Supporting Information

H_2Me-do2pa: An attractive chelator with fast, stable and inert $^{nat}Bi^{3+}$ and $^{213}Bi^{3+}$ complexation for α-radioimmunotherapy applications

Luís M. P. Lima,[a,b] Maryline Beyler,[a] Fatima Oukhatar,[a] Patricia Le Saec,[c] Alain Faivre-Chauvet,[c] Rita Delgado,*[,b] Carlos Platas-Iglesias*[,d] and Raphaël Tripier*[,a]

[a] Université de Bretagne Occidentale, UMR-CNRS 6521, UFR des Sciences et Techniques, 6 avenue Victor le Gorgeu, C.S. 93837, 29238 Brest Cedex 3.

[b] Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Av. da República, 2780-157 Oeiras, Portugal.

[c] Centre de Recherche en Cancérologie Nantes-Angers (CRCNA), Unité INSERM 892 - CNRS 6299, 8 quai Moncousu, BP 70721 44007 Nantes Cedex, France

[d] Departamento de Química Fundamental, Facultade de Ciencias, Universidade da Coruña, Campus da Zapateira-Rúa da Fraga 10, 15008 A Coruña, Spain.

Contents

Experimental section

Synthesis. $Bi(NO_3)_3.5H_2O$ was purchased from ACROS Organics. H_2**Me-do2pa** was synthesized as previously described.[1] NMR spectra (^1H and ^{13}C) were recorded at the "Services communs" of the University of Brest, with Bruker Avance 500 (500 MHz) or Bruker AMX-3 300 (300 MHz) spectrometers. The HR-MS analyses were performed at the Institute of Analytic and Organic Chemistry, ICOA in Orleans.

[Bi(Me-do2pa)](NO_3). The H_2**Me-do2pa**·4HCl (28 mg, 0.045 mmol) was dissolved in 3 mL of water and $Bi(NO_3)_3.5H_2O$ (22 mg, 1 eq.) was added. The mixture (pH = 1) was stirred at RT for 30 min and then the pH was raised to 4 by addition of KOH. A white precipitate was formed and the mixture was stirred for 16 h under reflux. The reaction mixture was cooled down to RT, and MeOH was added till formation of a precipitate, which was filtered off. The filtrate was evaporated and washed several times with a mixture of MeOH/acetone to give 32 mg (82 %) of a pale yellow solid [Bi(**Me-do2pa**)](NO_3). RMN ^1H: δ_H (solvent D_2O, 298 K, 300 MHz): 2.38 (s, 6 H, $-CH_3$), 2.99–3.47 (m, 12 H, $-CH_2-$), 3.60 (m, 4 H, $-CH_2-$), 4.86 (q, 4 H, $-CH_2-py$), 7.96 (d, 2 H, $^3J = 7.4$ Hz, py), 8.04 (d, 2 H, $^3J = 7.4$ Hz, py), 8.32 (t, 2 H, $^3J = 7.4$ Hz, py). RMN ^{13}C: δ_C (solvent D_2O, 298 K, 125.8 MHz): 46.65, 51.77 (primary C); 53.49, 55.94, 57.53, 59.61, 62.12 (secondary C); 129.39, 130.75, 145.47 (tertiary C); 151.55, 158.96, 174.00 (quaternary C). HRMS: *m/z* found 677.2280, calcd $C_{24}H_{32}BiN_6O_4$ (M^+) 677.2284.

Single crystal X-ray diffraction measurements. Single-crystal X-ray diffraction data were collected at 170 K on an X-CALIBUR-2 CCD 4-circle diffractometer (Oxford Diffraction) with graphite-monochromatized MoK_α radiation ($\lambda = 0.71073$). Crystal data and structure refinement details are given in Table S1. Unit-cell determination and data reduction, including interframe scaling, Lorentz, polarization, empirical absorption and detector sensitivity corrections, were carried out using attached programs of Crysalis software (Oxford Diffraction).[2] Structures were solved by direct methods and refined by full matrix least squares method on F^2 with the SHELXL suites of programs.[3] The hydrogen atoms were identified at the last step and refined under geometrical restraints and isotropic U-constraints. CCDC 1009076 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from the Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

