Journal of Organometallic Chemistry 694 (2009) 3806–3813

Contents lists available at ScienceDirect

Journal of Organometallic Chemistry

journal homepage: www.elsevier.com/locate/jorganchem

Insight into the mechanism of three component condensation leading to aminomethylenebisphosphonates

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ARTICLE INFO

Article history:
Received 8 May 2009
Received in revised form 7 July 2009
Accepted 10 July 2009
Available online 16 July 2009

The contribution is dedicated to Prof. Roman Tyka on his 85th anniversary

Keywords:
Aminomethylenebisphosphonates
Reaction mechanism
Organophosphorus chemistry

ABSTRACT

Three-component reaction of a primary amine, diethyl phosphite and triethyl orthoformate followed by acid hydrolysis of the adduct provides N-substituted aminomethylenebisphosphonic acids in good yields. Being extremely versatile, it is commonly utilized for preparation of compounds possessing potential antiosteoporotic, antibacterial, anticancer, antiparasitic or herbicidal activity. However, the mechanism of the reaction remains unknown. *p*-Nitroaniline has been found an interesting tool to shed light on this matter. Its use allowed to separate and identify four intermediates, both non-phosphorus and phosphorus containing, and subsequently suggest the mechanism of the whole process. The acquired knowledge was helpful in explanation the route and the final product structure obtained for more complex reaction proceeding with the use of 4-aminopyridine. Additional alkylation of the pyridine nitrogen atom, leading to unexpected *N*-(1-alkylpyridinium-4-amino)methylenebisphosphonic acids was unambiguously proved.

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1. Introduction

Bisphosphonic acids are hydrolytically stable analogues of pyrophosphate characterized by a common P–C–P fragment, in which the oxygen-to-phosphorus bonds are replaced by the carbon-to-phosphorus bonds. Bisphosphonates have been employed as therapeutic agents for treatment of bone disorders, hypocalcaemia of malignancy and osteoporosis for over two decades [1]. Despite this, the molecular mode of their action is still not clear and attracts considerable attention [2]. Recently, a promising drug delivery system using bisphosphonate moiety for targeting osseous tissues was also proposed [3]. Additionally, bisphosphonates have been found to have antibacterial [4] and anticancer [5] properties and to stimulate $\gamma\delta$ T cells of immune system, drawing interest in cancer immunotherapy [6].

A subclass of bisphosphonates, derivatives of aminomethylenebisphosphonic acid, has been also described to exhibit promising antiparasitic [7] and herbicidal activities [8]. It is worth to note that a representative of the latest generation of antiosteoporotic drugs – cycloheptylaminomethylenebisphosphonate, discovered in the early 90s [9] and commercialized as Incadronate, belongs to such modified bisphosphonic acids.

The simplest procedure for preparation of aminomethylenebisphosphonates relies on three-component reaction between a primary or secondary amine, triethyl orthoformate and diethyl phosphite, followed by acid hydrolysis (Scheme 1) [10]. Although this straightforward procedure affords desired products of a wide structural variety, the detailed route leading to the target structure is not recognized yet. The yield is usually satisfactory, however it happens unpredictably low. Moreover, unexpected by-products are obtained in those particular cases.

In this paper we wish to present the results of our studies on elucidation of the mechanism of the title three component condensation. The overall reaction route has been proposed on the basis of the structure of isolated intermediates and conversion reactions observed between them. These findings supported elucidation of the structure of unusual alkylated products obtained upon formation of pyridinylaminomethylenebisphosphonates.

2. Results and discussion

2.1. Reaction with p-nitroaniline and identification of intermediates

The analysis of the general mechanism of aminomethylenebisphosphonates formation in the condensation of dialkyl phosphite, trialkyl orthoformate and amine was performed using *p*-nitroaniline as the nucleophilic component. This compound was

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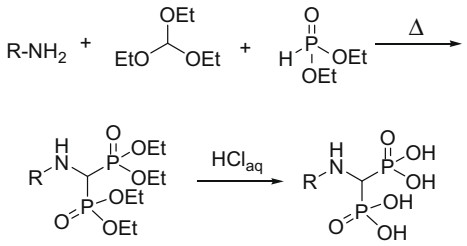
doi:10.1016/j.jorganchem.2009.07.025



Scheme 1. General reaction for the synthesis of derivatives of aminomethylenebisphosphonic acids.

chosen intentionally because of its characteristic features advantageous for this purpose. First, due to its relatively poor nucleophilicity the reaction proceeded slowly enough to detect and analyze intermediate products. Second, separation and purification processes (crystallization and chromatography) were greatly facilitated as compounds containing *p*-nitroaniline fragment are yellow colored, easily crystallizing solids. These properties were also used to obtain appropriate monocrystals and to analyze their molecular structure by X-ray diffraction.

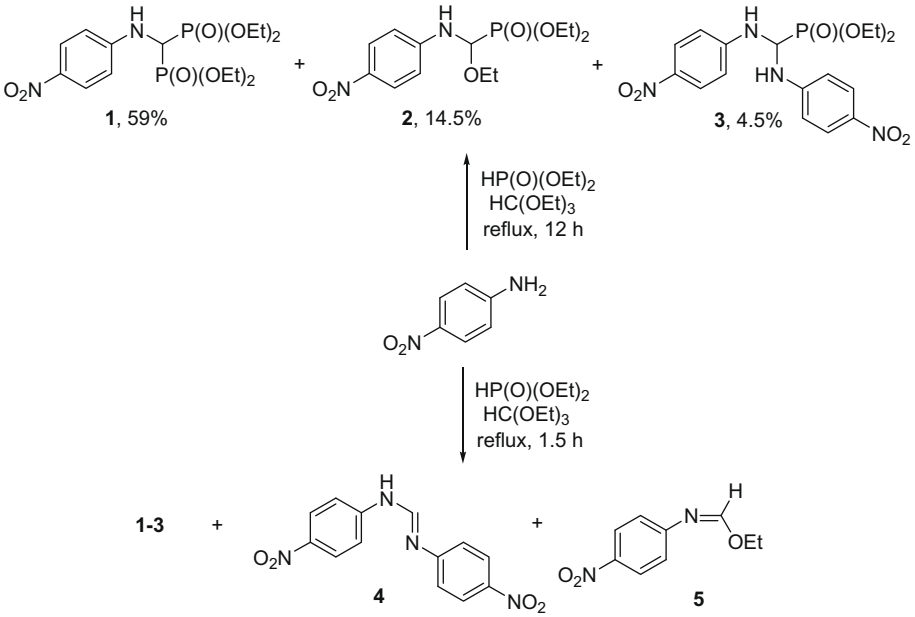
Standard reaction condition applied for the bisphosphonate synthesis involved 12 h of heating of three substrates under reflux. As the result, the formation of three organophosphorus compounds was observed (Scheme 2). Major portion of the target tetraethyl bisphosphonate **1** crystallized from the reaction mixture. This represented 56% of the total yield. The filtrate contained not reacted diethyl phosphite (50% relative yields obtained from ^{31}P NMR spectra of the residual liquid), non-separated amounts of the desired compound **1** (7%), and two other organophosphorus moieties.

