APPLIED ORGANOMETALLIC CHEMISTRY
*Appl. Organometal. Chem.* 2003; **17**: 226–231
Published online in Wiley InterScience (www.interscience.wiley.com). DOI:10.1002/aoc.425



# Pentavalent organoantimonial derivatives: two simple and efficient synthetic methods for meglumine antimonate

**Cynthia Demicheli[1]*, Rosemary Ochoa[1], Ivana Silva Lula[1], Fabio C. Gozzo[2], Marcos N. Eberlin[2] and Frédéric Frézard[3]**

[1]Departamento de Química, Universidade Federal de Minas Gerais–UFMG, Belo Horizonte 31270-901, Minas Gerais, Brazil
[2]Instituto de Química, Universidade Estadual de Campinas–UNICAMP, Campinas 13083-970, SP, Brazil
[3]Departamento de Biofísica e Fisiologia, Universidade Federal de Minas Gerais–UFMG, Belo Horizonte 31270-901, Minas Gerais, Brazil

Received 19 August 2002; Accepted 9 January 2003

**Two simple and efficient procedures for the preparation of pentavalent antimony derivatives are described, using either antimony pentachloride ($SbCl_5$) or potassium hexahydroxoantimonate ($KSb(OH)_6$) as sources of antimony(V). These two new methods are evaluated for the synthesis of an important anti-leishmanial drug: meglumine antimonate. Using elemental (carbon, hydrogen, nitrogen) and thermal analysis, atomic absorption (antimony), proton NMR spectroscopy and high-resolution positive-ion electrospray ionization mass spectrometry (ESI(+)-MS), products for the reaction with *N*-methyl-D-glucamine (NMG) using both the $SbCl_5$ and $KSb(OH)_6$ methods were characterized and found to be similar to a commercial sample of the drug. The only notable difference was observed for the ESI-MS spectrum of the $KSb(OH)_6$ product; it displays the same pattern of ESI-generated ions as those of both the $SbCl_5$ product and the commercial drug, but with significantly different abundance ratios. NMR data indicate that the NMG molecules coordinate antimony in two different fashions, which suggests either the coexistence of two different complexes or the existence of a single major complex in which two NMG molecules are coordinated with antimony in an asymmetrical geometry. Copyright © 2003 John Wiley & Sons, Ltd.**

**KEYWORDS:** meglumine antimonate; glucantime; antimony; leishmaniasis; chemotherapy

## INTRODUCTION

The pentavalent organoantimonials meglumine antimonate (antimony(V) *N*-methyl-glucamine) and sodium stibogluconate (sodium antimony(V) gluconate) are in common use for the treatment of leishmaniasis.[1,2] In spite of their wide use for over half a century, the mechanism of action[2–4] and molecular structures and binding sites[5–8] of these organoantimonials have, so far, not been fully elucidated. *Meglumine antimonate*, an amorphous solid susceptible to thermal degradation, readily transforms upon heating into involatile salts, and this property has limited its structural characterization. Fast-atom bombardment (FAB) mass spectrometry (MS) data on the commercially available *meglumine antimonate* (Glucantime) suggested a structure in which two molecules of *N*-methyl-D-glucamine (NMG) coordinate with a single antimony atom in a symmetrical geometry.[6] FAB-MS and positive-ion electrospray ionization (ESI(+)) MS indicated a polymeric structure with the general formula $(NMG–Sb)_n–NMG$.[7] Despite this recent progress in structural characterization using MS techniques, the exact structure and binding sites of NMG to antimony still need further investigation. In addition, one cannot completely exclude the possibility that species generated by FAB and ESI and detected by MS analysis may differ from those present in solution or in the solid phase.

