Heteroatom Chemistry
Volume 15, Number 5, 2004

^{13}C and ^{31}P High Resolution Solid State NMR Studies of Cyclophosphamide and Its Analogues

Elżbieta J. Tadeusiak, Sebastian Olejniczak, and Włodzimierz Ciesielski

Department of Structural Studies, Centre of Molecular and Macromolecular Studies, Polish Academy of Sciences, Sienkiewicza 112, 90-363 Łódź, Poland

Received 22 March 2004; revised 3 May 2004

ABSTRACT: *Three anticancer agents cyclophosphamide, iphosphamide, and bromophosphamide were studied using ^{13}C and ^{31}P SS NMR.* © 2004 Wiley Periodicals, Inc. Heteroatom Chem 15:388–394, 2004; Published online in Wiley InterScience (www.interscience.wiley.com). DOI 10.1002/hc.20028

INTRODUCTION

Cyclophosphamide (CP), iphosphamide (IF), and bromophosphamide (BF) (Fig. 1) are anticancer agents [1–4]. These compounds were studied in advance by X-ray crystallography [5–13], ODESSA experiment [14,15], and XRPD [16]. These oxazaphosphinanes are solid, so solid state NMR (SS NMR) study is very useful for them [17]. In this work we present ^{13}C and ^{31}P solid state NMR studies of these compounds.

Pharmaceutical drugs can crystallize in more than one crystallographic form (polymorph, crystalline modification). These differences in the crystalline structure can affect the physicochemical parameters of the substance (solubility, dissolution rate, hardness, etc.), which in turn can have an impact on important pharmaceutical properties of a drug (bioavailability, stability, dosage form). It is worthy to note that we observed two polymorphs in the case of IF crystallized from methylcyclohexane.

Correspondence to: Elżbieta J. Tadeusiak; e-mail: elatad@bilbo.cbmm.lodz.pl.

RESULTS AND DISCUSSION

^{31}P Solid State NMR

The ^{31}P nucleus is a very attractive probe for structural studies of phosphoroorganic compounds because of the high sensitivity and 100% natural abundance [18–20]. The room temperature ^{31}P CP/MAS spectra of the oxazaphosphinanes CP, IF, and BF show a set of spinning sidebands from the large chemical shielding anisotropy (CSA). The principal components of the ^{31}P chemical shift tensors δ_{ii} were calculated from spinning sidebands intensities employing WIN-MAS program that is based on the Berger–Herzfeld algorithm [21,22]. The calculated values of principal tensors elements δ_{ii} and shielding parameters are given in Table 1.

The accuracy of calculations was confirmed by comparison with experimental and theoretical spectra (Fig. 2).

The analysis of principal elements of ^{31}P chemical shift tensor is a source of structural information. It is well known that anisotropy parameter Ω reflects the distortion of molecular structure from ideal tetrahedral, whereas the asymmetry parameter κ corresponds to asymmetry of electron-density distribution about central atoms [23]. As

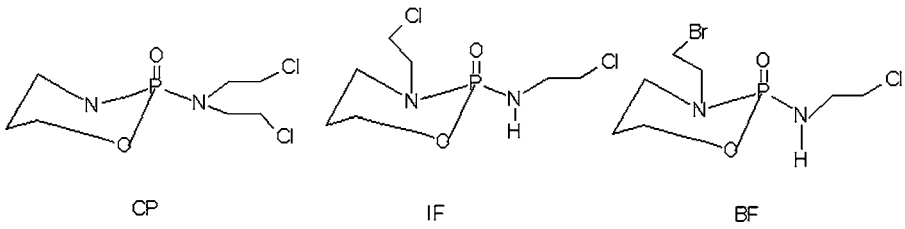

FIGURE 1 The anticancer drugs: CP, IF, and BF.

