

**A  $^1\text{H}$ ,  $^{13}\text{C}$ ,  $^{31}\text{P}$  and  $^{15}\text{N}$  NMR study of (pyrrolidine-2,2-diyl)bisphosphonic acid, tetraalkyl(pyrrolidine-2,2-diyl)bisphosphonates and acyclic tetraethyl bisphosphonates**

**Gilles Olive<sup>†</sup>, Marcel H.P. van Genderen<sup>‡\*</sup>**

<sup>†</sup> Laboratory of Polymer Chemistry and Coatings Technology

<sup>‡</sup> Laboratory of Macromolecular and Organic Chemistry

Eindhoven University of Technology, P.O. Box 513, 5600 MB Eindhoven, The Netherlands.

Tel: +31-40-2473139. Fax: +31-40-2451036.

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\* To whom correspondence should be addressed.

**ABSTRACT:**

A multinuclear NMR study ( $^1\text{H}$ ,  $^{13}\text{C}$ ,  $^{31}\text{P}$ ,  $^{15}\text{N}$ ) was performed on a series of new cyclic pyrrolidine bisphosphonates and acyclic bisphosphonates. Values are reported and discussed for the chemical shifts and coupling constants of the various nuclei.

**KEYWORDS:** NMR;  $^1\text{H}$  NMR;  $^{13}\text{C}$  NMR;  $^{31}\text{P}$  NMR;  $^{15}\text{N}$  NMR; tetraalkyl bisphosphonates; bisphosphonic acid

## INTRODUCTION

*Gem*-bisphosphonates or *gem*-bisphosphonic acids can complex well with calcium and magnesium.<sup>1</sup> As a result their use in various areas, for instance in the medical field<sup>2</sup>, as plant growth regulators<sup>3</sup> and in the nuclear industry<sup>2</sup>, has been known for some time. We report here a multinuclear NMR study, in particular nitrogen-15, of new tetraalkyl bisphosphonates in a cyclic series and acyclic forms. It is interesting that these compounds can be labeled with <sup>15</sup>N for a direct study in biological material, which has been a considerable problem for several decades.<sup>4</sup>

## EXPERIMENTAL

The synthesis of compounds **1** to **6** (Figure 1) has been described<sup>5,6</sup> and the synthesis and full characterization of **7** and **8** will be published in a forthcoming paper.

<sup>1</sup>H, <sup>13</sup>C and <sup>31</sup>P NMR spectra were measured at 25 °C on Bruker AC-100, AC-200 and AMX-400 spectrometers. <sup>1</sup>H spectra were recorded at 100, 200 and 400 MHz. <sup>13</sup>C spectra were recorded at 25.18, 50.32 and 100.65 MHz. <sup>31</sup>P spectra were recorded at 40.54 MHz. <sup>15</sup>N NMR spectra were measured at 25 °C on a Varian Unity Inova 500 spectrometer at 50.65 MHz. In all cases, a field-frequency lock on the solvent <sup>2</sup>H signal was used. Chemical shifts are reported in ppm downfield from the standards, which were: TMS ( $\delta = 0$ ) or residual HDO in aqueous NaOD ( $\delta = 4.81$ ) for <sup>1</sup>H spectra, TMS ( $\delta = 0$ ) for <sup>13</sup>C spectra, external 85% H<sub>3</sub>PO<sub>4</sub> ( $\delta = 0$ ) for <sup>31</sup>P spectra, and external CD<sub>3</sub>NO<sub>2</sub> ( $\delta = 0$ ) for <sup>15</sup>N spectra. Samples were prepared in C<sub>6</sub>D<sub>6</sub>, CDCl<sub>3</sub> or aqueous NaOD (50 mg Na/ml D<sub>2</sub>O), in concentrations of 50-100 mg/ml for <sup>1</sup>H, <sup>13</sup>C and <sup>31</sup>P spectra in 5-mm tubes, and in a 80:20 (v/v) ratio of compound and solvent for natural abundance <sup>15</sup>N measurements in 10-mm tubes. Acquisition parameters for <sup>1</sup>H spectra were: pulse width 2-6  $\mu$ s (45° pulse), 16 transients, 4 s acquisition time, 2 s relaxation delay, spectral width 10 ppm (1-4 kHz), 8-32 K data points, 0.24 Hz resolution. <sup>1</sup>H spectra were obtained with line broadening (lb = 0.1). Acquisition parameters for <sup>13</sup>C spectra were: pulse width 3  $\mu$ s (45° pulse), 100 transients, 0.7-1.4 s acquisition time, 5 s relaxation delay, spectral width 6-24 kHz, 16-32 K data points, 0.74-1.45 Hz resolution. <sup>13</sup>C spectra