Until now, little has been known about the methods used in industry to prepare pentavalent organoantimonials, and the procedures described in the literature are generally

*Correspondence to: Cynthia Demicheli, Departamento de Química, Instituto de Ciências Exatas, Universidade Federal de Minas Gerais, Av. Antônio Carlos 6627, Pampulha, Belo Horizonte 31270-901, MG, Brazil.
E-mail: demichel@dedalus.lcc.ufmg.br

lengthy and relatively complex.[5,9–12] Inadequate manufacture may also have occurred, as evidenced by the serious side effects produced by some commercial forms of pentavalent organoantimonials.[13] Hence, there is certainly a need for more direct, ideally simple and efficient, synthetic routes for organoantimonials, which would also help to improve the quality control and reproducibility of the industrial process. Moreover, the availability of synthetic products should facilitate more extensive academic studies of this important drug, and also help to accelerate the development of new alternative drugs.

Herein, we present two novel, simple and efficient methods for the synthesis of *meglumine antimonate* in aqueous solution. The amorphous solid products so obtained were then structurally characterized and compared with a commercial sample of the drug by elemental composition (carbon, hydrogen, nitrogen) and thermal analysis, atomic absorption (antimony), proton NMR techniques and high-resolution ESI(+)-MS.

## EXPERIMENTAL

### Reagents

NMG and antimony pentachloride ($SbCl_5$, 99%) were obtained from Aldrich Chemical Co. (Milwaukee, WI, USA). Potassium hexahydroxoantimonate ($KSb(OH)_6$) was obtained from Fluka Chemie GmbH (Switzerland). A commercial sample of meglumine antimonate (Glucantime, also known as RP2168) was obtained from Rhône-Poulenc SA (Paris, France) in powder form.

### Preparation[11] of meglumine antimonate from $KSb(OH)_6$

0.004 mol of NMG was dissolved in about 25 ml of water under stirring at 55 °C. 0.004 mol of $KSb(OH)_6$ was then added to the solution and the mixture was kept at 55 °C at pH 7 until the mixture remained clear. After cooling, precipitation was induced by the addition of 75 ml of acetone. The precipitate was filtered and dried. The resulting white amorphous solid (1.42 g, 89% yield) was found to contain three water of crystallization molecules. Anal. Found: C, 19.01; H, 5.08; N, 3.22; Sb, 29.35%.

### Preparation[12] of meglumine antimonate from $SbCl_5$

0.004 mol of NMG was dissolved in about 25 ml of water under stirring at 55 °C. Freshly precipitated and hydrated antimony pentoxide, obtained from $SbCl_5$ hydrolyzed in water, was added in equimolar amounts and the mixture was stirred at 55 °C pH 7, which was maintained by adding KOH. The reaction was completed when the mixture remained clear. After cooling, precipitation was induced by the addition of 75 ml of acetone. The precipitate was filtered and dried. The resulting white amorphous solid (1.59 g, 95% yield) was found to contain three water of crystallization molecules. Anal. Found: C, 20.15; H, 5.40; N, 3.50; Sb, 29.00. Calc. for $C_7H_{17}NO_5 \cdot HSbO_3 \cdot 3H_2O$ (1 : 1 Sb–NMG complex): C, 20.1; H, 5.72; N, 3.34; Sb, 29.00%.

### General experimental techniques

The thermogravimetric analysis was carried out under nitrogen atmosphere on a Shimadzu TGA-50. Carbon, hydrogen and nitrogen analyses were carried out using a Perkin–Elmer 240 Elemental Analyzer. Antimony content was determined by atomic absorption using a Hitachi Z8200 spectrophotometer.

