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Enantiodifferentiation of aminophosphonic and aminophosphinic acids with α- and β-cyclodextrins

Łukasz Berlicki, Ewa Rudzińska and Paweł Kafarski*

Institute of Organic Chemistry, Biochemistry and Biotechnology, Wrocław University of Technology, Wybrzeże Wyspiańskiego 27, 50-370 Wrocław, Poland

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Abstract—Cyclodextrins were used as chiral selectors for the ^{31}P NMR determination of the enantiomeric excess of aminoalkanephosphonic and aminoalkanephosphinic acids. Most of these acids form inclusion complexes with α- and/or β-cyclodextrin and upon increasing the cyclodextrin to aminophosphonic acid molar ratio ^{31}P NMR signals for (*R*)- and (*S*)-enantiomers separate. ROESY spectra allowed the determination of structures of the inclusion complexes.

1. Introduction

Aminoalkanephosphonic and aminoalkanephosphinic acids constitute a class of interesting mimetics of amino acids, whose importance derives from their promising and diverse biological activity.[1] This activity is usually based on the action of only one enantiomer of these acids and therefore it is of importance to determine reliably their enantiomeric excess. The most often applied and the easiest to perform method is the comparison of the specific rotation with the highest value reported in literature. However, this method is not applicable to new compounds and often not reliable for already known ones. There are numerous methods for the determination of enantiomeric excesses of aminophosphionic acids, which require their derivatisation prior to analysis,[2] but few methods have been developed, which enable their direct determination. These are HPLC with chiral stationary phase,[3] capillary electrophoresis[4] and NMR analysis of chiral complexes with palladium(II).[5]

^{31}P NMR spectroscopy is a very convenient tool for the determination of the enantiomeric purity of organophosphorus compounds because of the large chemical dispersion and the simplicity of the broad band ^{1}H decoupled spectra.[6] Determination of the enantiomeric excess might be achieved by application of either chiral derivatizing agents or chiral solvating agents. Indeed, these two approaches have been successively used for the determination of the enantiomeric excess of dialkyl amino- and hydroxyalkanephosphonates.[7] It is also known that aromatic amino acids form inclusion complexes with cyclodextrins.[8] This phenomenon has been studied by several methods, however NMR spectroscopy appeared to be the most informative technique,[9] which is well illustrated by the recent use of ^{19}F NMR for studying enantiomeric recognition of an organofluorine derivative complexed by γ-cyclodextrin.[10] As established by capillary electrophoresis, aminophosphonic acids bearing aromatic substituent in their side chain are also able to form inclusion complexes with cyclodextrins.[4]

The objective of this paper is to describe a new method for the determination of the enantiomeric composition of aminoalkanephosphonic and aminoalkanephosphinic acids by means of ^{31}P NMR spectroscopy with the use of cyclodextrins as chiral discriminators. The optimisation of the method was performed taking into account the influence of cyclodextrin to guest ratio and the pH of the solution. In order to understand better the observed phenomenon some more detailed studies of selected cyclodextrin-aminophosphonate inclusion complexes were performed by means of 2D ROESY spectra. Additionally molecular modelling studies allowed solution structures of the described complexes to be proposed.

* Corresponding author. Tel.: +40-71-320-36-82; fax: +48-71-328-40-64; e-mail: kafarski@kchf.ch.pwr.wroc.pl

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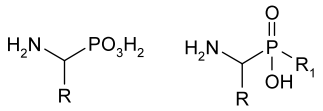
2. Results and discussion

We have recently shown that the formation of inclusion complexes between aromatic aminoalkanephosphonic **1** and aminoalkanephosphinic **2** acids with cyclodextrins forms a basis for their enantiomeric resolution.[4]



1 **2**

When dissolving a racemic mixture of an aminophosphonic acid in solution containing cyclodextrin two diastereomeric complexes are formed and in most cases two signals were observed in the ^{31}P NMR spectra. In order to optimise the analytical procedure titration of the phosphonic acid analogue of phenylglycine **1a** with α-cyclodextrin was performed. Results presented in Figure 1 clearly show that when the cyclodextrin to guest ratio exceeded 4, a concentration dependent separation of two signals was observed. This results from the fact that under equilibrium on the NMR time scale the free form of **1a** is in a dynamic exchange with its complexed form. Therefore, the highest possible cyclodextrin to guest ratio is recommended for analysis. For further experiments we have chosen a 1:10 guest to cyclodextrin molar ratio, optimal in consideration of the limited solubility of β-cyclodextrin and limited sensitivity of the NMR spectrometer.