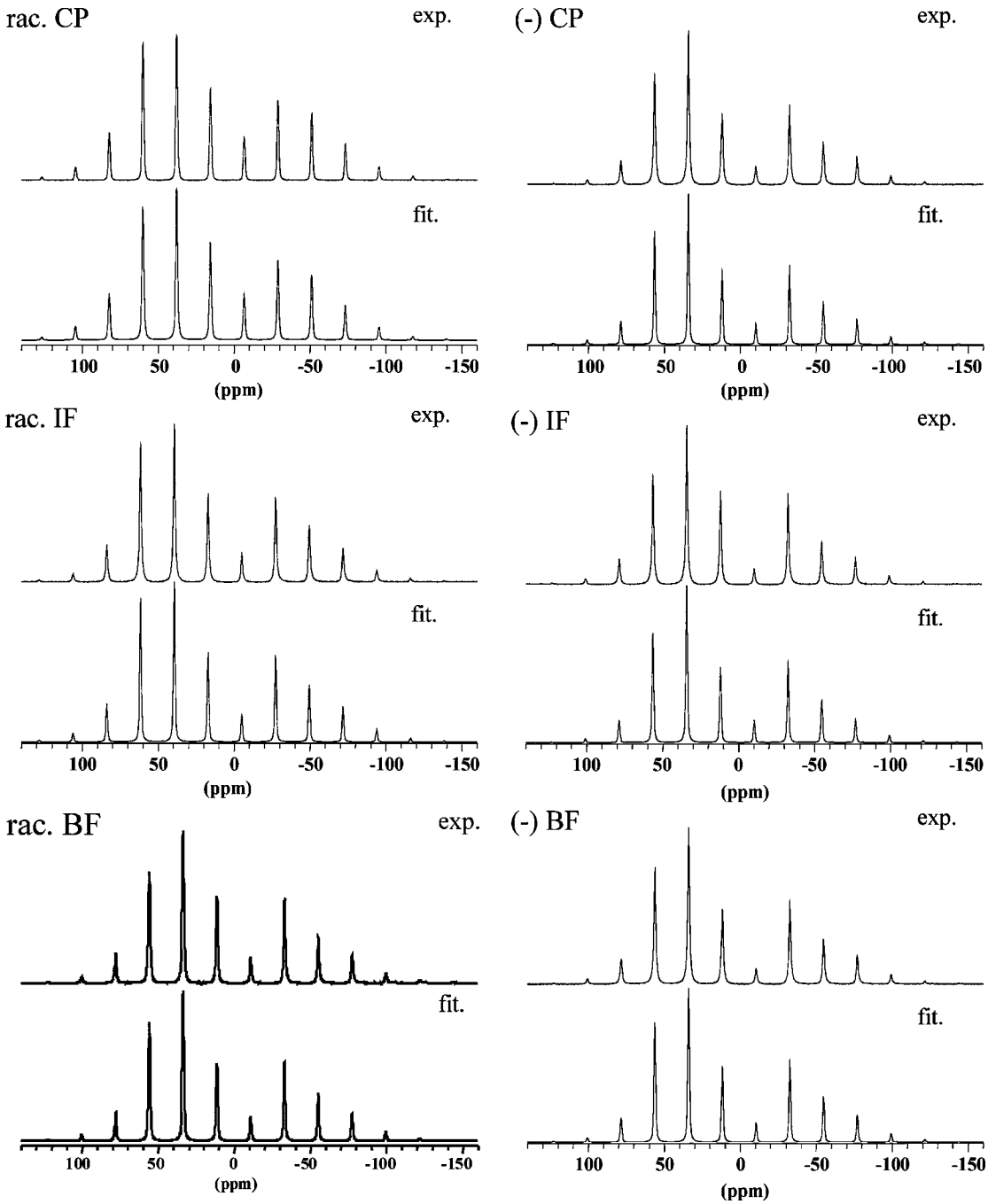

FIGURE 2 The theoretical and experimental ^{31}P SS NMR spectra of compounds CP, IF, and BF.