### NMR spectra

$^1H$ NMR spectra were measured on a Bruker DRX400–*AVANCE* spectrometer operating at 400.129 MHz using $D_2O$ as solvent. Samples were prepared 24 h before measurements at an antimony concentration of 0.1 mol $l^{-1}$. One-dimensional $^1H$ spectra were acquired under standard conditions, using a direct detection 5 mm $^1H/^{13}C$ dual probe with 90° pulse lengths of 7.2 μs for $^1H$. For all routine experiments, the same relaxation delay $d_1 = 2$ s was used. Homodecoupling experiments were carried out using the standard pulse sequence, with 16 scans and 60 dB in the pulse of irradiation for each selected frequency. Nuclear Overhauser enhancement (NOE) difference experiments were performed with 62 dB for the NOE build-up in a selected $^1H$ resonance and 50 ms for the irradiation time and eight scans for each experiment cycle; a line broadening of 0.3 Hz was used in the processing. Two-dimensional $^1H$ homonuclear correlation spectroscopy (COSY) with homospoil used 512 time increments for each data set and processed to the same number of points. One transient was collected for each time increment. The $f_1$ and $f_2$ spectral widths were also reduced in separate experiments, for better resolution of very close or overlapped correlations. Sine bell windows were used along both the $f_1$ and $f_2$ axes in the processing.

Data processing was carried out on an SGI workstation computer with Bruker DRX400 microprograms (XWIN-NMR 1.3).

### ESI(+)-MS spectra

ESI(+) mass spectra were collected on a Qtof (Micromass, UK) high-resolution and high mass-accuracy hydrid quadrupole orthogonal time-of-flight mass spectrometer using an ESI source of Z-configuration (Z-spray). Samples were run using the same instrumental conditions and parameters and within short time intervals so as to allow reliable comparison of the two synthetic products with the commercial sample of the drug. ESI(+) was performed in 1 : 1 acidic water–methanol solutions using the following main instrument parameters: capillary voltage 3 kV; cone voltage 60 V; desolvation gas temperature 120 °C. Lower cone voltages and desolvation gas temperatures were avoided as they favor a series of water clusters with the Sb–NMG complex ions.[7]

## RESULTS AND DISCUSSION

Two novel, simple and efficient synthetic methods for pentavalent antimony derivatives are presented herein. The first one uses $SbCl_5$ as a source of antimony (V) and consists of the following steps: (i) hydrolysis of $SbCl_5$ in water; (ii) isolation of hydrated antimony pentoxide; (iii) reaction of hydrated antimony pentoxide with NMG in water at neutral pH; and (iv) isolation of the antimony complex(es) after precipitation with acetone. The second method uses $KSb(OH)_6$ as the source of antimony(V) and consists of the following steps: (i) mixing $KSb(OH)_6$ and NMG in water at neutral pH; (ii) heating the mixture; and (iii) precipitating the antimony complex(es) by addition of acetone. For both procedures, amorphous white solids were obtained whose elemental, thermal, and atomic absorption analyses showed nearly the same chemical composition for both products. There results, especially those obtained for the $SbCl_5$ product, are consistent with the formation of a 1 : 1 Sb–NMG complex, as already established for Glucantime (Merck Index).

Both new methods employed here to prepare meglumine antimonate can be compared with those previously reported in the literature. Two processes proposed in a Rhône-Poulenc patent[9] start either with $SbCl_5$ or $SbCl_3$. In the $SbCl_5$ process, $SbCl_5$ is dissolved in chloroform at low temperature and the organic phase is then mixed with an aqueous solution of NMG. In the $SbCl_3$ process, $SbCl_3$ is first oxidized by $H_2O_2$ and the resulting antimony(V) product is mixed with NMG. Bazaco and Coca[10] recently proposed a method that starts with $Sb_2O_3$ and which is subsequently oxidized to antimony(V) by $H_2O_2$. The two methods we describe herein, therefore, are simpler, as they require less steps, they avoid the use of organic solvents (often toxic and sometimes difficult to remove), and residual and toxic antimony(III) products are expected to be substantially reduced. The two methods are also efficient, as yields vary within 85–95%.

