Rapid decomplexation of *bis*(thiosemicarbazonato)zinc(II) complexes using citric acid

Jason P. Holland[a,b]*, Jennie A. Hickin[a], Emma Grenville-Mathers[a], ThaoNguyen Nguyen[a] and Josephine M. Peach[a]*

[a]*Department of Chemistry, University of Oxford, Chemistry Research Laboratory, 12 Mansfield Road, Oxford OX1 3TA, UK*

[b]*Memorial Sloan-Kettering Cancer Centre, 1275 York Avenue, New York, NY10021, USA*

Facile and quantitative dissociation of *bis*(thiosemicarbazonato)zinc(II) complexes to give the corresponding neutral proligands in solution has been achieved by reaction with citric acid. This reaction provides an elegant solution to a problem encountered in the design, synthesis and purification of potential copper-based radiopharmaceuticals.

Keywords: radiopharmaceuticals, bis(thiosemicarbazonato) complexes, citric acid, copper, zinc

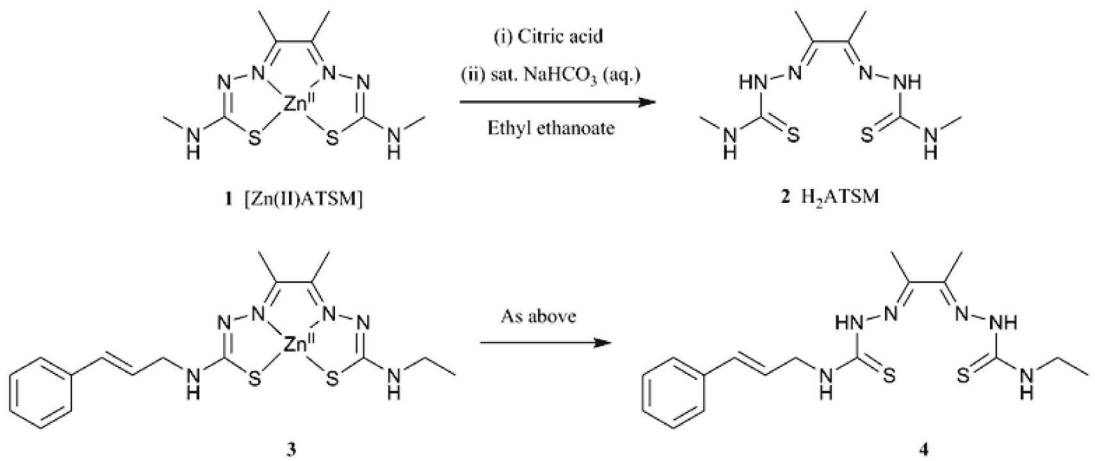
In the modern clinical environment, *in vivo* molecular imaging plays a vital role in drug discovery and in the diagnosis and treatment of disease.[1-4] In recent years, there has been intense research into the development of copper-based radiopharmaceuticals for imaging tumour hypoxia (tissue oxygenation <10% of normal levels).[5,6] In particular, the diacetyl-2,3-*bis*(4-*N*-methyl-3-thiosemicarbazonato)copper(II) complex, [Cu(II)ATSM] has emerged as the leading hypoxia-selective agent for positron emission tomography (PET) imaging and radiotherapy.[7-9] Recently, Donnelly *et al.*[10] have reported that selective intracellular release of copper and zinc ions from *bis*(thiosemicarbazonato) complexes reduce levels of amyloid-β peptide, which is an important factor in the regulation of Alzheimer's Disease (AD). These metal chelates have potential to be used in the diagnosis and treatment of patients with AD.