TABLE 1 The Calculated Values of Principal Tensors Elements δ_{ii} and Shielding Parameters of Compounds CP, IF, and BF

Compound	δ_{11} *(ppm)*	δ_{22} *(ppm)*	δ_{33} *(ppm)*	δ_{iso}[a] *(ppm)*	Ω[b] *(ppm)*	κ[c] *(ppm)*
Rac. CP	93	45	−92	15	185	0.49
(−) CP	70	56	−91	12	161	0.82
Rac. BF	76	52	−93	11	169	0.71
(−) BF	68	60	−92	12	160	0.90
Rac. IF	85	58	−91	17	176	0.70
(−) IF	71	55	−90	12	160	0.80
Rac. IF/methylcyclohexane	84	59	−90	17	174	0.70
	66	59	−87	12	153	0.89
Rac. IF/methylcyclohexane after 24 h	94	49	−95	16	188	0.51

Estimated errors in δ_{11}, δ_{22}, δ_{33} are ±3 ppm.
[a] $\delta_{iso} = 1/3(\delta_{11} + \delta_{22} + \delta_{33})$.
[b] $\Omega = \delta_{11} - \delta_{33}$.
[c] $\kappa = 3(\delta_{22} - \delta_{iso})/\Omega$.

seen, the anisotropy parameters (Ω) are different for racemates and enantiomers in each case, and they are always smaller for enantiomers. The values of κ are between 0.49 and 0.9 for a series of oxazaphosphorinanes. This indicates that P shielding is not localized to a particular bond but is averaged out over the entire tetrahedral.

Although a drug substance may exist in two or more polymorphic forms, only one form is thermodynamically stable at given temperature and pressure. The other forms would convert to the stable form. Different polymorphs of the same compound will possess different sets of ^{31}P chemical shift values. We have been using solid state ^{31}P NMR spectroscopy with cross polarization and magic-angle spinning (CP/MAS) to investigate polymorphism in racemate of IF, which was crystallized from methylcyclohexane.

The room-temperature ^{31}P CP/MAS spectra of rac. IF crystallized from methylcyclohexane and those of rac. IF crystallized from methylcyclohexane after 24 h are displayed in Fig. 3. Both spectra show a set of spinning sidebands from the large CSA. In the spectra we can see two polymorphs, which after 24 h converted into one thermodynamically stable form. The ^{31}P δ_{ii} parameters established from spinning sideband analysis for both rac. IF are given in Table 1.

^{13}C Solid State NMR

High resolution solid state ^{13}C NMR spectra for racemates and enantiomers of the studied compounds are shown in Fig. 4. Most of these resonances are singlets but some of them are broadened and split by ^{13}C–^{14}N dipole–dipole coupling [24,25]. We used the two-dimensional phase-adjusted spinning sidebands (2D PASS) experiment [26,27] with the aim of correctly analyzing these spectra.

The 2D PASS technique offers good sensitivity compared to other techniques [28–31] that are used for the separation of isotropic and anisotropic part of spectra with heavily overlapped systems.

Figure 5 displays the 2D PASS spectra of samples rac. BF and (−) BF recorded with a spinning rate of 500 Hz. Due to the separation of the spinning sidebands for each carbon and by employing a calculation procedure, establishing the ^{13}C δ_{ii} parameters was possible by proper data shearing. In this presentation the F2 projection corresponds to the TOSS (Total Suppression of Sidebands) [32,33] spectrum while F1 represents CSA.

The magnitudes of the principal elements of the CSA were obtained from the best-fitting simulated spinning patterns. Simulations of the spinning CSA sidebands spectra were performed on a PC; to calculate the ^{13}Cδ_{ii} parameters we used the SIMPSON program [34].