### MS data

Figure 1 displays the ESI(+)-MS spectrum of the $SbCl_5$ and $KSb(OH)_6$ products. The $SbCl_5$ product (Fig. 1a) is nearly identical to that of the commercial sample (not shown) and displays the same series of Sb–NMG major complex ions of $m/z$ 314, 507, 625, 818, and 1129 as those recently reported in the literature.[7] The corresponding isotopomers that characterize $^{121}Sb/^{123}Sb$- and $^{12}C/^{13}C$(NMG)-containing ions are also clearly seen due to the high resolution of mass analysis; see, for instance, the inset in Fig. 1a for the ion of $m/z$ 507 and its main isotopomers of $m/z$ 508, 509, and 510. Note also the major ion of $m/z$ 196, which corresponds to the free NMG in its protonated form ($NMG + H^+$). Note, however, that owing to ion suppression effects, ESI-MS abundances do not necessarily reflect quantitative ratios of solution

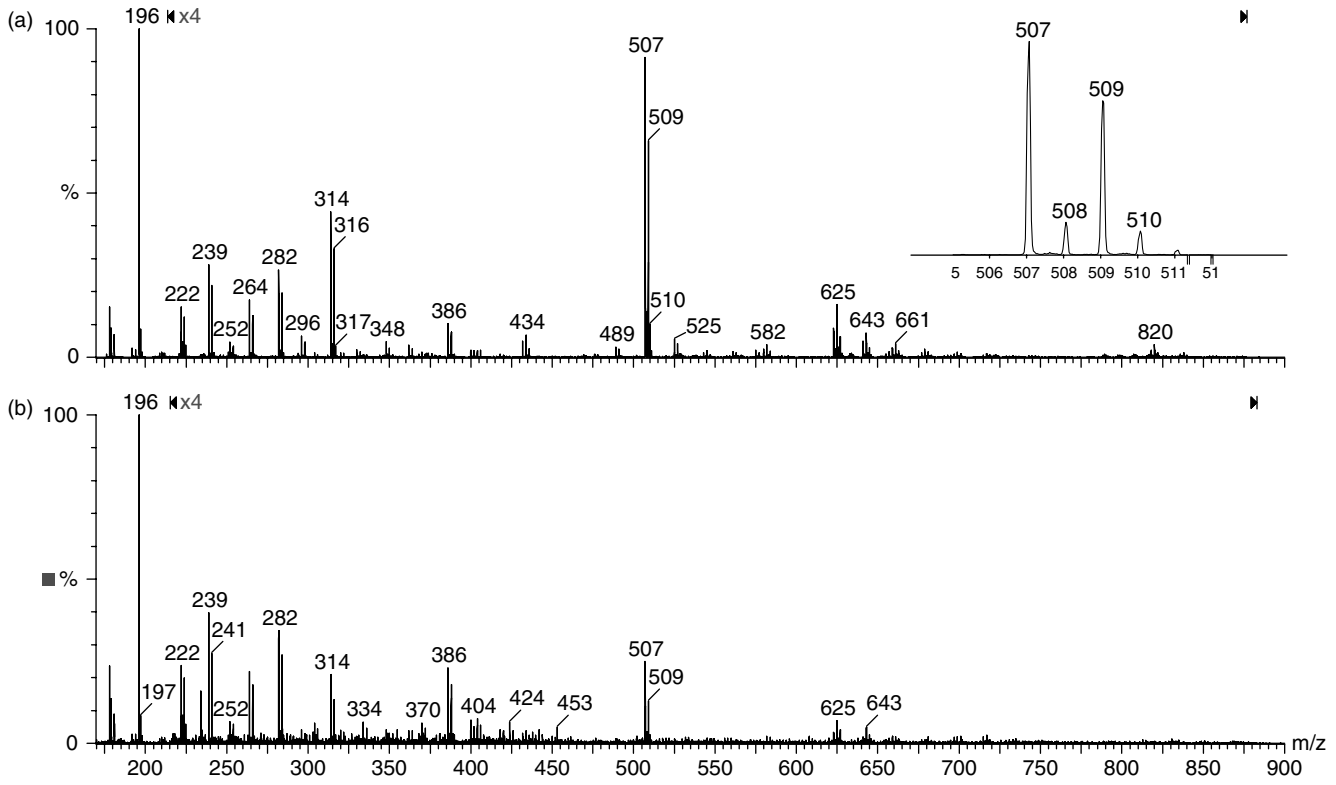


**Figure 1.** Positive ion high-resolution electrospray mass spectrum of meglumine antimonate formed via the $SbCl_5$ (a) and $KSb(OH)_6$ (b) methods. The inset shows the isotopic pattern of *m/z* 507, which characterizes an antimony-containing ion.

