

Supporting Information

Synthesis, Conformational Analysis and Complexation Study of an Iminosugar-Aza-Crown, a Sweet Chiral Cyclam Analog

Alexandra Bordes,^a Ana Poveda,^b Thibault Troadec,^c Antonio Franconetti,^b Ana Ardá,^b Flavie Perrin,^a Mickaël Ménand,^d Matthieu Sollogoub,^d Jérôme Guillard,^a Jérôme Désiré,^a Raphaël Tripier,^c Jesús Jiménez-Barbero,^{b,e,f,*} Yves Blériot^{a,*}

^a Université de Poitiers, IC2MP, UMR CNRS 7285, Équipe « Synthèse organique », Groupe Glycochimie, 4 Rue Michel Brunet, 86073 Poitiers Cedex 9, France.

^b CIC bioGUNE, Parque Tecnológico de Bizkaia, Edif. 801A-1°, derio-Bizkaia 48160, Spain.

^c Univ Brest, UMR-CNRS 6521 CEMCA, 6 Avenue Victor le Gorgeu, 29200 Brest, France.

^d Sorbonne Université, CNRS, Institut Parisien de Chimie Moléculaire, UMR 8232, 4 place Jussieu, 75005 Paris, France.

^e IKERBASQUE, Basque Foundation for Science, 48009 Bilbao, Spain; Dept. Organic Chemistry II

^f Faculty of Science and Technology, University of the Basque Country, 48940 Leioa, Spain.

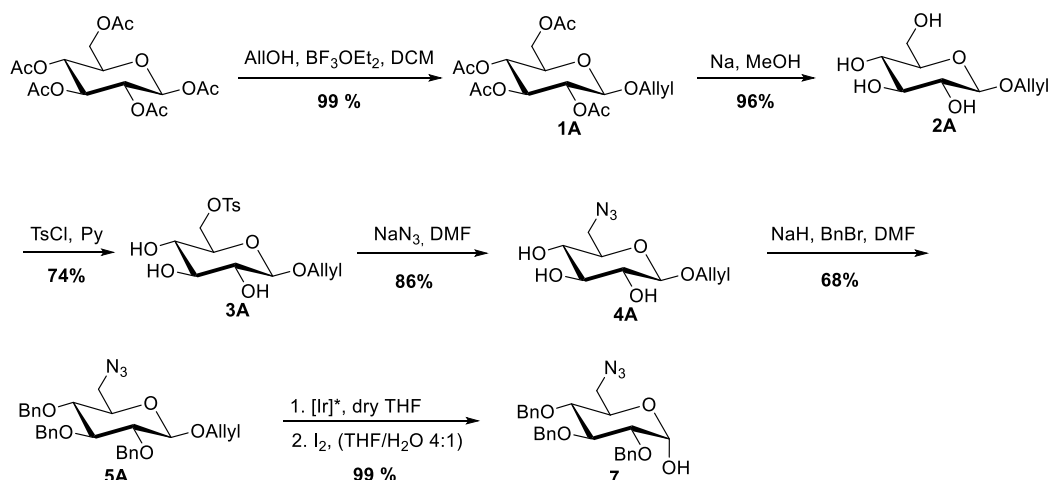
General methods	S2
Synthesis - Experimental procedures	S2
Copies of NMR spectra of 12, 13 and 6	S10
Monitoring of the Staudinger-aza-Wittig reaction for 12	S14
ISAC 6 - Cu²⁺ complex	S15
NMR conformational study of ISAC 6 and Cd²⁺ complex:	S18
1. Free ISAC 6 study	S19
2. ISAC 6 - Cd complex	S22

