ELSEVIER

Journal of Chromatography A, 678 (1994) 299–312

JOURNAL OF CHROMATOGRAPHY A

Carboxylic anhydrides–orthoesters —novel reagent systems for derivatization of aminoalkanephosphonic acids for characterization by gas chromatography and mass spectrometry. III☆

Zbigniew H. Kudzin[a,*], Marek Sochacki[b], Józef Drabowicz[b]

[a]*Department of Organic Chemistry, University of Łódź, Narutowicza 68, Łódź 90-136, Poland*

[b]*Centre of Molecular and Macromolecular Studies, Polish Academy of Sciences, Sienkiewicza 112, Łódź 90-363, Poland*

First received 26 January 1994; revised manuscript received 3 May 1994

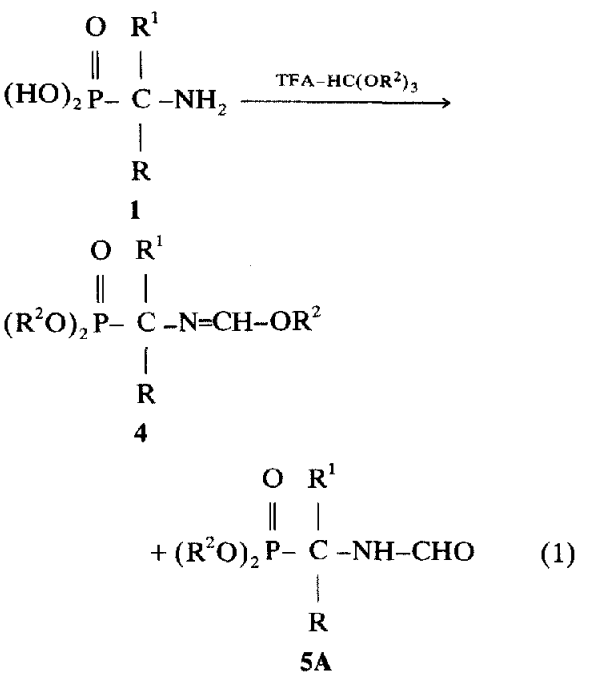
Abstract

Studies of the derivatization of aminoalkanephosphonic acids by means of carboxylic anhydrides–trialkyl orthoester systems are reported. Gas chromatographic separations of mixtures of derivatized 1-aminoalkanephosphonic acids and low-resolution electron impact mass spectra for the derivatives studied are presented.

1. Introduction

Phosphonic acids bearing amino functions have been found to possess substantial importance in pure, applied and environmental chemistry owing to their chelating [1,2] and biological properties [3,4]. For these reasons, their determination, mainly based on chromatographic methods, is of current interest in the analytical chemistry of organophosphorus compounds [5–7].

Recently we reported on the scope of the derivatization of 1-aminoalkanephosphonic acids (**1**) by means of trialkyl orthoformates [8,9], according to the equation



(1)

* Corresponding author.

☆ Dedicated to Professor E. Wenschuh on the occasion of his 60th birthday. Presented at the *3rd Symposium of ECOBAPHOS, Düsseldorf, April 1994.*

0021-9673/94/$07.00
SSDI 0021-9673(94)00455-I

where TFA = trifluoroacetic acid. The volatile derivatives **4** and **5** have been found to be the suitable models for analysis by gas chromatography–mass spectrometry (GC–MS), similarly to their amino ester analogues **3** [10]. However, the formation of two sets of derivatives **4** and **5** during the derivatization of **1** is a limitation of this procedure, especially in the analysis of more complex mixtures of **1**.

In this paper, we report another approach, involving the derivatization of 1-aminoalkanephosphonic acids (**1**) into the volatile N-acylaminoalkanephosphonates (**5**), which is based on the use of carboxylic anhydride–trialkyl orthoformate systems. This two-step, one-pot procedure is described by the general scheme presented in Eq. 2.

{The 1-(N-formylamino)alkanephosphonates **2AA*** and **2AB*** were described in previous papers [8,9].} An illustration of the GC–MS characteristics of the N-acylaminoalkanophosphonates **5** is also presented.

2. Experimental

2.1. Materials

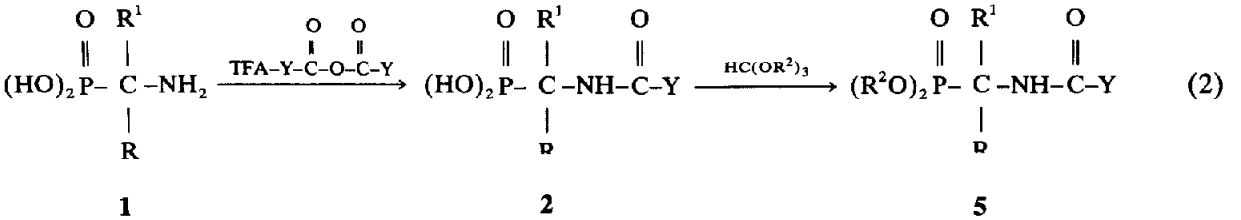
Acetic acid, acetic anhydride, trimethyl orthoacetate, triethyl orthoformate, trimethyl orthoformate, trifluoroacetic acid and trifluoroacetic anhydride were purchased from Aldrich (Milwaukee, WI, USA). 1-Aminoalkanephosphonic acids (**1**) were prepared according to Ref. [11] and phosphonoglycine (**1a**) according to Ref. [12]. All aminoalkanephosphonic acids were of the purity reported previously [11,12].

2.2. Preparation of derivatives 5

The conversions of 1-aminoalkanephosphonic acids (**1**) into the volatile derivatives **5** were carried out in a Wheaton 1-ml micro product V-vial, equipped with a spin vane, placed in a thermostated oil-bath.

In order to prepare a series of 1-(N-acetylamino)alkanephosphonates (**5B**), samples of **1** (0.1–5 mg) were dissolved in acetic acid (0.05 ml)–acetic anhydride (0.05 ml) and the resulting solutions were heated with stirring at 100°C for 10 min. Then the reaction mixture was cooled to ca. 40–50°C and trialkyl orthoformate (0.4 ml) or trialkyl orthoacetate (0.4 ml) was added. The resulting mixtures were stirred at 100°C for 1.5 h (the derivatizations were usually completed after 1 h) and after this time the reaction mixtures were analysed by GC and/or GC–MS.

