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Aust. J. Chem. **2016**, *69*, 1033–1048
http://dx.doi.org/10.1071/CH16044

Full Paper

Heteronuclear NMR Spectroscopic Investigations of Gallium Complexes of Substituted Thiosemicarbazones Including X-Ray Crystal Structure, a New Halogen Exchange Strategy, and ^{18}F Radiolabelling

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Five thiosemicarbazone ligands have been synthesized, and their coordination chemistry with gallium was investigated. The reaction of these thiosemicarbazones with gallium chloride in alcohol solutions in the presence of a base yielded the corresponding penta-coordinated Ga-Cl metal complexes. In contrast, the reaction of gallium nitrate with the ligands in the presence of alkoxides resulted in the formation of the corresponding Ga-alkoxides, rather than the anticipated Ga-nitrate complex. The crystal structures of gallium chloride and gallium methoxide complexes of diphenylthiosemicarbazone comprise a planar configuration of the tetradentate-coordinated thiosemicarbazone with Ga^{3+} ion, with the chloride or methoxide groups occupying the apical coordination site. The corresponding ethoxido complex was also prepared in an identical fashion, and NMR analysis confirmed structural similarity to the methoxido complex. Facile halogen exchange reactions of the gallium chloride complexes were achieved by treatment with silver nitrate, followed by addition of KF or KI to generate the gallium fluoride and iodide complexes, respectively. This method of exchange using halogenated inorganic salts aids the preparation of group 13 fluorides, which are notoriously insoluble in organic solvents, for complexation with organic ligands. All compounds have been fully characterized by NMR, and the X-ray crystal structures of two of the complexes are reported. Additionally, the positron-emitting isotope ^{18}F was introduced in the structure of the diphenyl gallium thiosemicarbazone complex.

Manuscript received: 25 January 2016.
Manuscript accepted: 3 March 2016.
Published online: 12 April 2016.

Introduction

Thiosemicarbazones are a unique class of versatile compounds that coordinate various metal ions to form stable complexes using their S- and N-donor atoms.[1,2] Thiosemicarbazones can form a 'masked thiolate' precursor, which facilitates their ability to coordinate a variety of 3d transition metal ions.[3–7] Cu^{II} and Ga^{III} complexes with thiosemicarbazones have been prepared in light of their biological activity, and the coordination of metal ions has been demonstrated to increase the cytotoxicity of tridendate thiosemicarbazones.[8–20] Copper complexes of pyridine derivatives of thiosemicarbazones also show improved biological activity compared with the parent ligand.[21] The radio-metal complexes (most notably ^{64}Cu) of these ligands have been investigated for their applications in molecular imaging agents.[22–27] Pascu et al.[28–30] have synthesized a series of ^{68}Ga complexes of bis-thiosemicarbazones to evaluate their imaging potential and report that the reaction of $GaCl_3$ with these ligands results in the formation of a square-based pyramidal mono-chloride metal complex. This prompted us to explore the potential of exchanging this chloride with other ligands such as other halogens. Potential applications of coordination complexes of group 13 metal ions with fluoride have recently been described. McBride et al. reported the formation of stable inorganic aluminium complexes with the radioisotope ^{18}F and highlighted that these provide simple moieties that can be incorporated in a wide range of biomolecules with potential as positron emission tomography imaging agents.[31–37] Recently, Bhalla et al. have demonstrated that coordination complexes containing Ga-^{18}F can be generated from the corresponding chloride complexes by facile halogen exchange.[38–40]

Here, we describe the preparation and characterization of several bis-thiosemicarbazone ligands and report the synthesis of their resulting complexes with Ga^{3+}, resulting in the formation of complexes with an N_2S_2Ga-X core (where X = Cl, I, F, OMe (methoxy), OEt (ethoxy)). In particular, we demonstrate that N_2S_2Ga-Cl complexes can be readily and simply exchanged with iodide and fluoride.

Experimental

All chemicals were obtained from Sigma-Aldrich and were used without further purification. NMR spectra were recorded in dimethyl sulfoxide (d_6-DMSO), and the chemical shifts were referenced to DMSO (proton: 2.5 ppm and carbon: 39.5 ppm). The NMR data were acquired on a Bruker 900 MHz NMR spectrometer equipped with a cryoprobe. The proton spectra were acquired with a sweep width of 18 ppm centred at 7 ppm. The carbon spectra were acquired with a sweep width of 220 ppm centred at 110 ppm. The COSY experiments were acquired with a sweep width of 18 ppm using a 90° pulse of 9 μs with 256 increments. The ^{13}C HSQC spectrum was acquired with sweep widths of 18 and 160 ppm for proton and carbon, respectively, and the carbon centred at 80 ppm. HMBC spectral data were acquired to establish the structures of the compounds (^{13}C sweep width of 220 ppm). The ^{15}N spectra were acquired using a sweep width of 400 ppm. The raw data were typically multiplied by an exponential or shifted sine-squared function before performing the Fourier transform. The ^{71}Ga NMR spectrum was acquired on a 500 MHz Bruker NMR machine with 350 ppm sweep width. Ga $(NO_3)_3$ in D_2O was used as a standard at 0 ppm before running the sample spectrum.

X-Ray Crystal Structure Determination

X-ray quality single crystals were obtained by slow diffusion of ether into tetrahydrofuran solution. Crystallographic data were acquired at 190 K on an Oxford Diffraction Gemini CCD diffractometer employing graphite-monochromated Cu Kα radiation (1.5418 Å) and operating within the range 2 Å $< 2\theta <$ 125 Å. Temperature control was achieved with an Oxford Cryosystems Desktop Cooler. Data reduction and empirical absorption corrections (multiscan) were performed with Oxford Diffraction *CrysAlisPro* software. The structure was solved by direct methods with *SHELXS* and refined by full-matrix least-squares analysis with *SHELXL-97*[41] within the *WinGX* graphical user interface.[42] All non-H atoms were refined with anisotropic thermal parameters. The molecular structure diagram was produced with *ORTEP-3*.[43] The data in CIF format have been deposited for the gallium chloride and methoxide complexes, respectively, with deposition numbers CCDC 1410428 and 1410429. The data can be obtained from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; Fax: +44 1223 336033; Email: deposit@ccdc.cam.ac.uk or from http://www.ccdc.cam.ac.uk/products/csd/request.

General Synthetic Procedure

Thiosemicarbazone

Concentrated hydrochloric (2 mL) was added to a flask containing glyoxal (1.16 g, 20 mmol) and methanol (50 mL). The mixture was stirred at room temperature to form a homogenous solution. To this mixture, a solution of thiosemicarbazide (3.6 g, 40 mmol) dissolved in methanol containing 2 N hydrochloric acid was added, and the contents stirred at room temperature for 3 days, resulting in the formation of a white precipitate. The precipitated thiosemicarbazone was filtered, washed with methanol, and dried under vacuum.

All thiosemicarbazones **1–5** were synthesized using the above experimental procedure.

1. Yield, 2.15 g. δ_H (d_6-DMSO) 11.67 (2H, br s), 8.30 (2H, br s), 7.87 (2H, br s), 7.71 (2H, s). δ_C (d_6-DMSO) 178.1, 140.6. δ_N (d_6-DMSO) 110.3 (NH_2), 175.5 (NH).

2. Yield, 2.4 g. δ_H (d_6-DMSO) 10.20 (2H, br s), 8.39 (2H, br s), 7.84 (2H, br s), 2.13 (6H, s). δ_C (d_6-DMSO) 178.9, 148.3, 11.6. δ_N (d_6-DMSO) 110.8 (NH_2), 168.3 (NH), 317.6 (C=N).

3. Yield, 4.98 g. δ_H (d_6-DMSO) 10.32 (2H, br s), 8.36 (2H, br s), 7.73 (2H, br s), 2.79 (4H, q, *J* 7.4), 0.87 (6H, t, *J* 7.4). δ_N (d_6-DMSO) 111.0 (NH_2), 166.0 (NH), 313.5 (C=N).

4. Yield, 4.5 g. ν_{max} (KBr)/cm^{-1} 3417, 3203, 3149, 2984, 1598, 1506, 1446, 1365, 1289, 1243, 1154, 1085, 1057, 987, 865, 841, 792. δ_H (d_6-DMSO) 10.33 (1H, s), 10.20 (1H, s), 8.40 (1H, s), 8.39 (1H, s), 7.82 (1H, s), 7.74 (1H, s), 2.84 (2H, q), 2.14 (3H, s), 0.89 (3H, t). δ_C (d_6-DMSO) 178.9, 178.8, 152.2, 147.3, 16.9, 11.7, 11.0. δ_N (d_6-DMSO) 317.2, 315.7, 167.8, 165.9, 110.8, 110.2.

5. Yield, 6.8 g. ν_{max} (KBr/cm^{-1} 3415, 3226, 3136, 2950, 1584, 1474, 1442, 1240, 1133, 1069, 1005, 922, 906, 865, 828, 763. δ_H (d_6-DMSO) 9.84 (2H, br s), 8.67 (2H, br s), 8.38 (2H, br s), 7.72 (4H, m), 7.43–7.38 (6H, m). δ_C (d_6-DMSO) 179.1, 140.5, 133.1, 130.2, 128.4, 126.7. δ_N (d_6-DMSO) 167.8, 113.8.

Synthesis of Gallium Complexes

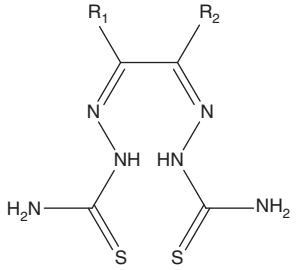
*Thiosemicarbazone-Gallium Chloride Complex (**6**)*

A suspension of thiosemicarbazone (1.23 g, 5 mmol) in methanol (40 mL) was added to a round-bottom flask and the contents were stirred. Sodium methoxide solid (0.540 g, 10 mmol) was introduced, resulting in a yellow solution. After 10 min of stirring, gallium chloride 0.845 g (5 mmol) was added, resulting in an exothermic reaction and the solution turned to a deep orange colour. The contents were refluxed for 8 h, and the mixture was cooled to room temperature. The product, which precipitated out as an orange solid during the reaction, was filtered and washed with methanol (1.44 g, 82 %). ν_{max} (KBr)/cm^{-1} 3444, 3402, 3365, 3293, 3171, 1622, 1599, 1580, 1549, 1484, 1465, 1325, 1302, 1243, 1220, 1187, 1144, 1056, 1021, 997, 942, 848, 793. δ_H (d_6-DMSO) 8.00 (1H, br s), 7.97 (1H, s), 2.77 (2H, q), 2.35 (3H, s), 1.08 (3H, t). δ_C (d_6-DMSO) 173.9, 173.6, 148.2, 143.8, 21.0,14.2, 10.0. δ_N (d_6-DMSO) 251.2, 249.5, 103.7, 103.4. *m*/*z* (electron impact (EI)) 351 (M^+), 315 (M – HCl).

