzyl alcohol and 1 equiv of benzylamine. TLC R_f 0.5 (AcOEt/hexane 8:2). Analytical HPLC ($MeOH/H_2O/Et_3N$ 70:30:0.1) t_R 7.1 min. Mp 86−87 °C. ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 7.34 (10 H, m); 7.26 (5 H, m); 5.05 (4 H, AB part of ABX syst, J_{AB} = 11.9, J_{AX} = 7.6, J_{BX} = 7.5 Hz, υ_A = 5.07, υ_B = 5.02); 4.06 (2 H, dd, J = 6.9, J = 10.0 Hz); 3.21 (1 H, td, J = 5.2, J = 10.5 Hz). ^{13}C-NMR ($CDCl_3$, 50 MHz) δ 139.49 (d, J = 6.5 Hz); 136.47 (d, J = 7.2 Hz); 128.48; 128.44; 128.17; 127.74; 127.35; 127.28; 68.01 (d, J = 5.1 Hz); 45.35. ^{31}P-NMR ($CDCl_3/H_3PO_4$, 81.015 MHz) δ 9.48 (s). IR (CH_2Cl_2): 3413; 3231; 3010; 1497; 1455; 1415; 1217; 998.

Dibenzyl *N*-Benzyl-*N*-[benzoxy(benzylamino)phosphinyl]phosphoramidic Acid (12). Benzylimidodiphosphoric acid tribenzyl ester (**9**) (0.86 g, 1.60 mmol, 1.0 equiv) in anhydrous toluene (10 mL) was cooled at 0 °C, and oxalyl chloride (280 μl, 3.20 mmol. 2.0 equiv) and DMF (1 μL) were added. The solution was stirred at room temperature for 3 h and reduced *in vacuo* to 5 mL. Anhydrous toluene (10 mL) was added and once again the solution was reduced to 5 mL. Intermediate chloride **10** was not further characterized, and the solution was cooled at 0 °C prior to addition of anhydrous triethylamine (268 mL, 1.92 mmol, 1.2 equiv) and benzylamine (175 μL, 1.60 mmol, 1.0 equiv). The mixture was warmed at room temperature and stirred for 2 h. The solvent was removed under reduced pressure, and the residue was purified by chromatography (Et_2O/hexane 8:2 to 10:0) to yield **12** (0.65 g, 65%) as a colorless oil. TLC R_f 0.5 (Et_2O). Analytical HPLC ($MeOH/H_2O/Et_3N$ 65:35:0.1) t_R 12.1 min. ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 7.50−7.46 (2 H, m); 7.34−7.23 (19 H, m); 7.15−7.06 (4 H, m); 5.04 (2 H, d, J = 7.2 Hz); 4.86 (2 H, AB part of ABX syst, J_{AB} = 12.0, J_{AX} = 6.9, J_{BX} = 7.2 Hz, υ_A = 4.89, υ_B = 4.83); 4.60 (2 H, AB part of ABX syst, J_{AB} = 12.4, J_{AX} = 7.5, J_{BX} = 7.5 Hz, υ_A = 4.65, υ_B = 4.50); 4.59 (2 H, t, J = 14.4 Hz); 4.21 (2 H, AB part of ABX syst, J_{AB} = 6.3, J_{AX} = 6.3, J_{BX} = 3.3 Hz, υ_A = 4.23, υ_B = 4.17); 4.03 (1 H, td, J = 6.3, J = 6.2 Hz). ^{31}P-NMR ($CDCl_3/H_3PO_4$, 81.015 MHz) δ 13.98 (1 P, d, J = 23.2 Hz); 5.12 (1 P, d, J = 23.2 Hz). MS (CI, NH_3): 644.1 (5) [$MNH4^+$]; 627.2 (3) [MH^+]; 504.1 (4); 395.1 (95); 288.2 (58); 215.2 (82); 198.3 (100).