Thermodynamic stability studies. The setup for potentiometric titrations has been described before.[4] The titrant was a carbonate-free KOH solution at *ca.* 0.50 M prepared from a commercial ampoule of analytical grade, and the exact concentration was obtained by application of the Gran's method[5] upon titration of a standard HNO_3 solution. A stock solution of H_2**Me-do2pa** was prepared at *ca.* 2×10^{-3} M. An analytical solution of $Bi(NO_3)_3$ was prepared at 0.025 M in 1.0 M aqueous HCl to avoid metal hydrolysis, and was standardized by complexometric titration against a standard K_2H_2edta (ethylenediaminetetraacetic acid) solution.[6] Potentiometric titrations were run with *ca.* 0.04 mmol of ligand in a total volume of 30.00 mL at 25.0 ± 0.1 ºC and with the ionic strength kept at 0.50 ± 0.01 M using KCl as background electrolyte. The $[H^+]$ of the solutions was determined by measurement of the electromotive force of the cell, $E = E^0 + Q \log[H^+] + E_j$. The term pH is defined as $-\log [H^+]$, and a value of $K_w = [H^+][OH^-] = 10^{-13.72}$ was taken from the literature for our experimental conditions.[7] The terms E^0 and Q were determined by titrating a solution of known hydrogen-ion concentration at the same ionic strength. The liquid-junction potential, E_j, was found to be negligible under the experimental conditions used. Each titration consisted of 80–100 equilibrium points in the range of pH 2.5−11.5, and at least two replicate titrations were performed for each individual system. The potentiometric data were refined with the Hyperquad software,[8] and speciation diagrams were plotted using the Hyss software.[9]

Spectrophotometric competition titrations were measured on a Unicam UV4 spectrophotometer at 25.0 ± 0.1 ºC using a Huber CCE-K6 circulating thermostatic bath. A stock solution of pre-formed bismuth(III) complex of H_2**Me-do2pa** at neutral pH was prepared at *ca.* 2.0×10^{-3} M, by addition of the acidic solution of $Bi(NO_3)_2$ to an aqueous solution of H_2**Me-do2pa** (in exact 1:1 molar ratio) followed by very slow neutralization of the mixture with aqueous KOH solution under constant heating (80–90 ºC). Each batch titration consisted of a set of 12 individual points distributed in an estimated pH range of 12.0–13.3, prepared under nitrogen atmosphere and kept in tightly sealed vials. Individual points were prepared by addition of precise volumes of the titrant KOH solution to samples containing a mixture of the pre-formed complex solution with *ca.* 2×10^{-3} mmol of complex and a concentrated KCl solution to keep each sample at 0.50 M in KCl. The points were incubated at 25.0 ºC until reaching equilibrium, which happened after two weeks. UV absorbance spectra were measured after quick transfer of each sample solution from its vial to a semi-micro UV cuvette while avoiding contact with atmosphere. Measurements used for the determinations were centered in the absorption band of $[Bi($**Me-dodpa**$)]^+$ (350 nm), and the molar absorptivity of the $[Bi($**Me-dodpa**$)]^+$ species was determined from the stock solutions of pre-formed complexes. The spectroscopic data were refined with the HypSpec software,[10] assuming a model containing the species [Bi(**Me-dodpa**)]$^+$ and [Bi(**Me-dodpa**)(OH)], as well as the competing $Bi(OH)_3$ and $Bi(OH)_4^-$ species. The hydrolysis constants of the bismuth(III), $Bi(OH)_3$ and $Bi(OH)_4^-$, used in the refinements were taken from the literature.[11]

The overall equilibrium (formation) constants β_{H_hL} and $\beta_{M_mH_hL_l}$ are defined by $\beta_{M_mH_hL_l}$ = $[M_mH_hL_l]/[M]^m[H]^h[L]^l$ and $\beta_{MH_{-1}L} = \beta_{ML(OH)} \times K_w$, while stepwise equilibrium constants are given by $K_{M_mH_hL_l} = [M_mH_hL_l]/[M_mH_{h-1}L_l][H]$ and correspond to the difference in log units between overall constants of sequentially protonated (or hydroxide) species. The pM values for metal complexes were calculated from the full set of stability constants for each system at pH = 7.4 with [L] = 1.0×10^{-5} M and [M] = 1.0×10^{-6} M.