components. The exact masses (mass accuracy of 5 ppm) and isotopomeric cluster distribution identify the following ionic species: $[Sb(NMG) - 2H]^+$ for $m/z$ 314, $[Sb(NMG)_2 - 4H]^+$ for $m/z$ 514, $[Sb_2(NMG)_2 - 7H]^+$ for $m/z$ 625, $[Sb_2(NMG)_3 - 9H]^+$ for $m/z$ 818, and $[Sb_3(NMG)_4 - 14H]^+$ for $m/z$ 1129. In future studies, MS/MS analysis of these ions, together with B3LYP/6-31G(d,p) calculations, should allow us to re-interpret the structure of these complexes. A series of minor antimony-containing species of $m/z$ 222, 239, 252, 264, 282, 304, 334, 348, 370, and 386 (and their corresponding isotopomers) is also detected.

The ESI(+)-MS spectrum of the $KSb(OH)_6$ product (Fig. 1b) displays the same complex set of ions as that of the $SbCl_5$ product, and both spectra are therefore qualitatively equivalent. The two spectra are, however, quantitatively different, as that of the $KSb(OH)_6$ product displays lower relative abundances for the series of antimony-containing ions of $m/z$ 314, 507, 625, and 818, i.e. those ions that are the most characteristic for the meglumine antimonate complexes.[7] For the $KSb(OH)_6$ product, the series of antimony-containing ions of $m/z$ 222, 239, 252, 264, 282, 304, 334, 348, 370, and 386 are much more prominent. These data suggest that the antimony products might be a mixture of several compounds produced in different proportions by the two routes.

## NMR data

Figure 2 shows the proton NMR spectra obtained for NMG ligand and the $SbCl_5$ product in $D_2O$. The $KSb(OH)_6$ product produces nearly the same spectrum as that of $SbCl_5$, which is not shown. For the NMG ligand (Fig. 3), the resonances of the $CH_3$ protons are observed at 2.66 ppm, those for $H_2$ and $H_{2'}$ are in the region of 3.11 to 3.22 ppm, those for $H_4$, $H_5$, $H_6$, $H_7$, $H_{7'}$ resonances are from 3.59 to 3.79 ppm and that for $H_3$ is present as a multiplet at about 4.06 ppm.

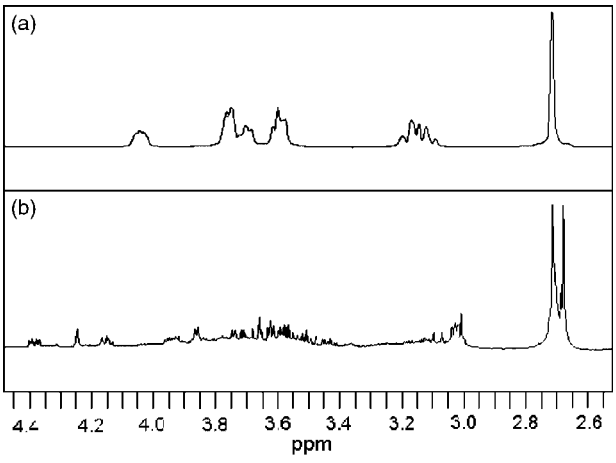


**Figure 2.** Proton NMR (400.129 MHz) spectra of NMG ligand (a) and of meglumine antimonate (b) formed via the $SbCl_5$ method. Nearly identical spectra were obtained for the $KSb(OH)_6$ product.