[2-[[Bis-(benzyloxy)phosphoryl]amino]ethyl]phosphoramidic Acid Dibenzyl Ester (13). Dibenzyl chlorophosphate (**6**) (9.87 g, 33.28 mmol, 2.0 equiv) in THF (10 mL) was added dropwise to ethylenediamine (1.00 g, 16.64 mmol, 1.0 equiv) and anhydrous triethylamine (4.87 mL, 34.94 mmol, 2.1 equiv) in THF (60 mL) at 0 °C. The mixture was warmed at room temperature and stirred for 1 h. The precipitate was removed by filtration and the filtrate was reduced *in vacuo*. The residue was chromatographed (AcOEt/EtOH 100:0 to 90:10) to give **13** (8.40 g, 87%) as a white powder. TLC R_f 0.5 (AcOEt/EtOH 9:1). Analytical HPLC ($MeOH/H_2O/Et_3N$ 60:40:0.1) t_R 8.6 min. Mp 62−63 °C. ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 7.31 (20 H, s); 4.99 (8 H, d, J = 7.8 Hz); 3.10 (2 H, m); 2.92−2.80 (4 H, m). ^{13}C-NMR ($CDCl_3$, 50 MHz) δ 136.21 (d, J = 7.4 Hz); 129.01; 128.00; 127.53; 67.77 (d, J = 5.2 Hz); 42.43 (d, J = 5.5 Hz). ^{31}P-NMR ($CDCl_3/H_3PO_4$, 81.015 MHz) δ 10.20 (s). MS (CI, NH_3): 598.1 (25) [MNH_4^+]; 581.2 (56) [MH^+]; 470.1 (33); 386.2 (100); 280.1 (51). IR (CH_2Cl_2): 3190; 3032; 2883; 1497; 1456; 1238; 1209; 1128; 1081; 1008.

[2-(Benzyloxy)-3-[bis(benzyloxy)phosphoryl][1,3,2]-diazaphospholidin-1-yl]phosphonic Acid Dibenzyl Ester (14). Benzyl dichlorophosphite (5.76 g, 27.56 mmol, 4.0 equiv) in THF (27 mL) was added dropwise to a mixture of compound **13** (4.00 g, 6.89 mmol, 1.0 equiv), triethylamine (1.92 mL, 13.78 mmol, 2.0 equiv), and 4-DMAP (0.20 g, 0.89 mmol, 0.13 equiv) in THF (40 mL) at 0 °C. The mixture was stirred at room temperature for 12 h. The precipitate was removed by filtration, and the filtrate was reduced *in vacuo*. The residue was chromatographed (AcOEt/hexane 80:0 to 100:0) to give **14** (3.21 g, 65%) as a colorless oil. TLC R_f 0.5 (AcOEt). Analytical HPLC ($MeOH/H_2O/Et_3N$ 60:40:0.1) t_R 11.3 min. ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 7.37−7.24 (25 H, m); 5.07−4.96 (8 H, m); 4.87 (2 H, d, J = 8.0 Hz); 3.49−3.19 (4 H, m). MS (CI/NH_3): 734.4 (41) [MNH_4^+]; 717.4 (39) [MH^+]; 280.2 (100).