Kinetic determinations. UV spectra were measured in the spectrophotometer setup described above. The formation of the bismuth(III) complexes of H_2**Me-do2pa** was studied in buffered aqueous solutions at 25.0 ºC. Stock solutions of potassium citrate (pH = 3.0) and potassium acetate (pH = 5.0) buffers were prepared at 1.0 M in water. A stock solution of $Bi(NO_3)_3$ was prepared at 2.5×10^{-3} M in 0.5 M aqueous HCl. The increasing intensity of the complex [Bi(**Me-do2pa**)]$^+$ UV absorption band (350 nm) was followed at pH = 3.0 (0.5 M citrate buffer) and pH = 5.0 (0.5 M acetate buffer), under pseudo-first order conditions at $C_{H_2\mathbf{Me\text{-}do2pa}} = 10\times C_{Bi^{3+}} = 1$ mM.

The acid-assisted dissociation of the bismuth(III) complex of H_2**Me-do2pa** was studied under pseudo-first order conditions at 25.0 ºC in 1.0 M HCl aqueous solution. Concentrated acid was added to sample solutions containing preformed complex for a starting complex concentration of 0.13 mM without control of ionic strength, and the reaction was followed by the decreasing intensity of the UV absorption band of complex.

General procedures for ^{213}Bi radiolabelling. All experiments with radioactive ^{213}Bi isotope were performed in a secured and dedicated room with all safety precautions related to the use of a α-emitters. The ^{225}Ac-^{213}Bi generator was supplied by the Institute for Transuranium Elements (Karlsruhe, Germany). The generator was eluted approximately every 2 h with a 1:1 HCl/NaI solution (0.1 M, 600 µL), following a standard protocol.[12] The initial concentration of ^{213}Bi was determined by radioactivity counting (^{213}Bi activity was measured with a calibrated NaI(Tl) scintillator (RayTest, France)). The average ^{213}Bi concentration was 0.3 nM.

Typical protocol for ligand labeling. To a 38 µM solution of H_2**Me-do2pa** (0.115 to 3.5 nmol) buffered at pH 7.4 with 2.0 M TRIS solution were added 3 to 12 MBq of bismuth-213 eluent (100 to 500 µL in 0.1 HCl / 0.1 M NaI 1:1) and a calculated volume of potassium ascorbate pH 7.4 solution in order to obtain a final concentration of 50 mM. The mixtures were incubated 15 min at 90 °C or at RT and analyzed both by reverse phase HPLC (C18 Kromasil column) and TLC with silicagel. HPLC analysis were performed with a 20 mn linear gradient of TFA (0.01% in water)-ACN. The TLC plates were developed in a mixture of methanol / 0.5 M ammonium chloride (20 %) 1:1. HPLC and TLC chromatograms were analyzed with gamma NaI scintillator. Percentages of ^{213}Bi incorporation were deduced from the ratio of the radioactivity intensities at $R_f \sim 0.5$ (which corresponds to [^{213}**Bi(Me-do2pa)**]$^+$) and $R_f \sim 0$ (which corresponds to the remaining ^{213}Bi salts). They are average values of at least two experiments (error estimated: ± 5%).

Serum stability study. 1 MBq (200 µL) of radiolabeled H_2**Me-do2pa** (radiochemical purity: 95%) was added to 800 µL of controlled human plasma pool. After 1 and 2 h of incubation time (about 1 and 2 decay period) at RT, 400 µL of the mixture were analyzed both by gel-filtration chromatography on PD-10 column (Pharmacia) and by TLC on silicagel plates after protein precipitation by ammonium sulfate.

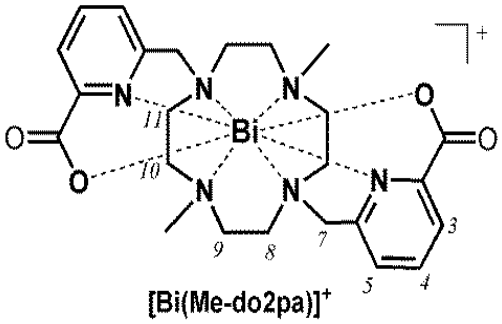
Supplementary Tables

Table S1. Crystal data and refinement details for [Bi(**Me-do2pa**)](NO_3).