For the preparation of 1-(N-trifluoroacetylamino)alkanephosphonates (**5C**), samples of **1** (0.1–5 mg) were dissolved in trifluoroacetic acid (TFA) (0.05 ml)–trifluoroacetic anhydride (0.05 ml) and the solutions were heated with stirring for 10 min at 30–40°C. Then trialkyl orthoformate (0.40 ml) was carefully added and the resulting mixtures were stirred at 100°C for 1.5 h (the derivatizations were usually completed after 1 h).



(2)

1

2

2B (Y = Me)

2C (Y = CF_3)

5

5BA* (Y = Me, R^2 = Me)

5BB* (Y = Me, R^2 = Et)

5CA* (Y = CF_3, R^2 = Me)

5CB* (Y = CF_3, R^2 = Et)

2.3. Gas chromatography–mass spectrometry

A Finnigan MAT 95 mass spectrometer was used for GC–MS analysis of the multi-component mixture of derivatives. Sample introduction was via a Varian 3400 gas chromatograph equipped with a 30 m × 0.25 mm I.D. capillary column coated with BP-17. The column temperature was 100°C for 3 min, then increased at 10°C min^{-1} to 250°C. The injector temperature was maintained at 200°C and the transfer line temperature was 250°C. The column was introduced directly into the ion source of the mass spectrometer. Mass spectra were recorded at an electron energy of 70 eV.

2.4. ^{31}P NMR

^{31}P NMR spectra were recorded on a Bruker AC 200 spectrometer operating at 81.01 MHz.

3. Results and discussion

3.1. Derivatization

The esterification of aminoalkanephosphonic acids (**1**) by means of trialkyl orthoformates afforded a mixture of forminoalkoxy (**4**) and N-formyl derivatives (**5**) according to Eq. 1 [8,9]. It was found that both the structure of **1** and the nature of the acidic catalyst exert a substantial influence on the course and the rate of derivatization [9]. Thus, 1-aminoalkanephosphonic acids with primary and secondary amino groups reacted with the TFA–trialkyl orthoformate system smoothly, with formation of corresponding amido esters. On the other hand, the esterification of 1-(N,N-dialkylamino)alkanephosphonic acids occurs much more slowly [8], which suggests that the formylation of the amino group in **1** constitutes the key step in the whole esterifica-

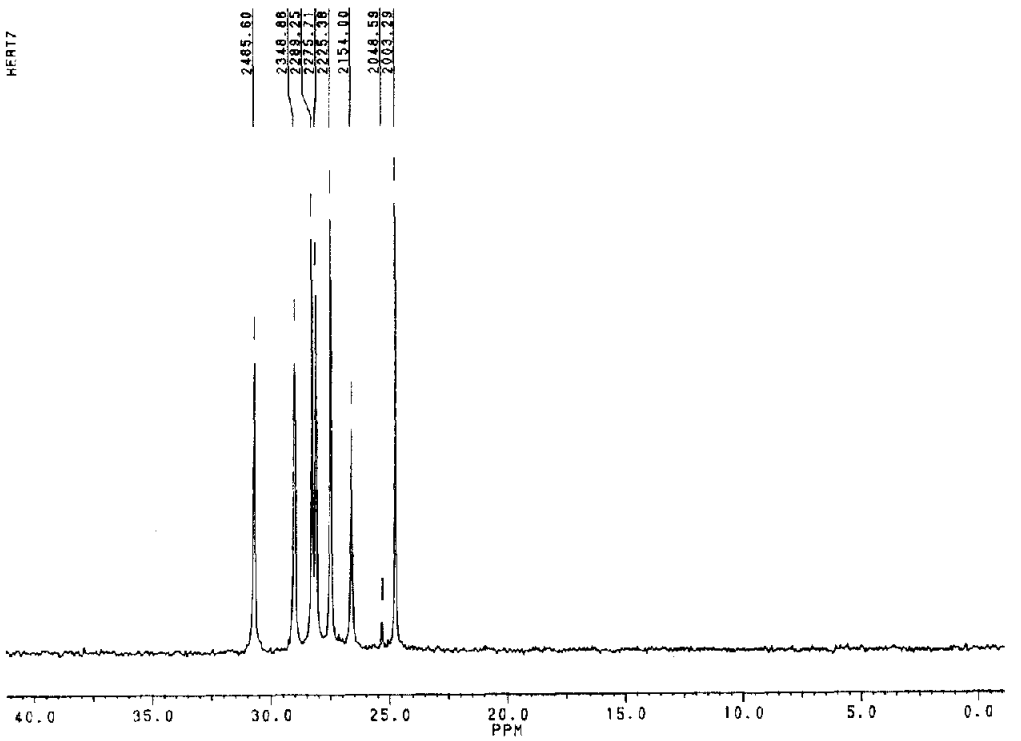


Fig. 1. ^{31}P NMR spectrum of the derivatization products **5BA*** (**a–g**) of the mixture of aminophosphonic acids [aminomethylenephosphonic (**1a**), 1-aminoethanephosphonic (**1b**), 1-aminopropanephosphonic (**1c**), 1-amino-1-methylethanephosphonic (**1d**), 1-aminopentanephosphonic (**1e**), 1-aminophenylmethanephosphonic (**1f**) and 1-amino-2-phenylethanephosphonic acid (**1g**)] obtained by means of acetic acid–acetic anhydride and trimethyl orthoacetate. Conditions as described under Experimental.

tion process of 1-aminoalkanephosphonic acids by means of orthoformates. Based on this observation (the results on the reaction course of various types of aminophosphonic acids with trialkyl orthoformates will be presented elsewhere) and in order to facilitate the conversion of **1** into their **5**, we pretreated **1** with carboxylic acid–acid anhydrides mixtures to induce the in situ formation of the N-acyl derivatives **2**. Subsequent treatment of the intermediates **2** with orthoesters afforded the desired 1-amidoalkanephosphonates (**5**) with almost quantitative NMR yields (Figs. 1 and 2).

