Using Solid-State ^{13}C NMR to Follow up the Synthesis of a New Bioactive *N*-acylhydrazone

E. T. Silva, C. A. Câmara, R. A. S. San Gil*
Departamento de Química Orgânica, Instituto de Química da UFRJ, Ed.CT, bloco A, Cid.Universitária, C.P. 068556 CEP 21941-972 Rio de Janeiro, Brazil
rsangil@iq.ufrj.br

C. A. M. Fraga, E. J. Barreiro
LASSBio, Faculdade de Farmácia, Universidade Federal do Rio de Janeiro. Centro de Ciências da Saúde, Bloco D, Ilha do Fundão-Rio de Janeiro, 21949-900 Brazil

***Keywords**: acylhydrazone, solid state ^{13}C NMR, CPMAS*

***Abstract:** In this communication, solid state ^{13}C NMR is used as a follow up technique for the synthesis of a new N-acylhydrazone with very low solubility in most common solvents. Both cross-polarization magic angle spinning without and with interrupted decoupling were employed. It was possible to confirm that the coupling reaction furnished the desired new hydrazone.*

Introduction

The importance of the hydrazone function (CH=N-NH-) as a pharmacophoric group is well established in several classes of therapeutically useful substances.[1] We have developed a series of useful substances with such functionality, as part of a research program focusing on the synthesis of new isosters of arachidonic acid cascade enzyme inhibitors.[2] We have also identified acylhydrazone derivatives as possessing analgesic profiles, as illustrated by compounds with structure (1) in Figure 1.[3]

In our ongoing research, we promote the isosteric replacement of the pyridine nucleus in (1) by a thiophene ring, resulting in series (2) in order to investigate the importance of the parameters of lipophilicity of such compounds.[4]

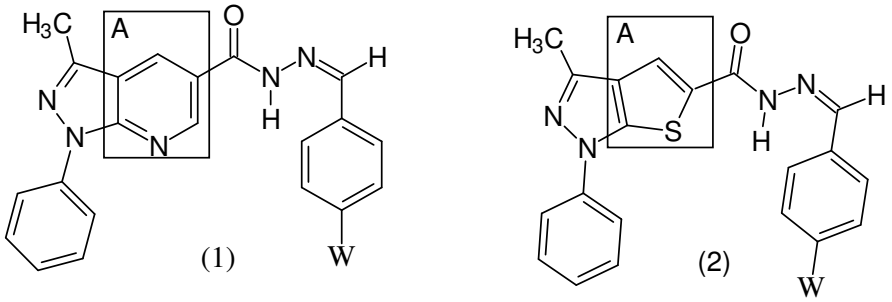

Figure 1. Chemical structures of the series of compounds (1) and (2).

These substances were prepared by the classical condensation reaction between a suitable acylhidrazide derivative (3) and specified aromatic aldehydes[4], as shown in the synthesis of compound (4) in Figure 2.

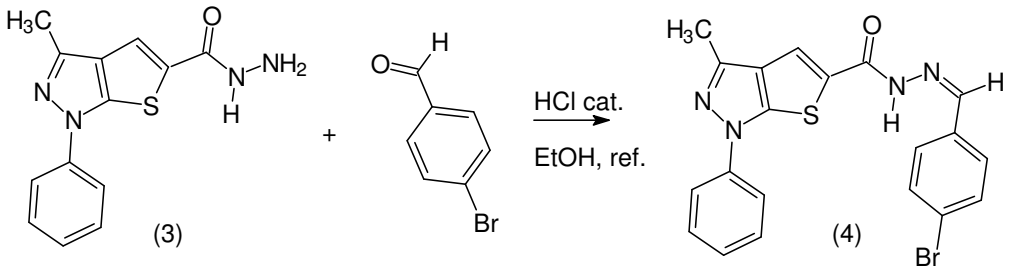

Figure 2. Scheme for preparation of (4'-bromo-benzylidene)-3-methyl-1-phenyl-1*H*-thieno[2,3*c*]pyrazolo-5-acyl hydrazone (compound 4).

Solution ^{1}H and ^{13}C NMR spectroscopy is a powerful follow up technique for the synthesis of biological compounds, both to control the purity of reagents and confirm the chemical structure of the product. However, when the compound of interest has a very low solubility, the characterization by solution NMR becomes a difficult task.