Synthetic work has lead to the development of new intermediates for the facile conjugation of *bis*(thiosemicarbazonato)zinc(II) complexes to a range of biologically active molecules.[11,12] Transmetallation of *bis*(thiosemicarbazonato) zinc(II) complexes with copper diacetate, $Cu(OAc)_2$(aq.) has also been shown to be an efficient and rapid method for the preparation of copper-64 radiolabelled complexes for PET imaging.[11]

Despite the potential advantages of using zinc(II) complexes as precursors for copper radiolabelling, during recent efforts towards the synthesis of *bis*(thiosemicarbazonato)metal(II) complexes conjugated to fluorophores such as pyrene, dansyl chloride and fluorescein, the zinc(II) complexes were found to be less reactive than their corresponding metal-free proligands. However, synthesis of the ligands was found to be best achieved using zinc(II) ions as a templating agent. Purification of the zinc(II) complexes was also found to be easier due to their increased solubility in common organic solvents in comparison to the corresponding proligands. Therefore, in order to facilitate these conjugation reactions we investigated several potential solution and solid-phase methods for the removal of zinc(II) ions from *bis*(thiosemicarbazonato)zinc(II) complexes.

Initial work focused on the use of imidodiacetate resins to remove the zinc(II) ions from complex **1**, [Zn(II)ATSM] to give the neutral proligand **2**, H_2ATSM. Although the reaction was found to be successful at room temperature in dimethyl sulfoxide (DMSO), proligand **2** could only be isolated on the milligram scale and the kinetics were found to be very slow (>24 h for the purification approx. 2 mg of **2**; the reaction was found to be even slower in ethyl acetate or methanol). In addition, recovery of the proligand from DMSO was found to be difficult. Solid-phase attempts were subsequently abandoned in favour of more rapid and high yielding solution-phase methods (*vide infra*).

It was noted that the characteristic bright yellow colour of complex **1** (λ_{max}(DMF) = 431 nm, with molar absorption coefficient, ε = 11266 M^{-1} cm^{-1}),[12] was lost on addition of strong acids such as HCl, HNO_3, H_2SO_4 and HBF_4 to a solution of complex **1** in ethyl acetate, ethanol, methanol, DMSO, or dimethyl formamide (DMF), giving a colourless/pale yellow solution. Protonation of complex **1** with strong acids leads to



1 [Zn(II)ATSM] **2** H_2ATSM **3** **4**

Scheme 1 Facile and quantitative removal of the zinc(II) ions from *bis*(thiosemicarbazonato)zinc(II) complexes to give the corresponding proligands.

* Correspondent. E-mail: hollanj3@mskcc.org

initial demetallation. However, it was not possible to isolate proligand **2** after reaction with these strong acids. Therefore, the reactions of complex **1** with weaker acids, especially citric acid, were investigated (Scheme 1).

Reaction of a solution of complex **1** or **3** in ethyl acetate with 10% citric acid (aq.) at room temperature, followed by neutralisation with saturated sodium hydrogen carbonate solution, organic-layer extraction, drying and removal of the solvent under reduced pressure, was found to give proligands **2** and **4**, respectively, in quantitative yield. The reaction to give proligand **4** has been successfully scaled up to >400 mg of complex **3**.

In conclusion the facile demetallation of zinc(II) ions from *bis*(thiosemicarbazonato)zinc(II) complexes by the reaction with citric acid will facilitate the synthesis and purification of new ligands for use as potential copper-based radiopharmaceuticals for PET imaging and radiotherapy. This reaction is being used frequently in the synthesis of functionalised bis(thiosemicarbazonato) ligands at Oxford.

Experimental

General experimental apparatus used has been described elsewhere.[11,12] High performance liquid chromatography was conducted by using an acetonitrile/water gradient elution method and unless otherwise stated a flow rate of 1.0 ml/min.[11]