[Bi(**Me-do2pa)**] $^+$	
formula	$C_{24} H_{36} N_7 O_9 Bi$
MW	775.58
crystal system	Orthorhombic
space group	Pbcn
T/K	297(2)
a/Å	13.9796(2)
b/Å	15.2329 (2)
c/Å	13.2072 (2)
β/deg	90
V/Å^3	2812.47(7)
F(000)	1536
Z	4
λ, Å (MoK_α)	0.71073
D_{calc}/g cm^{-3}	1.832
μ/mm^{-1}	6.333
θ range/deg	2.91 to 28.28
R_{int}	0.0294
reflns measd	23558
unique reflns	2128
reflns obsd	3496
GOF on F^2	0.967
$R_1{}^a$	0.0175
wR_2 (all data)^b	0.0450
Largest differences peak and hole /eÅ^{-3}	0.816 and -0.502

^{a} $R_1 = \Sigma||F_0|-|Fc||/\Sigma|F_0|$. ^{b} $wR_2 = \{\Sigma[w(||F_0|^2-|Fc|^2)^2]/\Sigma[w(F_0{}^4)]\}^{1/2}$

Table S2. ^{1}H shifts for the [Bi(**Me-do2pa**)]$^+$ complex at 298 K (pD ~ 7.0).[a]



^{1}**H**	chemical shift
H3	8.13
H4	8.40
H5	8.04
H7a	4.87
H7b	4.94
H8ax	3.15
H8eq	3.53
H9ax	3.69
H9eq	3.10
H10ax	3.44
H10eq	3.36
H11ax	3.42
H11eq	3.73
CH_3	2.54

[a] Assignment supported by 2D COSY, HSQC and HMBC experiments at 298 K; $^3J_{3,4}$ = 7.7 Hz; $^3J_{5,4}$ = 7.9 Hz; $^2J_{7a,7b}$ = 16.2 Hz; $^2J_{8eq,8ax}$ = 10.2 Hz.

Table S3. Overall (log $\beta_{\mathrm{MH_hL}}$) and stepwise (log $K_{\mathrm{MH_hL}}$) thermodynamic protonation constants of H_2**Me-do2pa** and its stability constants with Bi^{3+} in aqueous solution at 25.0 °C and I = 0.50 M in KCl. Literature data for $dota^{4-}$ are provided for comparison. Values of pBi were calculated from these constants at pH = 7.4 and $C_{H_2\mathbf{Me\text{-}do2pa}} = 2\times C_{Bi^{3+}} = 20\ \mu M$.

Species	H_2Me-do2pa		H_4dota
	log $\beta_{\mathrm{MH_hL}}$	log $K_{\mathrm{H_hL}}$	log $K_{\mathrm{H_hL}}$
HL	11.45(3)	11.45	9.01[a]
H_2L	20.40(4)	8.95	9.08[a]
H_3L	24.57(5)	4.17	4.44[a]
H_4L	27.63(5)	3.06	3.74[a]
H_5L	–	–	1.72[a]
BiL	34.2(1)	34.2	30.3[a]
BiHL	36.23(3)	2.0	–
BiLOH	22.20(1)	12.0	–
pBi	28.6		27.0

[a] These values were taken from ref. 13 at I = 1.0 M NaBr.

Supplementary Figures

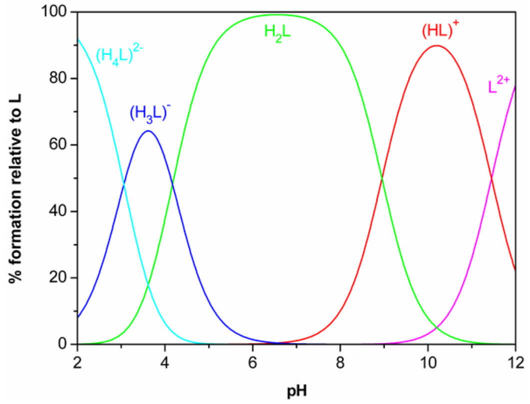


Figure S1. Species distribution diagram of H_2**Me-do2pa**, at $C_{H_2\mathbf{Me\text{-}do2pa}}$ = 1.0 mM.