[2-(Benzyloxy)-3-[bis(benzyloxy)phosphoryl]-2-oxo-2-λ⁵-[1,3,2]diazaphospholidin-1-yl]phosphonic Acid Dibenzyl Ester (15). A solution of *m*CPBA (0.82 g, 4.74 mmol, 1.7 equiv) in CH_2Cl_2 (10 mL) was added dropwise to compound **14** (2.00 g, 2.79 mmol, 1.0 equiv) in CH_2Cl_2 (18 mL) at −40 °C. The mixture was warmed at room temperature and stirred for 4 h. Excess *m*CPBA was decomposed by addition of a saturated aqueous solution of $Na_2S_2O_5$ (10 mL). The mixture was neutralized with a saturated aqueous $NaHCO_3$ solution (10 mL), and the aqueous phase was extracted with AcOEt. The organic layer was dried over sodium sulfate, reduced *in vacuo*, and chromatographed (AcOEt/hexane/EtOH 80/20/0 to 95/0/5) to yield **15** (2.03 g, 98%) as a colorless oil. TLC R_f 0.5 (AcOEt/EtOH 97:3). Analytical HPLC ($MeOH/H_2O/Et_3N$ 40:60:0.1) t_R 7.6 min. ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 7.37−7.24 (25 H, m); 5.06−4.95 (8 H, m); 3.51−3.21 (4 H, m). MS (CI/NH_3): 750.8 (28) [$MNH4^+$]; 733.4 (23) [MH^+]; 280.2 (100).

{2-[[[Bis(benzyloxy)phosphoryl]amino]methyl]benzyl}-phosphoramidic Acid Dibenzyl Ester (17). Compound **17** (7.89 g, 96%) was obtained as a white crystalline powder starting from *o*-xylenediamine and dibenzyl chlorophosphate (**6**), following the same procedure as for **13**. TLC R_f 0.5 (AcOEt/EtOH 95:5). Analytical HPLC ($MeOH/H_2O/Et_3N$ 60:40:0.1) t_R 9.0 min. Mp 85−86 °C. ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 7.32−7.15 (24 H, m); 4.93 (8 H, AB part of ABX syst, J_{AB} = 11.8, J_{AX} = 7.5, J_{BX} = 7.5 Hz, υ_A = 4.98, υ_B = 4.88); 4.06−3.96 (6 H, m). ^{13}C-NMR ($CDCl_3$, 50 MHz) δ 137.21 (d, J = 6.2 Hz); 136.33 (d, J = 7.3 Hz); 129.10; 128.24; 127.93; 127.58; 67.79 (d, J = 5.1 Hz); 42.24. ^{31}P-NMR ($CDCl_3/H_3PO_4$, 81.015 MHz) δ 9.72 (s). MS (CI/CH_4): 657 (100) [MH^+]; 216 (19). IR (CH_2Cl_2): 3220; 3033; 2948; 2884; 1497; 1455; 1379; 1226; 1080; 999.

[3-(Benzyloxy)-4-[bis(benzyloxy)phosphoryl]-1,3,4,5-tetrahydrobenzo[*e*][1,3,2]diazaphosphepin-2-yl]phosphonic Acid Dibenzyl Ester (18). Compound **18** (4.16 g, 45%) was obtained as a colorless oil starting from **17** and benzyl dichlorophosphite, following the same procedure as for compound **14**. TLC R_f 0.5 (AcOEt/hexane 7:3). Analytical HPLC ($MeOH/H_2O/Et_3N$ 80:20:0.1) t_R 16.2 min. ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 7.37−7.09 (29 H, m); 5.25−4.79 (10 H, m); 4.61 (2 H,

(20) Lebeau, L.; Oudet, P.; Mioskowski, C. Helv. Chim. Acta **1991**, *74*, 1697.

dd, J = 6.5, J = 11.6 Hz); 4.12 (2 H, m). ^{31}P-NMR ($CDCl_3$/H_3PO_4, 81.015 MHz) δ 8.36 (2 P, s); 7.51 (1 P, s). MS (CI/CH_4): 793 (71) [MH^+]; 525 (16); 463 (15); 353 (28); 280 (33); 263 (100); 201 (45).