Diacetyl-bis(4-N-methyl-3-thiosemicarbazonato) zinc(II) [Zn(II) ATSM] **(1)**: Synthesised in accordance with a previously reported procedure.[13] Proligand **2** (1.50 g, 5.78 mmol) was reacted with 1.1 equiv. $Zn(OAc)_2.2H_2O$ (1.40 g, 6.3 mmol) in ethanol (30 ml) under reflux for 4 h. Complex **1** was isolated as a yellow powder (1.57 g, 4.85 mmol, 84%). Elemental analysis (%) for $C_8H_{14}N_6S_2Zn$ (323.77 g mol^{-1}) Calcd C 29.7, H 4.4, N 26.0, S 19.8 and Zn 20.2; found C 29.8, H 4.4, N 25.9, S 19.5 and Zn 20.4. ^{1}H NMR (300 MHz, DMSO-d_6): δ/ppm 7.21 (2H, br, CH_3NH); 2.82 (6H, d, CH_3NH); 2.20 (6H, s, CH_3C=N). MS (ES$^+$): *m/z* (Calcd) 323.0104 (323.0091) = {M + H$^+$} 100%. λ_{max}(DMF)/nm 431 (ε/M^{-1} cm^{-1} 11266), 312 (11978). HPLC: R_t 10.40 min (Flow rate: 0.9 ml/min).

Diacetyl-bis(4-N-methyl-3-thiosemicarbazone) H_2ATSM **(2)**: Proligand was synthesised in accordance with a previously reported procedure.[13] Proligand **2** was isolated as a white powder (1.88 g, 7.2 mmol, 76%). Elemental analysis (%) for $C_8H_{16}N_6S_2$ (260.38 g mol^{-1}) Calcd C 36.9, H 6.2, N 32.3 and S 24.6; found C 36.9, H 6.3, N 32.1 and S 24.5. ^{1}H NMR (300 MHz, DMSO-d_6): δ/ppm 10.31 (2H, br s, 2 × C=SN*H*); 8.46 (2H, br m, 2 × CH_3NH); 3.09 (6H, d, $^3J_{HH}$ = 4.4 Hz, 2 × CH_3NH); 2.29 (6H, s, 2 × CH_3C=N). ^{13}C{^{1}H} NMR (75.5 MHz, DMSO-d_6): δ/ppm 178.96 (2 × *C*=*S*), 148.52 (2 × *C*=*N*), 31.77 (2 × CH_3NH), 12.25 (2 × CH_3C=N). MS (ES$^+$): *m/z* (Calcd) 261.0949 (261.0956) = {M + H$^+$} 100%. λ_{max}(DMF)/nm 344 (ε/mol^{-1} dm^3 cm^{-1} 36814), 334 (37813) and 273 (12174). HPLC: R_t 10.31 min.

Diacetyl-2-(4-N-(1-phenylprop-1(E)-enyl)-3-thiosemicarbazonato)-3-(4-N-ethyl-3-thiosemicarbazonato) zinc(II) **(3)**: Synthesised in accordance with a previously reported procedure.[12] Complex **3** was isolated as a yellow powder (0.16 g, 0.37 mmol, 93%). Elemental analysis (%). $C_{17}H_{22}N_6S_2Zn$ (438.06 g mol^{-1}) Calcd C 46.2, H 4.9, N 18.9, S 15.1 and Zn 15.3; found C 46.4, H 5.0, N 19.1, S 14.6 and Zn 14.9. ^{1}H NMR (300 MHz, DMSO-d_6): δ/ppm 7.50 (1H, br, (Ph)CH=CHCH$_2$N*H*); 7.38 (2H, d, $^3J_{HH}$ = 7.1 Hz, *o-Ph*); 7.31 (2H, app. t, $^3J_{HH}$ = 7.2 Hz, *m-Ph*); 7.25 – 7.18 (2H, m, *p-Ph* and CH_3CH_2NH, assigned by 2D-COSY); 6.51 (1H, d, $^3J_{HH}$ = 15.9 Hz, (Ph)CH=CHCH$_2$); 6.33 (1H, dt, $^3J_{HH}$ = 15.9 and 5.8 Hz, (Ph)CH=CHCH$_2$); 4.13 (2H, m, (Ph)CH=CHCH_2); 3.37 (2H, assigned by 2D-COSY, CH_3CH_2NH); 2.20 and 2.18 (6H, two overlapping singlets, CH_3C=N); 1.10 (3H, t, $^3J_{HH}$ = 7.1 Hz, CH_3CH_2NH). ^{13}C{^{1}H} NMR (75.5 MHz, DMSO-d_6): δ/ppm 175.49 (*C*=*S*); 144.75 (*C*=*N*) (NB: only two weak quaternary resonances were observed); 136.72 ((Ph)*CH*=CHCH$_2$); 130.10 (*i-Ph*); 128.56 (*m-Ph*); 127.47 (*p-Ph*); 127.22 ((Ph)CH=CHCH$_2$); 126.00 (*o-Ph*); 44.08 ((Ph)CH=CHCH$_2$); 36.88 (CH_3CH_2NH); 14.60 (CH_3CH_2NH); 13.92 (CH_3C=N); 13.78 (CH_3C=N). MS (ES$^+$): *m/z* (Calc.) 439.0707 (439.0717) = {M + H$^+$} 100%. λ_{max}(DMSO)/nm 437 (ε/M^{-1} cm^{-1} 11328) and 319 (13900). HPLC: R_t 15.91 min.