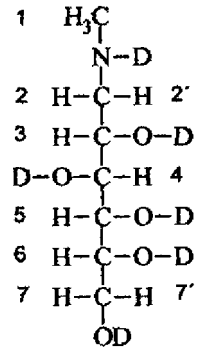


**Figure 3.** Numbering scheme for the NMG ligand.

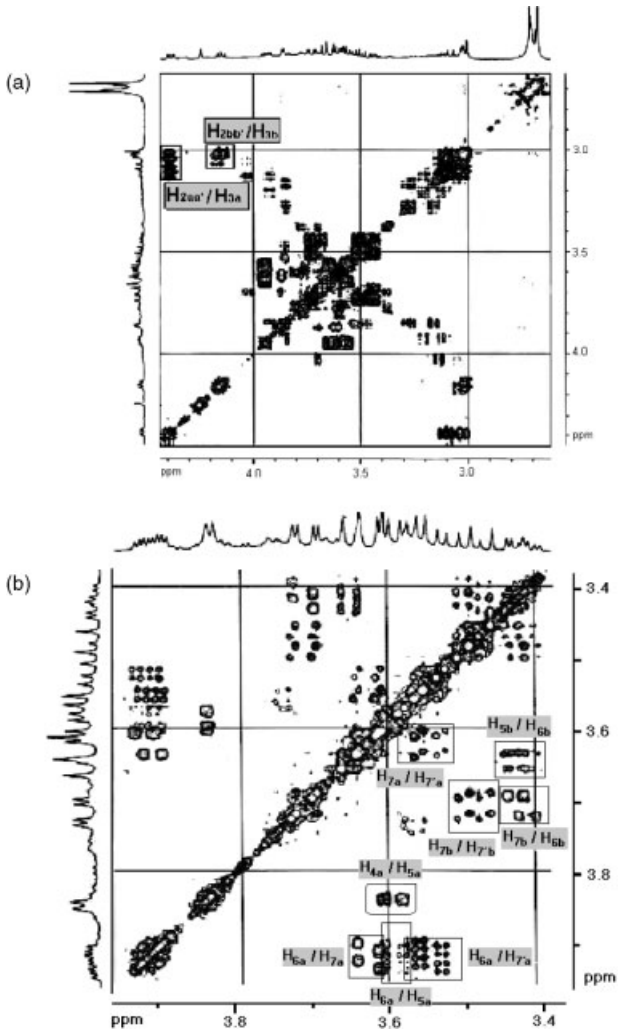


**Figure 4.** COSY NMR spectra in $D_2O$ of meglumine antimonate formed via the $SbCl_5$ method, in the 2.5–4.5 ppm (a) and the 3.4–4.0 ppm (b) regions. Nearly identical spectra were obtained for the $KSb(OH)_6$ product.

Therefore, the region from 3.2 to 4.0 ppm in the NMR spectrum is much more complex for the antimony derivatives (Fig. 2) than the free NMG ligand. The COSY experiment

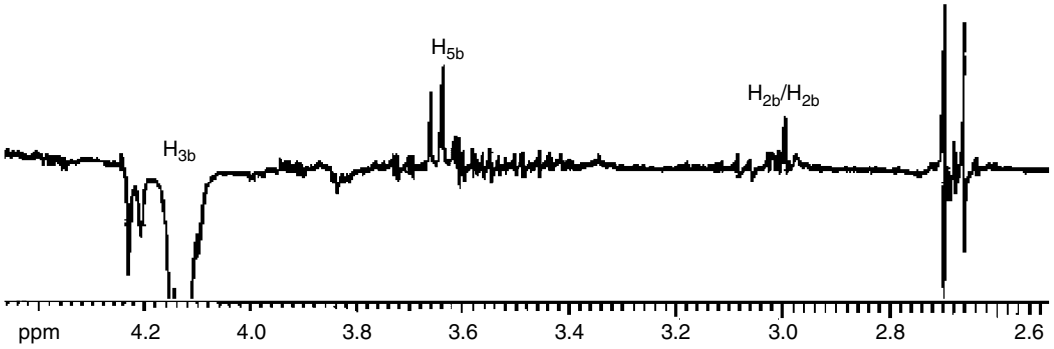


**Figure 5.** Homodecoupling and NOE-difference experiment, after presaturation of $H_{3b}$.

**Table 1.** $^1$H (400.129 MHz) NMR spectral assignments in $D_2O$ for *meglumine antimonate* prepared using the $SbCl_5$ method