On the basis of further studies they were identified as diethyl ethoxy(*p*-nitrophenylamino)methanephosphonate **2** (33%) and diethyl di(*p*-nitrophenylamino)methanephosphonate **3** (10%). Estimated overall yields of all three organophosphorus products, calculated by summarizing the solid and the liquid fraction contents are given in Scheme 2.

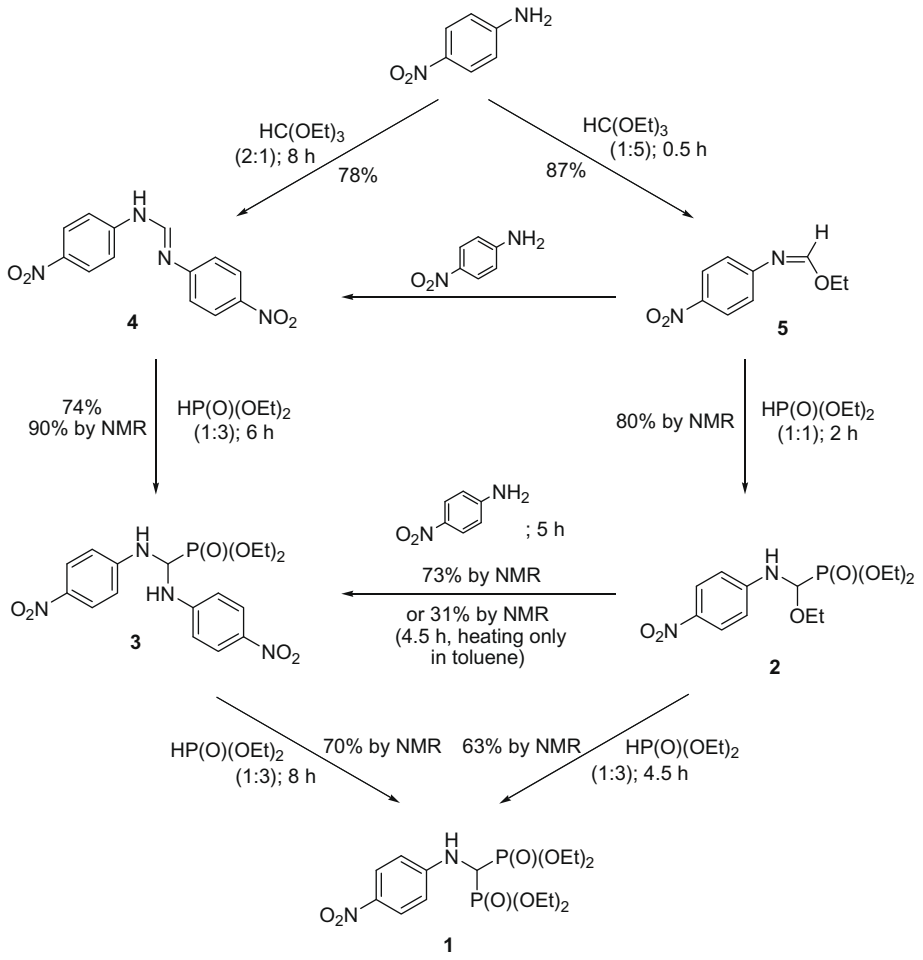
Shortening of the reaction time to 1.5 h not only allowed to obtain different ratios of the products but enabled to isolate and characterize individual components of the reaction mixture (Scheme 2). Thus, the major fraction of the main product **2** (62%) precipitated directly from the reaction mixture upon cooling, and it was obtained by filtration. The residual solution contained unreacted phosphite (68% of the filtrate, by ^{31}P NMR) and three phosphonate products **1–3** (1.5%, 26% and 4.5% of the filtrate, respectively). They were separated by column chromatography, characterized and identified, together with two non-phosphorus imine type derivatives: *N*,*N*′-di(*p*-nitrophenyl)formamidine **4** and ethyl *N*-(*p*-nitrophenyl)formimidate **5**. Dedicated synthetic procedures were additionally established (for the details see the Section 3) to obtain preparative quantities of **3–5** used in further study of the intermediates reactivity towards other components of the reaction mixture (Scheme 3).

Reaction of *p*-nitroaniline with triethyl orthoformate, depending on the molar ratio of the two substrates provided either compound **4** or **5** as the major product with satisfactory yield. As formimidate **5** is thermally unstable the reaction had to be stopped after 30 min. Its further subjection to elevated temperature induced decomposition partially leading to compound **4**. Conversion of **5** into **4** could be also achieved in preparative manner upon action of *p*-nitroaniline. When each of both compounds (**4** or **5**) was reacted with diethyl phosphite, the product of the addition reaction was obtained (**3** and **2**, respectively). Finally, phosphonates **2** and **3** reacted with excess of diethyl phosphite and yielded bisphosphonate **1**. Similarly to **4** and **5**, conversion of phosphonate **2**–**3** could be performed by reaction of the ethoxy derivative **2** with *p*-nitroaniline or, to some extend, by its thermal decomposition. Thus, the reaction is quite complex and relies on the kinetics of formation and thermodynamic stability of all the intermediates. This also shows that the optimization of the reaction conditions can be difficult and dependent on the structure of individual amino component since the ratio of formation of all intermediates is determined by its reactivity.