General Methods

All starting materials and reagents were purchased from commercial sources, and used as received without further purification. Cu-cyclam was prepared as previously reported¹. Air and H₂O sensitive reactions were performed in oven dried glassware under Ar atmosphere. Moisture sensitive reagents were introduced *via* a dry syringe. Anhydrous solvents were supplied over molecular sieves, and used as received. Petroleum ether (PE) refers to the 40-60 °C boiling fraction. Powdered molecular sieves were activated before use by heating with a heat gun for ~5 min under high vacuum. Reactions were monitored by thin-layer chromatography (TLC) with silica gel 60 F₂₅₄ 0.25 mm pre-coated aluminum foil plates. For reactions that require heating, the heat source used was heating mantle. Compounds were visualized by using UV₂₅₄ and/or phosphomolybdic acid stain [3 g 12MoO₃.H₃PO₄.xH₂O in 100 mL EtOH] followed by heating with a heat gun. Flash column chromatography was performed using Macherey-Nagel silica gel 60 (15–40 µm). NMR experiments were recorded with a Bruker Avance 400 spectrometer at 400 MHz for ¹H nuclei and at 100 MHz for ¹³C nuclei. The chemical shifts are expressed in part per million (ppm) relative to TMS (δ = 0 ppm) and the coupling constant *J* in Hertz (Hz). NMR multiplicities are reported using the following abbreviations: ls = large singlet, s = singlet, d = doublet, t = triplet, q = quadruplet, m = multiplet. HRMS were recorded on a Bruker micrOTOF spectrometer. The melting points were recorded with a SMP50 Stuart Scientific melting point apparatus. Optical rotations were measured using an Anton Paar MCP100 Polarimeter. Analytical RP-HPLC was carried out on a Water alliance system equipped with a UV/Visible variable wavelength detector and with a reverse-phase column chromatography (C18, 150x4.6 mm, 5 µm, 120 Å) at 30°C and 1mL.min⁻¹. **Method 1** used a linear gradient composed of A (0.2% TFA in water) and B (CH₃CN) beginning with A/B = 95/5 v/v and reaching A/B = 0/100 v/v within 20 min. All chromatograms were recorded at 210 nm.

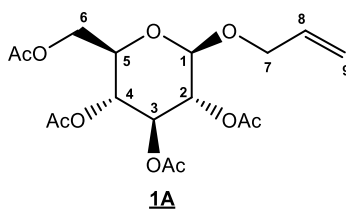
Synthesis - Experimental procedures

Synthesis of azidolactol 7



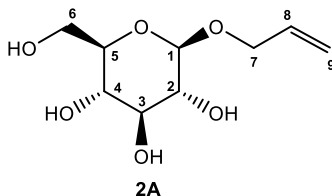
¹ A. Prevedello, I. Bazzan, N. Dalle Carbonare, A. Giuliani, S. Bhardwaj, C. Africh, C. Cepek, R. Argazzi, M. Bonchio, S. Caramori, M. Robert, A. Sartorel, *Chem. Asian J.* **2016**, *11* (8), 1281-1287.

Allyl 2,3,4,6-tetra-*O*-acetyl- β -D-glucopyranoside **1A**



This procedure was adapted from Litt². To a solution of β -D-Glucose pentaacetate (10.00 g, 25.60 mmol) in dry CH_2Cl_2 (100 mL) was added 4Å molecular sieves (200 mg). $\text{BF}_3 \cdot \text{Et}_2\text{O}$ (5 mL, 40.80 mmol, 1.6 eq) was then added dropwise at 0°C and the mixture was stirred for 10 min at 0°C. Allyl alcohol (2.5 mL, 33.20 mmol, 1.3 eq) was added and the reaction mixture was allowed to warm to room temperature and stirred for 24h (monitored by TLC, PE/Acetone: 7/3). NaHCO_3 sat was added (50 mL) at 0°C and the organic layer was washed with water (3x100 mL). After separation, the aqueous layer was further extracted with CH_2Cl_2 (2x50 mL). The combined organic extracts were dried (MgSO_4) and concentrated to afford compound **1A** as a colorless oil (9.90 g, 99%). R_f : 0.65 (PE/Acetone: 7/3); $[\alpha]_D^{25}$: +43.0 (c 1, MeOH); $^1\text{H NMR}$ (400 MHz, CDCl_3): δ (ppm) = 5.76 (m, 1H, H-8); 5.19 (dd, 1H, J = 17.2 Hz, J = 1.5 Hz, H-9a); 5.15-5.10 (m, 2H, H-3, H-9b); 5.00 (t, 1H, J = 9.6 Hz, H-4); 4.93 (dd, 1H, J = 9.5 Hz, J = 7.2 Hz, H-2); 4.49 (d, 1H, J = 7.9 Hz, H-1); 4.25 (dd, 1H, J = 13.2 Hz, J = 4.8 Hz, H-7a); 4.18 (dd, 1H, J = 12.4 Hz, J = 4.8 Hz, H-6a); 4.05 (dd, 1H, J = 12.4 Hz, J = 2.5 Hz, H-6b); 4.02 (dd, 1H, J = 13.1 Hz, J = 6.1 Hz, H-7b); 3.62 (ddd, 1H, J = 9.9 Hz, J = 4.8 Hz, J = 2.5 Hz, H-5); 2.00-1.90 (4xs, 12H, 4xOAc); $^{13}\text{C NMR}$ (100 MHz, CDCl_3): δ (ppm) = 170.5-169.2 (4x C=O); 133.3 (C-8); 117.5 (C-9); 99.1 (C-1); 72.8 (C-5); 71.7 (C-3); 71.2 (C-2); 69.9 (C-6); 68.3 (C-4); 61.9 (C-7); 20.6-20.5 (4xOAc); HRMS (ESI+) m/z : $[\text{M}+\text{Na}^+]$ calcd for $\text{C}_{17}\text{H}_{24}\text{NaO}_{10}$: 411.1444; found: 411.1463.