[3-(Benzyloxy)-4-[bis(benzyloxy)phosphoryl]-3-oxo-1,3,4,5-tetrahydro-3-λ^5-benzo[*e*][1,3,2]diazaphosphepin-2-yl]phosphonic Acid Dibenzyl Ester (19). Compound **19** (3.62 g, 98%) was obtained as a colorless oil from **18** following the same procedure as for compound **15**. TLC R_f 0.5 (AcOEt/hexane 8:2). Analytical HPLC (MeOH/H_2O/Et_3N 65:35:0.1) t_R 13.9 min. ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 7.29–7.08 (29 H, m); 5.04 (2 H, d, J =7.5 Hz); 4.99–4.83 (8 H, m); 4.81–4.52 (4 H, m). ^{13}C-NMR ($CDCl_3$, 50 MHz) δ 135.84; 135.67; 135.06; 128.92; 128.44; 128.40; 128.28; 128.00; 127.91; 127.83; 69.16; 69.09; 68.83; 49.01. ^{31}P-NMR ($CDCl_3$/H_3PO_4, 81.015 MHz) δ 8.11 (1 P, t, J = 18.9 Hz); 4.09 (2 P, d, J = 18.9 Hz). MS (CI/CH_4): 809.0 (85) [MH^+]; 611.0 (18); 391.2 (23); 369.1 (31); 245.0 (29); 229.0 (25); 201.2 (100). IR (CH_2Cl_2): 3050; 3045; 2990; 1889; 1812; 1719; 1498; 1455; 1375; 1310; 1286; 1020; 882.

Diimidotriphosphoric Acid (1). Compound **19** (223 mg, 0.27 mmol) and 20% $Pd(OH)_2$/C (220 mg) were suspended in *tert*-butyl alcohol/water (20 mL). The mixture was stirred under hydrogen atmosphere (70 psi) at room temperature for 12 h. Catalyst was filtered off, and the residue was lyophilized to yield **1** (61 mg, 87%) as a white hygroscopic powder. Prior to NMR studies the powder was lyophilized in aqueous triethylammonium bicarbonate 1 M (10 mL). ^{31}P-NMR (D_2O/H_3PO_4, 81.015 MHz) δ 4.01 (2 P, m); 0.23 (1 P, m).[21] MS (FAB$^-$, TEA): 254.9 (42) [M − H^-]; 246.1 (100); 237.9 (52); 228.0 (28); 209.0 (37).

[4-[Bis(benzyloxy)phosphoryl]-3-methoxy-3-oxo-1,3,4,5-tetrahydro-3-λ^5-benzo[*e*][1,3,2]diazaphosphepin-2-yl]phosphonic Acid Dibenzyl Ester (20). Compound **17** was cyclized with methyl dichlorophosphite following the same procedure as for **18**. The mixture was filtered, and the crude was oxidized in the same conditions as described for **15** to yield **20** (2.23 g, 40%) as a colorless oil. TLC R_f 0.5 (AcOEt). Analytical HPLC (MeOH/H_2O/Et_3N 75:25:0.1) t_R 8.0 min. ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 7.35–6.81 (24 H, m); 5.03 (4 H, AB part of ABX syst, J_{AB} = 11.8, J_{AX} = 7.9, J_{BX} = 7.7 Hz, υ_A = 5.08, υ_B = 4.99); 4.86 (4 H, m); 4.77–4.47 (4 H, td, J = 31.0, J = 16.3 Hz); 3.69 (3 H, d, J = 11.7 Hz). ^{13}C-NMR ($CDCl_3$, 50 MHz) δ 135.90 (d, J = 7.5 Hz); 135.69 (d, J = 7.9 Hz); 135.05; 128.85; 128.38; 128.32; 128.25; 128.19; 127.95; 127.79; 69.11 (d, J = 5.7 Hz); 68.88 (d, J = 5.5 Hz); 53.86 (d, J = 5.5 Hz); 48.91. ^{31}P-NMR ($CDCl_3$/H_3PO_4, 81.015 MHz) δ 9.25 (1 P, t, J = 18.9 Hz); 4.14 (2 P, d, J = 18.9 Hz). MS (CI/NH_3): 750.1 (30) [MNH_4^+]; 733.1 (6) [MH^+]; 279.1 (100); 216.4 (80). IR (neat): 3528; 3485; 3065; 3035; 2956; 2356; 1498; 1456; 1378; 1271; 1215; 1020.