To rationalize the optimization process, behavior of formimidate **5** in the reaction with increasing excess of diethyl phosphite



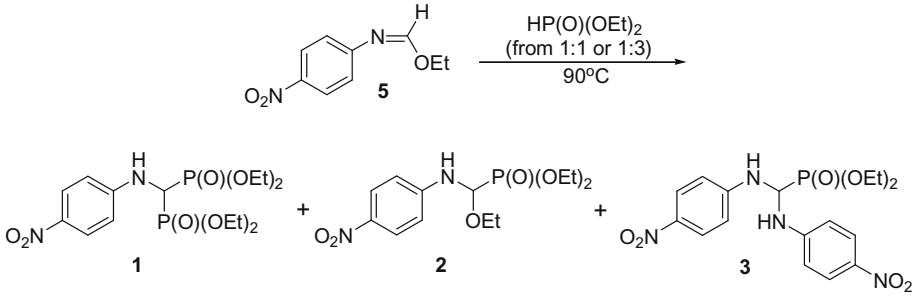
Scheme 2. Structures of phosphorus containing products (**1–3**) obtained in the reaction of *p*-nitroaniline with ethyl orthoformate and diethyl phosphite in standard conditions (1:1:2 molar ratio of the reagents, reflux, 12 h) and non-phosphorus intermediates (**4** and **5**) identified in the reaction mixture when stopped after 1.5 h of the reaction.



Scheme 3. General reaction scheme with conditions of selective transformations between non-phosphorus intermediates (**4** and **5**) and phosphorus containing products (**1–3**) (reactions carried out at 90 °C, in toluene or *o*-xylene when needed for solubility, the ratio of substrate to reagent indicated in parentheses).

was studied in some more details (Table 1). Similarly as stated before, ethoxyphosphonate **2** appeared overwhelming product at the early stages of the reaction (<4 h) when substrates in 1:1 molar ratio were heated together. Along the time **2** was slowly consumed being converted equally into bisphosphonate and bisamino derivatives (**1** and **3**), however, still remaining the major component of the mixture after 8 h of the reaction. Addition of greater excess of diethyl phosphite (3 eq.) and increasing the reaction time caused

Table 1
Monitoring the course of the reaction of formimidate **5** with increasing amount of diethyl phosphite (as relative ratio of phosphonates **1–3** calculated from ^{31}P NMR spectra).



Reaction time (h)	Diethyl phosphite	Ratio of the products (by ^{31}P NMR) (%)		
		1	**2**	**3**
0.5	1 eq.	7	79	14
2	1 eq.	8	80	12
4	1 eq.	11	76	13
8	1 eq.	19	55	26
0.5	3 eq.	6	48	46
4	3 eq.	23	0	77
8	3 eq.	69	0	31

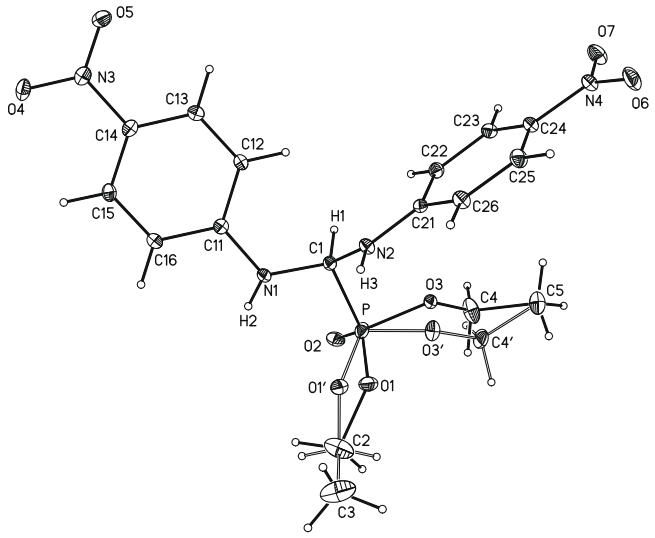


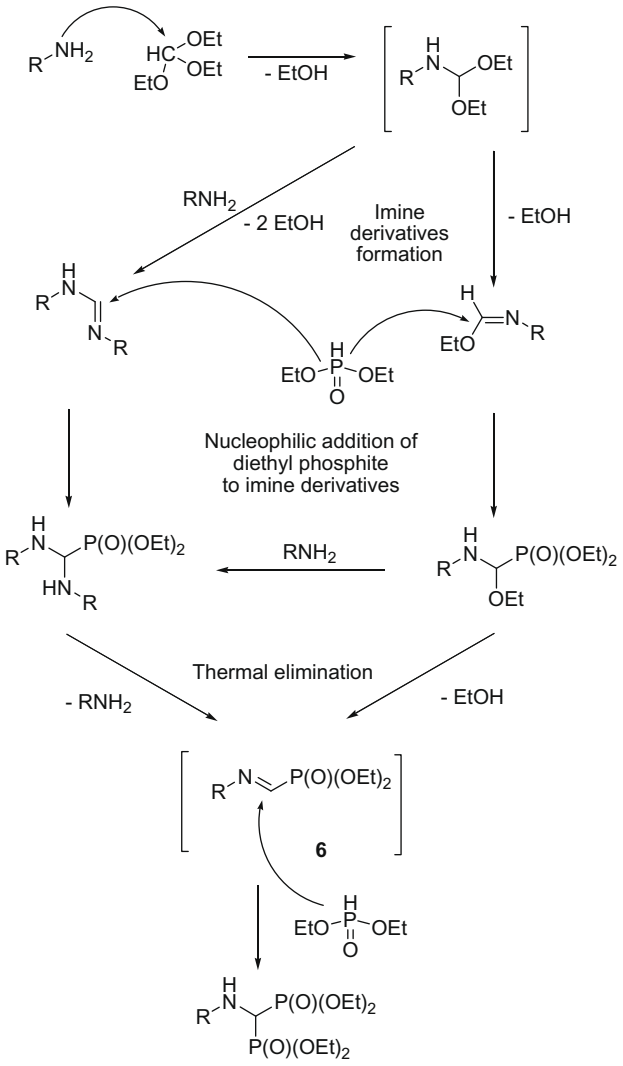
Fig. 1. The molecular structure and atom-numbering scheme for compound **3**. Displacement ellipsoids are shown at 45% probability level. Disorder of ethyl phosphonate groups is shown.

visible enhancement of the final product yield while **2** disappeared immediately. However, the obvious conclusion that the increase in the yield of target bisphosphonate should be achieved by increase of molar ratio of diethyl phosphate used is not so apparent when considering that this usually causes substantial difficulties in separation of pure final products.