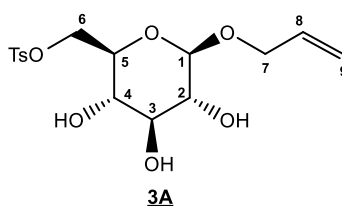
Allyl β -D-glucopyranoside **2A**



This procedure was adapted from Litt². Sodium (1.18 g, 51.4 mmol, 2 eq), previously washed three times with petroleum ether, was added under argon at 0°C to a solution of compound **1A** (9.90 g, 25.70 mmol) in dry MeOH (82 mL). The reaction mixture was then stirred at room temperature for 1 h (monitored by TLC, EtOAc/MeOH: 9/1). Amberlite IR15 was added to neutralize the media. Then the mixture was filtered and concentrated under reduced pressure to afford compound **2A** as a white solid (5.39 g, 96%); R_f : 0.82 (CH_2Cl_2 /MeOH: 7/3); $[\alpha]_D^{25}$: +35.0 (c 1, MeOH); m.p: 98-99°C; $^1\text{H NMR}$ (400MHz, CD_3OD): δ (ppm) = 5.99 (m, 1H, H-8); 5.35 (dd, 1H, J = 17.4 Hz, J = 1.7 Hz, H-9a); 5.18 (dd, 1H, J = 10.6 Hz, J = 1.6 Hz, H-9b); 4.40 (dd, 1H, J = 12.9 Hz, J = 5.1 Hz, H-7a); 4.32 (d, 1H, J = 7.9 Hz, H-1); 4.17 (dd, 1H, J = 12.9 Hz, J = 6.0 Hz, H-7b); 3.94 (dt, 1H, J = 12.5 Hz, J = 6.2 Hz, H-5); 3.89 (dd, 1H, J = 12.0 Hz, J = 1.7 Hz, H-6a); 3.68 (dd, 1H, J = 12.0 Hz, J = 5.5 Hz, H-6b); 3.40-3.18 (m, 3H, H-2, H-3, H-4); $^{13}\text{C NMR}$ (100MHz, CD_3OD): δ (ppm) = 134.7 (C-8); 116.6 (C-9); 102.3 (C-1); 77.0 (C-4); 76.9 (C-3); 74.0 (C-2); 70.6 (C-5); 70.1 (C-7); 61.7 (C-6); HRMS (ESI+) m/z : $[\text{M}+\text{Na}^+]$ calcd for $\text{C}_9\text{H}_{16}\text{NaO}_6$: 243.0924; found: 243.0938.

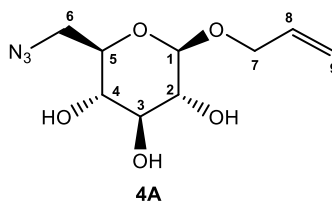
² R. D. Brisco, F. Ronchetti, A. Mangoni, V. Costantino, F. Compostella, *Carbohydr. Res.*, **2012**, 348, 27-32.