Because of the ionic structure of aminophosphonates the pH of the solution is considered as another factor possibly influencing their separation. Therefore, the dependence of separation efficiency for a 1:10 mixture of **1a** and α-cyclodextrin versus pH of the solution was studied (Fig. 2). The separation efficiency did not change drastically with pH, however, better results were obtained for solutions of low pH. Probably equilibrium of free versus complexed aminophosphonic acid is shifted more towards complex formation in acidic solution than under basic conditions and the protonated forms of the acids in acidic solutions yield more stable complexes with α-cyclodextrin

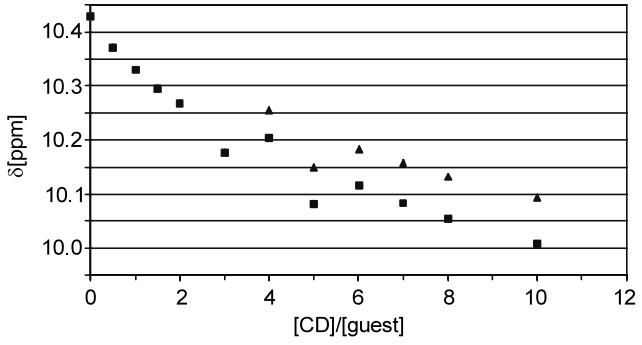


Figure 1. ^{31}P NMR shift change upon titration of **1a** with α-cyclodextrin.

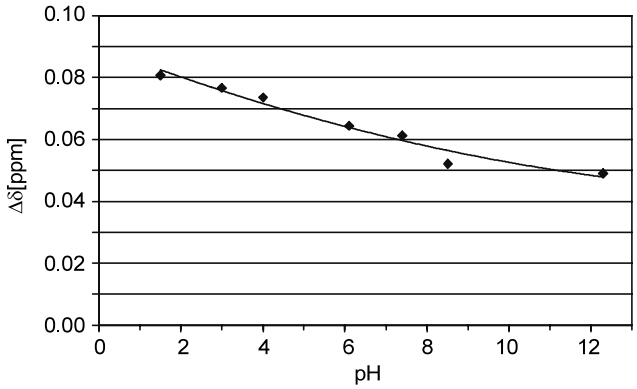


Figure 2. Dependence of separation of ^{31}P NMR signals for 1:10 mixture of α-cyclodextrin and **1a** versus pH.

In order to check the utility of our method we have used a set of structurally diverse aminophosphonic and aminophosphinic acids and have done spectroscopic measurements on their 1:10 mixtures with α- and β-cyclodextrins as chiral discriminators. ^{31}P NMR data presented in Table 1 revealed that most of the studied compounds form inclusion complexes with α-cyclodextrin, while β-cyclodextrin was less efficient. This was shown by comparison of the separation efficiencies obtained for both cyclodextrins used at the same concentration (10 mM). Elevation of the α-cyclodextrin concentration up to 100 mM resulted in better signal separation, usually base-line separation of signals deriving from each enantiomer (see representative example in Fig. 3). Quite interestingly, it also resulted in good separation of ^{31}P NMR signals derived from aminophosphinic acids **2**. Thus, our procedure is far simpler, more efficient and a more general method for the determination of enantiomeric composition of aminophosphonic and aminophosphinic acids than currently described in the literature.[5]

The stability of the complex is visibly dependent on the structure of the studied aminophosphonic acid. For nonfunctionalised aromatic and aliphatic acids the most important factor is the fit of their side chains in cyclodextrin cavity. Smaller side chains fit better into the α-cyclodextrin cavity, whereas bigger ones prefer β-cyclodextrin. The introduction of additional functionality into the side chain quite dramatically influences the binding affinity. For example, strongly polar groups such as -OH, -NH_2, -OCH_3 attached to the phenyl ring of aromatic aminophosphonic acids, compounds **1i**, **1j**, **1k**, did not allow the formation of the complex with cyclodextrins and thus no signal separation was observed. More neutral chlorine compounds **1e**–**h**, did not inhibit complex formation and good separation of signals corresponding to the two enantiomers was achieved. It is worth noting that position of the substituent on the phenyl ring of the chlorine atom did not influence significantly the signal separation.

For compounds **1e**, **1f**, **1g**, and **1j** more detailed structural studies were done. The formation of inclusion

Table 1. Separation of ^{31}P NMR signals for mixture of aminophosphonic **1** or aminophosphinic **2** acids with α-(αCD) and β-cyclodextrin (βCD) applied 1:10 ratio. NS: not separated, BS: broad signal