Formation of compound **3** was unambiguously shown by X-ray studies. The molecular structure and atom numbering of **3** is given in Fig. 1. We have found that O1–CH_2–CH_3 and O3–CH_2–CH_3 moieties are disordered into two position and their two positions (O1/O1′, C2/C2′ and O3/O3′, C4/C4′) were refined with s.o.f. = 0.56, 0.44 and 0.52, 0.48, respectively. This behavior of phosphonate ester group was reported earlier and thus is not surprising [11].

2.2. Reaction mechanism

Taking into consideration all the observation and accounts given above, a mechanism of the whole process of bisphosphonate formation could be proposed (Scheme 4). Its initial steps seem to be similar to the Kabachnik-Fields reaction in which a carbonyl component is replaced by triethyl orthoformate of corresponding electrophilic character. Imines are commonly suggested as intermediates of the Kabachnik-Fields reaction. Here, formation of ethyl *N*-(*p*-nitrophenyl)formimidate **5** and *N*,*N*′-di(*p*-nitrophenyl)formamidine **4** has been clearly evidenced. Respectively, the compounds are the products of single or double nucleophilic substitution of *p*-nitroaniline associated with ethanol elimination taking place on the electrophilic carbon of orthoformate. Both imine type intermediates can easily undergo nucleophilic addition of diethyl phosphite yielding secondary intermediate phosphonates. The following steps are not fully clear. We suggest a slow thermal elimination of ethanol or an amine leading to common hypothetical iminephosphonate **6**. It has not been separated nor spectroscopically identified, however appearance of trace phosphorus containing impurities has been observed during the study of the reaction course. Structure **6** seems to represent a logical substrate for subsequent hydrophosphonylation linking together two separate synthetic pathways. Sequential substitution in the phosphonate system leading to the thermodynamically most stable target compound can represent an alternative option for the last step of the reaction.

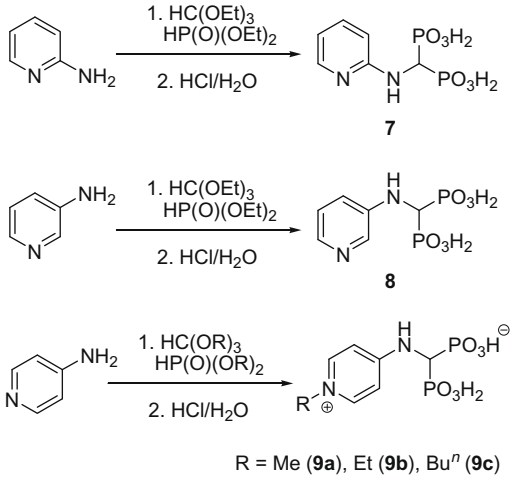


Scheme 4. Suggested general mechanism of aminomethylenebisphosphonate formation.

The mechanism described above can be additionally influenced by other substrate structural or reactivity factors complicating furthermore aminomethylenebisphosphonate formation. The use of aminopyridine derivatives as nucleophilic components of the condensation can represent such an example. The discussion on the reactions that do not proceed in a typical way is greatly facilitated by the general knowledge presented above.

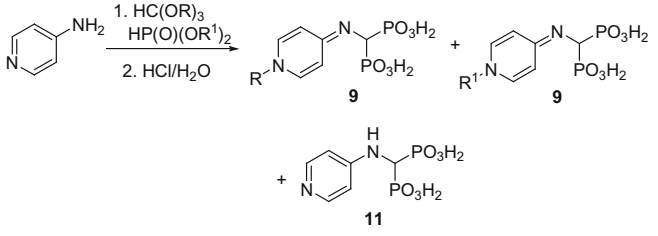
2.3. Alkylation of the pyridyl nitrogen atom

A series of *N*-pyridylaminomethylenebisphosphonates, derivatives of structurally variable aminopyridines chosen as substrates, were obtained in order to study their herbicidal activity [8]. Most of these products had been already described in the literature [8a,12]. The reactions with 2-amino- and 3-aminopyridine afforded, as expected, the desired products **7** and **8** with satisfactory yields, whereas in the reaction with 4-aminopyridine, surprisingly and in contrast to literature, the product possessing additional ethyl group was obtained. Similarly alkylated pyridinebisphosphonates were also identified as single products of the condensation proceeding in trimethyl orthoformate/dimethyl phosphite and tri(*n*-butyl) orthoformate/di(*n*-butyl) phosphite systems (compounds **9**, Scheme 5; see also Table 2).



Scheme 5. Reaction of trialkyl orthoformate and dialkyl phosphite with aminopyridines.

Table 2
Ratio of N-alkylated and non-alkylated products in the reaction of 4-aminopyridine with various orthoformates and phosphates.



Entry	Substrates		Products (%)		
	$HC(OR)_3$	$HP(O)(OR^1)$	**9**, R	**9**, R^1	**11**
1	Me	Et	50	50	0
2	Me	Pr^i	100	0	0
3	Et	Pr^i	50	0	50
4	Pr^n	Me	0	100	0
5	Pr^n	Et	0	100	0
6	Pr^n	Pr^i	20	0	80
7	Bu^n	Bu^n	100	100	0

Two possibilities of introduction of the ethyl group into bisphosphonate molecule can be considered, namely alkylation of the pyridine nitrogen atom or alkylation of the aminomethylenebisphosphonate nitrogen atom. Although the latter possibility was favored by Szabo et al. [13] our results strongly support the former option.