Allyl 6-(4-methylbenzenesulfonate)- β -D-glucopyranoside **3A**



This procedure was adapted from Litt². To a solution of allyl β -D-Glucopyranoside **2A** (5.40 g, 24.70 mmol) in dry pyridine (155 mL) was added *p*-toluenesulfonyl chloride (7.50 g, 39.50 mmol, 1.6 eq) at 0°C. The reaction was stirred at 0°C overnight (monitored by TLC, EtOAc/MeOH: 9/1). The reaction mixture was quenched with MeOH and the solvents were removed under reduced pressure. The residue was purified by flash chromatography (EtOAc/MeOH: 9/1) to afford compound **3A** as a colorless oil (6.81 g, 74%); *R_f*: 0.62 (EtOAc/MeOH: 9/1); $[\alpha]_D^{25}$: -21.0 (c 1, MeOH); ¹H NMR (CD₃OD, 400MHz): δ (ppm) = 7.85-7.79 (m, 2H, CH-Ar); 7.50-7.42 (m, 2H, CH-Ar); 5.90 (m, 1H, H-8); 5.35 (m, 1H, H-9a); 5.17 (m, 1H, H-9b); 4.36 (dd, 1H, *J* = 10.8 Hz, *J* = 1.7 Hz, H-6a); 4.28-4.18 (m, 2H, H-1, H-7a); 4.17 (dd, 1H, *J* = 10.8 Hz, *J* = 6.1 Hz, H-6b); 4.10-4.01 (m, 1H, H-7b); 3.45-3.39 (m, 1H, H-5); 3.31 (t, 1H, *J* = 9.2 Hz, H-3); 3.22 (t, 1H, *J* = 9.2 Hz, H-4); 3.16 (dd, 1H, *J* = 8.1 Hz, *J* = 9.2 Hz, H-2); 2.47 (s, 3H, CH₃); ¹³C NMR (CD₃OD, 100MHz): δ (ppm) = 145.1-135.4 (Cq-Ar); 129.6-128.9 (CH-Ar); 127.7 (C-8); 116.2 (C-9); 101.9 (C-1); 76.5 (C-4); 73.6 (C-3); 73.4 (C-2); 69.8 (C-5); 69.7 (C-7); 69.4 (C-6); 20.2 (CH₃); HRMS (ESI+) *m/z*: [M+Na⁺] calcd for C₁₆H₂₂NaO₈S: 397.1452; found: 397.1498.

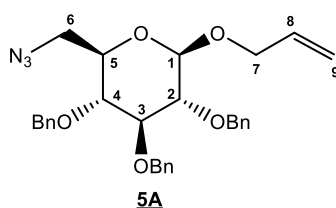
Allyl 6-azido-6-deoxy- β -D-glucopyranoside **4A**



This procedure was adapted from Litt². A mixture of compound **3A** (6.76 g, 18.10 mmol) and sodium azide (3.53 g, 54.3 mmol, 3 eq) in dry DMF (122 mL) was stirred at 80°C for 3h (monitored by TLC, EtOAc/MeOH: 9/1). DMF was removed under high vacuum and the residue was purified by flash-chromatography (CH₂Cl₂/MeOH: 9/1) to afford compound **4A** as a colorless oil (3.79 g, 86%); *R_f*: 0.65 (EtOAc/MeOH: 9/1); $[\alpha]_D^{25}$: +81.5 (c 1, MeOH); ¹H NMR (CD₃OD, 400MHz): δ (ppm) = 6.01-5.92 (m, 1H, H-8); 5.38-5.30 (m, 1H, H-9a); 5.23-5.16 (m, 1H, H-9b); 4.39-4.31 (m, 2H, H-1, H-7a); 4.21-4.15 (m, 1H, H-7b); 3.54-3.19 (m, 6H, H-2, H-3, H-4, H-5, H-6a, H-6b); ¹³C NMR (CD₃OD, 100MHz): δ (ppm) = 134.2 (C-8); 116.2 (C-9); 101.9 (C-1); 76.5 (C-4); 75.7 (C-3); 73.7 (C-2); 71.2 (C-5); 69.7 (C-7); 51.7 (C-6); HRMS (ESI+) *m/z*: [M+Na⁺] calcd for C₉H₁₅NaN₃O₅: 268.1047; found: 268.1082.

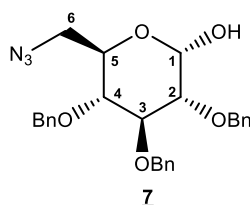
² R. D. Brisco, F. Ronchetti, A. Mangoni, V. Costantino, F. Compostella, *Carbohydr. Res.*, **2012**, 348, 27-32.