When the N-acylation of **1** was carried out in TFA (owing to the good solubility of **1** in TFA)–acetic anhydride mixture (TFA–Ac_2O, 10 min, 40–50°C), followed by O-alkylation of the intermediate **2** with triethyl orthoformate, N-acetylaminoalkanephosphonates (**5BB***) and N-trifluoroacetylaminoalkanephosphonates (**5CB***) were found in the reaction mixture, presumably owing to the formation of mixed trifluoroacetyl–acetic anhydrides:

$$CF_3C(O)OH + CH_3C(O)OC(O)CH_3 \longrightarrow$$

$$CH_3C(O)OH + CF_3C(O)OC(O)CH_3 \qquad (3)$$

$$\mathbf{1} \xrightarrow{\text{TFA–}Ac_2O} \begin{cases} \mathbf{2B} \xrightarrow{HC(OEt)_3} \mathbf{5BB}* \\ + \\ \mathbf{2C} \xrightarrow{HC(OEt)_3} \mathbf{5CB}* \end{cases} \qquad (4)$$

In order to determine the relative rate of esterification of amino acids **1** by means of trimethyl and triethyl orthoformates, the starting amino acids **1** were converted into 1-(N-TFA-amino)alkanephosphonic acids (**2**) and these were esterified with the mixture of trimethyl and triethyl orthoesters (1:1):

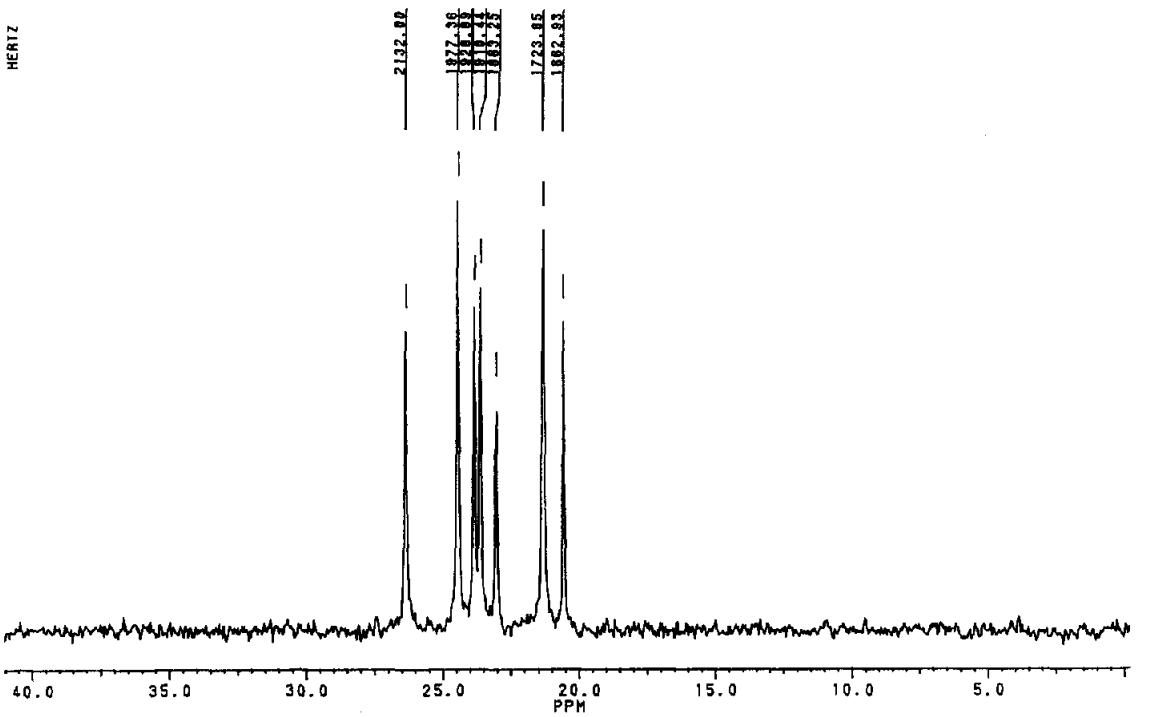
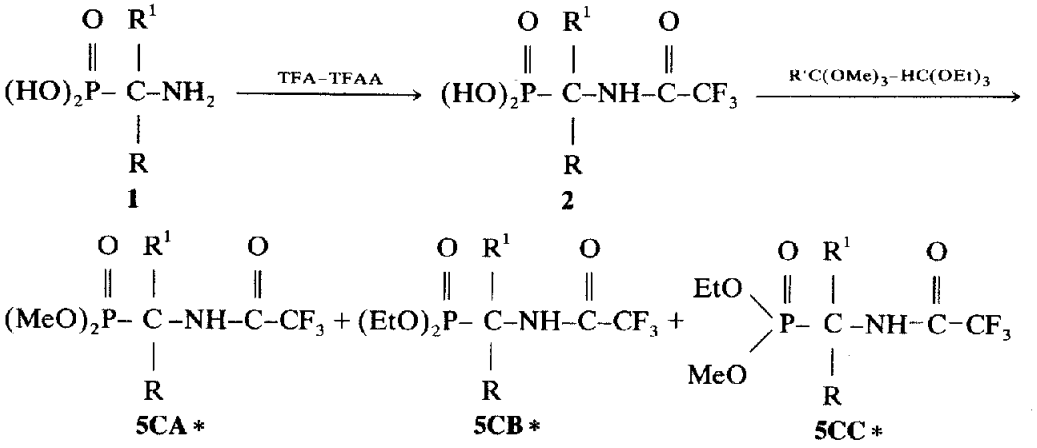


Fig. 2. ^{31}P NMR spectrum of the derivatization products **5CB*** (**a–g**) of the mixture of aminophosphonic acids **1a**, **1b**, **1c**, **1d**, **1e**, **1f** and **1g** obtained by means of trifluoroacetic acid–trifluoroacetic anhydride and triethyl orthoformate. Conditions as described under Experimental.



(5)

The relative amounts of the amido esters **5CA*** (O,O-dimethyl), **5CB*** (O,O-diethyl) and mixed **5CC*** (O-methyl-O-ethyl) formed reflected the alkylation capability of all the types of orthoesters applied towards the phosphonic function. Thus, the alkylation rate of derivatives **2** by means of these orthoesters generally exhibit the orders $HC(OMe)_3 < HC(OEt)_3 \approx MeC(OMe)_3$ for N-TFA-aminoalkanephosphonic acids (**2C**) and $HC(OMe)_3 \approx MeC(OMe)_3 < HC(OEt)_3$ for N-acetylaminoalkanephosphonic acids (**2B**).