*Diphenylthiosemicarbazone Gallium Chloride Complex (**7**, **C1**)*

The method as that employed to prepare compound **6** was used. Yield: 1.98 g, 86 %. ν_{max} (KBr)/cm^{-1} 3447, 3326, 3273, 3067, 1627, 1582, 1521, 1493, 1462, 1428, 1335, 1315, 1288, 1273, 1210, 1166, 1105, 1069, 1051, 1025, 1001, 981, 887, 783.

Table 1. Structures of thiosemicarbazones ligands 1–5

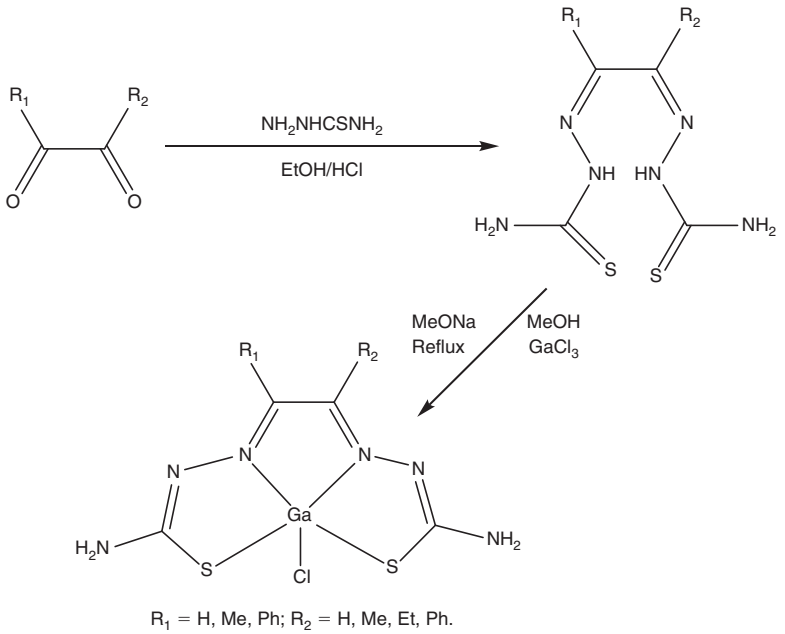


Compound no.	R_1	R_2
1	H	H
2	CH_3	CH_3
3	CH_3CH_2	CH_3CH_2
4	CH_3	CH_3CH_2
5	Ph	Ph

δ_H (d_6-DMSO) 8.14 (4H, br s), 7.26 (6H, m), 7.18 (4H, m). δ_C (d_6-DMSO) 174.8, 142.7, 131.8, 129.5, 129.1, 127.7. δ_N (d_6-DMSO) 304.5, 253.7, 107.9. *m*/*z* (EI) 461 (M^+).

*Diphenylthiosemicarbazone Gallium Methoxide Complex (**8**, **C2**)*

A suspension of thiosemicarbazone (0.445 g, 1.25 mmol) in methanol (40 mL) was added to a round-bottom flask and the contents were stirred. Sodium methoxide solid (0.270 g, 5 mmol) was introduced, resulting in a deep yellow solution. After 10 min of stirring, gallium nitrate 0.32 g (1.25 mmol) was added, resulting in an exothermic reaction and the solution turned to a deep orange colour. The contents were refluxed for 8 h, and the mixture was cooled to room temperature. The product, which precipitated out as an orange red solid during the reaction, was filtered and washed with methanol. (Note: the methoxide complex in d_6-DMSO showed multiple peaks for the elimination of methanol (MeOH) from the coordinating complex, see X-ray data for further details.) δ_H (d_6-DMSO) 8.0–7.18 (10H, m), 4.10–4.07 (q, MeOH, hydrogen-bonded), 3.36–3.31 (d, MeO hydrogen-bonded to MeOH), 3.17–3.16 (3H, d, hydrogen-bonded MeOH). δ_C (d_6-DMSO) 175.9, 143.2, 132.0, 129.5, 128.7, 127.6, 127.5, 127.2, 51.6, 48.6.



Scheme 1. Synthesis of gallium complexes of thiosemicarbazones with ligands **2**–**4**.

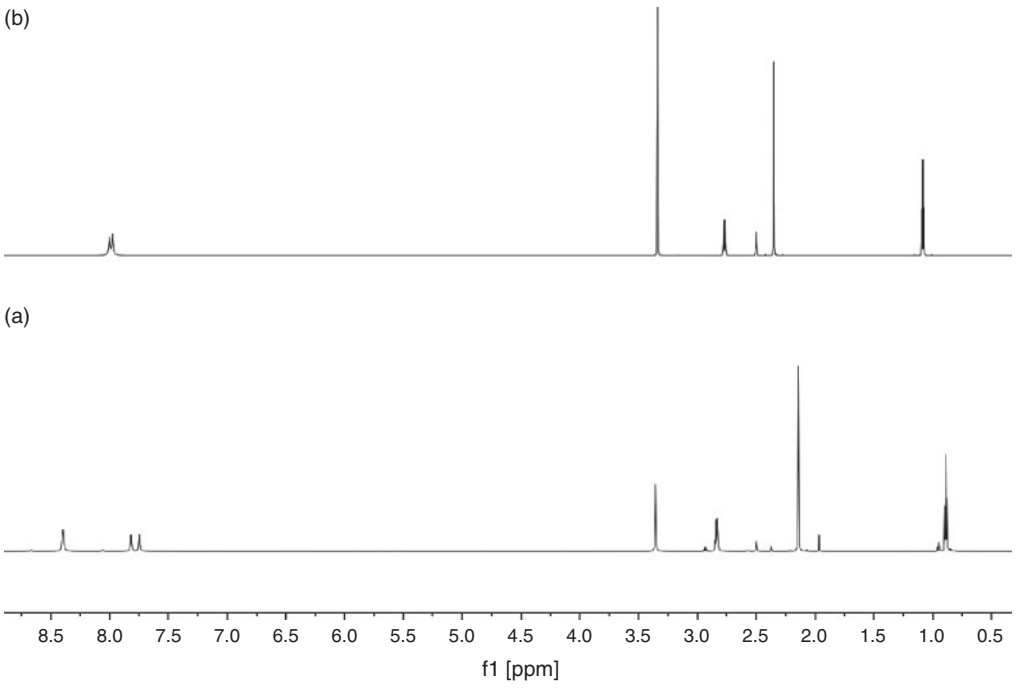


Fig. 1. ^{1}H NMR spectra of (a) unsymmetrical thiosemicarbazone (ligand **4**) and (b) gallium-complexed thiosemicarbazone (compound **6**) in d_6-DMSO.

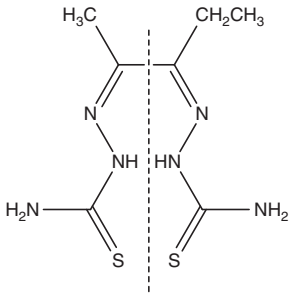
*Diphenylthiosemicarbazone Gallium Ethoxide Complex (**9**)*

The method as that employed to prepare compound **8** was used. δ_H (d_6-DMSO) (note: the ethoxide complex in d_6-DMSO showed multiple peaks for the elimination of ethanol (EtOH) from the coordinating complex) 7.6–7.0 (10H, m), 4.32 (EtOH, OH), 3.55 (q, EtOH), 3.44 (q, EtO), 1.24 (t, CH_3CH_2OH), 1.04 (3H, t, EtO). δ_C (d_6-DMSO) 181.2, 142.8, 141.8, 137.7, 133–126.0 (multiple peaks), 58.7 (EtO), 56.02 (EtOH), 18.6 (EtO), 14.9 (EtOH).

Halogen Exchange Reactions

*Diphenylthiosemicarbazone Gallium Fluoride (**10**)*

Diphenylthiosemicarbazone gallium chloride **7** (0. 0459 g, 0.1 mmol) was suspended in methanol (15 mL) in a 20-mL vial. The blood red suspension was stirred for 10 min followed by the addition of silver nitrate (0.017 g, 0.1 mmol), and the contents were shaken initially for 2 min and then vigorously stirred for 30 min, resulting in the formation of an orange solution and precipitation of silver chloride. The contents were allowed to settle, and the orange supernatant was transferred to another vial. To this solution-containing vial was added, with stirring, potassium fluoride (0.0058 g, 0.1 mmol), resulting in a deep red solution. The contents were stirred for 10 min at room temperature, and the solvent was evaporated to obtain an orange-red residue (0.021 g, 47.7 %). An NMR spectrum of the product revealed the formation of a fluoride complex. δ_H (d_6-DMSO) 8.07–7.15 (10H, m), 4.10–4.09 (t, MeOH, hydrogen-bonded with F), 3.17–3.16 (d, MeO hydrogen-bonded). δ_C (d_6-DMSO) 175.6, 141.0, 132.2, 131.9, 129.1, 127.8, 48.7. δ_F (d_6-DMSO) –93.7 (relative to TFA 1 % standard capillary insert standard at 0 ppm).



(Dashed line depicting the asymmetry of ligand **4**)

Chart 1.

*Diphenylthiosemicarbazone Gallium Iodide (**11**)*

The method as that employed to prepare compound **10** was used except that potassium iodide was used instead. Yield: 0.033 g, 61.0 %. δ_H (d_6-DMSO) 8.07 (br s), 7.15–7.28 (m, Ar), 4.10 (br s, MeO hydrogen-bonded), 3.16 (s, MeO). δ_C (d_6-DMSO) 175.8, 141.2, 131.9, 129.5, 127.8, 48.6.