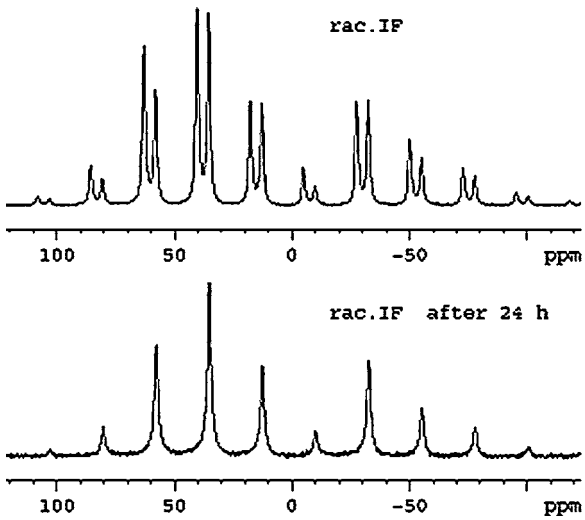

FIGURE 3 The room-temperature ^{31}P CP/MAS spectra of rac. IF crystallized from methylcyclohexane and rac. IF crystallized from methylcyclohexane after 24 h.

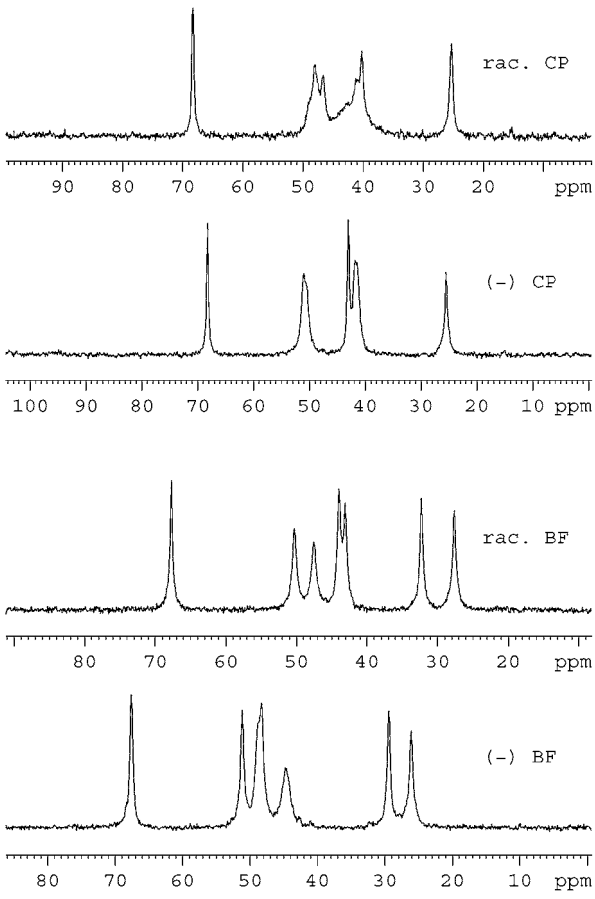

FIGURE 4 High resolution solid state ^{13}C NMR spectra for racemate and enantiomer compounds CP and BF.

The experimental and the best-fitting simulated 1D spinning CSA sideband pattern for selected carbons of rac. BF are given in Fig. 6.

The same procedures (as in the case of rac. BF) were employed to obtained data from the 2D PASS cross-sections of compounds (−) BF, (−) CP, and rac. CP. The obtained principal elements of chemical shift tensors for these samples are given in Table 2.

In conclusion, we have demonstrated 1D and 2D techniques in interpretating the spectra of very important anticancer agents. These results obtained from SS NMR are complementary with those from liquid phase [35–38] and X- ray crystallography [39–44].

EXPERIMENTAL

Solid State NMR Spectroscopy

CP/MAS solid state ^{31}P NMR spectra were run on a spectrometer Bruker, model Avance DSX 300, at 121.46 MHz. All spectra were recorded with a contact time of 1 ms and a repetition rate of 6s; 120 scans were accumulated in the presence of high-power proton decoupling. Powdered samples were placed in a 4 mm ZrO_2 rotor and spun at 2.0–4.5 kHz. ^{31}P chemical shifts were calibrated indirectly through bis(dineopentoxyphosphorothioyl)disulfide resonance signal set at 84.0 ppm.