were obtained with line broadening ( $lb = 1$ ) and  $^1\text{H}$  broadband decoupling. Acquisition parameters for  $^{31}\text{P}$  spectra were: pulse width  $5\ \mu\text{s}$  ( $90^\circ$  pulse), 80 transients, 5 s acquisition time, 3 s relaxation delay, spectral width 16 kHz, 16 K data points, 2 Hz resolution.  $^{31}\text{P}$  spectra were obtained with  $^1\text{H}$  broadband decoupling. Acquisition parameters for  $^{15}\text{N}$  spectra were: pulse width  $10\ \mu\text{s}$  ( $90^\circ$  pulse:  $20.5\ \mu\text{s}$ ), 400 transients, 1.9 s acquisition time, 10 s relaxation delay, spectral width 8 kHz, 30000 data points, 0.5 Hz resolution.  $^{15}\text{N}$  spectra were obtained with line broadening ( $lb = 1$ ) and inverse-gated  $^1\text{H}$  broadband decoupling.

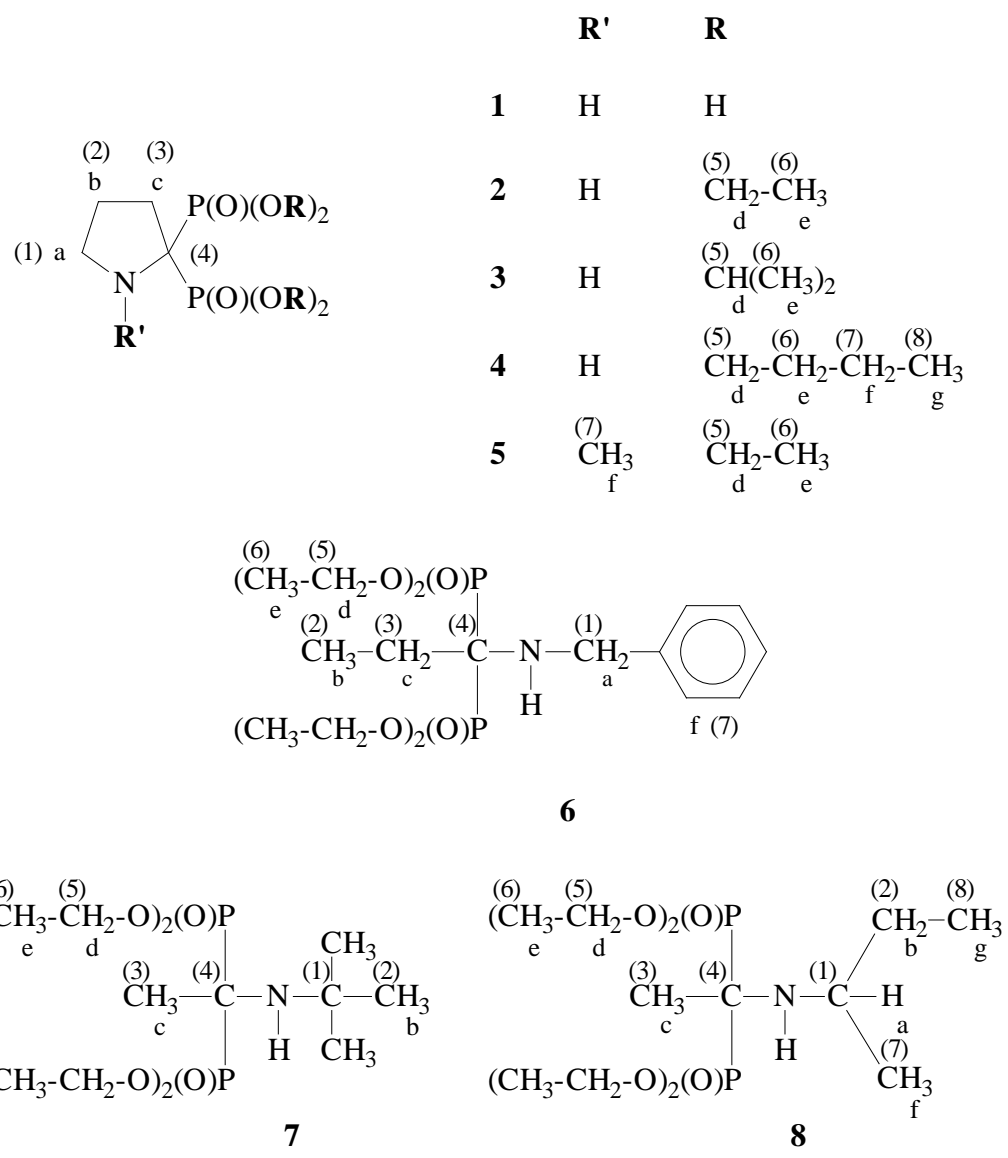
## RESULTS AND DISCUSSION

For all bisphosphonates, except **1**, the  $^{15}\text{N}$  spectrum (Table 1) shows a triplet, indicating that the two phosphorus nuclei are equivalent. This means that the ring pseudorotation is very fast for **2** to **5**, as previously reported for the nitroxide<sup>7</sup> (confirmed by the fact that we obtained triplets in  $^{13}\text{C}$  NMR). The two-bond coupling constants between phosphorus and nitrogen are in the range 2-9 Hz, which agrees with the few instances of  $^2J_{\text{NP}}$  reported in the literature.<sup>8,9</sup> It is evident that the linear compounds **6**, **7** and **8** have smaller coupling constants (6.3, 6.1 and 5.2 Hz respectively) than the cyclic structures.

Except for **1** (due to a different solvent) and for the picrate of **2**, the  $^{15}\text{N}$  chemical shifts of the cyclic forms **2-4** are all similar (max. change 0.5 ppm) and no correlation with the structure was found. Cyclic **5** has a different chemical shift because it is a tertiary amine (the donating methyl group induces an upfield shift). All  $^{15}\text{N}$  chemical shifts (Table 1) for amines **2-8** are very different from the starting materials (respectively -264.3 ppm for the pyrrolidine-2-one, -271.9 ppm for *N*-methyl pyrrolidone, -262.1 ppm for the precursor of **6**, -242.0 ppm for the precursor of **7**, and -244.5 ppm for the precursor of **8**).