Cold Thin Layer Chromatography Determination of Formation of Ga-F Complex

After preparation of the complex using the exchange method, the Ga-F complex was spotted on a thin layer chromatography silica gel plate and eluted with 10 % ammonium acetate/MeOH (1 : 1) and the observed R_F value of the product was 0.86. The chloro compound and the iodo compound of the gallium complex showed an R_F value very similar to that obtained for the gallium fluoride complex. However, the NMR spectroscopy showed the ^{19}F NMR signal for the fluoro compound and was absent for the other two compounds, thus confirming the formation of the fluoro compound.

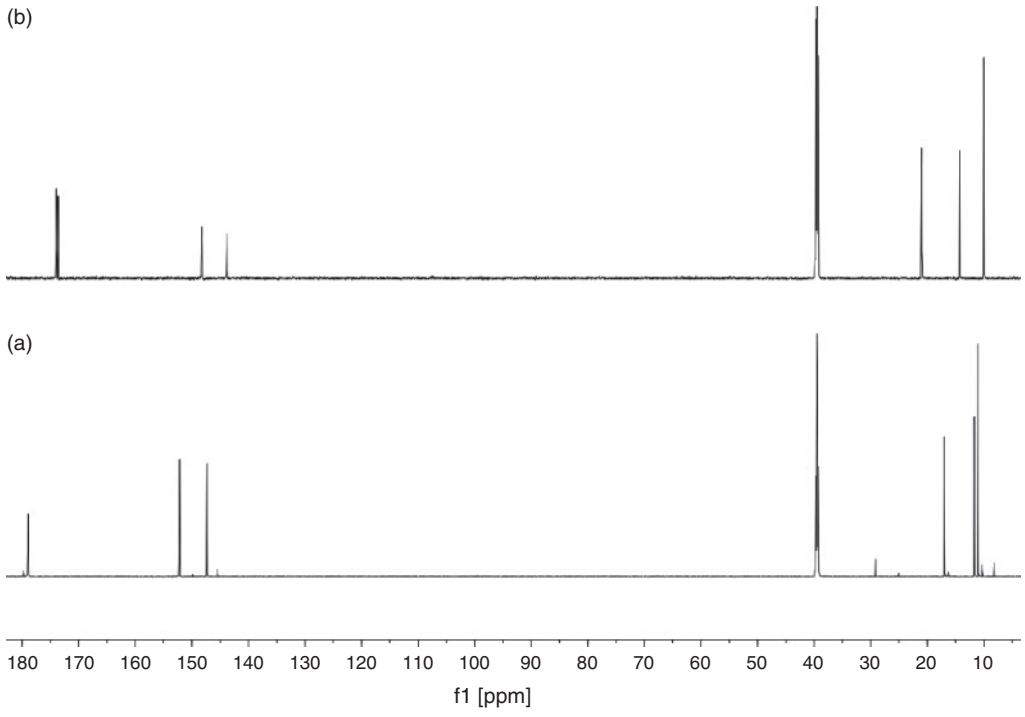


Fig. 2. ^{13}C NMR spectra of (a) unsymmetrical thiosemicarbazone (ligand **4**) and (b) gallium-complexed thiosemicarbazone (compound **6**) in d_6-DMSO.

Production of Fluorine-18

No-carrier-added fluorine-18 was manufactured via the $^{18}O(p,n)^{18}F$ nuclear reaction using a Cyclone® 18 Twin (IBA, Belgium) dual ion source cyclotron. Pure water (~0.7 mL) enriched to >98 % $H_2{}^{18}O$ was irradiated with 18 MeV protons at a beam current of 14 μA for 8 min to produce 4.5 GBq of fluorine-18. The aqueous [^{18}F] fluoride was transferred from the cyclotron target to a 10-mL glass-receiving vial located in

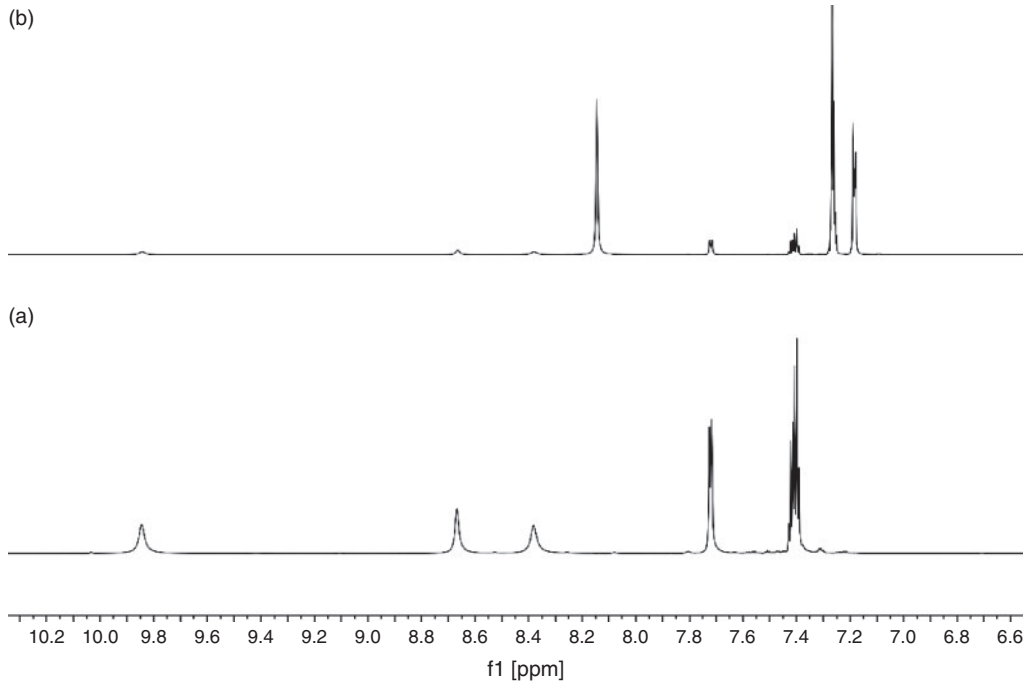


Fig. 3. ^{1}H NMR of (a) diphenylthiosemicarbazone (ligand **5**) and (b) gallium-complexed diphenylthiosemicarbazone (compound **7**) in d_6-DMSO.

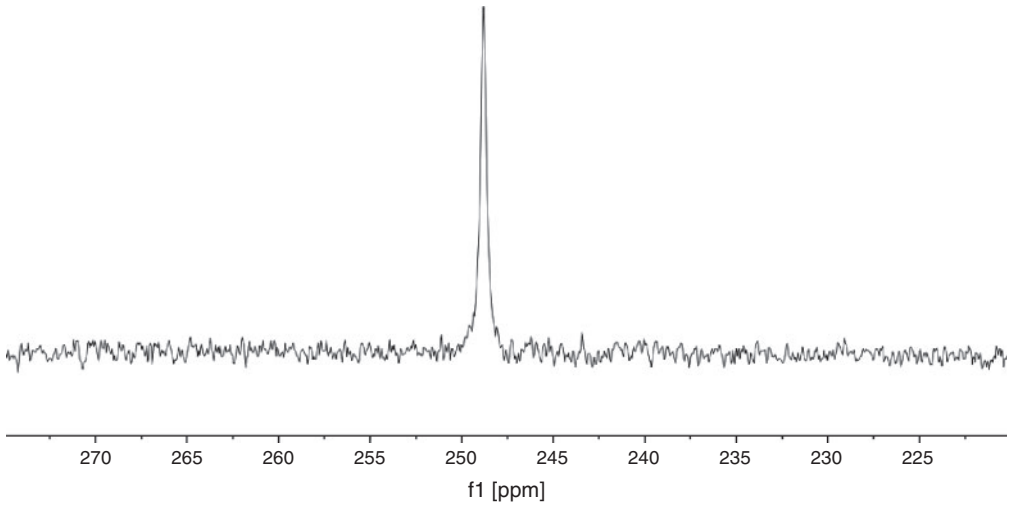


Fig. 4. ^{71}Ga NMR spectrum of the diphenylthiosemicarbazone gallium chloride (compound **7**) in d_6-DMSO.

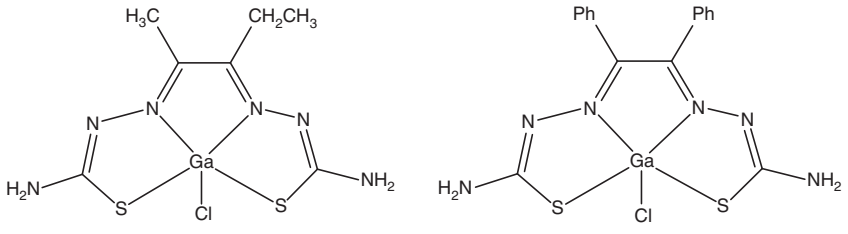


Chart 2.