Allyl 6-azido-6-deoxy-2,3,4-tri-*O*-benzyl- β -D-glucopyranoside **5A**



This procedure was adapted from Litt³. Sodium hydride (60% in mineral oil, 2.83 g, 118.0 mmol, 5 eq), previously washed three times with n-hexane, was added under argon at 0°C to a solution of compound **4A** (5.75 g, 23.6 mmol) in dry DMF (80 mL), and the foaming gray mixture was then allowed to warm to room temperature. Benzyl bromide (14.0 mL, 118.0 mmol, 5 eq) was slowly added, and the mixture was stirred at room temperature for 6 h (monitored by TLC, EtOAc/MeOH: 95/5). MeOH was added to destroy the excess of NaH, then the mixture was concentrated under reduced pressure, and diluted with EtOAc (100 mL). The organic layer was washed with water (200 mL). After separation, the aqueous layer was further extracted with EtOAc (2x50 mL). The combined organic extracts were dried (Na₂SO₄) and concentrated. The residue was purified by flash-chromatography (PE/EtOAc: 9/1 then 5/5) to afford compound **5A** as a colorless oil (8.25 g, 68%); *R*_f: 0.65 (PE/EtOAc: 8/2); [α]_D: 7.1 (c 1, CHCl₃); ¹H NMR (400MHz, CDCl₃): δ (ppm) = 7.40-7.28 (m, 15H, CH-Ar); 5.98 (m, 1H, H-8); 5.35 (m, 1H, H-9a); 5.23 (m, 1H, H-9b); 5.00-4.05 (m, 6H, OCH₂-Ph); 4.50 (d, 1H, *J* = 7.8 Hz, H-1); 4.45 (m, 1H, H-7a); 4.15 (m, 1H, H-7b); 3.81 (t, 1H, *J* = 9.2 Hz, H-3); 3.50 (m, 2H, H-2, H-6a); 3.45 (m, 1H, H-4); 3.35 (m, 2H, H-5, H-6b); ¹³C NMR (100MHz, CDCl₃): δ (ppm) = 138.4-137.7 (Cq-Ar); 133.7 (C-8); 128.5-127.6 (CH-Ar); 117.7 (C-9); 102.4 (C-1); 84.5 (C-3); 82.3 (C-2); 79.9 (C-5); 75.7 (C-4); 75.2-74.7 (OCH₂-Ph); 70.3 (C-7); 51.4 (C-6); HRMS (ESI+) *m/z*: [M+Na⁺] calcd for C₃₀H₃₃N₃NaO₅: 538.2412; found: 538.2425.

6-Azido-6-deoxy-2,3,4-tri-*O*-benzyl- α -D-glucopyranose **7**



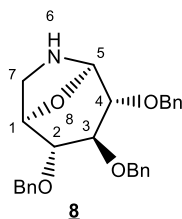
This procedure was adapted from Litt⁴. A suspension of Iridium catalyst, (1,5-Cyclooctadiene)bis(methyldiphenylphosphine)iridium(I) hexafluorophosphate (405 mg, 0.479 mmol, 0.05 eq), in dry THF (80 mL) under Ar at room temperature was placed under H₂. When the red suspension has turned to a slight yellow solution, H₂ was replaced by Ar using vacuum, and a solution of allyl compound **6A** (4.93 g, 9.57 mmol, 1 eq) in dry THF (80 mL) was added dropwise. The reaction mixture was stirred at room temperature for 2h. Then a solution of iodine (4.86 g, 19.1 mmol, 2 eq) in a mixture of THF/H₂O: 4/1 (65 mL) was added dropwise. The mixture was stirred for 1h at room temperature under argon (monitored by TLC, PE/EtOAc: 8/2). Na₂S₂O₃ sat was added (200 mL). The organic layer was washed with water (3x100 mL). After separation, the aqueous layer was further extracted with CH₂Cl₂ (3x50 mL). The combined organic extracts were dried (MgSO₄) and concentrated. The residue was purified by flash-chromatography (PE/EtOAc: 9/1 then 5/5) to afford compound **7** as

³ A. A. Sherman, O. N. Yudina, A. S. Shashkov, V. M. Menshov, N. E. Nifant'ev, *Carbohydr. Res.*, **2001**, 330, 445.

⁴ (a) J. J. Oltvoort, C. A. A. Van Boeckel, J. H. De Koning, J. H. Van Boom, *Synthesis*, **1981**, 1981, 305 ; (b) J. Lucchetti, A. Krief, *J. Chem. Soc., Chem. Commun.*, **1982**, 127 ; (c) R. Patnam, J. M. Juárez-Ruiz, R. Roy, *Org. Lett.*, **2006**, 8, 2691 ; (d) T. Nakamura, M. Shiozaki, *Tetrahedron Lett.*, **2001**, 42, 2701.