The solid state CP/MAS ^{13}C experiments were performed on the same spectrometer, Bruker DSX 300, at a frequency of 75.47 MHz , that was equipped

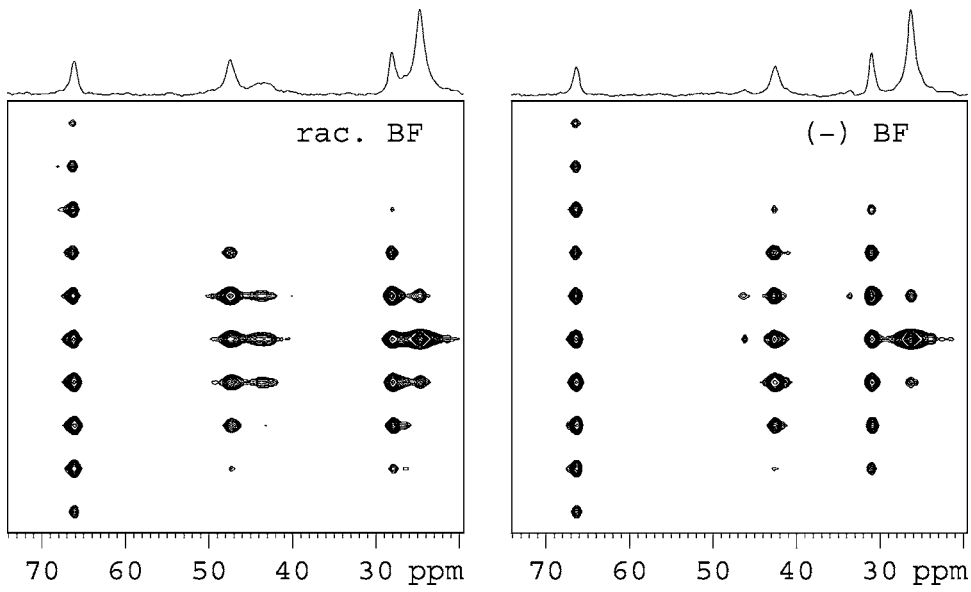

FIGURE 5 2D PASS spectrum of compound BF.