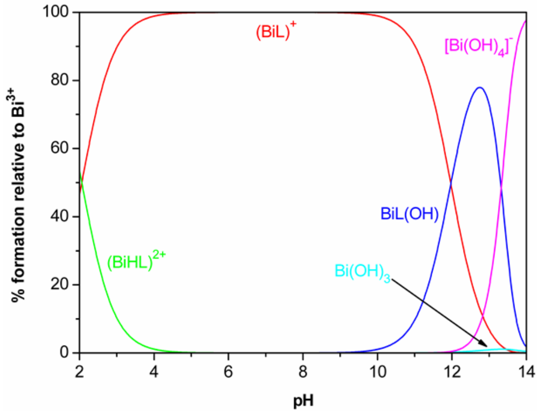


Figure S2. Species distribution diagram of bismuth(III) in presence of H_2**Me-do2pa**, at $C_{Bi^{3+}}$ = $C_{H_2\mathbf{Me\text{-}do2pa}}$ = 1.0 mM.

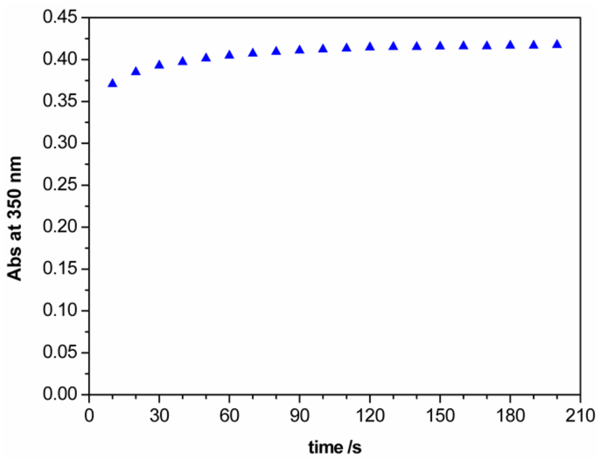


Figure S3. Time course (maximum absorbance versus time) of the UV spectra of bismuth(III) in presence of H_2**Me-do2pa** in aqueous solution at pH = 5.0 and 25.0 °C ($C_{H_2\mathbf{Me\text{-}do2pa}}$ = 10×$C_{Bi^{3+}}$ = 1.0 mM, 0.5 M potassium acetate buffer).