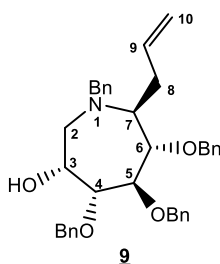
a white solid (4.54 g, 99%); R_f : 0.46 (PE/EtOAc: 8/2); $[\alpha]_D^{25}$: +22.4 (c 1, CHCl₃); $^1\text{H NMR}$ (400MHz, CDCl₃): δ (ppm) = 7.32-7.24 (m, 15H, CH-Ar); 5.21 (d, J = 3.6 Hz, 1H, H-1); 4.95 (d, J = 11.0 Hz, 1H, OCH₂-Ph); 4.90 (d, J = 10.9 Hz, 1H, OCH₂-Ph); 4.84 (d, J = 11.0 Hz, 1H, OCH₂-Ph); 4.74 (qAB, J = 11.9 Hz, 2H, OCH₂-Ph); 4.60 (d, J = 11.0 Hz, 1H, OCH₂-Ph); 4.07-4.03 (m, 1H, H-5); 3.97 (t, J = 9.4 Hz, 1H, H-3); 3.57 (dd, J = 9.4 Hz, J = 3.6 Hz, 1H, H-2); 3.51 (m, 2H, H-4, H-6a); 3.54 (m, 1H, H-6b); $^{13}\text{C NMR}$ (100MHz, CDCl₃): δ (ppm) = 138.0-137.7 (Cq-Ar); 128.7-127.1 (CH-Ar); 91.3 (C-1); 81.6 (C-3); 80.2 (C-2); 78.2 (C-4); 75.8; 75.3; 73.5 (OCH₂-Ph); 70.2 (C-5); 51.4 (C-6); **HRMS (ESI+)** m/z : [M+Na⁺] calcd for C₂₇H₂₉N₃NaO₅: 498.2105; found: 498.2118.

(1R,2R,3S,4R,5R)-2,3,4-tris(benzyloxy)-8-oxa-6-azabicyclo[3.2.1]octane 8



A solution of trimethylphosphine (1M/toluene, 12.6 mL, 12.6 mmol, 3eq.) was added to a solution of azidolactol 7 (2.0 g, 4.2 mmol) in anhydrous THF (60 mL). The reaction mixture was stirred at 90°C for 2h, then cooled to rt and the solvents removed under reduced pressure. The crude compound 8 was directly used for the next step. Analytical data were in agreement with those previously reported.⁵

(3R,4R,5R,6S,7S)-7-Allyl-1-benzyl-4,5,6-tris(benzyloxy)azepan-3-ol 9



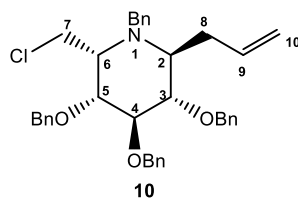
A solution of the crude compound 8 diluted in anhydrous THF (11 mL) was added dropwise to a solution of allylmagnesium bromide (1M/Et₂O, 11.6 mL, 11.6 mmol, 5 eq) at 0°C. The reaction mixture was stirred at room temperature for 2h. The crude was quenched by NH₄Cl sat (30 mL) and extracted twice with EtOAc. The combined organic layers were washed by NaCl sat (100 mL), dried over MgSO₄ and concentrated to give the crude azepane, directly used for the next step.

To a solution of the crude azepane in DMF (20 mL) were added at 0°C, K₂CO₃ (0.96 g, 6.96 mmol, 3 eq) and BnBr (413 μ L, 3.48 mmol, 1.5 eq). The reaction mixture was stirred for 15h under Argon at room temperature (monitored by TLC, PE/EtOAc: 8/2). After filtration of K₂CO₃, the mixture was washed with NH₄Cl sat (30 mL) and extracted with EtOAc (2x50 mL). The organics layers were washed by NaCl sat (80 mL), dried over MgSO₄ and concentrated in vacuo. The residue was purified by flash chromatography (PE/EtOAc: 9/1) to give compound 9 as a colorless oil (1.14 g, 53%, 3 steps, from 7); R_f : 0.65 (PE/EtOAc: 8/2); $[\alpha]_D^{25}$: -14.0 (C 0.9, DCM); $^1\text{H NMR}$ (400MHz, CDCl₃): δ (ppm) = 7.32-7.23 (m, 20H, CH-Ar); 5.88-5.78 (m, 1H, H-9); 5.08-5.00 (m, 2H, H-10); 4.70 (qAB, J = 11.7 Hz, 2H, OCH₂-Ph); 4.61 (s, 2H, OCH₂-Ph); 4.42 (qAB, J = 11.6 Hz, 2H, OCH₂-Ph); 4.12 (dd, J = 2.4 Hz, J = 6.9 Hz, 1H, H-5); 4.05-

⁵ M. Mondon, N. Fontelle, J. Désiré, F. Lecornué, J. Guillard, J. Marrot, Y. Blériot, *Org. Lett.*, **2012**, 14 (3), 870-873.