Table 2. Crystal data and structure refinement for gallium complexes of diphenylthiosemicarbazone

Identification code	**C1**		**C2**	
Empirical formula	$C_{16}H_{14}ClGaN_6S_2$		$C_{18}H_{21}GaN_6O_2S_2$	
Formula weight	459.62		487.25	
Temperature [K]	190(2)		190(2)	
Wavelength [Å]	1.54180		1.54184	
Crystal system	Triclinic		Monoclinic	
Space group	$P\,\bar{1}$		$P\,2_1/n$	
Unit cell dimensions	$a = 8.0530(7)$ Å	$\alpha = 83.241(8)°$	$a = 12.721(2)$ Å	
	$b = 11.2761(11)$ Å	$\beta = 73.961(8)°$	$b = 10.1135(9)$ Å	$\beta = 106.71(1)°$
	$c = 11.4160(10)$ Å	$\gamma = 69.217(9)°$	$c = 17.297(2)$ Å	
Volume [Å^3]	931.24(15)		2131.5(4)	
Z	2		4	
Density (calculated) [Mg m^{-3}]	1.639		1.518	
Absorption coefficient [mm^{-1}]	5.553		3.830	
$F(000)$	464		1000	
Crystal size [mm^3]	$0.2 \times 0.01 \times 0.01$		$0.4 \times 0.2 \times 0.1$	
Theta range for data collection [°]	4.03–62.45		3.84–62.49	
Index ranges	$-8 \le h \le 9, -12 \le k \le 12, -13 \le l \le 13$		$-14 \le h \le 14, -7 \le k \le 11, -19 \le l \le 19$	
Reflections collected	6311		9215	
Independent reflections	2920 ($R_{int} = 0.0758$)		3348 ($R_{int} = 0.0553$)	
Completeness to theta = 62.45° [%]	98.2		98.4	
Absorption correction	Semi-empirical from equivalents		Semi-empirical from equivalents	
Max. and min. transmission	1 and 0.80489		1 and 0.47098	
Refinement method	Full-matrix least-squares on F^2		Full-matrix least-squares on F^2	
Data/restraints/parameters	2920/0/235		3348/0/265	
Goodness-of-fit on F^2	1.092		1.055	
Final R indices ($I > 2\sigma(I)$)	$R1 = 0.0448$, $wR2 = 0.1092$		$R1 = 0.0371$, $wR2 = 0.0931$	
R indices (all data)	$R1 = 0.0675$, $wR2 = 0.1482$		$R1 = 0.0446$, $wR2 = 0.0988$	
Largest diff. peak and hole [e Å^{-3}]	0.622 and –0.874		0.619 and –0.411	

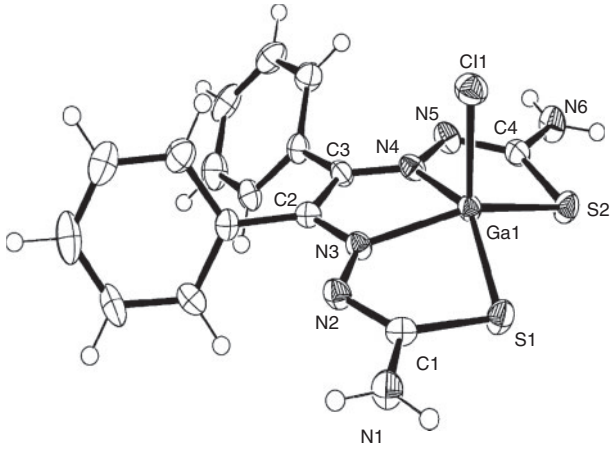


Fig. 5. Single-crystal X-ray structure of diphenylthiosemicarbazone gallium chloride complex (compound **7**).

a hot-cell via a 1/16″ diameter ETFE tubing with a length of ~20 m under helium pressure. The receiving vial was measured for radioactive content in a dose calibrator housed within the hot-cell before delivery into a lead pot. The lead pot was then manually transferred to a fume cabinet where aliquots of the aqueous [^{18}F] fluoride were taken for radiolabelling.

Radiolabelling

For the radiolabelling experiments, we initially formed the nitrate complex in situ and then evaporated the solvent methanol

Table 3. Selected bond lengths (Å) for diphenylthiosemicarbazone gallium chloride complex

N2–N3	1.370(7)
N3–Ga1	2.052(4)
N4–N5	1.348(7)
N4–Ga1	2.042(5)
S1–Ga1	2.3080(15)
S2–Ga1	2.3032(15)
Cl1–Ga1	2.2345

Table 4. Selected bond angles (°) for diphenylthiosemicarbazone gallium chloride complex

C2–N3–Ga1	117.1(4)
N2–N3–Ga1	121.2(4)
C3–N4–N5	120.4(5)
C3–N4–Ga1	118.3(4)
N5–N4–Ga1	121.2(4)
C4–N5–N4	112.7(5)
C1–S1–Ga1	94.41(19)
C4–S2–Ga1	94.2(2)
N4–Ga1–N3	76.48(18)
N4–Ga1–Cl1	99.02(14)
N3–Ga1–Cl1	99.23(13)
N4–Ga1–S2	83.04(14)
N3–Ga1–S2	146.55(14)
Cl1–Ga1–S2	110.0(6)
N4–Ga1–S1	149.84(14)
N3–Ga1–S1	82.47(13)

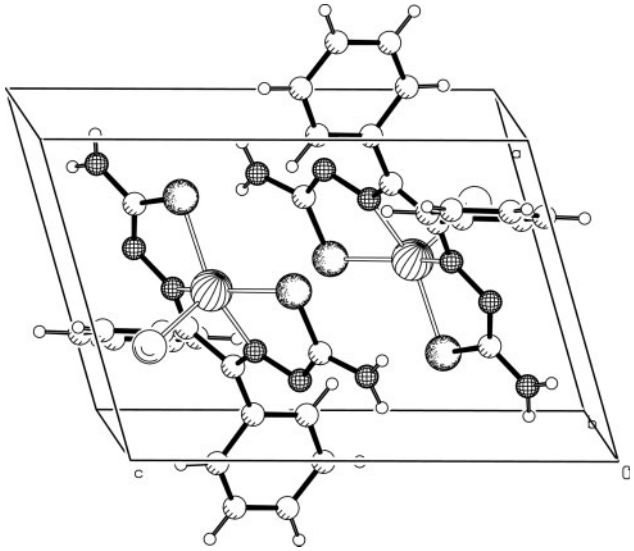


Fig. 6. Crystal packing diagram of diphenyl gallium thiosemicarbazide chloride (compound **7**).

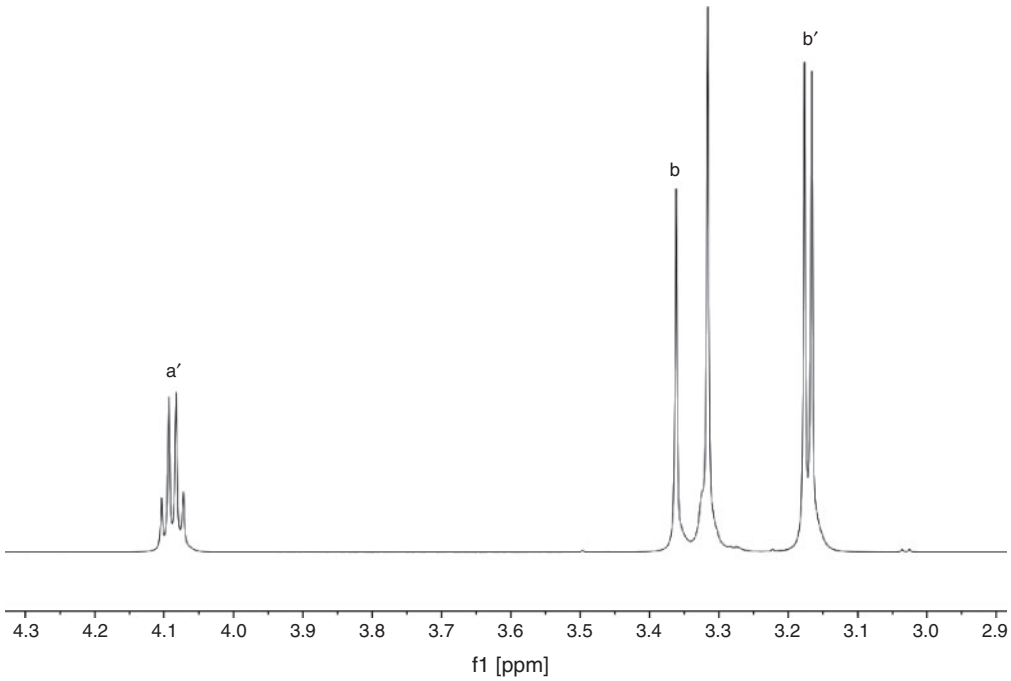


Fig. 7. ^{1}H NMR spectrum of diphenylthiosemicarbazone gallium methoxide (compound **8**) with a molecule of coordinated methanol. (Note: only the resonance of the region of interest is shown; peaks labelled as a′ and b′ represent a quartet and a doublet, respectively, for the hydrogen bonding between methoxide and methanol; peak labelled as b represents the doublet peak in the spectrum.)

and redissolved the residue in dimethyl sulfoxide (we found previously that the fluoride complex is very stable in dimethyl sulfoxide solvent). The following quantities of reagents were used to prepare the nitrate complex.

A 20-mL scintillation vial was charged with compound **7** (10 mg), and methanol (4 mL) was added and the contents were stirred. To this red suspension was added silver nitrate (4 mg), and the mixture was shaken for 3 min, followed by stirring at room temperature for 30 min when an orange yellow solution was obtained and a white precipitate appeared. The silver chloride precipitate was allowed to settle (5 min) and two aliquots of 0.5 mL of the above solution were transferred into two scintillation vials. The solvent was evaporated under nitrogen atmosphere to yield a dark red residue, which was immediately dissolved in dimethyl sulfoxide (0.5 mL) to yield an orange-red solution.

In the meantime, to a 10-mL vial was added potassium carbonate (0.7 mg), followed by Kryptofix 222 (1.2 mg). To this mixture was added acetonitrile (200 µL) and ^{18}F aqueous solution (10 µL; 88 MBq). The vial was sealed and heated on a hot plate under nitrogen atmosphere and allowed to dry. To this dry mixture was added, using a syringe, the gallium nitrate complex (0.5 mL), pre-dissolved in dimethyl sulfoxide. The contents were stirred and heated for 3 min at 40–50°C resulting in a deep red solution of the ^{18}F complex.