It is also not possible to make a correlation between the structure and the chemical shift in the  $^{31}\text{P}$  spectra (Table 1). In all cases we see only one line in the  $^{31}\text{P}$  spectra. Hence the two phosphorus nuclei are equivalent and the ring pseudorotation is very fast for **1-5**. For  $^1\text{H}$  (Table 2) and  $^{13}\text{C}$  NMR (Table 3) there are some cases (for example for **3**) where the methyl groups are not equivalent,

presumably free rotation around the P-C bond is prevented.<sup>10</sup> The analysis of the <sup>13</sup>C spectra (Table 3) for the  $\alpha$  and  $\beta$ -carbons of the phosphonate ester groups, that is an AA'X system, has been described in reference 5.



**Figure 1.** Structures and numbering of the compounds studied.  
Letters refer to protons and numbers to carbons

**Table 1.**  $^{31}\text{P}$  and  $^{15}\text{N}$  NMR spectral data for bisphosphonates **1-8**:  $\delta(^{31}\text{P})$  and  $\delta(^{15}\text{N})$  (ppm)

(Parentheses indicate coupling constants in Hz)

	$^{31}\text{P}$			$^{15}\text{N}$	
	$\delta$	Solvent	Ref	$\delta$	Solvent
<b>1</b>	15.9	Na/D <sub>2</sub> O <sup>d</sup>	5	-312.8	Na/D <sub>2</sub> O <sup>d</sup>
<b>2</b>	22.5	CDCl <sub>3</sub>	5	-340.7 (8.7) <sup>a</sup>	C <sub>6</sub> D <sub>6</sub>
<b>Picrate of 2</b>	16.2	CDCl <sub>3</sub>	5	-12.2 <sup>b</sup> -15.4 <sup>c</sup> -322.1 (2.2)	CDCl <sub>3</sub>
<b>3</b>	21.2	CDCl <sub>3</sub>	5	-340.3 (9.3)	C <sub>6</sub> D <sub>6</sub>
<b>4</b>	22.7	CDCl <sub>3</sub>	6	-340.5 (8.5)	C <sub>6</sub> D <sub>6</sub>
<b>5</b>	21.8	CDCl <sub>3</sub>	6	-344.8 (8.3)	C <sub>6</sub> D <sub>6</sub>
<b>6</b>	21.5	CDCl <sub>3</sub>	6	-340.4 (6.3)	C <sub>6</sub> D <sub>6</sub>
<b>7</b>	22.5	CDCl <sub>3</sub>		-324.3 (6.1)	C <sub>6</sub> D <sub>6</sub>
<b>8</b>	23.3	CDCl <sub>3</sub>		-330.8 (5.2)	C <sub>6</sub> D <sub>6</sub>