| H | $\delta$ | $J$ (Hz) | H | $\delta$ | $J$ (Hz) |
|---|---|---|---|---|---|
| 1a | 2.67(s) | – | 1b | 2.71(s) | – |
| 2a | 3.09(m) | – | 2b | 3.01(m) | – |
| 2a′ | 3.09(m) | – | 2b′ | 3.01(m) | – |
| 3a | 4.36(dd) | 9.7; 3.4 | 3b | 4.13(dd) | 7.6; 4.9 |
| 4a | 3.84(d) | 3.9 | 4b | 4.23(s) | – |
| 5a | 3.60(dd) | 10.2; 3.9 | 5b | 3.62(d) | 9.2 |
| 6a | 3.92(ddd) | 10.2; 4,7; 2.6 | 6b | 3.42(ddd) | 9.2; 7.1; 2.9 |
| 7a | 3.64(dd) | 11.8; 2.6 | 7b | 3.67(dd) | 11.8; 2.9 |
| 7a′ | 3.53(dd) | 11.8; 4.7 | 7b′ | 3.46(dd) | 11.8; 7.1 |

indicates that each proton resides in two distinct chemical environments, represented here as $H_{1a}$, $H_{2a}$, $H_{2'a}$, $H_{3a}$, $H_{4a}$, $H_{5a}$, $H_{6a}$, $H_{7a}$ and $H_{7'a}$ (first environment) and as $H_{1b}$, $H_{2b}$, $H_{2'b}$, $H_{3b}$, $H_{4b}$, $H_{5b}$, $H_{6b}$, $H_{7b}$ and $H_{7'b}$ (second environment). The homodecoupling and NOE-difference experiments, using a selective irradiation of the methyl-group protons $H_{1a}$ and $H_{1b}$, allowed the assignment of $H_{2a}/H_{2'a}$ and $H_{2b}/H_{2'b}$ resonances (data not shown). As illustrated in Fig. 4a, COSY analysis allowed us to establish correlations between $H_{2a}/H_{3a}$, $H_{2'a}/H_{3a}$, $H_{2b}/H_{3b}$ and $H_{2'b}/H_{3b}$. From these data, one can also infer that the $H_3/H_4$ coupling observed for the free NMG is not found for the antimony complexes ($H_{3a}/H_{4a}$ and $H_{3b}/H_{4b}$). To achieve the assignment of the $H_{4b}$ resonances, a new homodecoupling and NOE-difference experiment was performed, with the selective irradiation of $H_{3b}$. As shown in Fig. 5, the irradiation of the $H_{3b}$ resonance induced a significant enhancement of the resonances corresponding to $H_{5b}$ and $H_{2b}/H_{2'b}$. Moreover, since the multiplicity of the $H_{5b}$ resonance is a doublet then there is no coupling between $H_{4b}/H_{5b}$, and the singlet observed around 4.2 ppm can only be assigned to the $H_{4b}$ resonance. Another NOE experiment was performed with the selective irradiation of the $H_{3a}$ signal. A significant enhancement of the $H_{4a}$ and $H_{6a}$ resonances was observed (data not shown). Fig. 4b also shows the COSY analysis specifically in the region of 3.4 to 4.0 ppm. This analysis allowed us to establish correlations between $H_{4a}/H_{5a}$, $H_{6a}/H_{5a}$, $H_{7a}/H_{6a}$, $H_{7'a}/H_{6a}$ and $H_{7a}/H_{7'a}$ in one NMG molecule and between $H_{5b}/H_{6b}$, $H_{7b}/H_{6b}$, $H_{7'b}/H_{6b}$ and $H_{7b}/H_{7'b}$ in a second NMG molecule. Table 1 shows the NMR resonances of all protons in the Sb–NMG complex. Comparison of these resonances with those of the free NMG ligand indicates large shifts for $H_{3a}$, $H_{5a}$ and $H_{6a}$ in one of the two NMG molecules and for $H_{3b}$ and $H_{4b}$ in the other. These data indicate that NMG molecules coordinate antimony in two different fashions, and suggest either the coexistence of at least two different complexes or the existence of a major complex in which two NMG molecules are coordinated with antimony in an asymmetrical geometry. Considering that previous attempts to characterize meglumine antimonate solutions by NMR were not successful,[7] this study represents a major advance toward the elucidation of the meglumine antimonate structure.