[4-[Bis(benzyloxy)phosphoryl]-3-hydroxy-3-oxo-1,3,4,5-tetrahydro-3-λ^5-benzo[*e*][1,3,2]diazaphosphepin-2-yl]phosphonic Acid Dibenzyl Ester (21). Potassium cyanide (39 mg, 0.60 mmol, 1.0 equiv) was added to compound **20** (0.44 g, 0.60 mmol, 1.0 equiv) in anhydrous DMF (5 mL). The mixture was stirred at 70 °C for 4 h. The solvent was removed *in vacuo*, and the residue was solved in methanol/water 70:30 (10 mL) and gently stirred with an ion-exchange resin (Dowex 50 × 8, H^+ form) for 12 h. The polymer beads were removed by filtration, and the filtrate was coevaporated with toluene (3 × 10 mL) to yield **21** (0.41 g, 95%) as a slightly yellow. Analytical HPLC (MeOH/H_2O/Et_3N 50:50:0.1) t_R 6.7 min. ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 7.26–7.03 (24 H, m); 4.90 (8 H, AB part of ABX syst, J_{AB} = 11.7, J_{AX} = 5.6, J_{BX} = 8.0 Hz, υ_A = 4.91, υ_B = 4.86); 4.66 (4 H,*t* , J = 15.2 Hz). ^{13}C-NMR ($CDCl_3$, 50 MHz) δ 135.38; 135.22; 135.07; 129.07; 128.37; 128.32; 127.88; 69.06 (d, J = 5.2 Hz); 48.69. ^{31}P-NMR ($CDCl_3$, 81.015 MHz) δ 6.36 (1 P, *t*, J = 23.4 Hz); 4.57 (2 P, *d*, J = 23.4 Hz). IR (CH_2Cl_2): 3740–3033; 1659; 1248; 1216; 1016. MS (CI/NH_3): 735.5 (12) [MNH_4^+]; 418.0 (22); 326.0 (37); 280.1 (45); 218.0 (100).

{2-[[[(Benzyloxy)methoxyphosphoryl]amino]methyl]-benzyl}phosphoramidic Acid Benzyl Ester Methyl Ester (23). Compound **23** (11.20 g, 93%) was obtained as a slightly yellow oil (mixture of diastereomers) from *o*-xylenediamine **16** and benzyl methyl chlorophosphate **22** following the procedure described for **13**. Diastereomers did not separate by TLC or analytical HPLC. TLC R_f 0.5 (AcOEt/EtOH 85:15). Analytical HPLC (MeOH/H_2O/Et_3N 65:35:0.1) t_R 10.1 min. ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 7.40–7.11 (14 H, m); 4.96 (8 H, AB part of ABX syst, J_{AB} = 11.7, J_{AX} = 7.7, J_{BX} = 8.6 Hz, υ_A = 5.01, υ_B = 4.91); 4.08 (4 H, dd, J = 6.9, 9.9 Hz); 3.64 (3 H, d, J = 11.3 Hz); 3.63 (3 H, d, J = 11.3 Hz); 3.56–3.50 (2 H, m). ^{13}C-NMR ($CDCl_3$, 50 MHz) δ 137.25 (d, J = 6.4 Hz), 136.45 (d, J = 7.3 Hz); 129.13; 128.44; 128.17; 128.00; 127.74; 67.95 (d, J = 4.9 Hz); 53.07 (d, J = 5.5 Hz); 42.42. ^{31}P-NMR ($CDCl_3$/H_3PO_4, 81.015 MHz) δ 10.65 (s). MS (CI/NH_3): 523.1 (96) [MNH_4^+]; 505.1 (100) [MH^+]; 450.1 (31). IR (CH_2Cl_2): 3411; 3239; 3060; 3036; 2953; 2892; 2851; 1607; 1456; 1414; 1286; 1218; 1186; 1069; 912; 866.