Diacetyl-2-(4-N-ethyl-3-thiosemicarbazone)-3-(4-N-(1-phenyl-prop-1(E)-enyl)-3-thiosemicarbazone) **(4)**: Synthesised in accordance with a previously reported procedure.[12] Compound **4** was isolated as a cream/white powder (0.43 g, 1.1 mmol, 79%). Elemental analysis (%) for $C_{17}H_{24}N_6S_2$ (376.15 g mol^{-1}) Calcd C 54.0, H 6.4, N 21.9 and S 17.5; found C 54.2, H 6.4, N 22.3 and S 17.0. ^{1}H NMR (300 MHz, $CDCl_3$): δ/ppm 10.34 (1H, s, NHC(= S)N*H*N =); 10.17 (1H, s, NHC(= S)N*H*N =); 8.66 (1H, br m, (Ph)CH=CHCH$_2$N*H*); 8.43 (1H, br m, CH_3CH_2NH); 7.41 (2H, d, $^3J_{HH}$ = 7.4 Hz, *o-Ph*); 7.32 (2H, app. t, $^3J_{HH}$ = 7.3 Hz, *m-Ph*); 7.21 (1H, m, $^3J_{HH}$ = 7.3 Hz, *p-Ph*); 6.52 (1H, d, $^3J_{HH}$ = 16.0 Hz, (Ph)CH=CHCH$_2$NH); 6.35 (1H, dt, $^3J_{HH}$ = 16.0 and 5.4 Hz, (Ph)CH=CHCH$_2$); 4.41 (2H, m, (Ph)CH=CHCH_2); 3.60 (2H, m, $^3J_{HH}$ = 6.6 Hz, CH_3CH_2NH); 2.23 (6H, two overlapping singlets, CH_3C=N); 1.14 (3H, t, $^3J_{HH}$ = 6.9 Hz, CH_3CH_2NH). ^{13}C{^{1}H} NMR (75.5 MHz, DMSO-d_6): δ/ppm 177.82 (*C*=*S*); 177.31 (*C*=*S*); 148.27 (*C*=*N*); 147.85 (*C*=*N*); 136.45 ((Ph)*CH*=CHCH$_2$); 130.40 (*i-Ph*); 128.57 (*m-Ph*); 127.37 (*p-Ph*); 126.40 ((Ph)CH=CHCH$_2$); 126.01 (*o-Ph*); 45.54 ((Ph)CH=CHCH$_2$); 38.50 (CH_3CH_2NH); 14.32 (CH_3CH_2NH); 11.74 (CH_3C=N); 11.69 (CH_3C=N). MS (ES$^+$): *m/z* 399 = {M + Na$^+$} 100%. HPLC: R_t 15.72 min.