A similar approach based on the prior trifluoroacetylation of **1** in the two-stage procedures for their esterification was also applied in the methods elaborated by Ruppel *et al.* [13] and

Table 1
Comparison with other methods of aminoalkanephosphonic acid derivatization

Conversion to derivative	Reagent	Time (h)	Temperature (°C)	Range (mg)	Ref.
(I) O,O-Dibutyl 1-(N-TFA-amino)alkane phosphonate	(Ms. pr.)	ca. 3		0.25–10	[13]
	(1) TFA–TFAA	1	40		
	(2) Diazobutane	0.2	20		
(II) O,O-Diethyl 1-(N-TFA-amino)alkane phosphonate	(Ms. pr.)	ca. 10		10	[14]
	(1) TFA–TFAA	1	20		
	(20 $HC(OEt)_3$	8	>130		
(III) O,O-Diethyl 1-(N-formylamino)alkane phosphonate	(One-pot pr.)				[9]
	TFA–$HC(OEt)_3$	2	120	0.1–5	
(IV)	(One-pot pr.)	ca. 2		0.1–5	This work
(IVa) O,O-Dialkyl 1-(N-acetylamino) alkanephosphonate	(1) $AcOH–Ac_2O$	0.2	100		
	(1a) $HC(OR)_3$ or $MeC(OR)_3$	1.5	100		
(IVb) O,O-Dialkyl 1-(N-TFA-amino)alkane phosphonates	(1) TFA–TFAA	0.2	30–40		
	(1a) $HC(OR)_3$	1.5	100		

Abbreviations: Ac = acetyl; Ac_2O = acetic anhydride; TFA = trifluoroacetic acid; TFAA = trifluoroacetic anhydride; N-TFA = N-trifluoroacetyl; Ms. pr. = multi-stage procedure; One-pot pr. = one-pot procedure.

Huber [14]. The comparison of the present method with those procedures is given in Table 1.

Optimization of the reaction conditions was carried out using ^{31}P NMR monitoring. These investigations revealed the complete conversion of aminoalkanephosphonic acids **1** into the corresponding diester derivatives **5**. The ^{31}P NMR spectra of the derivatization products (**5BA*** and **5CB***) of **1** obtained with the carboxylic anhydride–orthoester systems are presented in Figs. 1 and 2. The phosphorus chemical shifts for all the derivatives obtained, **5BA***, **5BB***, **5CA*** and **5CB***, are given in Tables 2 and 3.

Table 2
^{31}P NMR chemical shifts (δ, ppm) of N-acetylaminoalkanephosphonates **5B** (**5BA*** and **5BB***)

Starting amino acid **1**		**5B** derivatives	
		$P(O)(OMe)_2$	$P(O)(OEt)_2$
No.	R ($R^1 \neq H$)		
1a	H	26.6	23.2
1b	Me	29.1	25.6
1c	Et	28.3	25.0
1d	Me (Me)	31.0	27.6
1e	Bu	28.1	24.7
1f	Ph	24.8	21.6
1g	$PhCH_2$	27.5	24.1

Table 3
^{31}P NMR chemical shifts (δ, ppm) of N-trifluoroacetylaminoalkanephosphonates **5C** (**5CA*** and **5CB***)

Starting amino acid **1**		**5C** derivatives	
		$P(O)(OMe)_2$	$P(O)(OEt)_2$
No.	R ($R^1 \neq H$)		
1a	H	25.4	21.3
1b	Me	26.8	24.4
1c	Et	26.3	23.8
1d	Me (Me)	28.5	26.3
1e	Bu	26.0	23.6
1f	Ph	22.6	20.5
1g	$PhCH_2$	24.6	23.0

3.2. Chromatographic properties of derivatives 5

The derivatives **5** can be stored for several weeks at 0°C without extensive decomposition (^{31}P NMR) and have been found to be suitable for characterization by means of GC. These compounds give reproducible retention data. The separation of derivatives **5** from derivatization of the mixture of 1-aminoalkanephosphonic acids **1a**, **1b**, **1c**, **1d**, **1e**, **1f** and **1g**, achieved on a DB-17 column, is presented in Figs. 3 and 4.

The DB-17 column was suitable for the separations of all the N-acylaminoalkanephosphonates **5BA*** and **5BB***. The higher polarity of N-trifluoroacetylamino derivatives **5CA*** and **5CB*** had the result that, in two cases (Fig. 4) (**5CB*d** and **5CB*b**; **5CB*a** and **5CB*c**), the overlapping effect occurred. This suggests that, for better separation, a column of higher polarity should be used (e.g., DB-225).

The elution of the amidoalkanephosphonates **5** did not follow the order of their molecular masses. The derivatives **5d** appeared first, then the others in the order **5b**, **5c**, **5a**, **5e**, **5f** and **5g** (in all four series of derivatives **5**, i.e. **5BA***, **5BB***, **5CA*** and **5CB***). The comparison of the retention times of acetyl (**5B**) and trifluoroacetyl derivatives (**5C**), and also different O-alkyl amidoalkanephosphonates (**5CA***, **5CB*** and **5CC***) revealed the following orders of increasing retention times: **5B** > **5C** and **5CA*** < **5CC*** < **5CB***.

3.3. Mass spectral properties of N-acylaminoalkanephosphonates 5

The partial mass spectra of N-acyl derivatives **5** are summarized in Tables 4–8.

The N-acylaminoalkanephosphonates **5** present distinct structural differences caused by (a) the constitution of the acyl group, (b) the type of O-alkyl phosphonate function and (c) the type of hydrocarbon side-chain attached to C_α. However, in spite of these structural differences, the whole group present some common features, which are reflected in their fragmentation patterns, and consequently determine the shape of all the mass spectra.