Entry	R	R^1	Δδ (ppm) for αCD applied at concentration of 100 mM and *10 mM*	Δδ (ppm) for βCD applied at concentration of 10 mM
1a	C_6H_5	OH	0.085 *0.015*	NS
1b	$C_6H_5CH_2$	OH	NS *NS*	0.058
1c	$C_6H_5CH_2CH_2$	OH	0.126 *0.046*	NS
1d	2-Naphtyl	OH	0.107 *0.070*	0.071
1e	*o*-Cl-C_6H_4	OH	0.070 *NS*	NS
1f	*m*-Cl-C_6H_4	OH	0.366 *0.297*	NS
1g	*p*-Cl-C_6H_4	OH	0.065 *0.067*	NS
1h	1,3-Di-Cl-C_6H_3	OH	0.179 *0.350*	NS
1i	*p*-NH_2-C_6H_4	OH	NS *NS*	NS
1j	*p*-HO-C_6H_4	OH	NS *NS*	NS
1k	*p*-CH_3O-C_6H_4	OH	NS *NS*	NS
1l	$(CH_3)_2CH$	OH	BS	NS
1m	$(CH_3)_2CHCH_2$	OH	0.081 *0.010*	NS
1n	Cyclopropyl	OH	BS	NS
1o	Cyclopentyl-CH_2	OH	0.631 *0.205*	NS
1p	Cyclohexyl-CH_2	OH	0.074 *0.044*	0.055
2a	C_6H_5	H	0.438 *BS*	NS
2b	$C_6H_5CH_2$	H	NS *NS*	BS
2c	$C_6H_5CH_2CH_2$	H	0.022 *BS*	0.291
2d	C_6H_5	CH_3	0.019 *BS*	NS
2e	C_6H_5	C_2H_5	0.041 *BS*	NS
2f	$(CH_3)_2CHCH_2$	H	0.426 *BS*	NS

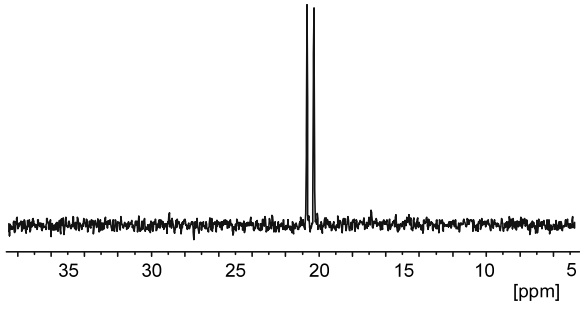


Figure 3. ^{31}P NMR spectrum of 1:10 mixture of compound **1m** with α-cyclodextrin.

complexes is visible not only by separation of ^{31}P signals but also by the observed shift of signals of protons affected by complex formation. We have found that application of ROESY spectra enabled the determination of the mode of binding of aminophosphonic acids inside the hydrophobic cyclodextrin cavity. The representative region of the ROESY spectrum of a 1:1 mixture of **1e** and α-cyclodextrin is presented in Figure 4. The proposed structure of this complex and visualisation of observed contacts are shown schematically in Figure 5a. Similar experiments performed for compounds **1f**, **1g**, and **1j** allowed their binding modes by α-cyclodextrin as shown in Figure 5b, c and d to be proposed. Uncharged, hydrophobic fragments of the molecules (aromatic or aliphatic) are placed inside the cyclodextrin cavity, while the charged aminophosphonic fragment is directed towards the aqueous solution. It is worth noting that no uniform mode of binding was obtained and that the structure of the complex between aminophosphonic acid and cyclodextrin depends on features of both compounds.

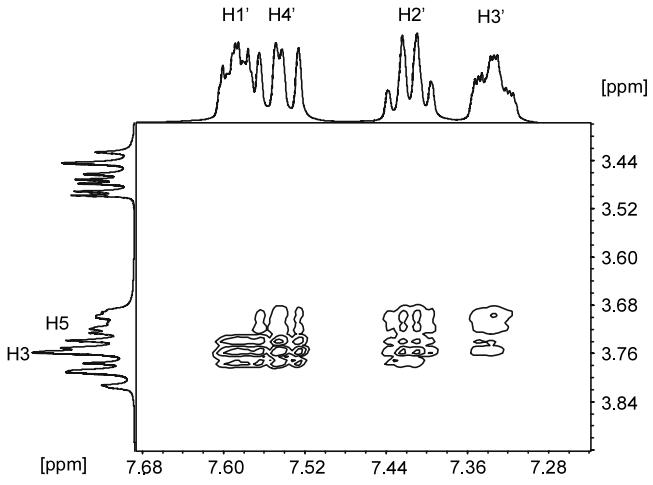


Figure 4. Selected region of ROESY spectrum for 1:1 mixture of compound **1e** and α-cyclodextrin.

Crosspeaks between aromatic protons of the guest molecule and H3 and H5 protons of cyclodextrin define close contacts between these protons. However, not all aromatic protons of **1e** are close to both H3 and H5 protons of cyclodextrin. H1′ proton of the guest molecule is far from the H5 proton of cyclodextrin (no crosspeak was observed) and the H3′ proton yielded a relatively small ROE so it has to be further from H3 than the H1′, H2′ and H4′ protons.