First of all, the crystal structure determined for *N*-methyl derivative **9a** unambiguously reveals the formation of product with methylated N2 atom (Fig. 2). This compound crystallizes as a zwitterion with the negative charge on the phosphonate $P(1)O_3H^-$ group, the positive charge on the alkylated N2 atom and the phosphonic $P(2)O_3H_2$ group being neutral. It is worth to note that despite similarity of the pyridinium N2–C2 and N2–C6 bond lengths (1.358(2) and 1.356(2) Å, respectively) to those found in the related compounds [14], the value of the endocyclic C–N–C bond angle of 119.5(1)° is slightly below the generally accepted limit (120°) for the endocyclic C–N–C bond angle in pyridinium salts. This can be explained by a displacement of the charge within

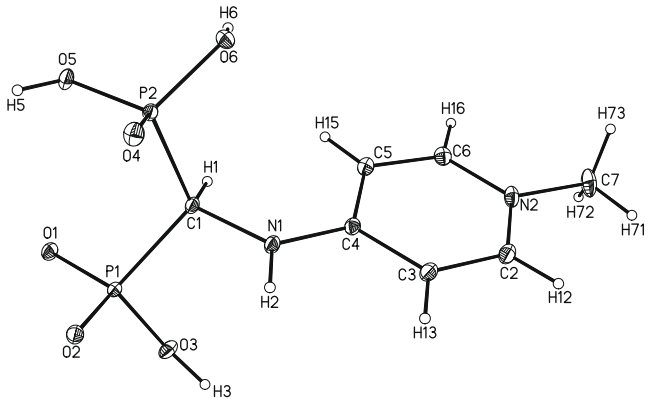
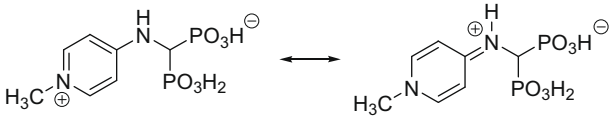


Fig. 2. The molecular structure and atom-numbering scheme in **9a**. Displacement ellipsoids are shown at 45% probability level.



Scheme 6. Mesomeric forms of compound **9a**.

9a and indicates that this compound can exist in two mesomeric forms (Scheme 6) with the pyridinium-type resonance form (N^+–CH_3) being the major contributor to the true molecular structure. Consistent with previous observations [14] the N1 atom of **9a** is formally sp^2 hybridized and therefore both N1 and C1 atoms are nearly coplanar with the pyridinium ring. This results in a partially-double bond characters of the N1–C4 linkage, which is reflected in its length being 1.347(1) Å, as compared to C1–N1 of 1.463(1) Å, which is a typical value for the single C–N bond. Details of crystal packing in **9a**, determined mainly by hydrogen bonds involving phosphonic (PO_3H_2) and phosphonate (PO_3H^-) groups, are provided in Supplementary materials.

Second, similarity of the ^{31}P NMR titration curves presented in Fig. 3 clearly indicates that the pyridinium atom N2 is alkylated in all compounds **9a–c**. This is reflected in relatively small chemical shifts changes detected upon changing the pH, ca. 0.6 ppm upfield in the pH range ~2–5 and *ca.* 0.5 ppm downfield in the pH range ~5–10. Given the fact that the $P(2)O_3H_2$ group is very acidic and releases the first proton well below pH 2, the observed changes can be attributed to subsequent release of protons from the

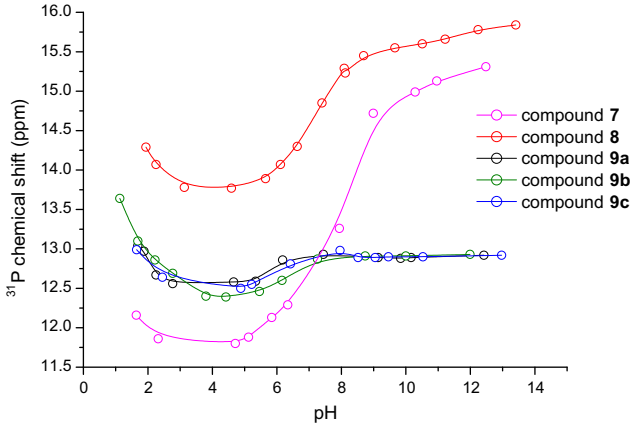
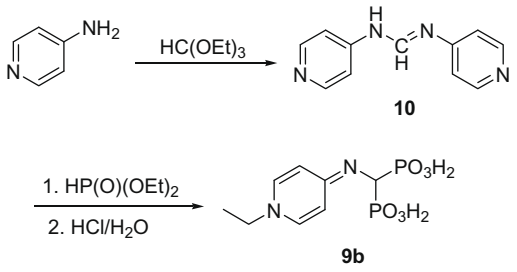


Fig. 3. The ^{31}P NMR titration curves of **9a–c** compared with those of **7** and **8**.



Scheme 7. Two-step procedure for synthesis of **9b**.

$P(1)O_3H^-$ and $P(2)O_3H^-$ groups. This behavior is very similar to that previously reported for piperid-1-ylmethane-1,1-diphosphonic acids, characterized by the presence of the protonated piperidine nitrogen atom over the broad range of pH [15]. Otherwise, as demonstrated for **7** and **8**, the proton dissociation from the pyridyl nitrogen atom should lead to much significant changes in the ^{31}P NMR chemical shifts versus pH [16].

Finally, an evidence for alkylation of the pyridinium nitrogen is provided from the ^{1}H and ^{13}C NMR spectra of **9a–c**. A strong deshielding effect exerted on the proton and carbon resonances of the CH_3 (**9a**) and $C(1')H_2$ groups (**9b**, **9c**) (see Supplementary materials) may be explained by their close proximity to the positively charged pyridinium atom N2.

In order to study the route of this reaction in some more details, two sets of experiments were designed. First, the reaction of 4-aminopyridine with orthoformate was found to give corresponding intermediate *N*,*N*′-di(pyridin-4-yl)formamidine **10** with good isolated yield, accordingly to the reaction mechanism described above. The amidine **10** when reacted with diethyl phosphite followed by acid hydrolysis afforded compound **9b** in a nearly quantitative yield (Scheme 7). This suggested that phosphite must have acted as an agent alkylating the pyridine nitrogen atom. Similar alkylation of pyridines and other nitrogen containing heterocycles by pentavalent phosphorus esters (phosphates or phosphites) were reported previously [17]. In such substitution reactions pyridines play a role of attacking nucleophiles whereas phosphorus monoacids are appropriate leaving groups.