## CONCLUSIONS

Two simple and efficient new methods have been reported for the synthesis of pentavalent antimony derivatives using either $SbCl_5$ or $KSb(OH)_6$ as a source of antimony(V), and their efficacy has been demonstrated for the synthesis of the important commercial drug *meglumine antimonate*. Carbon, hydrogen, nitrogen and antimony analyses, as well as NMR and ESI(+)-MS spectra, suggest that both methods yield products rather similar to the commercial drug. The NMR spectra of the $SbCl_5$ product, which are nearly identical to those of the commercial drug, indicate that NMG molecules coordinate antimony in two different fashions, with either the coexistence of two different complexes or the existence of oligomeric species in which two NMG molecules are coordinated with antimony in an asymmetrical geometry. Additionally, the ESI-MS spectra indicate that the $SbCl_5$ product is nearly identical to the commercial drug; however, whereas both the $SbCl_5$ and $KSb(OH)_6$ products show distributions of the same species that are qualitatively similar, they are quantitatively distinct. These results suggest that

these products might be a mixture of several compounds produced in different proportions by the two routes.

## Acknowledgements

This work was supported by the Brazilian agencies CNPq, FAPEMIG (CD, FF) and FAPESP (MNE, FCG). RO is the recipient of a studentship from Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (Capes). We thank Professor Oliver Howarth for his invaluable advice during the course of the NMR experiments.

## REFERENCES

1. Marsden PD. *Rev. Soc. Bras. Med. Trop.* 1985; **18**: 187.
2. Berman JD. *Rev. Infect. Dis.* 1988; **10**: 560.
3. Frézard F, Demicheli C, Ferreira CS, Costa MAP. *Antimicrob. Agents Chemother.* 2001; **45**: 913.
4. Demicheli C, Frézard F, Lecouvey M, Garnier-Suillerot A. *Biochim. Biophys. Acta* 2002; **1570**: 192.
5. Steck EA. *Prog. Drug Res.* 1974; **18**: 289.
6. Headley JV, Yong MS, Brooks PW, Phillips A. *Rapid Commun. Mass Spectrom.* 1995; **9**: 372.
7. Roberts WL, McMurray WJ, Rainey PM. *Antimicrob. Agents Chemother.* 1998; **42**: 1076.
8. Demicheli C, Figueiredo TL, Carvalho S, Sinesterra RD, Lopes JC, Frézard F. *Biometals* 1999; **12**: 63.
9. Gailliot PL. Procédé de préparation de dérivés de l'antimoine. FR 868 747, Usines Chimiques Rhône-Poulenc.
10. Bazaco F, Coca JYM. Nuevo procedimiento para la preparación de derivados del ácido antimónico aplicable en el tratamiento de la Leishmaniosis canina. ES 2 050 614, Iteve, S.A.
11. Demicheli C. 1999; Síntese de derivados de antimônio utilizados no tratamento de protozoonoses. *Patent* PI 9907575-0 (pending).
12. Demicheli C, Frézard F. 2001; Novo processo para preparação de derivados de antimônio. *Patent* PI 0106305-7 (pending).
13. Sundar S, Sinha PR, Agrawal NK, Srivastava R, Rainey PM, Berman JD, Murray HW, Singh VP. *Am. J. Trop. Med. Hyg.* 1998; **59**: 139.

 