Using molecular modelling we computed the solution structures of investigated complexes.[11] With information from the ROESY spectra the structure of aminophosphonic acid and α-cyclodextrin complex in a box of water was minimised. The structures obtained in that way were consistent with these presented in Figure 5a–d. Then all restraints were removed and molecular dynamics during 60 ps was done. This step of simulations did not result in any significant change of the structures of these complexes thus enforcing our NMR based determination of their architecture.

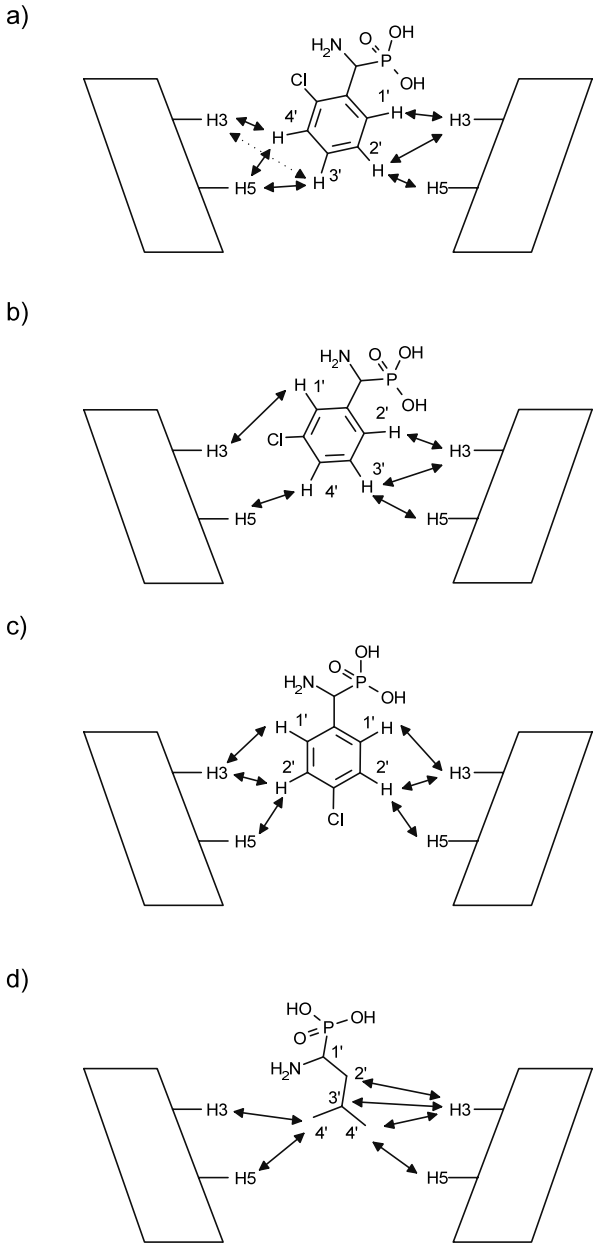


Figure 5. Proposed models of inclusion complexes of studied compounds according to ROE restraints: (a) **1e**; (b) **1f**; (c) **1g**; (d) **1j**.

3. Experimental

D_2O was purchased from Dr. Glaser, AG Basel, α-cyclodextrin and β-cyclodextrin were from Aldrich. All aminophosphonic and aminophosphinic acids were synthesised according to standard procedures.[12] The 1D NMR measurements were done using Bruker Avance DRX 300 instrument, operating at 300.13 MHz (^{1}H) and 121.499 MHz (^{31}P), respectively, at 298 K. Measurements were made in D_2O (99.8 at.% D) solutions. 10 mM solutions of aminophosphonic acids and 100 mM of α-cyclodextrin or 1 mM of these guest compounds and 10 mM α- or β-cyclodextrin in D_2O were used. TMS for ^{1}H spectra or 85% phosphoric acid in H_2O for ^{31}P spectra were used as external standards. ROESY experiments were recorded on Bruker AMX 500 MHz instrument at 298 K using 100 mM solution of guest compound and 100 mM of α-cyclodextrin. The experiments were carried out at 700 ms mixing time.

Calculations were done with the Accelrys Insight 2000[13] molecular modelling package on a Silicon Graphics Onyx workstation. All minimisations and molecular dynamics simulations were done with the program Discover[14] using cff97[15] molecular force field. The conjugate gradient algorithm was applied for minimisation. Default values were assumed for all parameters. The initial structures of aminophosphonic acids and cyclodextrin were built in Builder module from standard fragments and initially minimised in vacuo. The

complex was placed in box of water of dimension: 30 Å×30 Å×30 Å. The whole system was minimised with restrains from ROESY spectra up to RMS derivative equal or less then 0.1 kcal/mol. Molecular dynamics was performed on minimised system during 60 ps with 1 fs time step at 300 K (with 100 steps of equilibration). The co-ordinates were saved every 0.1 ps.

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