Second, reactions of 4-aminopyridine with various trialkyl orthoformates and dialkyl phosphites as substrates were carried out. Results of these studies (Table 2, relative yields of products are given in percents as determined directly from ^{31}P NMR spectra of the reaction mixtures) indicated that the alkylation seems to be a simple nucleophilic S_N2 substitution [17a]. This is seen from that only primary alkyl groups are substituting pyridine nitrogen atom and the substitution yields follow general rules of S_N2 reaction (for example, N-methylation is favoured against N-ethylation). These studies are somewhat complicated by the fact that phosphites react with orthoformates interchanging their ester groups, and thus providing the complex mixtures of mixed phosphite esters [18], which in turn alkylate pyridine nitrogen.

2.4. Conclusions and summary

The analysis of the general mechanism of three component condensation of aminomethylenebisphosphonate formation was carried out using *p*-nitroaniline as the nucleophilic substrate. The amine was proven to be a valuable tool to follow appearance of various intermediates, both non-phosphorus and phosphorus containing. They were isolated, their structures were identified and characterized what in turn allowed suggestion the overall reaction route. The synthetic pathway leads to aminomethylenebisphosphonates via a series of imine and phosphonate products existing in kinetic and thermodynamic equilibrium. Thus, their ratio depends on the specific conditions applied for an individual amine substrate. The nucleophilicity seems to determine mostly the reaction rate progress. For weak nucleophiles an excess of phosphite and time elongation is recommended. The aforementioned factor can effect, however, with some difficulties in the final product separation. Additional reactive groups present in the amine structure might also be troubleshooting. Here, not typical observation concerned unexpected alkylation of the pyridyl nitrogen, uniquely in the 4-amino derivatives, was definitely evidenced and discussed.

3. Experimental

3.1. General

All reagents and solvents were purchased from commercial suppliers. The reaction products were identified and characterized by their melting points with a Boetius apparatus, by analysis of their ^{1}H, ^{31}P and ^{13}C NMR, IR and MS data. NMR spectra were recorded on a Bruker Avance DRX-300 spectrometer operating at 300.13 MHz for 1H, 75.46 MHz for ^{13}C and 124.50 MHz for ^{31}P at 25 °C. Chemical shifts (δ, [D6]DMSO or D_2O) are reported in ppm relative to TMS and 85% H_3PO_4. The standard Bruker programs were used to perform inverse detected [^{1}H–^{13}C] HMQC experiments that verified the ^{1}H and ^{13}C NMR chemical shift assignments. The NMR samples for titration studies were prepared in deuterated water. The experiments were carried out at 300 K at ligand concentrations 2×10^{-2} mol dm^{-3} for ^{1}H and ^{31}P NMR, and 1×10^{-1} mol dm^{-3} for ^{13}C experiments. The pH was measured using a Radiometer pHM 83 instrument equipped with a Mettler Toledo INLAB 422 combined electrode and is given as readings without correction for pD.

IR spectra were recorded on Perkin–Elmer 2000 FT spectrometer in KBr pellets. Mass spectra were obtained on Bruker micrOTOF-Q mass spectrometer with ESI source. Reaction progress was monitored by thin layer chromatography (TLC) on silica gel 60F254 coated aluminum. Silica gel 60 (70–230 mesh) was used for column chromatography.

3.2. Tetraethyl (*p*-nitrophenylamino)methylenebisphosphonate (**1**): general synthetic procedure for synthesis of methylenebisphosphonate esters

p-Nitroaniline (30 mmol, 4.14 g), triethyl orthoformate (30 mmol, 5.0 mL) and diethyl phosphite (60 mmol, 7.7 mL) were refluxed for 12 h. Then volatile components were removed under reduced pressure and the residue was worked up with acetone. Precipitated solid was filtered and recrystallized from ethyl acetate/methanol (1:5) to give **1** as yellow crystals. Yield 7.1 g, 56%; m.p. 205 °C; δ_H ([D6]DMSO) 1.10 (6 H, t, *J* 7.0, 2 × CH_3), 1.16 (6 H, t, *J* 7.0, 6H, 2 × CH_3), 4.01 (8 H, m, 4 × CH_2), 4.88 (1 H, dt, $^{2}J_{HP}$ 22.9, $^{3}J_{HH}$ 10.6, NCH), 7.05 (2 H, d, *J* 9.2, C_6H_4), 7.40 (1 H, d, *J* 10.6, HNC), 7.94 (2 H, d, *J* 9.2, C_6H_4); δ_C 154.01 (t, $^{3}J_{CP}$ 4.0, CNH), 137.42 (CNO_2), 126.06 ($CHCNO_2$), 112.81 (CHCNH), 63.3 (d, $^{2}J_{CP}$ 11.3, CH_2), 48.44 (t, $^{1}J_{CP}$ 145.1, NCH), 16.70 (CH_3); δ_P 17.53; IR (KBr): ν = 3268, 3088, 2982, 1596, 1506, 1477, 1314, 1287, 1235, 1113, 1022, 857 cm^{-1}; MS (ESI): *m*/*z*: 447.10 [M+Na]$^{+}$, 425.1 [M+H]$^{+}$; elemental Anal. Calc. for $C_{15}H_{26}N_2O_8P_2$: C, 42.46; H, 6.18; N, 6.60; P, 14.60. Found: C, 42.39; H, 6.21; N, 6.57; P, 14.60%.