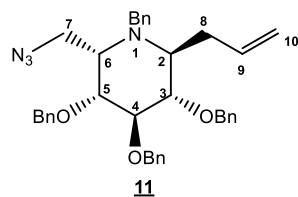
4.01 (m, 1H, H-3); 3.99 (dd, $J = 2.8$ Hz, $J = 7.0$ Hz, 1H, H-4); 3.89-3.79 (m, 2H, $\text{NCH}_2\text{-Ph}$); 3.56 (dd, $J = 2.5$ Hz, $J = 6.4$ Hz, 1H, H-6); 3.23 (dd, $J = 6.4$ Hz, $J = 13.8$ Hz, 1H, H-7); 2.87 (m, 2H, H-2a, H-2b); 2.56-2.51 (m, 1H, H-8a); 2.40-2.38 (m, 1H, H-8b); ^{13}C NMR (100MHz, CDCl_3): δ (ppm) = 139.7-137.9 (Cq-Ar); 136.6 (C-9); 128.9-127.1 (CH-Ar); 116.6 (C-10); 86.1 (C-4); 83.8 (C-5); 79.9 (C-6); 73.1-71.5 ($\text{OCH}_2\text{-Ph}$); 69.1 (C-3); 62.4 (C-7); 58.5 ($\text{NCH}_2\text{-Ph}$); 52.9 (C-2) 30.1 (C-8); HRMS (ESI+) m/z : $[\text{M}+\text{Na}^+]$ calcd for $\text{C}_{37}\text{H}_{41}\text{NO}_4\text{Na}$: 586.2933; found: 586.2929.

(2*S*,3*S*,4*R*,5*R*,6*R*)-2-Allyl-1-benzyl-3,4,5-tris(benzyloxy)-6-(chloromethyl)piperidine 10



This procedure was adapted from Litt⁵. To a solution of compound 9 (1.70 g, 3.03 mmol) in CH_2Cl_2 (46 mL) were added Et_3N (816.0 μL , 6.06 mmol, 2 eq) and MsCl (366.0 μL , 4.54 mmol, 1.5 eq) at 0°C . The reaction mixture was stirred for 1h under Argon at 0°C (monitored by TLC, PE/EtOAc: 95/5). The residue was diluted with CH_2Cl_2 , then few drops of 1N HCl were added. The mixture was washed with H_2O and extracted with in CH_2Cl_2 . The organics layers were dried over MgSO_4 and concentrated in vacuo. The residue was purified by flash chromatography (PE/EtOAc: 95/5) to give compound 10 as a colorless oil (1.34 g, 76%); R_f : 0.88 (PE/AcOEt : 95/5); $[\alpha]_D^{25}$: -35.2 (C 0.8, CH_2Cl_2); ^1H NMR (400MHz, CDCl_3): δ (ppm) = 7.36-7.22 (m, 20H, CH-Ar); 5.98-5.87 (m, 1H, H-9); 5.12-5.07 (m, 2H, H-10); 4.90 (d, $J = 10.8$ Hz, 2H, $\text{OCH}_2\text{-Ph}$); 4.77 (d, $J = 10.7$ Hz, 1H, $\text{OCH}_2\text{-Ph}$); 4.62 (d, $J = 11.7$ Hz, 1H, $\text{OCH}_2\text{-Ph}$); 3.84 (s, 2H, $\text{OCH}_2\text{-Ph}$); 3.82 (d, $J = 13.6$ Hz, 1H, $\text{NCH}_2\text{-Ph}$); 3.82 (dd, $J = 5.7$ Hz, $J = 9.5$ Hz, 1H, H-5); 3.81-3.63 (m, 2H, H-7a, H-7b); 3.70 (d, $J = 13.6$ Hz, 1H, $\text{NCH}_2\text{-Ph}$); 3.65 (dd, $J = 8.4$ Hz, $J = 9.5$ Hz, 1H, H-4); 3.47 (dd, $J = 8.4$ Hz, $J = 9.7$ Hz, 1H, H-3); 3.25 (dt, $J = 4.7$ Hz, $J = 5.7$ Hz, $J = 8.6$ Hz, 1H, H-6); 3.05 (ddd, $J = 4.5$ Hz, $J = 8.4$ Hz, $J = 9.7$ Hz, 1H, H-2); 2.77-2.73 (m, 1H, H-8a); 2.44-2.38 (m, 1H, H-8b); ^{13}C NMR (100MHz, CDCl_3): δ (ppm) = 139.1-137.9 (Cq-Ar); 135.3 (C-9); 128.8-127.2 (CH-Ar); 116.7 (C-10); 83.7 (C-4); 80.6 (C-3); 77.9 (C-5); 75.5-72.6 ($\text{OCH}_2\text{-Ph}$); 57.9 (C-6); 56.7 (C-2); 50.4 ($\text{NCH}_2\text{-Ph}$); 40.1 (C-7); 32.7 (C-8); HRMS (ESI+) m/z : $[\text{M}+\text{H}^+]$ calcd for $\text{C}_{37}\text{H}_{41}\text{NO}_3\text{Cl}$: 582.2775; found: 582.2782.