The use of NMR in solid-state is little explored as a structural characterization technique of organic substances. In solid form one can notice the absence of molecular movements which, in solution, allow us to consider as zero the interactions between nuclear dipoles. Therefore, as scalar coupling constants (from the order of Hz) are the determinant factors of the unfoldings observed in the NMR spectra in solution, the couplings due to dipolar interactions (from the order of kHz) and chemical shift anisotropy dominate the NMR spectra of samples with spin ½ nuclei in solid-state. However, magic angle spinning (MAS) technique overcomes broadening in solids due to these couplings. The development of high-resolution solid-state NMR, through MAS and high power decoupling (HPD) on one hand and cross polarization (CP) on the other, reduce dipolar interactions, which are responsible for the enlargement of NMR signals, and ^{13}C signal intensification, respectively. However signal widths are generally larger, when compared to those observed for the organic compound in solution. Thus, solid-state NMR allows the characterization of materials that because of their own nature, cannot be analyzed in solution. This is true for characterization of polymers and heterogeneous catalysts or for studies on conformations in solid-state, such as polymorphism in pharmaceutical products.[5]

This communication presents the results obtained with the use of ^{13}C solid-state NMR to follow up the synthesis of compound (4), which

presents a very low solubility. This feature precludes structural characterization by routine solution NMR. For this purpose, cross polarization magic angle spinning without and with interrupted decoupling were employed[6], as shown in Figure 3.

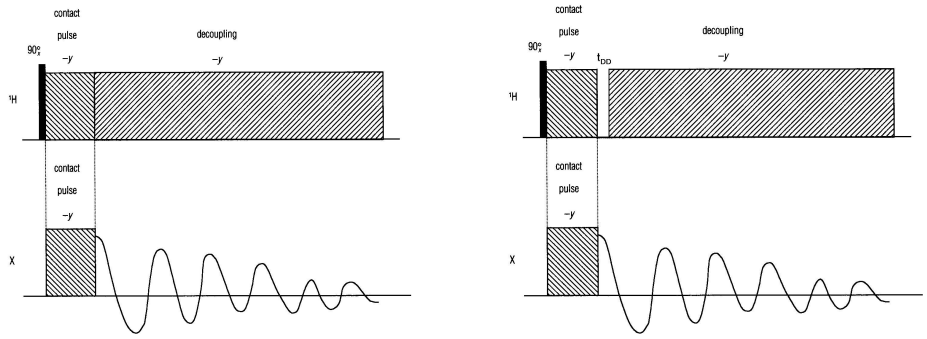

Figure 3. Cross-polarization pulse sequences, without (left) and with (right) interrupted decoupling, used for dipolar dephasing experiments (adapted from ref. 6).

Experimental

Melting points were determined with a Thomas-Hoover apparatus and were uncorrected. Infrared spectra were obtained with a Nicolet-550 Magna spectrophotometer by using potassium bromide plates. Mass spectra (MS) were obtained by electron impact (70 eV) with a GC/VG Micromass 12 spectrometer. Prior to concentration, under reduced pressure, all organic extracts were dried over anhydrous sodium sulfate powder. The progress of all reactions was monitored by thin-layer chromatography (TLC) on 2.0 cm X 6.0 cm aluminum sheets precoated with silica gel 60 (HF-254, Merck) with a thickness of 0.25mm. The chromatograms were analyzed under an ultraviolet light. For column chromatography Merck silica gel (70-230 mesh) was used. The solvents used in the reactions were redistilled prior to use. The reactions were stirred under a dry nitrogen atmosphere.

Synthesis of (4'-bromo-benzylidene)-3-methyl-1-phenyl-1*H*-thieno [2,3*c*] pyrazolo -5- acyl hydrazone (compound 4): stoichiometric quantities of 4-bromo-benzaldehyde (185 mg, 1 mmol) and a few drops of hydrochloric acid as a catalyst were added to an ethanolic solution of hydrazide derivative (3) (288.36 mg, 1 mmol). . At the end of the reaction, monitored by tlc analysis, the compound (4) was isolated by concentrating the reaction mixture under reduced pressure, with cooling and filtration under vacuum. A white amorphous solid, was obtained, yielding 395 mg (90%), m.p.>300^{o}C. IR (KBr): 3141; 2924; 1643; 1504; 1335; 1000-500 cm^{-1}; m/z (%): 439 (M^{+}, 3), 257 (15), 241 (100), 128 (29), 77 (17).

Solid-state ^{13}C NMR spectra were obtained on a BRUKER Avance DRX-300 (7.05 Tesla) spectrometer, operating at 75.47 MHz at ambient temperature and equipped with a supersonic probe and rotors of zirconium oxide with 4mm outer diameter. The pulse sequence used was cross-polarization combined with high power decoupling and magic angle spinning at 6 and 7 kHz (CP/MAS) with contact time of 1000µs, repetition times of 2s and 500 scans, and cross-polarization dipolar dephasing experiments, with contact time of 1000µs, repetition times of 2s and dephasing time of 40 µs. A sample of hexamethylbenzene was used as secondary reference (CH_3 at 17.3 ppm).

Results and discussion

As can be seen in Figure 4, the ^{13}C CP/MAS spectrum of p-bromo benzaldehyde has two signals at 127 and 135 ppm corresponding to carbons of the aromatic ring, and a third one at 195 ppm, due to the carbonyl of the aldehyde group.