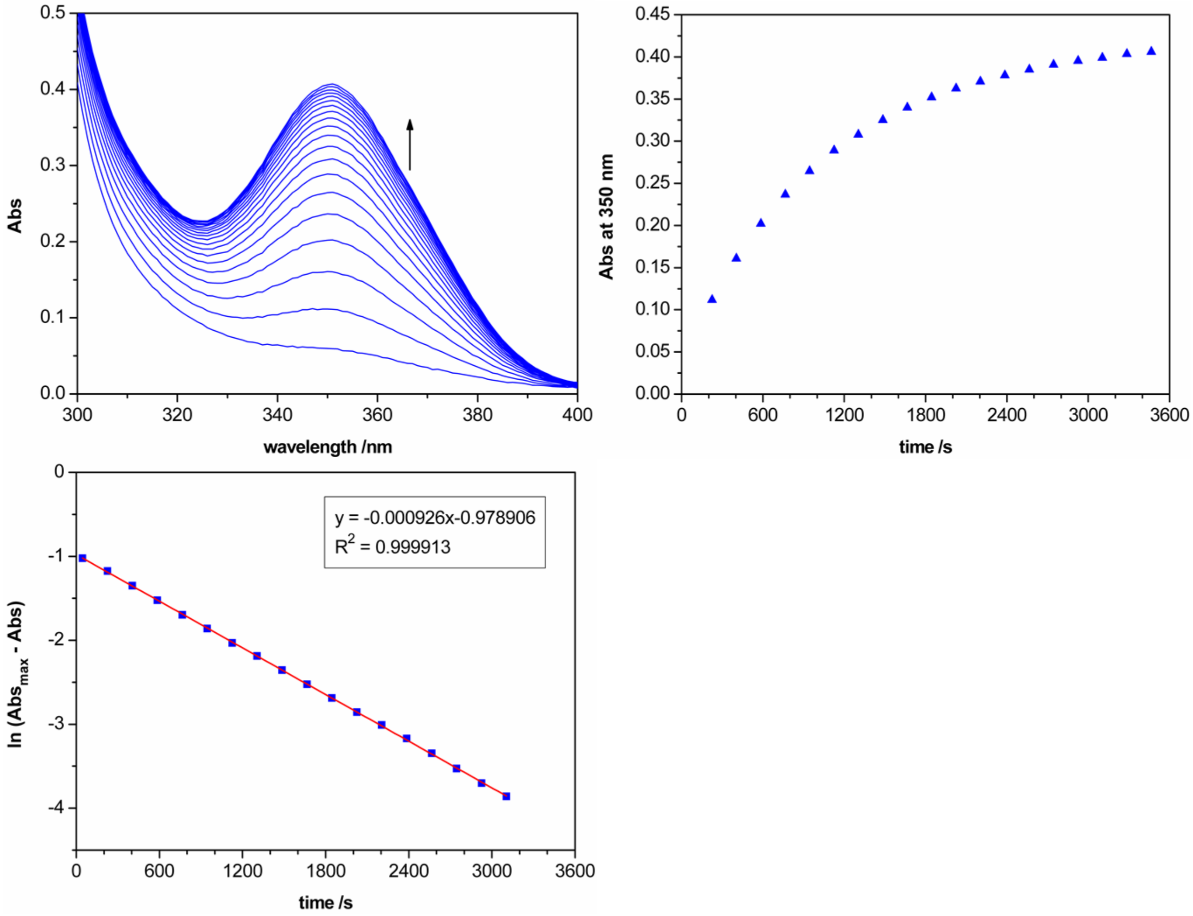


Figure S4. Time course of the UV spectra of bismuth(III) in presence of H_2**Me-do2pa** in aqueous solution at pH = 3.0 and 25.0 °C ($C_{H_2\textbf{Me-do2pa}} = 10 \times C_{Bi^{3+}} = 1.0$ mM, 0.5 M potassium citrate buffer): A) spectra obtained at 3 min intervals; B) absorbance at 350 nm vs. time; C) fitting of the absorbance at 350 nm to the observed first order rate constant.