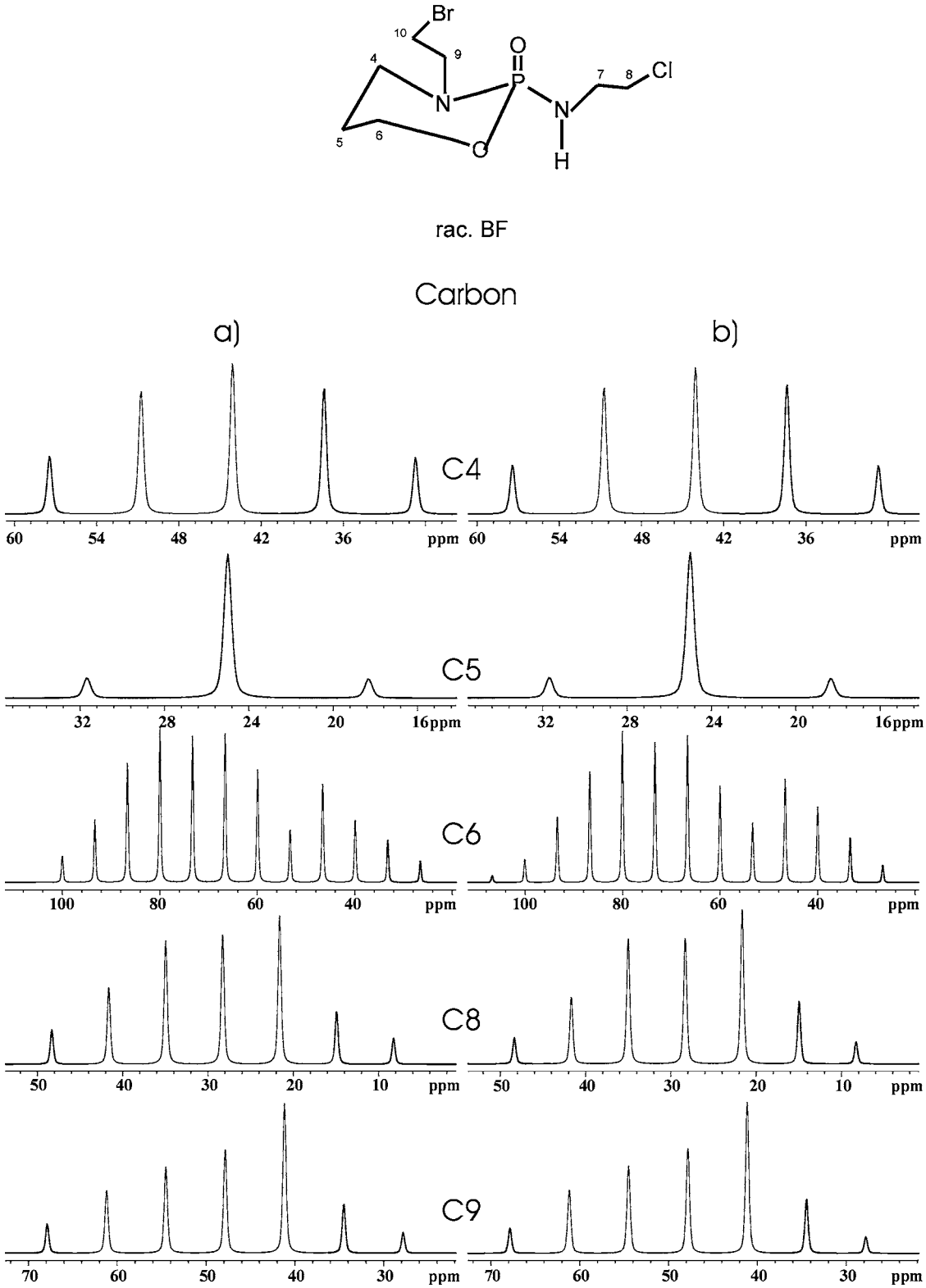

FIGURE 6 The experimental (a) and the best-fitting simulated (b) 1D spinning CSA sideband patterns for selected carbons of rac. BF.

TABLE 2 The Calculated Values of Principal Tensors Elements δ_{ii} of Compounds CP and BF by SIMPSON Program

Compound	δ_{11} *(ppm)*	δ_{22} *(ppm)*	δ_{33} *(ppm)*	δ_{iso}[a] *(ppm)*	Ω[b] *(ppm)*	κ[c] *(ppm)*
Rac. CP	99.0	72.4	29.6	67.0	69.4	0.23
	92.1	48.5	−0.46	46.7	92.5	0.05
	49.8	41.0	25.6	38.8	24.2	0.27
	33.9	23.3	14.2	23.8	19.7	−0.07
(−) CP	166.1	67.5	−33.2	66.8	199.3	0.01
	77.7	53.0	16.0	48.9	61.7	0.19
	88.4	39.6	−7.03	40.3	95.4	−0.02
	67.1	42.4	14.5	41.3	52.6	0.06
Rac. BF	98.7	72.7	27.3	66.2	71.4	0.27
	69.5	43.8	29.2	47.5	40.3	−0.27
	61.4	43.6	26.2	43.7	35.2	−0.01
	48.8	26.6	8.8	28.0	40.0	−0.10
	32.5	25.1	16.8	24.8	15.7	0.05
(−) BF	98.3	71.7	29.2	66.4	69.1	0.23
	61.4	45.3	21.4	42.7	40.0	0.19
	53.8	27.8	11.3	30.9	42.5	−0.21
	34.1	25.2	19.8	26.3	14.3	−0.23

Estimated errors in δ_{11}, δ_{22}, δ_{33} are ±3 ppm.
[a] $\delta_{iso} = 1/3(\delta_{11} + \delta_{22} + \delta_{33})$.
[b] $\Omega = \delta_{11} - \delta_{33}$.
[c] $\kappa = 3(\delta_{22} - \delta_{iso})/\Omega$.

with a MAS probehead, using 4-mm ZrO_2 rotor. A sample of glycine was used to set the Hartmann–Hahn condition and adamantane was used as a secondary chemical-shift reference ($\delta = 38.48$ and 29.46 ppm from external tetramethylsilane (TMS).