The spectrum of acylhydrazide (3) obtained with 6kHz of spinning rate (Figure 5, bottom) shows a more complex pattern, with signals in the aliphatic (at around 10, 30, 50 and 60 ppm) and aromatic regions. The presence of more than one signal in the aliphatic region is unexpected, since the molecule has only one methyl group. Indeed, this signal could be due to spinning side bands of aromatic carbons. On the other side, in the case of more than one conformation present in compound (3), a second signal due to methyl carbon would possibly be seen in the region of 10 ppm.

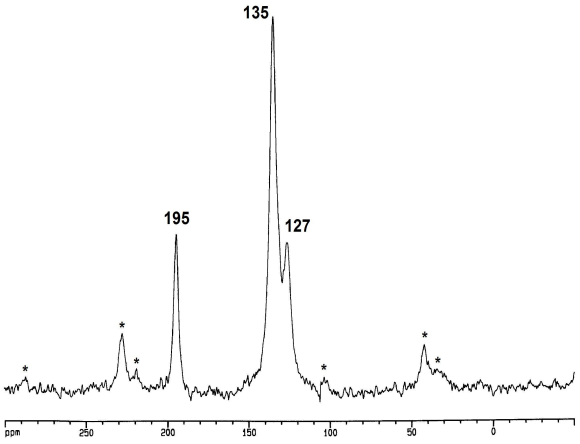

Figure 4. Solid-state ^{13}C CPMAS spectrum of p-bromobenzaldehyde. (*) denotes spinning sidebands

One of the easiest ways to confirm a signal in solid-state NMR is varying this parameter, but spinning sidebands shift.[7] Increase in the spinning rate to 7 kHz (Figure 5, top) did not shift the signals at around 30 and 60 ppm toward lower frequencies, indicating the presence of impurities. The dipolar dephasing spectrum (not shown) showed that the signals at 149, 145, 139 and 128 ppm are due to nonprotonated carbons. Also, the relative intensity of the signal at 128 ppm suggests the presence of two signals at this chemical shift.

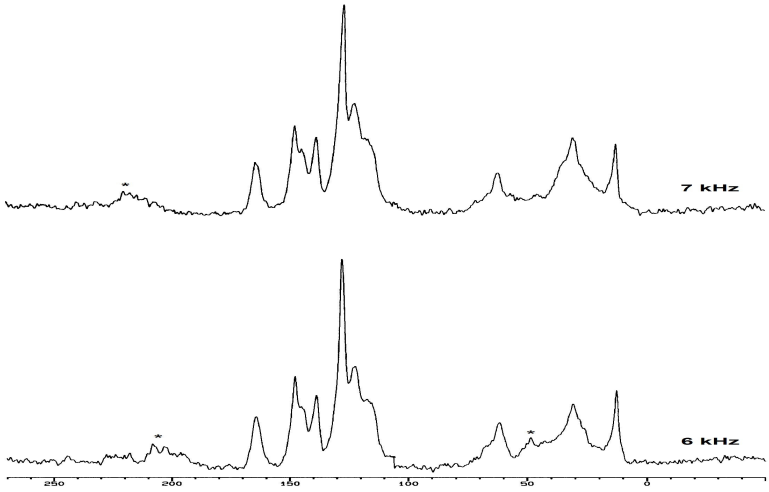

Figure 5. Solid-state ^{13}C CPMAS spectrum of acylhydrazide (3), at spinning rate of 6 kHz (bottom) and 7 kHz (top). (*) denotes spinning sidebands

The solid-state ^{13}C NMR spectrum of product 4 (Figure 6, bottom) shows a signal at 13 ppm, due to CH_3; a signal at 165 ppm, corresponding to the carbonyl group of hydrazide, and signals at 113, 120, 128, 133, 138, 142 and 148 ppm, corresponding to aromatic carbons. The dipolar dephasing spectrum (Figure 6, top) made it possible to distinguish between non protonated and protonated carbons and perform the assignment of the compounds (Table 1).

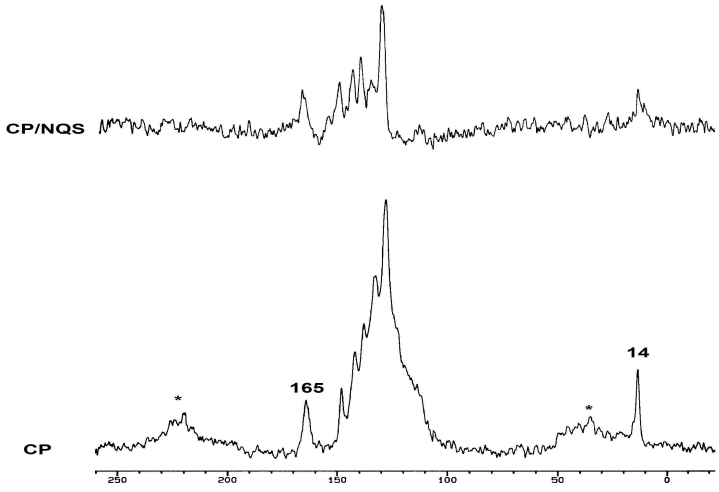

Figure 6. Solid-state ^{13}C CPMAS (bottom) and CP-NQS MAS (top) spectra of acylhydrazone (4). (*) denotes spinning sidebands.