[3-(Benzyloxy-4-[(benzyloxy)methoxyphosphoryl]-3-oxo-1,3,4,5-tetrahydro-3-λ^5-benzo[*e*][1,3,2]diazaphosphepin-2-yl]phosphonic Acid Benzyl Ester Methyl Ester (24). Compound **23** was cyclized with benzyl dichlorophosphite following the procedure described for **18**. The mixture was filtrated, and the crude was oxidized in the same conditions as described for **15** to yield **24** (6.62 g, 67%) as a slightly yellow oil (mixture of diastereomers). TLC R_f 0.5 (AcOEt/EtOH 97:3). Analytical HPLC (MeOH/H_2O/Et_3N 65:35:0.1) t_R 23.2, 24.5, 27.7 min. ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 7.50–7.14 (19 H, m); 5.44–4.46 (10 H, m); 3.68, 3.63, 3.56, and 3.44 (6 H, 4d, J = 11.8 Hz). ^{13}C-NMR ($CDCl_3$, 50 MHz) δ 135.97–135.11 (m); 129.01–128.90 (m); 128.5–127.73 (m); 69.15–68.88 (m); 54.10, 54.04, 53.85, and 53.80 (4d, J = 5.3 Hz); 48.96 (m). ^{31}P-NMR ($CDCl_3$/H_3PO_4, 81.015 MHz) δ 8.15, 7.67, 7.09 and 7.18 (1 P, 4t, J = 17.6, 19.1, 18.1 and 18.1 Hz resp.); 5.38, 5.36, 5.32 and 5.26 (2 P, 4d, J = 18.1, 19.1, 17.6 and 18.1 Hz resp.). MS (CI/NH_3): 674.1 (100) [MNH_4^+]; 657.1 (25) [MH^+]; 598.1 (55); 386.2 (25); 310.2 (71); 296.3 (24). IR (CH_2Cl_2): 3459; 3069–2853; 1719; 1498; 1456; 1378; 1310; 1215; 1074; 913; 856.

[3-(Benzyloxy)-4-[(benzyloxy)methoxyphosphoryl]-3-oxo-1,3,4,5-tetrahydro-3-λ^5-benzo[*e*][1,3,2]diazaphosphepin-2-yl]phosphonic Acid Monobenzyl Ester (25). Compound **25** (2.76 g, 94%) was obtained as a slightly yellow oil (mixture of diastereomers) from **24** following the same procedure as for **21**. Diastereomers did not separate by TLC or analytical HPLC. Analytical HPLC (MeOH/H_2O/Et_3N 60:40:0.1) t_R 12.7 min. ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 11.32 (1 H, s broad); 7.44–7.05 (19 H, m); 5.37–4.40 (10 H, m); 3.63–3.28 (3 H; m). ^{13}C-NMR ($CDCl_3$, 50 MHz) δ 135.86–134.80 (m); 128.97–127.51 (m); 69.02–68.29 (m); 54.07; 48.83–48.40 (m). ^{31}P-NMR ($CDCl_3$/H_3PO_4, 81.015 MHz) δ 11.19–10.83 (m); 9.46–7.76 (m); 7.03–5.85 (m); 5.54–5.04 (m); 4.85–3.13 (m); 2.70–2.41 (m); 1.57–1.42 (m). MS (FAB$^-$/HEDS): 641.0 (19) [M − H^-], 627.0 (64); 565 (23); 551.0 (67); 519.0 (58); 461.0 (39); 443.0 (55); 428.9 (69). IR (neat): 2956; 2352; 1722; 1498; 1455; 1379; 1257; 1016.