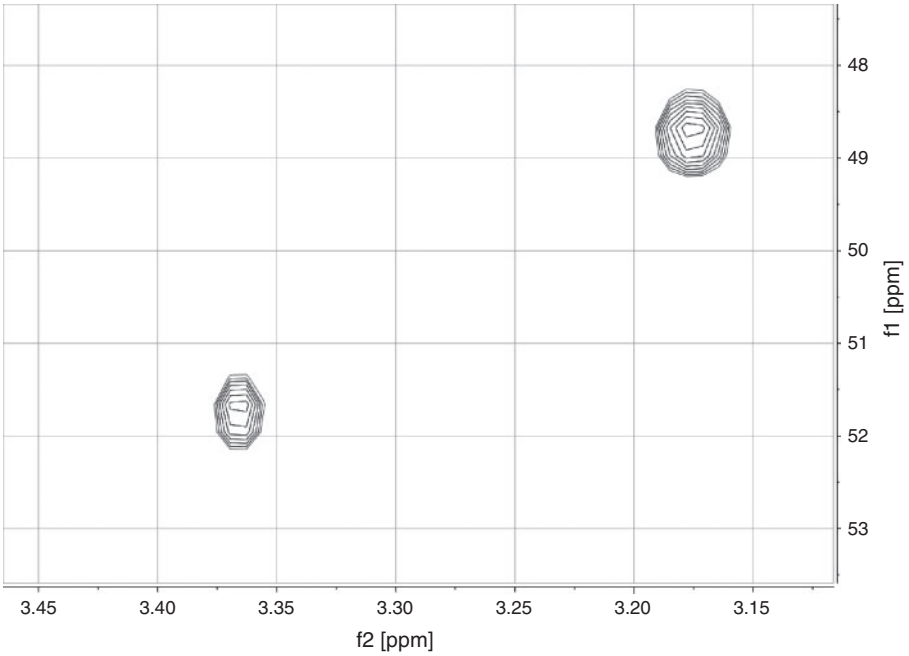
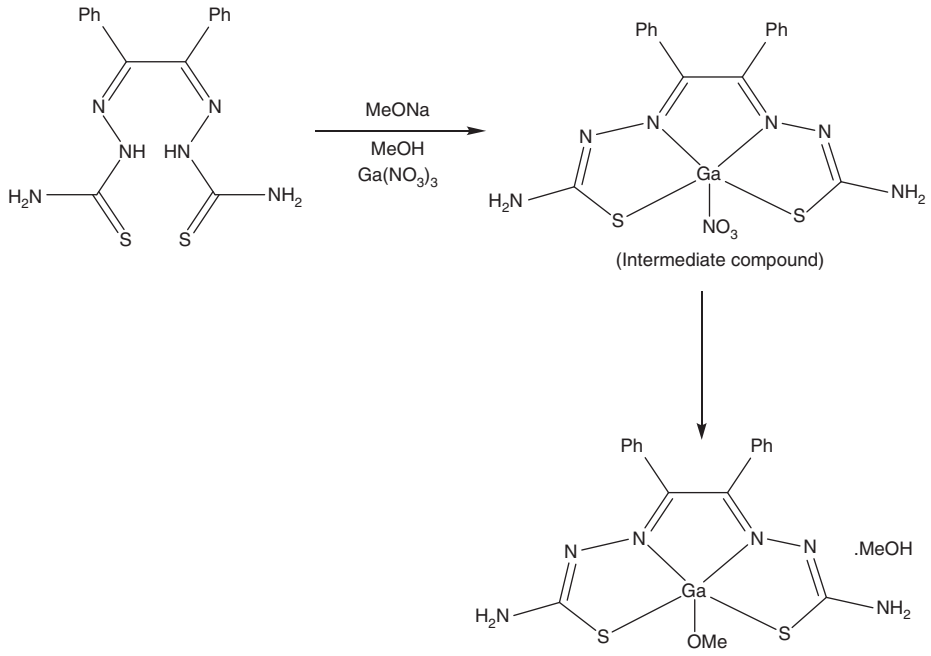


Fig. 8. HSQC spectrum of diphenylthiosemicarbazone gallium methoxide (compound **8**) in d_6-DMSO.



Scheme 2. Formation of diphenylthiosemicarbazone gallium methoxide (compound **8**).

HPLC Conditions for Determination of Radiolabelled Compound

HPLC analysis was performed using a Shimadzu LC 20AD instrument equipped with a diode ray detector, a quaternary pump, a thermostatic column compartment, and a radio detector (Bioscan Flow Count with a pin-diode detector) in conjunction with *LCsolutions* software computer-operated system. The analytical column used was a Zorbax 'Eclipse Plus C18' (Agilent) of dimensions of 4.6 mm (diameter) × 150 mm (length), 5 μ (particle size of column material). The temperature of the column was maintained at room throughout the analysis. The eluent used for eluting the radiolabelled compound was a mixture of acetonitrile and 0.1 % TFA/water (90 : 10). The flow rate was adjusted to 1 mL min^{-1} and the injection volume was 20 μL. Under these experimental conditions, the radiolabelled compound eluted at 15.58 min.

Determination of Partition Coefficient

To determine the liphophilicity of the molecule, a fresh batch of the fluoride gallium complex was made using the exchange method, and it was dissolved in an octanol (20 mL)/water (20 mL) mixture and was stirred vigorously for 15 min. The

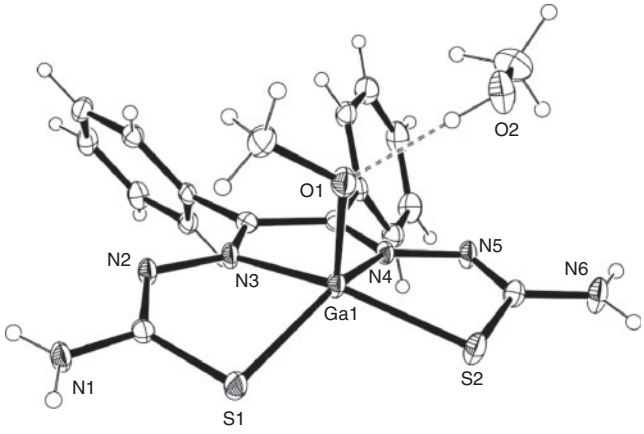


Fig. 9. Single-crystal X-ray structure of diphenylthiosemicarbazone gallium methoxide complex (compound **9**). (Note: a single methanol molecule also co-crystallized in the crystal.)

Table 5. Selected bond lengths (Å) for diphenylthiosemicarbazone gallium methoxide complex (C2)

N2–N3	1.374(3)
N3–Ga1	2.083(2)
N4–N5	1.366(3)
N4–Ga1	2.075(2)
O1–Ga1	1.829(2)
S1–Ga1	2.2990(8)
S2–Ga1	2.3168(7)

Table 6. Selected bond angles (°) for diphenylthiosemicarbazone gallium methoxide complex (C2)

C2–N3–Ga1	116.78(17)
N2–N3–Ga1	120.89(16)
C3–N4–N5	122.2(2)
C3–N4–Ga1	116.27(17)
N5–N4–Ga1	120.54(17)
C4–N5–N4	113.1(2)
C22–O1–Ga1	119.90(19)
C1–S1–Ga1	95.03(9)
C4–S2–Ga1	94.73(9)
O1–Ga1–N4	104.13(9)
O1–Ga1–N3	98.91(9)
N4–Ga1–N3	76.33(9)
O1–Ga1–S1	111.92(7)
N4–Ga1–S1	139.82(7)
N3–Ga1–S1	81.04(6)
O1–Ga1–S2	103.51(7)
N4–Ga1–S2	82.28(6)
N3–Ga1–S2	152.14(7)
S1–Ga1–S2	105.21(3)

resulting layers were separated and analyzed using a UV spectrometer. The ratio of the UV absorbance at 475 nm for each of the layers was used to determine the partition coefficient value. The aqueous solution after storing for 48 h still showed no significant decomposition, as determined by its UV spectral characteristics.

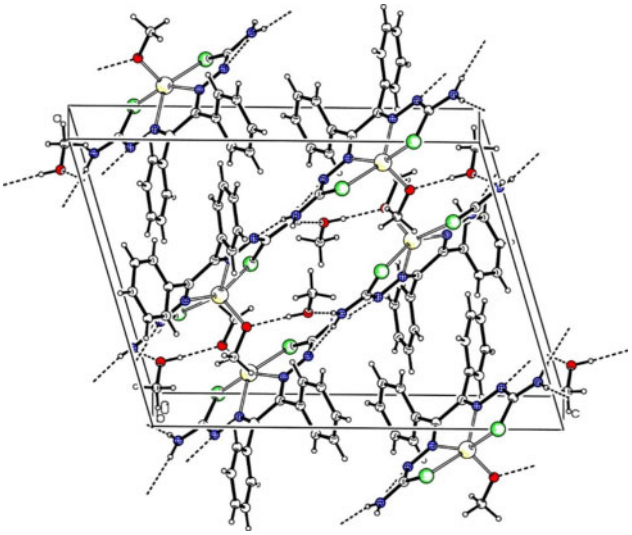


Fig. 10. Crystal packing diagram of the diphenylthiosemicarbazone gallium methoxide complex (compound **8**; **C2**).

Stability of Gallium Chloride and Fluoride Complexes

The prepared complexes were weighed (10 mg) and water was added (pH = 6.4) (10 mL), and the resulting mixture was stirred for 30 min. The compounds did not go into the solution. To further test the stability, the compounds were dissolved in dimethyl sulfoxide and left to stand undisturbed for 3 weeks, and ^{1}H NMR analysis showed no decomposition, thus demonstrating the stability of these complexes.

Results and Discussion

Five thiosemicarbazone ligands have been synthesized by the reaction of thiosemicarbazide with glyoxal and substituted diones. All ligands were prepared and have been fully characterized (Table 1).

Gallium Chloride Complexes

The synthesis of gallium chloride complexes of the thiosemicarbazones is summarized in Scheme 1. The synthesis involved treating the thiosemicarbazone ligands (**2**–**4**) with sodium methoxide, followed by reaction with gallium chloride. All gallium complexes were obtained in high yield. Surprisingly, we were unable to form the gallium chloride complex of ligand **1**.

The NMR spectra of the methyl-substituted symmetrical ligand **2** (Table 1) showed the methyl and NH_2 resonances as expected for the structure. The symmetrical diphenylthiosemicarbazone ligand displays four distinct singlets corresponding to the NH and NH_2 protons (see Fig. 1) on one side of the thiosemicarbazone structure as illustrated below (Chart 1).