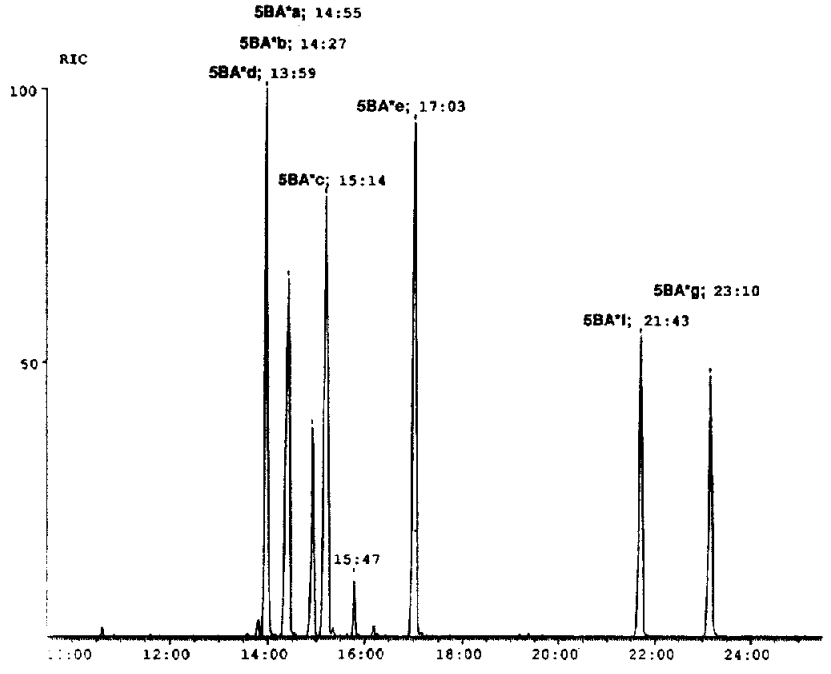


Fig. 3. GC–MS analysis of the derivatization products **5BA** * (**a–g**) of the mixture of aminophosphonic acids **1a**, **1b**, **1c**, **1d**, **1e**, **1f** and **1g** obtained by means of acetic acid–acetic anhydride and trimethyl orthoacetate. Conditions as described under Experimental. The ^{31}P NMR spectrum of the derivatization products of this reaction mixture is presented in Fig. 1.

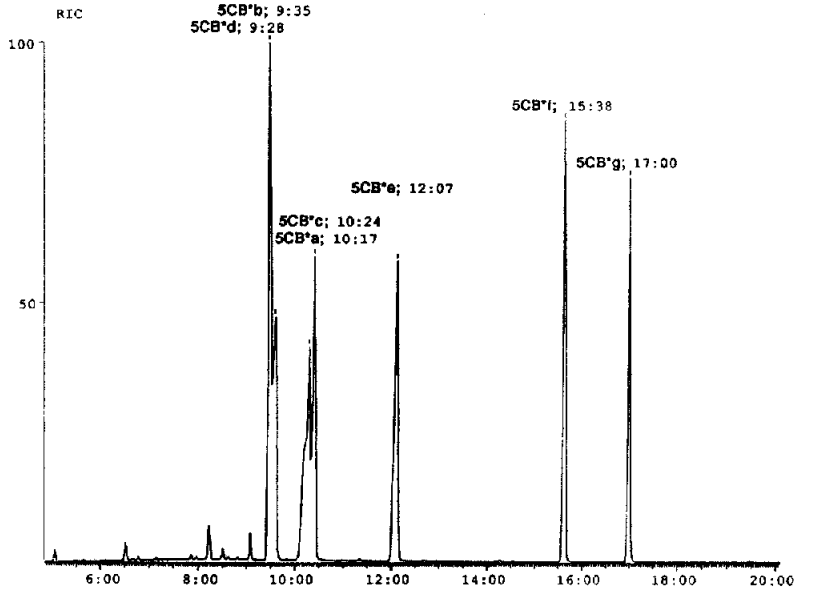


Fig. 4. GC–MS analysis of the derivatization products **5CB** * (**a–g**) of the mixture of aminophosphonic acids **1a**, **1b**, **1c**, **1d**, **1e**, **1f** and **1g** obtained by means of trifluoroacetic acid–trifluoroacetic anhydride and triethyl orthoformate. Conditions as described under Experimental. The ^{31}P NMR spectrum of the derivatization products of this reaction mixture is presented in Fig. 3.

Table 4
Reduced mass spectra of O,O-dimethyl 1-(acetylamino)alkanephosphonates **5BA***

No.	R ($R^1 \neq H$)	Molecular formula (mass)	Mass spectrometry: *m/z* (relative intensity, %)										
			M	M − MeO [M − 31]	M − R	[M − 42]	M − Ac [M − 43]	M − NHAc [M − 58]	M − $(MeO)_2PO$ [M − 109]	Phosphonyl-derived ions[a]			$RCH{=}NH_2^+$ [R + 29]
										[110]	[95]	[79]	
5BA*a	H	$C_5H_{12}NPO_4$	181	150	180	140	139	124	72	110	95	79	30
		(181.1)	(4.2)	(8.9)	(−)	(0.2)	(2.0)	(10)	(31)	(90)	(13)	(21)	(100)
5BA*b	CH_3	$C_6H_{14}NPO_4$	195	164	180	153	152	138	86	110	95	79	44
		(195.1)	(2.0)	(0.5)	(0.5)	(0.5)	(6.5)	(2.6)	(64)	(17)	(3.5)	(6.1)	(100)
5BA*c	C_2H_5	$C_7H_{16}NPO_4$	209	178	180	167	166	152	100	110	95	80	58
		(209.2)	(1.7)	(0.2)	(0.2)	(0.2)	(1.6)	(0.5)	(56)	(8.2)	(1.7)	(2.9)	(100)
5BA*d	$CH_3(CH_3)$	$C_7H_{16}NPO_4$	209	178	180	167	166	152	100	110	95	80	58
		(209.2)	(0.5)	(0.5)	(−)	(0.1)	(2.5)	(2.4)	(74)	(5.0)	(1.3)	(2.0)	(100)
5BA*e	*n*-C_4H_9	$C_9H_{20}NPO_4$	237	206	180[b]	195[c]	194	166	128	110	95	80	86
		(237.2)	(1.8)	(0.1)	(0.2)	(0.9)	(1.3)	(2.5)	(64)	(4.6)	(1.0)	(1.7)	(100)
5BA*f	C_6H_5	$C_{11}H_{18}NPO_4$	257	226	180	215[c]	214	200	148	110	95	80	106
		(257.2)	(7.2)	(0.1)	(0.1)	(2.9)	(5.1)	(1.6)	(61)	(3.0)	(0.9)	(1.3)	(100)
5BA*g	$C_6H_5CH_2$	$C_{12}H_{20}NPO_4$	271	240	180	229	228	214	162	110	95	80	120
		(271.2)	(2.2)	(0.2)	(4.8)	(0.1)	(0.5)	(2.9)	(26)	(4.5)	(0.2)	(1.8)	(100)

[a] $(MeO)_2POH$ [110], (MeO)P(O)(OH) [95], PO_3 [79].
[b] [181]; (1.3%).
[c] $[M - CH_2CO]$.