<sup>a</sup> -341.6 (8.6) in CDCl<sub>3</sub>, <sup>b</sup> nitro ortho, <sup>c</sup> nitro para, <sup>d</sup> 50 mg Na/ml D<sub>2</sub>O

**Table 2.**  $^1\text{H}$  NMR spectral data for bisphosphonates **1-8**:  $\delta(^1\text{H})$  (ppm)  
(Parentheses indicate the pattern of coupling and the coupling constants in Hz)

	a	b	c	d	e	f	g	Solvent Frequency	Ref
<b>1</b>	3.21 (t, 7.0)	1.85 (quint., 7.0)	2.17 (sept., 7.1 <sup>e</sup> ; 14.5 <sup>f</sup> )					Na/D <sub>2</sub> O <sup>c</sup> 200	5
<b>2</b>	2.88 (t, 6.5)	1.69 (quint., 6.8)	2.42 (tt, 7.2 <sup>e</sup> ; 17.7 <sup>f</sup> )	4.17 (m)	1.11 (t, 7.1 <sup>e</sup> ) 1.10 (t, 7.1 <sup>e</sup> )			C <sub>6</sub> D <sub>6</sub> 400	5
<b>Picrate of 2</b>	3.64 (t, 6.9)	2.15-2.67 (m)	4.24 (m)		1.36 (t, 7.1 <sup>e</sup> ) 1.33 (t, 7.1 <sup>e</sup> )	8.86 <sup>a</sup> (s)	7.99 <sup>b</sup> (s)	CDCl <sub>3</sub> 100	5
<b>3</b>	2.95 (t, 6.5)	1.75 (quint., 6.8)	2.37 (tt, 7.3 <sup>e</sup> ; 17.7 <sup>f</sup> )	5.00 (m) 4.87 (m)	1.31 (d, 6.2 <sup>e</sup> ) 1.28 (d, 6.3 <sup>e</sup> ) 1.27 (d, 6.4 <sup>e</sup> ) 1.22 (d, 6.1 <sup>e</sup> )			C <sub>6</sub> D <sub>6</sub> 400	5
<b>4</b>	2.97 (t, 6.5)	1.76 (quint., 6.9)	2.49 (tt, 7.3 <sup>e</sup> ; 17.8 <sup>f</sup> )	4.27 (m) 4.22 (m)	1.56 (m)	1.33 (sext., 7.5 <sup>e</sup> ) 1.32 (sext., 7.3 <sup>e</sup> )	0.83 (t, 7.4 <sup>e</sup> ) 0.82 (t, 7.4 <sup>e</sup> )	C <sub>6</sub> D <sub>6</sub> 400	6
<b>5</b>	2.84 (t, 6.6)	1.84 (quint., 6.6)	2.43 (m)	4.21 (m)	1.34 (t, 7.1 <sup>e</sup> )	2.79 (t, 1.7)		CDCl <sub>3</sub> 200	6
<b>6</b>	4.11 (m)	1.12 (t, 7.5 <sup>e</sup> )	2.13 (tq, 7.5 <sup>e</sup> ; 15.0 <sup>f</sup> )	4.21 (m)	1.32 (t, 7.1 <sup>e</sup> ) 1.31 (t, 7.1 <sup>e</sup> )	7.24 (m)		CDCl <sub>3</sub> 400	6
<b>7</b>		1.24 (s)	1.74 (t, 18.4 <sup>f</sup> )	4.1 - 4.3 (m)	1.33 (q, 7.0)			CDCl <sub>3</sub> 400	
<b>8</b>	2.9 (m)	1.3 <sup>d</sup> (m) 1.1 <sup>d</sup> (m)	1.41 (t, 17.4 <sup>f</sup> )	4.02 (m)	1.15 (t, 7.2 <sup>e</sup> )	0.87 (d, 6.4 <sup>e</sup> )	0.68 (t, 7.4 <sup>e</sup> )	CDCl <sub>3</sub> 400	