^{13}C NMR spectra obtained using a 500 MHz instrument showed only one peak at 178.9 ppm for the C=S carbon. However, at higher field (900 MHz), the improved resolution allows us to observe peaks at 178.8 and 178.9 ppm, demonstrating that we have synthesized an unsymmetrical molecule, with distinct peaks corresponding to the methyl-substituted arm and the ethyl-substituted arm of the molecule. Examination of the ^{15}N spectra using both ^{15}N HSQC and ^{15}N HMBC revealed chemical shift values at 317.9 and 315.7 ppm, representing the C=N nitrogens, 167.8 and 165.9 ppm, representing the NH nitrogen, and peaks at 110.8 and 110.2 ppm, indicating the presence of the two NH_2 groups in the structure of the molecule.

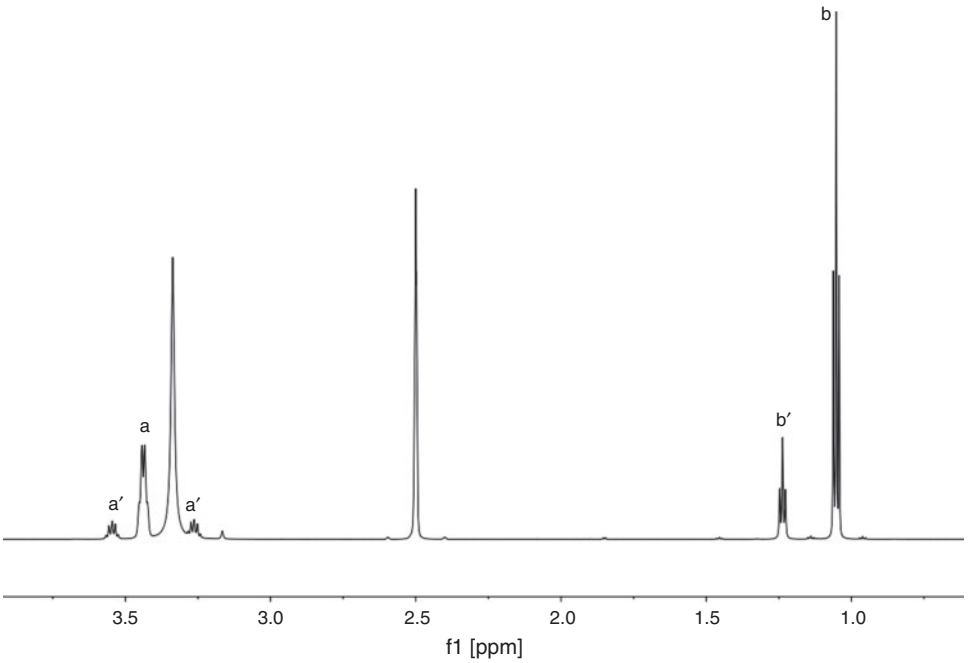


Fig. 11. ^{1}H NMR spectrum of diphenylthiosemicarbazone gallium methoxide (compound **9**) in d_6-DMSO (only resonances in the region of interest are shown; peaks labelled a and b represent a quartet and a triplet, respectively, corresponding to ethanol, whereas peaks labelled a′ and b′ can be ascribed to the ethoxide-coordinated gallium complex). (Note: the multiplicity of the signal shows a pentet due to hydrogen bond formation between the ethoxide and ethanol.)

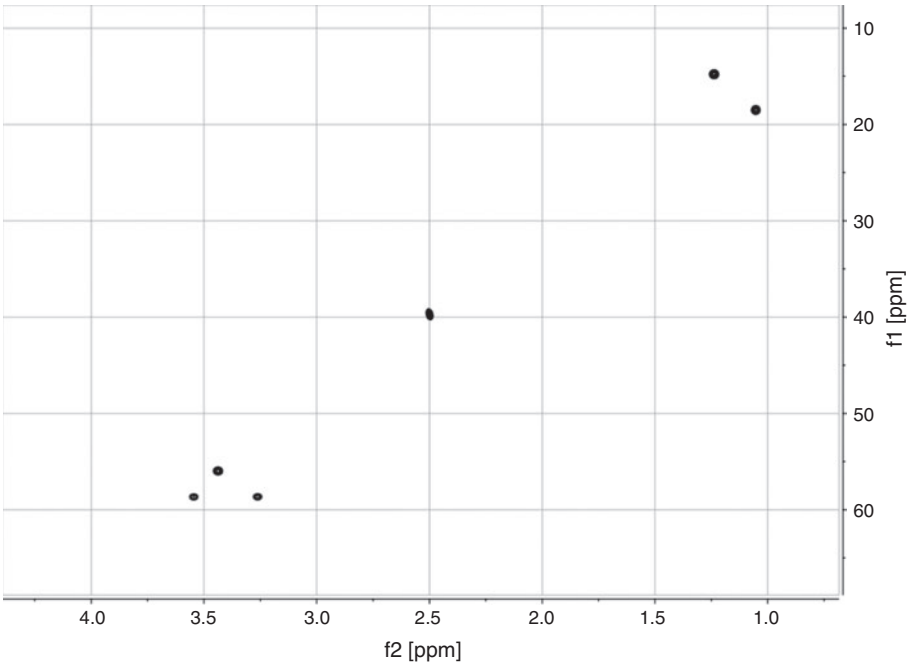
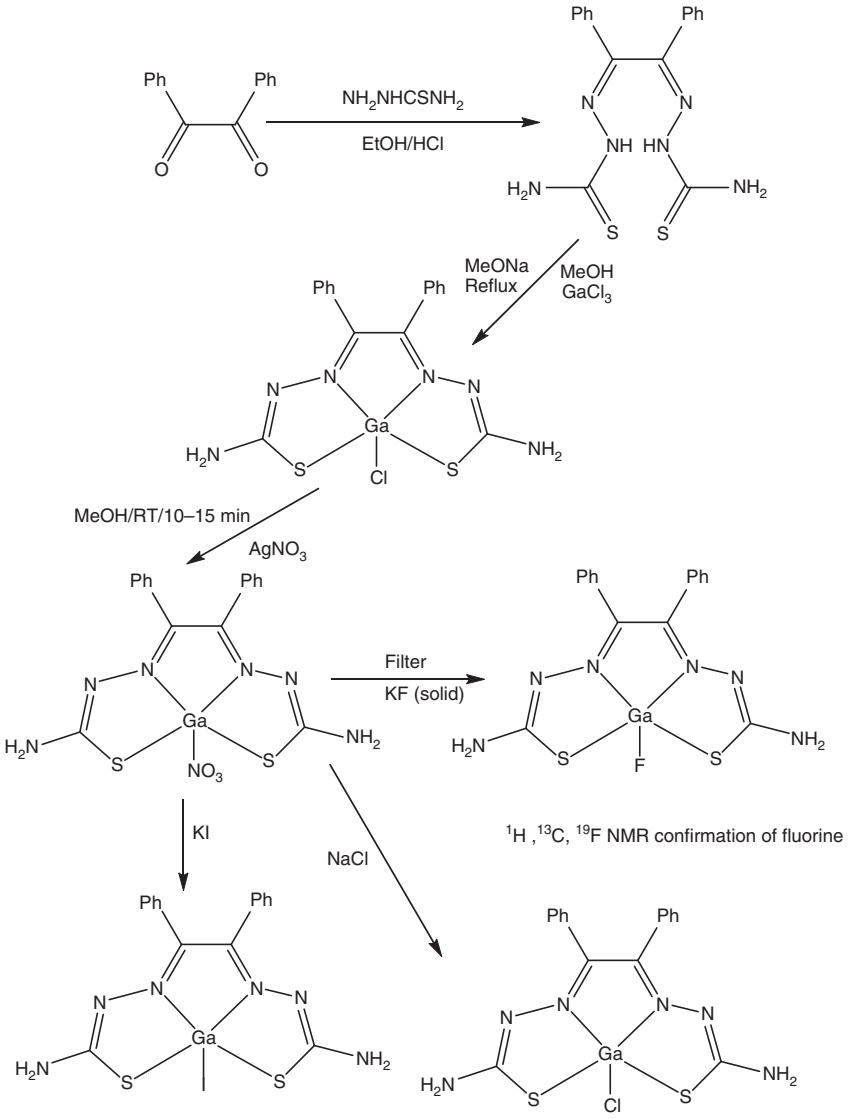


Fig. 12. HSQC spectrum of diphenylthiosemicarbazone gallium ethoxide (compound **9**) in d_6-DMSO showing the coordination of a molecule of ethanol (only resonances for the region of interest are shown).

The gallium chloride complex of ligand **4** showed two singlets very close to each other for the NH_2 protons in each side of the molecule. However, no NH protons of the free ligand were observed, confirming coordination of gallium to the ligand as described in Scheme 1. The ^{13}C NMR spectrum showed C–S signals at 173.9 and 173.6 ppm, respectively (Fig. 2). The ^{15}N NMR spectra showed peaks at 251.2 and 249.5 ppm, corresponding to N=C and peaks at 103.7 and 103.4 ppm, representing the NH_2 protons in the gallium complex. We were unable to obtain the ^{15}N NMR of the C=N nitrogens despite several hours of data acquisition. The NMR data provide clear evidence that the gallium complex showed considerable



Scheme 3. Exchange reactions involving the preparation of fluoro/iodo/chloro diphenylthiosemicarbazone gallium complexes using silver nitrate in methanol. RT, room temperature.