3.3. Diethyl ethoxy(*p*-nitrophenylamino)methanephosphonate (**2**)

p-Nitroaniline (30 mmol, 4.14 g), triethyl orthoformate (30 mmol, 5.0 mL) and diethyl phosphite (60 mmol, 7.7 mL) were refluxed gently under condenser for 1.5 h. Then volatile compo-

nents were removed under reduced pressure and the residue was worked up with diethyl ether. Precipitated solid was filtered to give **2** as yellow crystals. Yield 6.2 g, 62%; m.p. 134–135 °C; δ_H ([D6]DMSO) 1.18 (3 H, t, *J* 7.0, CH_3), 1.27 (3 H, t, *J* 7.0, CH_3), 1.35 (3 H, t, *J* 7.0, CH_3), 3.69 (2 H, m, CH_2), 4.19 (4 H, m, 2 × CH_2), 5.06 (1 H, dd, $^2J_{HP}$ $^3J_{HH}$ 10.0, NCH), 5.60 (1 H, m, HNC), 6.76 (2 H, d, *J* 9.1, C_6H_4), 8.09 (2 H, d, *J* 9.1, C_6H_4); δ_C 153.61 (d, $^3J_{CP}$ 10.1, CNH), 138.29 (CNO_2), 126.19 ($CHCNO_2$), 113.19 (CHCNH), 79.03 (d, $^1J_{CP}$ 198.4, NCH), 63.90 (d, $^2J_{CP}$ 11.4, CH_2), 63.11 and 63.03 (d each, $^2J_{CP}$ 11.8 and $^2J_{CP}$ 11.7, CH_2), 16.80 (d, $^3J_{CP}$ 4.3, CH_3), 15.49 (CH_3); δ_P 16.88; IR (KBr): ν = 3262, 3205, 3078, 2978, 1598, 1550, 1505, 1489, 1315, 1229, 1113, 1066, 1022, 844 cm^{-1}; MS (ESI): *m*/*z*: 355.10 $[M+Na]^+$, 333.10 $[M+H]^+$; elemental Anal. Calc. for $C_{13}H_{21}N_2O_6P$: C, 46.99; H, 6.37; N, 8.43; P, 9.32. Found: C, 46.52; H, 6.17; N, 8.67; P, 9.36%.

Diethyl ether solution obtained after filtration still contained not separated **2** and all the remaining intermediates (compounds **3–5**, R_f = 0.13 for **3**, R_f = 0.33 for **5**, R_f = 0.63 for **4**, TLC in ethyl acetate/hexane, 3:1). After removal of the solvent the residue was subjected to column chromatography in ethyl acetate/hexane gradient (1:3 to 1:0) to yield the analytical samples. For conditions of the preparative syntheses see below.

3.4. Diethyl bis(p-nitrophenylamino)methanephosphonate (*3*)

N,*N*′-Di(*p*-nitrophenyl)formamidine (**4**) (30 mmol, 8.6 g) and diethyl phosphite (90 mmol, 11.5 mL) were heated at 90 °C (an oil bath) under condenser for 6 h. Then volatile components were removed under reduced pressure and the residue was treated with acetone. Precipitated solid was filtered and recrystallized from dioxane/toluene (2:1) to give **3** as yellow crystals. Yield 9.4 g, 74%; m.p. 176 °C; δ_H ([D6]DMSO) 1.16 (6 H, t, *J* 7.1, 2 × CH_3), 4.06 (4 H, m, 2 × CH_2), 5.66 (1 H, dt, $^2J_{HP}$ 16.4, $^3J_{HH}$ 8.3, NCH), 6.94 (4 H, d, *J* 9.3, 2 × C_6H_4), 7.69 (2 H, dd, $^3J_{HP}$ 8.3, $^3J_{HH}$ 4.6, 2 × NHC), 7.99 (4 H, d, *J* 9.3, 2 × C_6H_4); δ_C 153.08 (d, $^3J_{CP}$ 8.6, CNH), 137.99 (CNO_2), 126.22 ($CHCNO_2$), 113.02 (CHCNH), 63.50 (d, $^2J_{CP}$ 6.9, CH_2), 58.83 (d, $^1J_{CP}$ 181.65, NCH), 16.79 (d, $^3J_{CP}$ 5.1, CH_3); δ_P 16.08; IR (KBr): ν = 3348, 3265, 3063, 2987, 1604, 1505, 1479, 1471, 1526, 1472, 1320, 1301, 1265, 1107, 1048, 1020, 838 cm^{-1}; MS (ESI): *m*/*z*: 447.10 $[M+Na]^+$, 425.1 $[M+H]^+$; elemental Anal. Calc. for $C_{17}H_{21}N_4O_7P$: C, 48.12; H, 4.99; N, 13.20; P, 7.30. Found: C, 47.77; H, 4.78; N, 13.04; P, 7.30%.

3.5. N,N′-Di(p-nitrophenyl)formamidine (*4*)

p-Nitroaniline (30 mmol, 4.14 g) and triethyl orthoformate (15 mmol, 2.49 mL) were heated at 90 °C (an oil bath) in *o*-xylene (10 mL) under condenser for 8 h. Then the solvent and other volatile components were removed under reduced pressure. The residue was worked up with toluene and precipitated solid was filtered to give **4** as yellow crystals. Yield 6.7 g, 78%; m.p. 241 °C; δ_H ([D6]DMSO) 6.68 (4 H, br d, 2 × C_6H_4), 8.11 (4 H, d, *J* 9.1, 2 × C_6H_4), 8.49 (1 H, br s, HNC), 10.75 (1 H, br s, CH); δ_C 153.75 (C═N), 150.48 (CNH), 143.40 (CNO_2), 125.61 ($CHCNO_2$), 122.30 (CHCNH), 117.58 (CHCNH); IR (KBr): ν = 3304, 3179, 3104, 3036, 1660, 1599, 1579, 1522, 1508, 1344, 1312, 1300, 1205, 846 cm^{-1}; MS (ESI): *m*/*z*: 287.10 $[M+H]^+$; elemental Anal. Calc. for $C_{13}H_{10}N_4O_4$: C, 54.55; H, 3.52; N, 19.57. Found: C, 54.50; H, 3.54; N, 19.54%.