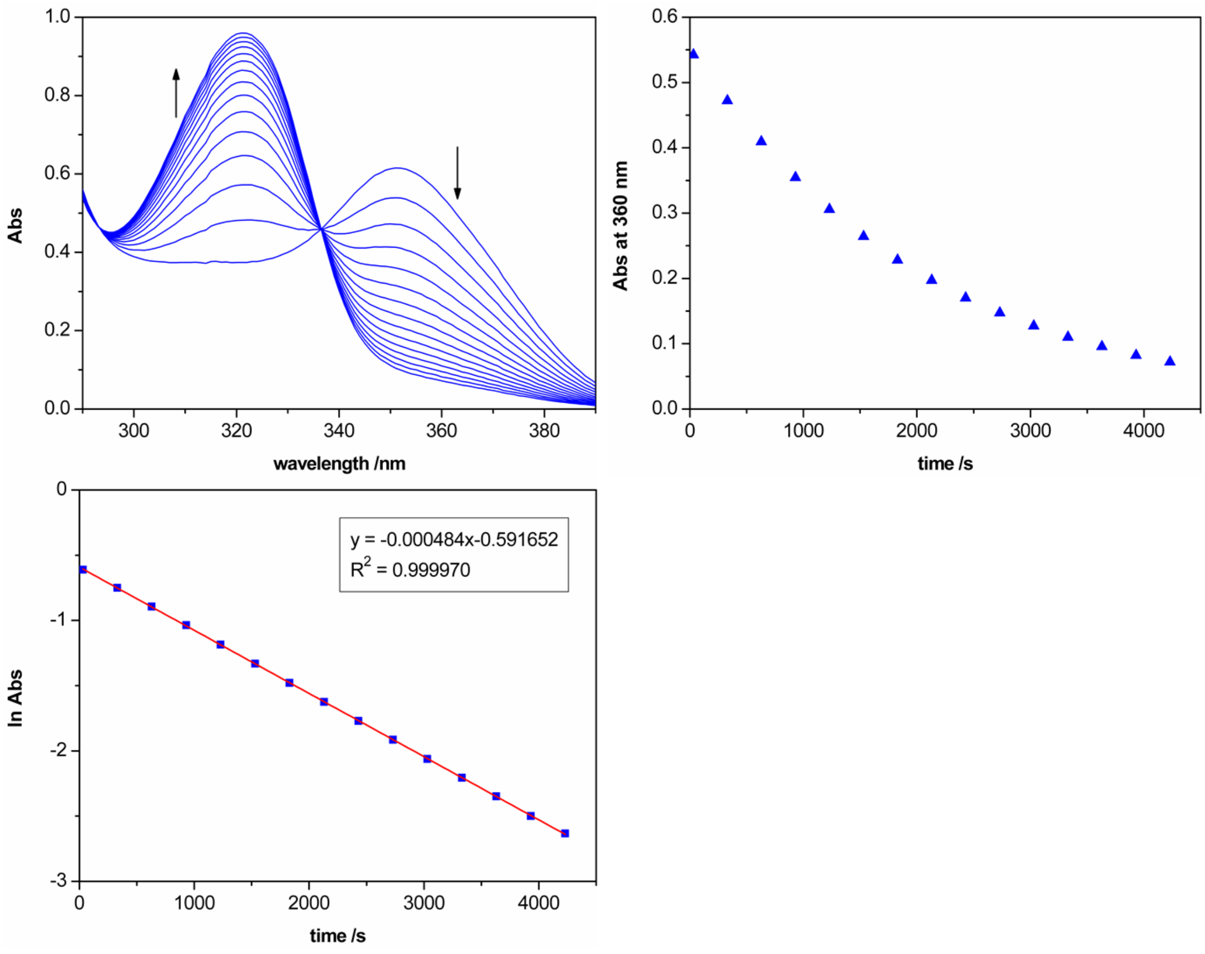


Figure S5. Time course of the UV spectra of the bismuth(III) complex of H_2**Me-do2pa** in 1.0 M HCl solution at 25.0 °C ($C_{complex}$ = 0.13 mM): A) spectra obtained at 5 min intervals; B) absorbance at 360 nm vs. time; C) fitting of the absorbance at 360 nm to the observed first order rate constant.

References

1. A. Rodríguez-Rodríguez, D. Esteban-Gomez, A. de Blas, T. Rodríguez-Blas, M. Fekete, M. Botta, R. Tripier, C. Platas-Iglesias, *Inorg. Chem.* 2012, **51**, 2509–2521.
2. (a) CrysAlis CCD, version 1.171.33.52; Oxford Diffraction Ltd.: Oxfordshire, UK, 2009; (b) *CrysAlis RED*, version 1.171.33.52; Oxford Diffraction Ltd.: Oxfordshire, UK, 2009.
3. G. M. Sheldrick, *Acta Crystallogr.* 2008, **A64**, 112–122.
4. M. Roger, L. M. P. Lima, M. Frindel, C. Platas-Iglesias, J.-F. Gestin, R. Delgado, V. Patinec, R.Tripier, *Inorg. Chem.* 2013, **52**, 5246–5259.
5. G. Gran, *Analyst* 1952, 661–671.
6. G. Schwarzenbach, W. Flaschka, *Complexometric Titrations*, Methuen & Co, London, 1969.
7. F. H. Sweeton, R. E. Mesmer, C. F. Baes Jr., *J. Sol. Chem.* 1974, **3**, 191–214.
8. P. Gans, A. Sabatini, A. Vacca, *Talanta* 1996, **43**, 1739–1753.
9. L. Alderighi, P. Gans, A. Ienco, D. Peters, A. Sabatini, A. Vacca, *Coord. Chem. Rev.* 1999, **184**, 311–318.
10. P. Gans, A. Sabatini, A. Vacca, *Annali di Chimica* 1999, **89**, 45–49.
11. N. J. Williams, N. E. Dean, D. G. VanDerveer, R. C. Luckay, R. D. Hancock, *Inorg. Chem.* 2009, **48**, 7853–7863.
12. M. R. McDevitt, R. D. Finn, G. Sgouros, D. Ma, D. A. Scheinberg, *Appl. Radiat. Isot.* 1999, **50**, 895–904.
13. E. Csajbók, Z. Baranyai, I. Bányai, E. Brücher, R. Király, A. Müller-Fahrnow, J. Platzek, B. Radüchel, M. Schäfer, *Inorg. Chem.* 2003, **42**, 2342–2349.