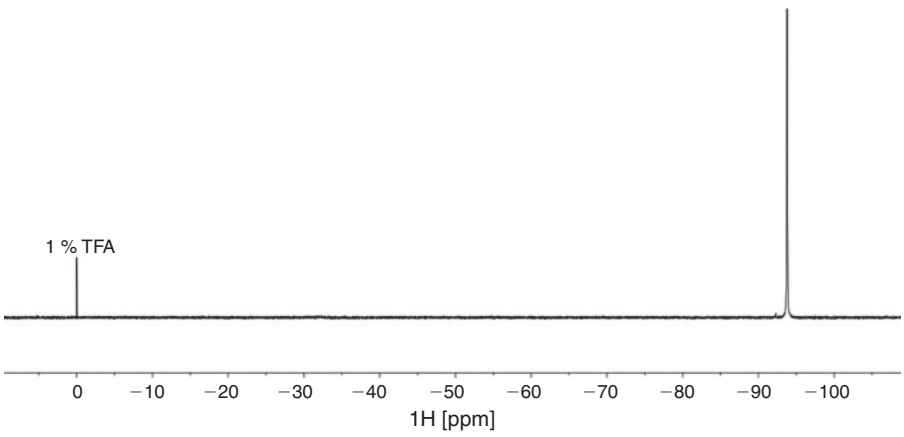
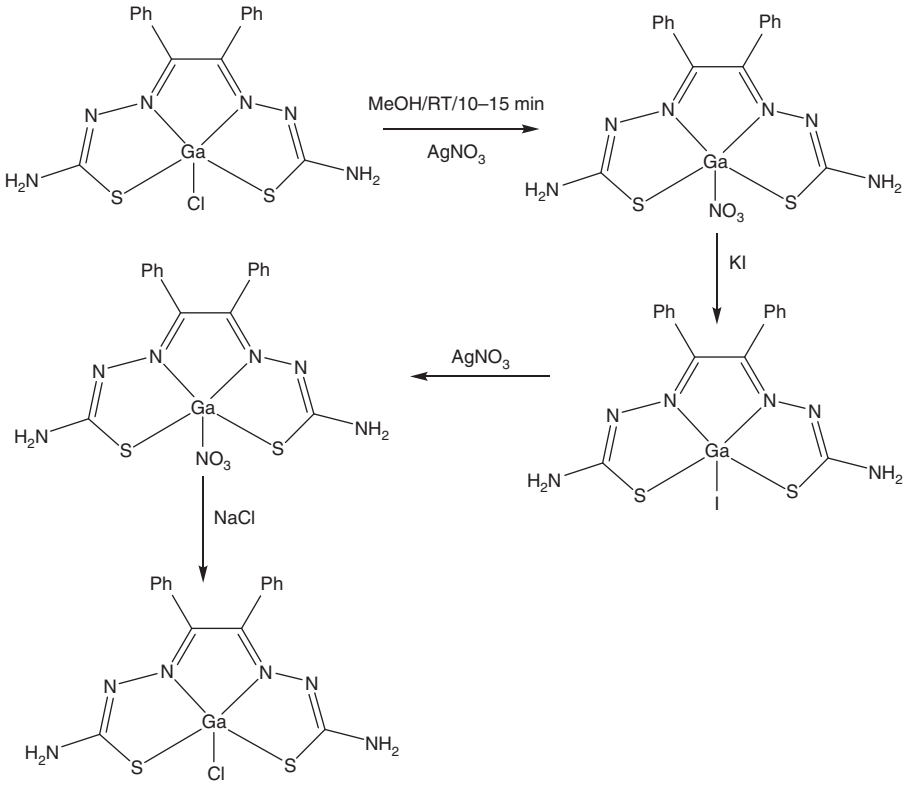
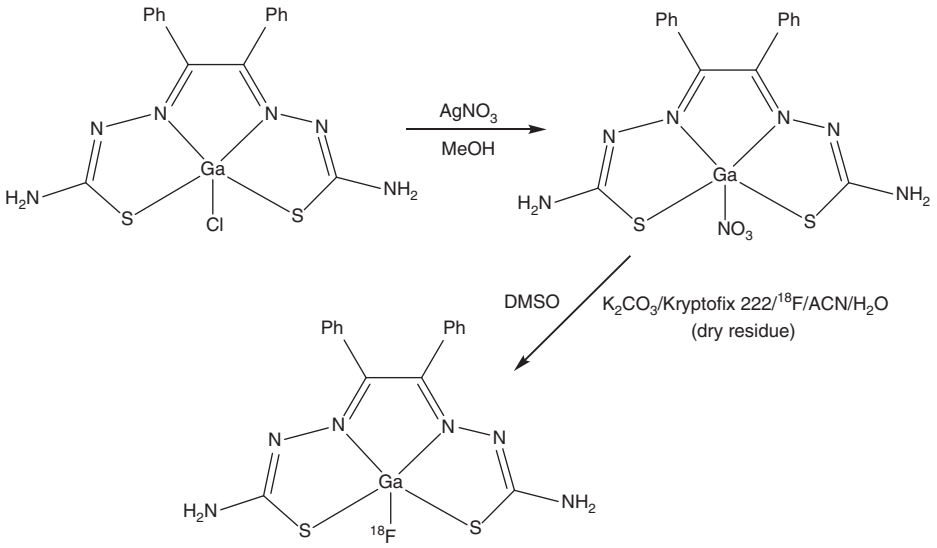


Fig. 13. ^{19}F NMR spectrum of diphenylthiosemicarbazone gallium fluoride complex (compound **10**) after exchange reaction in DMSO solvent (1 % TFA was used in the capillary as reference standard).



Scheme 4. Reversible cycle exchange of halogen in gallium thiosemicarbazone structural core.



Scheme 5. Radiolabelling of gallium-^{18}F thiosemicarbazone complex. ACN, acetonitrile.

difference in their chemical shifts when compared with the starting thiosemicarbazone, confirming complex formation as depicted in Scheme 1. Additional evidence for the presence of gallium in the complex was obtained using ^{71}Ga NMR, which showed a broad peak at 248.7 ppm after 7 h of NMR data acquisition.

The proton NMR of diphenyl-substituted thiosemicarbazone **5** (Fig. 3) shows three singlets with an integral ratio of 2 : 2 : 2, indicating two protons on each of the peaks. The most downfield broad singlet was assigned to one of the NH_2 groups, the next singlet was assigned to NH group protons, and the final singlet was assigned to the other NH_2 protons. ^{13}C NMR showed the C–S signal at 179.1 ppm as a singlet, indicative of a symmetrical compound (without the different chemical shift values observed in the unsymmetrical compound **4**. For this compound, ^{15}N NMR revealed two nitrogens i.e. one corresponding to the NH group at 167.8 ppm and the other corresponding to the NH_2 group at 113.9 ppm. We were unable to detect the signal for the third nitrogen atom associated with C=N of the phenyl group attached moiety in the molecule.

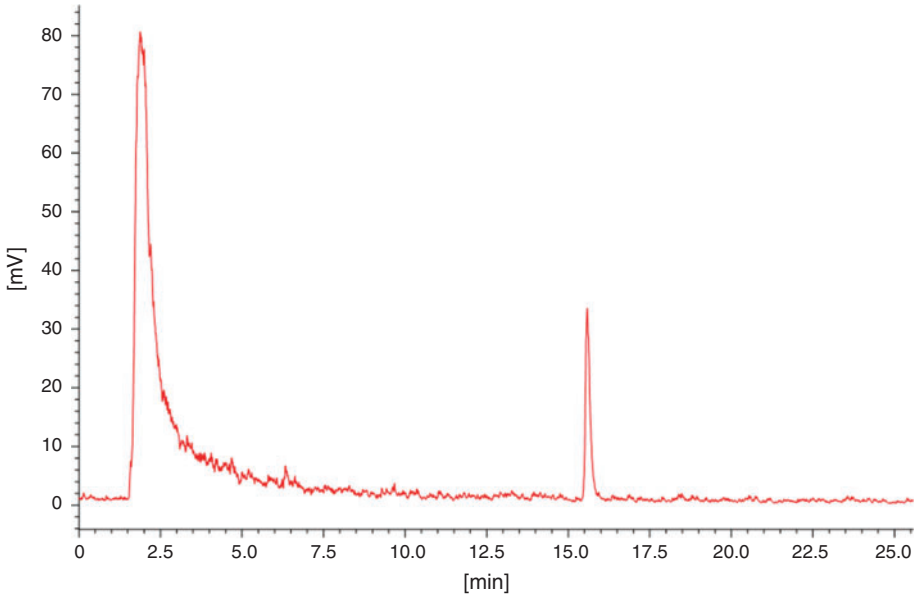


Fig. 14. Radio HPLC of diphenylthiosemicarbazone gallium fluoride (^{18}F).

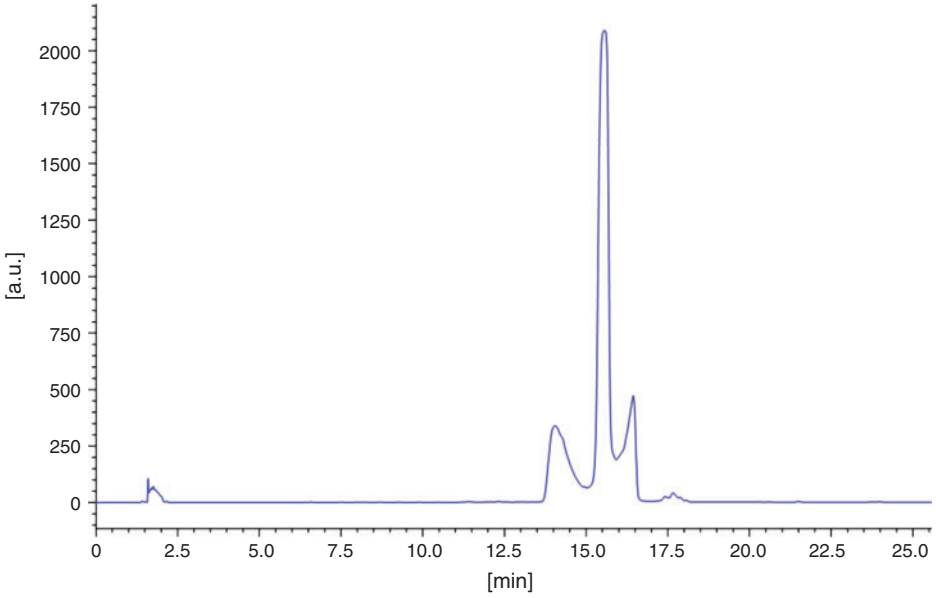


Fig. 15. HPLC of thiosemicarbazone gallium fluoride complex (UV trace).