3.6. Ethyl N-(p-nitrophenyl)formimidate (*5*)

p-Nitroaniline (30 mmol, 4.14 g) and triethyl orthoformate (150 mmol, 25.0 mL) were heated at 90 °C (an oil bath) under condenser for 0.5 h. The residue was cooled and worked up with acetone. Precipitated solid was filtered to give **5** as yellow crystals. Yield 5.1 g, 87%. IR, MS, elemental and melting point analyses gave false results because of rapid decomposition of the compound. Thus, the formamidate **5** was characterized only by NMR spectroscopy. δ_H ([D6]DMSO) 1.32 (3 H, t, *J* 7.1, CH_3), 4.28 (2 H, q, *J* 7.1, CH_2), 7.21 (2 H, d, *J* 8.8, C_6H_4), 8.09 (1 H, s, CH), 8.19 (2 H, d, *J* 8.8, C_6H_4).

3.7. General procedure for synthesis of methylenebisphosphonic acids, aminopyridine derivatives [10c]

Round-bottom flask (50 mL) was charged with aminopyridine (30 mmol, 2.82 g), triethyl orthoformate (30 mmol, 5.0 mL) and diethyl phosphite (60 mmol, 7.7 mL). The mixture was refluxed for 12 h. Volatile components of the reaction mixture were removed under reduced pressure and the oily residue was dissolved in 20 mL of concentrated hydrochloric acid and refluxed for 8 h. Then the solvent was removed *in vacuo* and the obtained product was purified by crystallization from water/ethanol mixture.

3.8. N-(1-Methylpyridinium-4-amino)methylenebisphosphonic acid (*9a*)

Yield 2.9 g, 43% (from reaction presented as an entry 2 in Table 2); m.p. 305–307 °C; δ_H (D_2O, pD = 6.64) 3.80 (3 H, s, CH_3), 3.89 (1 H, t, $^2J_{PH}$ 19.3, NCH), 6.84, 7.74, 7.89 (2 H, 1 H and 1 H, m each, 4 × CH_{py}); δ_C 156.71 (d, $^3J_{PC}$ 4.7, $C4_{py}$), 143.98, 141.66 ($C2_{py}$ and $C6_{py}$), 111.33, 105.99 ($C3_{py}$ and $C5_{py}$), 52.06 (t, $^1J_{PC}$ 123.8, NCH), 44.36 (CH_3); δ_P 13.53; MS (ESI): *m*/*z*: 281.8 $[M-H]^+$; elemental Anal. Calc. for $C_7H_{12}N_2O_6P_2$: C, 29.77; H, 4.25; N, 10.06; P, 22.89. Found: C, 29.35; H, 4.42; N, 10.06; P, 21.89%.

3.9. N-(1-Ethylpyridinium-4-amino)methylenebisphosphonic acid (*9b*)

Yield 5.4 g, 61% (from reaction presented in Scheme 5) and 81% (from reaction presented in Scheme 7); m.p. 287–289 °C; δ_H (D_2O, pD = 4.88) 1.37 (3 H, t, *J* 7.2, CH_3), 4.02 (1 H, t, $^2J_{PH}$ 19.8, NCH), 4.08 (2 H, q, *J* 7.2, CH_2), 6.86, 6.90, 7.86, 8.01 ppm (1 H each, m each, 4 × CH_{py}); δ_C 156.94 (d, $^3J_{PC}$ 4.4, $C4_{py}$), 142.94, 140.68 ($C2_{py}$ and $C6_{py}$), 111.61, 106.19 ($C3_{py}$ and $C5_{py}$), 53.28 (CH_2), 51.64 (t, $^1J_{PC}$ 129.5, NCH), 15.10 (CH_3); δ_P 13.18; MS (ESI): *m*/*z*: 297.1 $[M]^+$; elemental Anal. Calc. for $C_8H_{14}N_2O_6P_2$: C, 32.42; H, 4.73; N, 9.46; P, 20.94. Found: C, 31.84; H, 4.79; N, 8.98; P, 21.08%.

3.10. N-(1-n-Butylpyridinium-4-amino)methylenebisphosphonic acid (*9c*)

Yield 5.2 g, 54% (from reaction presented as an entry 7 in Table 2); m.p. 305–307 °C; δ_H (D_2O, pD = 5.52) 0.87 (3 H, t, *J* 7.2, CH_3), 1.27 (2 H, m, CH_2), 1.78 (2 H, m, CH_2), 3.98 (1 H, t, $^2J_{PH}$ 18.3, NCH), 4.08 (2 H, t, *J* 7.2, CH_2), 6.91, 6.92, 7.88, 8.02 (1 H each, m each, 4 × CH_{py}); δ_C 156.40 (d, $^3J_{PC}$ 2.1, $C4_{py}$), 143.31, 140.47 ($C2_{py}$ and $C6_{py}$), 111.58, 106.07 ($C3_{py}$ and $C5_{py}$), 57.89 (CH_2), 51.78 (t, $^1J_{PC}$ 124.5, NCH), 32.04 (CH_2), 18.72 (CH_2), 12.75 (CH_3); δ_P 12.48; MS (ESI): *m*/*z*: 325.0 $[M]^+$; elemental Anal. Calc. for $C_{10}H_{18}N_2O_6P_2$: C, 37.02; H, 5.55; N, 8.64; P, 19.013. Found: C, 36.73; H, 5.27; N, 8.86; P, 18.70%.

3.11. N,N′-Di(pyridin-4-yl)formamidine (*10*)

4-Aminopyridine (30 mmol, 2.82 g) and of triethyl orthoformate (20 mL) were refluxed under condenser for 12 h. Then volatile components were removed under reduced pressure and resulting yellow oil dissolved in water. Compound **10** of satisfactory purity crystallized as yellowish solid. Yield 4.9 g 82%, m.p. 105–107 °C; δ_H (D_2O) 6.21 (4 H, d, *J* 7.1, Py), 7.34 (4H, d, *J* 7.1, Py), 7.61 (1 H, s, N═CH–N); MS (ESI): *m*/*z*: 199.4 $[M+H]^+$; elemental

Anal. Calc. for $C_{11}H_{10}N_4$: C, 66.58; H, 5.04; N, 28.25. Found: C, 66.20; H, 5.27; N, 27.85%.

Acknowledgment

Financial support by Ministry of Science and Higher Education (Grant No. R05 034 03) is gratefully acknowledged.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jorganchem.2009.07.025.

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