Table 5
Reduced mass spectra of O,O-diethyl 1-(acetylamino)alkanephosphonates **5BB** *

No.	R ($R^1 \neq H$)	Molecular formula (mass)	Mass spectrometry: *m/z* (relative intensity, %)										
			M	M − EtO [M − 45]	M − R	[M − 42]	M − Ac [M − 43]	M − NAc [M − 57]	M − $(EtO)_2PO$ [M − 137]	Phosphonyl-derived ions[a]			$RCH{=}NH_2^+$ [R + 29]
										[138]	[111]	[82]	
5BB * a	H	$C_7H_{16}NPO_4$	209	164	208	167[b]	166	152	72	138	111	82	30
		(209.2)	(54)	(14.0)	(−)	(5.8)	(7.4)	(6.2)	(23)	(100)	(54)	(21)	(−)
5BB * b	CH_3	$C_8H_{18}NPO_4$	223	178	208	181	180	166	86	138	111	82	44
		(223.2)	(7.8)	(3.8)	(0.2)	(1.4)	(23.0)	(1.8)	(73)	(30)	(33)	(15)	(100)
5BB * c	C_2H_5	$C_9H_{20}NPO_4$	237	192	208	195	194	180	100	138	111	82	58
		(237.1)	(5.9)	(1.7)	(0.2)	(0.4)	(3.1)	(0.7)	(70)	(12)	(12)	(5)	(100)
5BB * d	$CH_3(CH_3)$	$C_9H_{20}NPO_4$	237	192	208	195	194	180	100	138	111	82	58
		(237.1)	(2.6)	(2.4)	(−)	(0.3)	(8.2)	(2.5)	(74)	(5.0)	(7.1)	(4.6)	(100)
5BB * e	*n*-C_4H_9	$C_{11}H_{24}NPO_4$	265	220	208	223[b]	222	208	128	138	111	82	86
		(265.3)	(3.2)	(1.2)	(0.4)	(2.3)	(2.8)	(0.4)	(64)	(5.4)	(5.6)	(3.7)	(100)
5BB * f	C_6H_5	$C_{13}H_{20}NPO_4$	285	240	208	243[b]	242	228	148	138	111	82	106
		(285.3)	(9.2)	(0.9)	(−)	(1.8)	(5.1)	(−)	(61)	(1.4)	(2.9)	(3.0)	(100)
5BB * g	$C_6H_5CH_2$	$C_{14}H_{22}NPO_4$	299	254	208	257	256	242[c]	162[d]	138	111	82	120
		(299.3)	(5.3)	(1.7)	(5.7)	(−)	(0.3)	(0.2)	(26)	(20)	(4.2)	(4.2)	(80)

[a] $(EtO)_2POH$ [138], $(EtO)P(H)(OH)_2$ [111], H_3PO_3 [82].
[b] M − CH_2CO.
[c] [241]; (0.2%) and [240]; (18%).
[d] [161]; (100%).

Table 6
Reduced mass spectra of O,O-dimethyl 1-(trifluoroacetylamino)alkanephosphonates **5CA***

No.	R ($R^1 \neq H$)	Molecular formula (mass)	Mass spectrometry: *m/z* (relative intensity, %)											
			M	M − MeO [M − 31]	M − R	M − CF_3 [M − 69]	M − TFA [M − 97]	M − $(MeO)_2PO$ [M − 109]	Phosphonyl-derived ions[a]				CF_3 [69]	$RCH{=}NH_2^+$ [R + 29]
									[110]	[95]	[80]	[79]		
5CA*a	H	$C_5H_9F_3NPO_4$	235	204	234	166	140	126	110[d]	95	80	79	69	30
		(235.1)	(16.0)	(2.3)	(−)	(66.0)	(4.7)	(26)	(100)	(17)	(22)	(44)	(19)	(−)
5CA*b	CH_3	$C_6H_{11}F_3NPO_4$	249	217	234	180	154	140	110	95	80	79	69	44
		(249.1)	(1.4)	(−)	(−)	(0.8)	(0.3)	(20)	(100)	(8.9)	(17)	(14)	(9.8)	(0.4)
5CA*c	C_2H_5	$C_7H_{13}F_3NPO_4$	263	232	234	194	166	154	110	95	80	79	69	58
		(263.1)	(2.7)	(0.2)	(1.1)	(1.1)	(0.3)	(22)	(100)	(5.0)	(11)	(9.7)	(5.1)	(0.2)
5CA*d	$CH_3(CH_3)$	$C_7H_{13}F_3NPO_4$	263	232	234[b]	194	166	154	110	95	80	79	69	58
		(263.1)	(4.6)	(0.2)	(−)	(0.7)	(4.0)	(91)	(100)	(7.8)	(18)	(13)	(11)	(0.5)[e]
5CA*e	*n*-C_4H_9	$C_9H_{17}F_3NPO_4$	291	260	234[c]	222	194	182	110	95	80	79	69	86
		(291.2)	(0.4)	(0.2)	(0.4)	(0.5)	(0.2)	(17)	(100)	(3.4)	(22)	(8.4)	(22)	(0.2)
5CA*f	C_6H_5	$C_{11}H_{15}F_3NPO_4$	311	280	234	242	214	202	110	95	80	79	69	106[f]
		(311.2)	(29.0)	(0.2)	(−)	(−)	(1.6)	(100)	(16)	(1.8)	(4)	(12)	(4.2)	(0.1)
5CA*g	$C_6H_5CH_2$	$C_{12}H_{17}F_3NPO_4$	325	294	234	256	228	215	110	95	80	79	69	120
		(325.2)	(25.0)	(2.5)	(2.5)	(−)	(−)	(100)	(11)	(1.6)	(2.3)	(6.1)	(1.7)	(1.3)

[a] $(MeO)_2POH$ [110], (MeO)P(O)(OH) [95], HPO_3 [80], PO_3 [79].
[b] [248]; (1.1%).
[c] [235]; (7.1%).
[d] [109]; (60%).
[e] [59]; (24%).
[f] [107]; (16%).