Table 1. Solid-state ^{13}C NMR chemical shifts and assignments of the compounds studied

$\delta^{13}C$, ppm			SIGNAL ASSIGNMENT
p-bromo benzaldehyde	acylhydrazide	acylhydrazone	
195	-	-	C=O aldehyde
-	165	165	NC=O amide, hydrazone
-	148	148	$C_{arom.}$
-	145	-	$C_{arom.}$
-	-	142	$C_{arom.}$
-	139	138	$C_{arom.}$
135	-	133	$C_{arom.}$
-	128	128	$C_{arom.}$
127	-	-	$CH_{arom.}$
-	122	-	$CH_{arom.}$
-	119	120	$CH_{arom.}$
-	117	-	$CH_{arom.}$
-	115	113	$CH_{arom.}$
-	62	-	Impurity
-	31	-	Impurity
-	13	14	CH_3

The absence of a signal at 195 ppm in this spectrum suggests that aldehyde was completely converted to the desired hydrazone. Additionally, the absence of signals at around 30 and 60 ppm indicates that the impurities present in the reagent were completely removed during the purification of the desired product.

Conclusion

The application of solid-state ^{13}C NMR spectroscopy showed to be a valuable tool for the structural determination of biologically important derivatives, as demonstrated by the analysis of compound (4), by confirming the correct features of the mentioned and correlated compounds, when solution spectra cannot be used for structural characterization purposes.

Acknowledgements

The authors would like to acknowledge FAPERJ, CNPq and CAPES for financial assistance.

References

1. C.A.M. Fraga, E.J. Barreiro, *Current Medicinal Chemistry* **13** (2006) 167; P. Melnyk, V. Leroux, C. Sergheraert, P. Grellier, *Bioorg. & Med. Chem. Lett.* **16** (2006) 31.; A.G. Silva, G. Zapata-Sudo, A.E. Kummerle, C.A.M. Fraga, E.J. Barreiro, R.T. Sudo, *Bioorg. & Med. Chem*. **13** (2005) 3431; I.N. Junior, M.C.S. Lourenço, M.G.M.O. Henrique, B. Ferreira, T.R.A. Vasconcelos, M.A. Peralta, P.S.M. de Oliveira, S.M.S.V. Wardel, M.V.N. De Souza, *Lett. Drug Design & Disc*. **2** (2005) 563; G.A. Silva, L.M.M. Costa, F.C.F. Brito, A.L.P. Miranda, E.J. Barreiro, C.A.M. Fraga, *Bioorg. & Med. Chem.* **12** (2004) 3149; Y. Zhang, R.-Z. Qiao, P.-F. Xu, Z.-Y. Zhang, Q. Wang, L-M. Mao, K.-B. Yu, *J. Chinese Chem.*

Soc. **49** (2002) 369; H.K. Wang, S. Y. Liu, K. M. Hwang, G. Taylor, K.H. Lee, Bioorg. & Med. Chem. Lett. **4** (1994) 579; D.W. Combs, M.S. Rampulla, J.P. Demer, R. Falocito, J.B. Moore, *J. Med. Chem.* **35** (1992) 172.

2. C. R. Rodrigues, M. P. Veloso, H. Verli, C. A. M. Fraga, A. L. P. Miranda, E. J. Barreiro, *Current Medicinal Chemistry* **9** (2002) 849; I.A.F.B. Silveira, L.G. Paulo, A.L.P. Miranda, S.O. Rocha, A.C.C. Freitas, E.J. Barreiro, *J. Pharm. Pharmacol.* **45** (1993) 646.
3. L.R.S. Dias, M.J.F. Alvim, A.C.C. Freitas, E.J. Barreiro, A.L.P. Miranda, *J. Pharm. Acta Helv.* **69** (1994) 163.
4. C.A. Câmara, E.T. da Silva, C.A.M. Fraga, E.J. Barreiro, unpublished results.
5. M. Wolniak, M. Tomczyk, J. Gudej, I. Wawer, J. Mol. Struct. **825** (2006) 26.
6. M.J. Duer, Introduction to Solid State NMR Spectroscopy. Blackwell Publishing. 349p. (2004).
7. S. Aime, I. Bertini, C. Luchinat, *Coordination Chem. Rev*. **150** (1996) 221.