Reactions with citric acid

Decomplexation of *bis*(thiosemicarbazonato)zinc(II) complexes (**1** or **3**), to give the corresponding neutral proligands (**2** or **4**) in solution was achieved using the following general procedure. *Bis*(thiosemicarbazonato)zinc(II) complex (Complex **1**, 50.0 mg, 0.015 mmol) was dissolved in ethyl acetate (100 ml) and 10% aqueous citric acid solution (100 ml) was added. The solution was stirred for 5 min at room temperature during which time the colour changed from bright yellow to colourless/pale yellow. Saturated $NaHCO_3$(aq.) (100 ml) was then added and the organic layer was extracted (3 × 50 ml ethyl acetate), dried over anhydrous magnesium sulfate, filtered and the solvent was removed under reduced pressure to give either proligands **2** or **4** as a white powder. (Proligand **2**, 40.1 mg, 100%). Analytical data were fully consistent with previous reports.[12,13] Removal of zinc(II) ions from complex **3** to give proligand **4** was also found to be quantitative.

J.P.H. thanks the Engineering and Physical Sciences Research Council (EPSRC) and Merton College (Oxford) for financial support. We thank Prof. Jonathan R. Dilworth and Prof. Jennifer C. Green for helpful discussions. We are indebted to Dr Nick Rees, Mr Colin Sparrow and Mrs Maria Marshall for technical support.

Received 22 July 2008; accepted 15 October 2008
Paper 08/0068 doi:10.3184/030823408X380731
Published online: 10 December 2008

References

1 M. Rudin and R. Weissleder, *Nature Rev. Drug Dis.*, 2003, **2**, 123-131.
2 P. Blower, *Dalton Trans.*, 2006, 1705-1711.
3 M.J. Adam and D.S. Wilbur, *Chem. Soc. Rev.*, 2005, **34**, 153-163.
4 C.J. Anderson and M.J. Welch, *Chem. Rev.*, 1999, **99**, 2219-2234.
5 S. V. Smith, *J. Inorg. Biochem.*, 2004, **98**, 1874-1901.
6 J.P. Holland, J.C. Green and J.R. Dilworth, *Dalton Trans.*, 2006, 783-794.
7 A.L. Vavere and J.S. Lewis, *Dalton Trans.*, 2007, 4893-4902.
8 J.S. Lewis, R. Laforest, T.L. Buettner, S.-K. Song, Y. Fujibayashi, J.M. Connett and M.J. Welch, *Proc. Nat. Acad. Sci.*, 2001, **98**, 1206-1211.
9 A. Obata, S. Kasamatsu, J.S. Lewis, T. Furukawa, S. Takamatsu, J. Toyohara, T. Asai, M.J. Welch, S.G. Adams, H. Saji, Y. Yonekura and Y. Fujibayashi, *Nucl. Med. Biol.*, 2005, **32**, 21-28.
10 P.S. Donnelly, A. Caragounis, T. Du, K.M. Laughton, I. Volitakis, R.A. Cherny, R.A. Sharples, A.F. Hill, Q.-X. Li, C.L. Masters, K.J. Barnham and A.R. White, *J. Biol. Chem.*, 2008, **283**, 4568-4577.
11 J.P. Holland, F.I. Aigbirhio, H.M. Betts, P.D. Bonnitcha, P. Burke, M. Christlieb, G.C. Churchill, A.R. Cowley, J.R. Dilworth, P.S. Donnelly, J.C. Green, J.M. Peach, S.R. Vasudevan and J.E. Warren, *Inorg. Chem.*, 2007, **46**, 465-485.
12 J.P. Holland, P.J. Barnard, S.R. Bayly, H.M. Betts, G.C. Churchill, J.R. Dilworth, R. Edge, J.C. Green and R. Hueting, *Eur. J. Inorg. Chem.*, 2008, 1985-1993.
13 A.R. Cowley, J. Davis, J.R. Dilworth, P.S. Donnelly, R. Dobson, A. Nightingale, J.M. Peach, B. Shore, D. Kerr and L. Seymour, *Chem. Commun.*, 2005, 845-847.