Table 7
Reduced mass spectra of O,O-diethyl 1-(trifluoroacetylamino)alkanephosphonates **5CB** *

No.	R ($R^1 \neq H$)	Molecular formula (mass)	Mass spectrometry: *m/z* (relative intensity, %)											
			M	M − R	M − EtO [M − 45]	M − CF_3 [M − 69]	M − TFA [M − 97]	M − NHTFA [M − 112]	M − $(EtO)_2PO$ [M − 138]	Phosphonyl-derived ions[a]			CF_3 [69]	$RCH{=}NH_2^+$ [R + 29]
										[138]	[111]	[82]		
5CB * a	H	$C_7H_{13}F_3NPO_4$	263	262	218	194	156	150	126	138	111	82	69	30
		(263.2)	(54.0)	(−)	(18.0)	(4.6)	(0.6)	(1.9)	(55)	(100)	(50)	(34)	(22)	(5.4)
5CB * b	CH_3	$C_8H_{15}F_3NPO_4$	277	262	232	208	180	164	140	138	111	82	69	44
		(277.2)	(3.5)	(−)	(5.0)	(1.0)	(1.7)	(0.1)	(29)	(100)	(56)	(34)	(10)	(2.0)
5CB * c	C_2H_5	$C_9H_{17}F_3NPO_4$	291	262[b]	246	222	194	179	154	138	111	82	69	58
		(291.1)	(6.1)	(0.3)	(3.7)	(0.7)	(0.3)	(0.2)	(32)	(100)	(61)	(19)	(6.6)	(0.5)
5CB * d	$CH_3(CH_3)$	$C_9H_{17}F_3NPO_4$	291	262[c]	246	222	194	179	154	138	111	82	69	58
		(291.1)	(4.0)	(−)	(4.2)	(1.0)	(2.4)	(1.5)	(18)	(100)	(68)	(29)	(6.6)	(0.6)
5CB * e	*n*-C_4H_9	$C_{11}H_{21}F_3NPO_4$	319	262[d]	274	250	222	207	182	138	111	82	69	86
		(319.3)	(1.0)	(0.2)	(2.7)	(0.2)	(0.2)	(3.1)	(6.4)	(100)	(43)	(13)	(2.3)	(0.2)
5CB * f	C_6H_5	$C_{13}H_{19}F_3NPO_4$	339	262	294	270	242	227	202	138	111	82	69	106[f]
		(339.3)	(53.0)	(−)	(2.6)	(0.3)	(2.0)	(0.3)	(100)	(56)	(40)	(15)	(8.5)	(0.5)
5CB * g	$C_6H_5CH_2$	$C_{14}H_{21}F_3NPO_4$	353	262	308	284	256	241[e]	215	138	111	82	69	120
		(353.3)	(25.0)	(2.8)	(2.5)	(0.2)	(0.1)	(4.4)	(100)	(66)	(37)	(12)	(3.8)	(2.0)

[a] $(EtO)_2POH$ [138], $(EtO)P(H)(OH)_2$ [111], H_2PO_3 [82].
[b] [263]; (1.3%).
[c] [276]; (0.3%).
[d] [263]; (8.2%).
[e] [240]; (13%).
[f] [107]; (15%).

Table 8
Reduced mass spectra of O-methyl-O-ethyl 1-(trifluoroacetylamino)alkanephosphonates **5CC** *

No.	R ($R^1 \neq H$)	Molecular formula (mass)	Mass spectrometry: *m/z* (relative intensity, %)													
			M	M − EtO[a] [M − 45]	M − R	M − CF_3 [M − 69]	M − TFA [M − 97]	M − HNTFA [M − 112]	M − $(RO)_2PO$ [M − 123]	Phosphonyl-derived ions[a]				CF_3 [69]	RCH=NH_2^+ [R + 29]
										[124]	[97]	[96]	[79]		
5CC*a	H	$C_6H_{11}F_3NPO_4$	249	204	248	180	152	137	126	124	97	96[f]	79	69	30
		(249.2)	(33.0)	(29.0)	(−)	(84.0)	(53.0)	(1.0)	(55)	(83)	(65)	(90)	(55)	(38)	(6.9)
5CC*b	CH_3	$C_7H_{13}F_3NPO_4$	263	218	248	194	166	151	140	124	97	96	79	69	44
		(263.2)	(2.3)	(4.3)	(−)	(1.2)	(4.2)	(0.2)	(36)	(100)	(54)	(61)	(9.6)	(12)	(1.6)
5CC*d	$CH_3(CH_3)$	$C_8H_{15}F_3NPO_4$	277	232	–[e]	208	180	165	154	124	97	96	79	69	58
		(277.1)	(10)	(4.7)	–	(1.6)	(6.7)	(1.4)	(85)	(100)	(64)	(62)	(7.7)	(10)	(0.6)
5CC*f	C_6H_5	$C_{12}H_{17}F_3NPO_4$	325	280	248	256	228	213	202	124	97	96	79	69	114
		(325.3)	(29.0)	(1.0)	(−)	(0.2)	(1.5)	(1.3)	(100)	(18)	(16)	(18)	(11)	(6.4)	(0.2)
5CC*g	$C_6H_5CH_2$	$C_{13}H_{19}F_3NPO_4$	339	294	248	270	242	227[d]	216[e]	124	97	96	79	69	128
		(339.3)	(8.3)	(1.2)	(1.5)	(0.2)	(−)	(2.4)	(14)	(19)	(18)	(13)	(13)	(3.2)	(1.6)

[a] The ions [M − MeO, (M − 31)] exhibited intensity <0.2%.
[b] (EtO)(MeO)POH [124], (MeO)P(H)$(OH)_2$ [97], (MeO)P$(OH)_2$ [96], PO_3 [79].
[c] [262]; (0.9%).
[d] [226]; (7.8%).
[e] [215]; (100%).
[f] [95]; (100%).

3.4. Mass spectral properties of N-acetylaminoalkanephosphonates 5B

The molecular ions $[M]^{+\cdot}$ of these derivatives were observed in low abundance, except the lowest homologue **5Ba** derived from phosphonoglycine (**1a**, Gly^P). Charge localization on the nitrogen atom produced ions $[M-(R^2O)_2P(O)]^+$ and $[M-R]^+$ ions (R is the alkyl chain at C_α) resulting from α-cleavage. The ions $[RCH{-}NH_2]^+$ or $[M-(R^2O)_2P(O)]^+$ constitute the base peaks for the majority of N-acyl derivatives **5BA*** and **5BB***. The ions $[M-1]^+$ and/or $[M-R]^+$, excluding compound **5BB*g**, derived from phosphonophenylalanine (**1f**, Phe^P) were not observed or were observed in very low abundances.