The spectra were recorded with a proton 90° pulse length of 4 μs and a contact time of 2.5 ms; the repetition delay was 6 s. The spectra data were processed using WIN NMR program [21,22].

A sample spinning speed of 500 Hz was used in 2D PASS experiments [26,27]. The magnitudes of the principal elements of the CSA were obtained from the best-fitting simulated spinning patterns. Simulations of the spinning CSA sidebands spectra were performed on a PC, using the SIMPSON program under the LINUX environment [34].

ACKNOWLEDGMENTS

The authors thank Dr. Konrad Misiura (CMMS PAS) for the generous gift of oxazaphosphinanes.

REFERENCES

[1] Brock, N. Cancer Res 1989, 49, 1.
[2] McGuigan, M.; Rumack, B.; Micromedex: Poisindex; Micromex: Denver, CO, 1998.
[3] McEnvoy, G. K.; Litvak, K.; Welsh, O. H. AHFS Drug Information; American Society of Health–System Pharmacists: Bethesda, MD, 1998; pp. 779, 824, 835.
[4] Misiura, K.; Kinas, R.; Stec, W. J.; Kuśnierczyk, H.; Radzikowski, C.; Sonoda, A. J Med Chem 1988, 31, 226.
[5] Garcia-Blanco, S.; Perales, A. Acta Crystallogr Sect B 1972, 28, 2647.
[6] Clardy, J. C.; Mosbo, J. A.; Verkade, J. G. J Chem Soc Chem Commun 1972, 24, 1163.
[7] Clardy, J. C.; Mosbo, J. A.; Verkade, J. G. Phosphorus 1974, 4, 151.
[8] Jones, P. G.; Thönnessen, H.; Fischer, A.; Neda, I.; Schmutzler, R.; Engel, J.; Kutscher, B.; Niemeyer, U. Acta Crystallogr Sect C 1996, 52, 2359.
[9] Karle, I. L.; Karle, J. M.; Egan, W.; Zon, G.; Brandt, A. J Am Chem Soc 1977, 99, 4803.
[10] Herbstein, F. H.; Marsh, R. E. Acta Crystallogr Sect B 1998, 54, 677.
[11] Brassfield, H. A.; Jacobsen, R. A.; Verkade, J. G. J Am Chem Soc 1975, 97, 4143.
[12] Adamiak, D. A.; Gdaniec, M.; Pankiewicz, K.; Stec, W. J. Angew Chem Int Ed Engl 1980, 19, 549.
[13] Karolak-Wojciechowska, J.; Wieczorek, M.; Grynkiewicz, G.; Kutner, A. Pol J Chem 1999, 73, 1877.
[14] Potrzebowski, M. J.; Tadeusiak, E.; Misiura, K.; Ciesielski, W.; Bujacz, G.; Tekely, P. Chem Eur J 2002, 8, 5007.
[15] Gerardy-Montouillout, V.; Malveau, C.; Tekely, P.; Olender, Z.; Luz, Z. J Magn Reson Ser A 1996, 123, 7.
[16] Tadeusiak, E. J.; Kruszyński, R.; Misiura, K. Polish J Chem 2004, 78, 431.
[17] Potrzebowski, M. Eur J Org Chem 2003, 8, 1367–1376.
[18] Gorenstein, D. G. Phosphorus-31P NMR. Principles and Applications; Academic Press: Orlando, 1984.
[19] Van Wazer, J. R. Phosphorus and Its Compounds; Wiley-Interscience: NY, 1961.
[20] Verkade, J. G.; Quin, L. D. Phosphorus-31 NMR Spectroscopy in Stereochemical Analysis; VCH Publishers: Deerfield Beach, FL, 1987.
[21] (a) WIN- NMR program, version 6.0; Bruker-Franzen Analytik GmbH: Bremen, 1993; (b) WIN-MAS version