The ^{1}H NMR chemical shifts for the diphenylthiosemicarbazone gallium chloride complex revealed a broad singlet at 8.14 ppm, corresponding to the NH_2 protons followed by two multiplets at 7.26 and 7.18 ppm, respectively, for the phenyl protons of the molecule (Fig. 3). These are due to the symmetrical nature of the compound. The ^{13}C NMR spectra showed a sharp peak at 174.8 ppm, which was assigned to the C–S carbon. This carbon was shifted upfield by 4 ppm relative to the signal generated by the staring thiosemicarbazone (179.1 ppm), demonstrating the coordination of gallium in the structure. There were five peaks corresponding to the five carbons in the structure of the molecule. ^{15}N spectra showed three nitrogen peaks at 107.9 ppm assigned to NH_2, 253.7 ppm assigned to N=C, and the third at 304.5 ppm assigned to the C=N group. The NH group showed a marked downfield shift in the gallium complex relative to the starting thiosemicarbazone (253.7 ppm versus 167.8 ppm). ^{71}Ga NMR was used to confirm the presence of gallium in the complex. A sharp peak at 248.8 ppm was observed in the gallium NMR spectrum of the compound in d_6-DMSO (Fig. 4). This chemical shift value is consistent with previous studies on metallopolymers by Mejia et al.[44] in which gallium complexes showed a sharp signal at 248 ppm for one compound and 250 ppm for another compound. Based on the NMR results, we assign the structures for the gallium complexes of the unsymmetrical-substituted compound **3** and the diphenyl-substituted compound **5** as shown in Chart 2.

The crystal parameters, including the size and other physical characteristics, are shown in Table 2. The gallium ion has a square pyramidal geometry, and the chloride atom occupies the apex of the coordination sphere (Fig. 5). The crystal structure showed

that one of the phenyl rings is slightly twisted relative to the other phenyl ring, and the sulfur atoms of the thiosemicarbazone moiety are coordinated. The lengths and angles of selective bonds are presented in Tables 3 and 4. The Ga–Cl bond is slightly longer than the Ga–S bond. Cheng et al.[45] reported the Ga–Cl bond distance in several diphosphane and diarsane complexes to be 2.3585 Å, as consistent with the value obtained in our study. The two amino arms were located in the opposite direction of the plane of the molecule. The crystal packing diagram for compound **7** is shown in Fig. 6. The crystal packing diagram shows absence of π–π stacking of the phenyl rings in the structure of the gallium complex molecule.

Gallium Alkoxide Complexes

Attempts to isolate the gallium nitrate complexes by reacting the deprotonated thiosemicarbazone ligands with $Ga(NO_3)_3$ in methanol proved unsuccessful. In fact, this reaction yielded the Ga-OMe complex. For the diphenyl thiosemicarbazone, the proton NMR spectrum showed the expected aromatic peaks in the region of 7.1–8.0 ppm (as observed for the analogous gallium chloride complex). We also observed a quartet centred at 4.08 ppm and a doublet centred at 3.165 ppm (Fig. 7). The quartet is due to the hydrogen bond between the methoxide and another molecule of methanol. In keeping with this, the methyl group showed a doublet. The ^{13}C NMR spectrum showed two peaks at 51.56 and 48.56 ppm, corresponding to the methoxide and methanol. Furthermore, the ^{13}C NMR indicated the presence of two types of MeO group in the product. The HSQC spectra (Fig. 8) revealed a correlation between the coordinated methoxide and the free methanol, demonstrating the presence of a molecule of methanol in the solid state. A single-crystal X-ray diffraction study confirmed the observation of the NMR studies, showing the presence of a gallium-methoxide complex. The formation of the gallium-methoxide complex may be explained by the presence of the sodium methoxide, a base used to deprotonate the ligand, which may have exchanged with a nitrate ion of the gallium-nitrate complex initially formed in the reaction. We propose the following reaction mechanism for the formation of the methoxide complex (Scheme 2).

The gallium nitrate complex has subsequently been synthesized in the absence of methoxide. NMR studies have demonstrated that this gallium nitrate complex is less stable in d_6-DMSO.

The X-ray crystal structure of the gallium methoxide complex showed that additional methanol forms hydrogen bonds with the methoxide in the structure. Hence, two methoxy signals would be expected in the NMR spectrum and that is consistent with the results obtained. The crystal parameters are presented in Table 2. Fig. 9 shows the X-ray crystal structure of the compound. The bond lengths and angles are shown in Tables 5 and 6, respectively, for this complex.

The crystal packing diagram for the methoxide complex is shown in Fig. 10. It is evident from the packing diagram that the methanol coordination with the methoxide of the complex forms infinite hydrogen bonds in the crystal lattice as expected. However, a closer look at the packing diagram reveals the absence of π–π stacking effect in this compound. This observation is consistent with the results obtained for the chloride complex **C1**.

Similarly, we have been able to generate the ethoxide analogue by preparation of the gallium complex using sodium ethoxide instead of sodium methoxide. In a typical synthesis, the diphenylthiosemicarbazone was reacted with gallium nitrate in the presence of sodium ethoxide in ethanol. The ^1H NMR spectrum of the resultant product in DMSO confirmed formation of the ethoxide complex with the gallium as two sets of quartets and triplets were present (see Fig. 11). Ethanol coordination was also observed with two additional sets of quartets and triplets. The ^{13}C NMR spectra also confirmed the ethoxide coordination to gallium along with a molecule of ethanol. The HSQC spectra (Fig. 12) showed the correlation between the coordinated ethoxide with a molecule of ethanol.

Gallium Fluoride and Iodide Complexes

Attempts to synthesize the gallium fluoride via direct reaction of the thiosemicarbazone with $GaF_3 \cdot 3H_2O$ proved difficult due to the poor solubility of the inorganic gallium starting material. As we had synthesized the gallium chloride complexes, we investigated the possibility of direct exchange of the Cl with F by reacting the chloride complex with potassium fluoride. Though NMR suggested that some exchange had occurred, the incorporation of fluoride was low. As a consequence, we developed an alternative approach where we have been able to use the preformed gallium chloride complexes of our thiosemicarbazone ligands successfully from the desired fluoride complexes simply and in high yields. Scheme 3 summarize these reactions.

The first step involves reaction of the chloride complex with an equimolar quantity of silver nitrate in methanol upon which the solution immediately changed from red to orange with precipitation of silver chloride. The solution was filtered and potassium fluoride was added and stirred at room temperature for 10 min, yielding a red solution, which was evaporated under nitrogen to yield an orange red residue. This was dissolved in d_6-dimethyl sulfoxide. The NMR spectrum in d_6-DMSO indicated formation of the fluoride complex. The ^{19}F NMR showed a strong peak indicating complex formation (Fig. 13).

To confirm that the exchange reaction occurring using this halogen exchange method had broader utility, we formed the nitrate compound in situ and then successfully regenerated the chloride complex in moderate yield by the addition of sodium chloride (instead of potassium fluoride, Scheme 4). Similar nitrate-mediated exchange reactions yielded the formation of the iodide derivatives in moderate yield. Thus, we have developed a method that can facilitate the synthesis of fluoro- and iodo-complexes from the chloride analogues. This strategy may be useful as a method of labelling group 13 metals with ^{18}F.

Radiochemistry of the Gallium Chloride Complex

Based on the successful strategy, treatment of the gallium nitrate formed in situ in methanol medium with $^{18}F/H_2O$, obtained directly from the cyclotron, did not furnish the required labelled compound. Repeated trials did not show any radiolabelling effects despite changing the temperature or ratio of reactants. Addition of potassium fluoride in methanol medium also did not yield the required labelled compound. These unsuccessful attempts could be rationalized owing to pH changes in the medium. However, adjustment of the pH with buffers also failed to yield the required compound. Finally, the radiolabelling of the thiosemicarbazone gallium chloride complex was performed following Scheme 5 shown below. In brief, the precursor was treated with silver nitrate in methanol medium to furnish the nitrate in situ, followed by the reaction of $K^{18}F$ in dimethyl sulfoxide to yield finally the Ga-^{18}F thiosemicarbazone complex as a red solution. The complete details of the reaction

conditions and the yield are described in the experimental section.

The following HPLC chromatograms show both the hot peak of ^{18}F as well as the UV trace of the complex (Figs 14 and 15). The retention time of the product was 15.7 min under our experimental conditions. The complex was stable for prolonged periods of time under vacuum in a sealed vial (Fig. 14 and Scheme 5).

Thus, we were able to radiolabel the gallium complex of diphenylthiosemicarbazone chloride with ^{18}F positron emitter in a short time under less harsh experimental conditions. This novel strategy may be applicable to introducing radio fluorine atom in complexes that are resistant to direct fluorination. Furthermore, it is important to note that the insoluble nature of group 13 metal fluorides in various organic solvents hampers the introduction of the fluorine atom directly. However, this halogen exchange method enables radiofluorination.

The ^{18}F-complex showed a peak at 15.5 min consistent with the cold fluoride complex. The observed retention time was similar to that obtained for dimethylthiosemicarbazone gallium chloride complex,[46] further confirming the formation of the gallium-^{18}fluoride complex.

The partition coefficient was also measured for the cold fluoro gallium complex using the absorbance at 475 nm in an octanol/water mixture, yielding a log *P* value of 1.1. The complex was stable at room temperature for more than 24 h in the presence of water. Further work is in progress to optimize radiolabelling including purification, stability, and in vivo efficacy to understand the usefulness of this compound as an imaging agent.

Conclusions

Several thiosemicarbazone gallium complexes have been synthesized, and their structural characteristics have been analyzed using multinuclear NMR techniques. The formation of these gallium complexes with thiosemicarbazones is further confirmed by single-crystal X-ray crystallographic methods. Diphenylthiosemicarbazone forms gallium chloride/methoxide/ethoxide/nitrate complexes. We provide evidence that, in the presence of a base, the methoxide or ethoxide forms the corresponding gallium-coordinated species, which are stable. The gallium methoxide and ethoxide complexes are formed through the intermediate nitrate complex by elimination of nitrate. The X-ray crystal structure shows that the gallium forms a penta-coordinated complex with chloride (or methoxide). Furthermore, we have demonstrated that the 'chloride ion' of the pre-formed gallium chloride complexes of our thiosemicarbazone ligands can be readily exchanged with fluoride (or iodide) via a nitrate-mediated exchange reaction, potentially opening future opportunities for the formation of inorganic gallium complexes with the radioisotope ^{18}F that may have potential application in positron emission topography imaging.

Acknowledgements

This work was partially supported by an ARC-Linkage grant LP 130100703 (D.R). We thank the colleagues of the Centre for Advanced Imaging for their help during the course of this investigation.

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