The ions resulting from the competitive charge localization on the phosphonate moiety were very abundant. Thus, the ions at *m/z* 138, 137, 110 and 82 were characteristic of the presence of the O,O-diethyl phosphonate system and had the mechanism of formation reported previously [15]. The ions at *m/z* 110, 109, 95 and 79 were characteristic of the presence of the O,O-dimethyl phosphonate system.

Cleavage of the acetate or acetamide group from the molecular ions of derivatives **5B** gave rise to the ions at $[M-43]^+$ or $[M-57]^+$, respectively. Ions at $[M-42]^{+\cdot}$ which were more abundant for derivatives of **5BB*** than for **5BA***, were produced by the loss of ketene (CH_2=C=O) from $[M]^{+\cdot}$. Considerable scrambling between the acetate methyl hydrogens and the amide hydrogen accompanied these eliminations.

Cleavage of the alkoxy group of the phosphonate moiety from the molecular ions of derivatives **5B** afforded the ions $[M-31]^+$ or $[M-45]^+$.

3.5. Mass spectral properties of N-trifluoroacetylaminoalkanephosphonates 5C

The molecular ions $[M]^{+\cdot}$ of these derivatives were observed in abundances varying from 0.4% (for **5CA*e**) to over 50% (for **5CB*a** and **5CB*f**). Generally the highest abundances occurred for O,O-diethyl derivatives **5CB*** and the lowest for O,O-dimethyl derivatives **5CA***. Charge localization on the nitrogen atom produced ions $[M-(R^2O)_2P(O)]^+$ and $[M-R]^+$ ions (R is the alkyl chain at C_α) resulting from α-cleavage. The ions $[M-(R^2O)_2P(O)]^+$ represent the base peaks for most of the N-trifluoroacetyl derivatives **5BA*** and **5BB***. The ions $[M-1]^+$ were not observed or were observed in very low abundances. The ions $[M-R]^+$ appeared for R > Et and R = $PhCH_2$, and were of low abundance.

The ions resulting from competitive charge localization on the phosphonate moiety were very abundant. Thus, the ions at *m/z* 138, 137, 110 and 82 were characteristic of the presence of the O,O-diethyl phosphonate system. The ions at *m/z* 124, 97, 96 and 81 indicated the presence of the O-ethyl-O-methyl phosphonate system and the ions at *m/z* 110, 109, 95 and 79 were characteristic of the presence of the O,O-dimethyl phosphonate system. The ions $[(R^2O)_2POH]^{+\cdot}$ at *m/z* 138, 124 and 110 constitute the base peaks of derivatives **5CB***, **5CC*** and **5CA***, respectively.

Cleavage of the trifluoromethyl, the trifluoroacetyl or the trifluoroacetamide group from the molecular ions of derivatives **5C** gave rise to ions at $[M-69]^+$, $[M-97]^+$ or $[M-112]^+$, respectively. The ions $[RCH{=}NH_2]^{+\cdot}$ appeared at low abundances, if they were observed at all.

Cleavage of the alkoxy group of the phosphate moiety from the molecular ions of derivatives **5CA*** afforded the ions $[M-31]^+$. The corresponding cleavage in the derivatives **5CB*** and **5CC*** gave rise to the ions $[M-45]^+$.

4. Conclusions

A general derivatization method for aminoalkanephosphonic acids using carboxylic anhydrides–trialkyl orthoesters has been developed. ^{31}P NMR spectroscopic investigations confirmed the complete conversion of 1-aminoalkanephosphonic acids into the volatile diester

derivatives **5** within 2 h. The ester products of this reaction, N-acetyl (**5B**) or N-trifluoroacetyl (**5C**) derivatives of aminoalkanephosphonates are satisfactory for characterization by both GC and GC–MS. The mass spectra of these derivatives were structurally informative. Ions characteristic of the phosphonate system were present in the mass spectra of all derivatives **5**, permitting rapid identification of these compounds.

References

[1] L. Maier, *Chimia*, 23 (1969) 323.
[2] K.A. Pietrov, V.A. Chauzov and T.S. Erokhina, *Usp. Khim.*, 43 (1974) 2045.
[3] P. Kafarski, B. Lejczak and P. Mastalerz, *Beitrage Wirkstofforschung*, 25 (1985) 1.
[4] P. Kafarski and P. Mastalerz, *Beitr. Wirkstofforschung*, 21 (1984) 1.
[5] F. Eisenberg in M. Halmann (Editor), *Analytical Chemistry of Phosphorous Compounds*, Wiley-Interscience, New York, 1972, p. 69.
[6] Y. Kiso, H. Kobayashi and Y. Kitaoka, in M. Halmann (Editor), *Analytical Chemistry of Phosphorus Compounds*, Wiley-Interscience, New York, 1972, p. 93.
[7] M. Horiguchi, in M. Halmann (Editor), *Analytical Chemistry of Phosphorus Compounds*, Wiley-Interscience, New York, 1972, p. 703.
[8] Z.H. Kudzin, M. Sochacki and W. Kopycki, *Phosphorus Sulfur Silicon*, 77 (1993) 207.
[9] Z.H. Kudzin, M. Sochacki and W. Kopycki, *J. Chromatogr. A*, 655 (1993) 346.
[10] Z.H. Kudzin and A. Kotyński, *Synthesis*, (1984) 1028.
[11] Z.H. Kudzin and W.J. Stec, *Synthesis*, (1978) 469.
[12] B. Gallenkamp, W. Hofer, B.W. Kruger, F. Haurer and T. Pfister, in M. Regitz (Editor), *Methoden der Organischen Chemie (Houben–Weyl)*, Vol. III, Georg Thieme, Stuttgart, 1982, p. 300.
[13] M.L. Ruppel, C.A. Suba and J.T. Marvel, *Biomed. Mass Spectrom.*, 9 (1976) 28.
[14] J.W. Huber, *J. Chromatogr.*, 152 (1978) 220.
[15] T. Nishiwaki, *Tetrahedron*, 23 (1967) 2187.