<sup>a</sup> aromatics, <sup>b</sup> -NH<sub>2</sub><sup>+</sup>, <sup>c</sup> 50 mg Na/ml D<sub>2</sub>O, <sup>d</sup> the two protons are not equivalent, <sup>e</sup> H-H coupling, <sup>f</sup> P-H coupling

**Table 3.**  $^{13}\text{C}$  NMR spectral data for bisphosphonates **1-8**:  $\delta(^{13}\text{C})$  (ppm)  
(Parentheses indicate coupling constants with  $^{31}\text{P}$  in Hz)

	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	Solvent Frequency	Ref
<b>1</b>	48.6	25.4	31.3	65.8 (t, 118.0)					Na/D <sub>2</sub> O <sup>i</sup> 50	5
<b>2</b>	47.7 (t, 4.0)	26.5 (t, 3.1)	31.2 (t, 3.0)	62.8 (t, 151.8)	63.4 (t, 5.3) <sup>f</sup> 62.7 (t, 5.8) <sup>f</sup>	16.6 (t, 5.5) <sup>g</sup> 16.5 (t, 7.2) <sup>g</sup>			C <sub>6</sub> D <sub>6</sub> 100	5
<b>Picrate of 2</b>	49.3 (t <sup>e</sup> )	24.2 (t <sup>e</sup> )	31.2 (t <sup>e</sup> )	63.3 (t, 145.4)	65.0 (d <sup>e</sup> )	16.4 (t <sup>e</sup> )	160.6 <sup>a</sup> 141.0 <sup>c</sup> 128.7 <sup>d</sup> 126.2 <sup>b</sup>		CDCl <sub>3</sub> 100	
<b>3</b>	47.7 (t, 4.6)	26.4 (t, 3.3)	31.0 (t, 3.4)	63.1 (t, 151.1)	71.7 (t, 6.2) <sup>f</sup> 70.8 (t, 6.7) <sup>f</sup>	24.7 24.4 24.0 (t, 6.2) <sup>g</sup> 23.8 (t, 6.7) <sup>g</sup>			C <sub>6</sub> D <sub>6</sub> 100	5
<b>4</b>	48.1 (t, 4.3)	26.8 (t, 3.2)	31.5 (t, 3.0)	63.2 (t, 152.0)	67.8 (t, 3.2) <sup>f</sup> 67.1 (t, 3.5) <sup>f</sup>	33.5 (t, 2.7) <sup>g</sup> 33.4 (t, 2.7) <sup>g</sup>	19.4	14.1	C <sub>6</sub> D <sub>6</sub> 100	6
<b>5</b>	55.3 (t, 4.7)	23.6 (t <sup>e</sup> )	32.2 (4.6)	64.2 (153.0)	62.2 (m)	16.1 (t <sup>e</sup> )	37.3 (t <sup>e</sup> )		CDCl <sub>3</sub> 50	6
<b>6</b>	47.9 (t, 6.5)	8.5 (t, 6.5)	23.5 (t <sup>e</sup> )	62.8 (t, 140.1)	63.1 (t, 3.2) <sup>f</sup> 62.8 (t, 3.1) <sup>f</sup>	16.5 (t <sup>e</sup> )	140.5 <sup>a</sup> 128.3 <sup>b</sup> 128.2 <sup>c</sup> 127.0 <sup>d</sup>		CDCl <sub>3</sub> 100	6
<b>7</b>	52.6 (t, 5.7)	32.4	15.8 (t, 5.5)	58.9 (t, 145.0)	64.0 (t, 3.7) <sup>f</sup> 62.5 (t, 4.1) <sup>f</sup>	16.3 (m)			CDCl <sub>3</sub> 100	
<b>8</b>	48.4 (t, 6.1)	32.1	15.6 (t, 4.2)	57.5 (t, 143.0)	63.2 (t, 3.8) <sup>f</sup> 62.3 (t, 3.8) <sup>f</sup>	16.2 (t, 3.0) <sup>g</sup>	10.1	22.3	CDCl <sub>3</sub> 100	

<sup>a</sup> quaternary aromatic, <sup>b</sup> meta aromatics, <sup>c</sup> ortho aromatics, <sup>d</sup> para aromatic, <sup>e</sup> coupling can not be resolved because they are too small, <sup>f</sup>  $^2J_{\text{CP}}(+^4J_{\text{CP}})$ , <sup>g</sup>  $^3J_{\text{CP}}$ , <sup>h</sup> see ref 5, <sup>i</sup> 50 mg Na/ml D<sub>2</sub>O

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