[3-(Benzyloxy)-4-[bis(benzyloxy)phosphoryl]-3-oxo-1,3,4,5-tetrahydro-3-λ^5-benzo[*e*][1,3,2]diazaphosphepin-2-yl]phosphonic Acid Benzyl Ester Methyl Ester (26). Diethyl azodicarboxylate (1.97 mL, 12.49 mmol, 2.5 equiv) was added dropwise to a mixture of compound **25** (3.21 g, 4.99 mmol, 1.0 equiv), triphenylphosphine (3.27 g, 12.49 mmol, 2.5 equiv), and benzyl alcohol (0.57 mL, 5.49 mmol, 1.1 equiv) in THF (25 mL) at room temperature. The mixture was stirred for 2 h before removal of the solvent *in vacuo*. The residue was chromatographed (AcOEt/hexane/EtOH 75/25/0 to 95/0/5) to yield **26** (3.16 g, 87%) as a colorless oil (mixture of diastereomers). Diastereomers did not separate by TLC or analytical HPLC. TLC R_f 0.5 (AcOEt). Analytical HPLC (MeOH/H_2O/Et_3N 70:30:0.1) t_R 9.9 min. ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 7.31–7.26 (24 H, m); 5.08–4.86 (12 H, m); 3.60 and 3.48 (3 H, 2d, J = 11.5 Hz). ^{13}C-NMR ($CDCl_3$, 50 MHz) δ 136.01; 135.81; 135.65; 135.35; 135.19; 135.07; 133.87; 132.21; 132.01; 128.99; 128.91; 128.57; 128.43; 128.37; 128.33; 128.30; 128.24; 128.09; 128.00; 127.90; 127.84; 69.21; 69.10; 69.03; 68.99; 68.93; 68.88; 53.98; 53.87; 53.32; 48.95. ^{31}P-NMR ($CDCl_3$/H_3PO_4, 81.015 MHz) δ 8.12–7.26 (1 P, m); 5.50–5168

(21) Poor resolution obtained in ^{31}P-NMR with that compound did not allow for measuring P–P coupling constants.

(1 P, m); 4.15–3.60 (1 P, m). MS (CI/NH_3): 750.2 (100) [MNH_4^+]; 733.1 (18) [M – H^+] 688.2 (69); 674.2 (78); 557.1 (93); 392.3 (85); 370.3 (72). IR (CH_2Cl_2): 3046; 2996; 1889; 1812; 1736; 1593; 1550; 1421; 1266; 1030; 885.

[3-(Benzyloxy)-4-[bis(benzyloxy)phosphoryl]-3-oxo-1,3,4,5-tetrahydro-3-λ^5-benzo[*e*][1,3,2]diazaphosphepin-2-yl]phosphonic Acid Monobenzyl Ester (27). Compound **27** (1.86 g, 97%) was obtained as an oil from **26** following the same procedure as described for **25** (mixture of diastereomers). Diastereomers did not separate by TLC or analytical HPLC. Analytical HPLC ($MeOH/H_2O/Et_3N$ 40:60:0.1) t_R 38.2 min. ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 7.37–7.01 (24 H, m); 5.12–4.82 (8 H, m); 4.80–4.57 (4 H, m). ^{13}C-NMR ($CDCl_3$, 50 MHz) δ 135.93–134.81 (m); 128.87–127.19 (m); 69.32–68.33 (m); 48.83–48.48 (m). ^{31}P-NMR ($CDCl_3$, 81.015 MHz)[22] δ 11.88 and 10.47 (1 P, 2 t, J = 19.7 Hz); 5.72 and 3.81 (1 P, 2 d, J = 20.4 Hz); –0.05 and –0.96 (1 P, 2 d, J = 19.7 Hz). MS (CI/NH_3): 735.5 (7) [MNH_4^+]; 418.0 (22); 326.0 (37); 280.1 (45); 218.0 (100).

(22) ^{31}P-NMR spectra of compound **27** refers to the potassium salt that offered better resolution than the acidic form.

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Supplementary Material Available: Compound characterization data for **1**, **16**, **17**, **19–21**, **and 24–27** (27 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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