940108, Bruker-Franzen Analytik GmbH: Bremen, 1993.
[22] Herzfeld, J.; Berger, A. J Chem Phys 1980, 73, 6021.
[23] (a) Fyfe, C. A. Solid State NMR for Chemists, CFC Press: Guelph, 1983; (b) Mehring, M. Principle of High Resolution NMR in Solids, 2nd ed.; Springer: Berlin, 1983; (c) Mason, J. Solid State Nuc Magn Reson 1993, 2, 285.
[24] Hexem, J. G.; Frey, M. H.; Opella, S. J. J Chem Phys Lett 1983, 99, 177.
[25] Olivier, A. C. Solid State Nuc Magn Reson 1992, 1, 345.
[26] Antzutkin, O. N.; Shekar, S. C.; Levitt, M. H. J Magn Reson A 1995, 115, 7.
[27] Antzutkin, O. N. Prog NMR Spectrosc 1999, 35, 203.
[28] Frydman, L.; Chingas, G. C.; Lee, Y. K.; Grandinetti, P. J.; Eastman M. A.; Barral, G. A.; Pines, A. J Chem Phys 1992, 97, 480.
[29] Hu, J.; Wang, W.; Liu, F.; Solum, M. S.; Alderman, D. W.; Pugmire, R. J. J Magn Reson Ser A 1995, 113, 210.
[30] Alderman, J. D.; McGeorge, G.; Hu, J. Z.; Pugmire, R. J.; Grant, D. M. Mol Phys 1998, 95, 1113.
[31] Kolbert, A. C.; Griffin, R. G. Chem Phys Lett 1990, 166, 87.
[32] Dixon, W. T.; Schaefer, J.; Sefcik, M. D.; Stejskal, E. O.; McKay, R. A. J Magn Reson 1982, 49, 341.
[33] Dixon, W. T. J Chem Phys 1982, 77, 1800.
[34] Bak, M.; Rasmussen, J. T.; Nielsen, N. C. J Magn Reson 2000, 147, 296.
[35] Misiura, K.; Kinas, R. W.; Stec, W. J.; Kuśnierczyk, H.; Radzikowski, C.; Sonoda, A. J Med Chem 1988, 31, 226.
[36] Gilard, V.; Martino, R.; Malet-Martino, M.-C.; Kutscher, B.; Müller, A.; Niemeyer, U.; Pohl, J.; Polymeropulos, E. E. J Med Chem 1994, 37, 3986.
[37] Struck, R. F.; Thorpe, M. C.; Coburn, W. C., Jr.; Laster, W. R., Jr. J Am Chem Soc 1974, 96, 313.
[38] Gilard, V.; Martino, R.; Malet-Martino, M.; Niemeyer, U.; Pohl, J. J Med Chem 1999, 42, 2542.
[39] Karle, I. L.; Karle, J. M.; Egan, W.; Zon, G.; Brandt, J. A. J Am Chem Soc 1977, 99, 4803.
[40] Clardy, J. C.; Mosbo, J. A.; Verkade, J. G. Phosphorus 1974, 4, 151.
[41] Herbstein, F. H.; Marsh, R. E. Acta Crystallogr Sect B 1998, 54, 677.
[42] Jones, P. G.; Thönnessen, H.; Fischer, A.; Nada, I.; Schmutzler, R.; Engel, J.; Kutscher, B.; Niemeyer, U. Acta Crystallogr Sect C 1996, 52, 2359.
[43] Karolak-Wojciechowska, J.; Wieczorek, M.; Grynkiewicz, G.; Kutner, A. Polish J Chem 1999, 73, 1877.
[44] Perales, A.; Garcia-Blanco, S. Acta Crystallogr Sect B 1977, 33, 1935.