(2*S*,3*S*,4*R*,5*R*,6*S*)-2-Allyl-6-(azidomethyl)-1-benzyl-3,4,5-tris(benzyloxy)piperidine 11

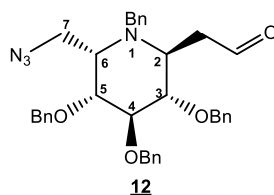


This procedure was adapted from Litt⁵. A mixture of compound 10 (922 mg, 1.58 mmol) and sodium azide (206 mg, 3.17 mmol, 2.0 eq) in dry DMF (18 mL) was stirred at 80°C for 3h (monitored by TLC, PE/EtOAc: 95/5), then DMF was removed under high vacuum. The reaction mixture was diluted with EtOAc (50 mL) and water (50 mL). The crude was washed with NaCl sat and extracted with Et_2O . The organics layers were dried over MgSO_4 and concentrated in vacuo. The residue was purified by flash chromatography (PE/EtOAc: 95/5) to afford compound 11 as a colorless oil (689 mg, 74%); R_f : 0.22

⁵ M. Mondon, N. Fontelle, J. Désiré, F. Lecornué, J. Guillard, J. Marrot, Y. Blériot, *Org. Lett.*, **2012**, 14 (3), 870-873.

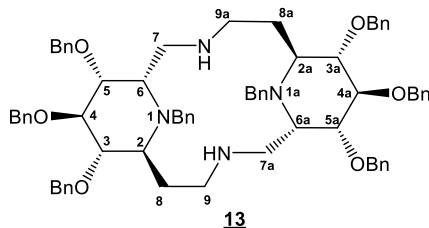
(PE/Et₂O : 95/5); [α]_D: +0.22 (C 1, CH₂Cl₂); ¹H NMR (400MHz, CDCl₃): δ (ppm) = 7.37-7.19 (m, 20H, CH-Ar); 5.95-5.91 (m, 1H, H-9); 5.11 (d, *J* = 4.8 Hz, 2H, H-10); 4.92 (dd, *J* = 4.6 Hz, *J* = 10.7 Hz, 2H, OCH₂-Ph); 4.78 (d, *J* = 10.7 Hz, 1H, OCH₂-Ph); 4.62 (d, *J* = 10.6 Hz, 1H, OCH₂-Ph); 4.41 (d, *J* = 6.1 Hz, 2H, OCH₂-Ph); 3.96 (d, *J* = 13.3 Hz, 1H, NCH₂-Ph); 3.83 (dd, *J* = 5.9 Hz, *J* = 9.8 Hz, 1H, H-5); 3.64 (dd, *J* = 8.8 Hz, *J* = 9.7 Hz, 1H, H-4); 3.61 (d, *J* = 13.2 Hz, 1H, NCH₂-Ph); 3.54 (dd, *J* = 13.1 Hz, *J* = 9.3 Hz, 1H, H-7a); 3.48 (dd, *J* = 8.8 Hz, *J* = 10.1 Hz, 1H, H-3); 3.35 (dd, *J* = 13.1 Hz, *J* = 3.6 Hz, 1H, H-7b); 3.14-3.08 (m, 2H, H-2, H-6); 2.82-2.78 (m, 1H, H-8a); 2.44-2.38 (m, 1H, H-8b); ¹³C NMR (100MHz, CDCl₃): δ (ppm) = 139.0-137.9 (Cq-Ar); 135.3 (C-9); 128.8-127.4 (CH-Ar); 116.7 (C-10); 84.2 (C-4); 80.5 (C-3); 76.8 (C-5); 75.6-72.7 (OCH₂-Ph); 57.9 (C-2); 56.5 (C-6); 50.2 (NCH₂-Ph); 46.3 (C-7); 33.0 (C-8); HRMS (ESI+) *m/z*: [M+H⁺] calcd for C₃₇H₄₁N₄O₃: 589.3178; found: 589.3176.

2-((2*S*,3*S*,4*R*,5*R*,6*S*)-6-(Azidomethyl)-1-benzyl-3,4,5-tris(benzyloxy)piperidin-2-yl)acetaldehyde **12**



To a solution of compound **11** (600 mg, 1.02 mmol) in 1,4-Dioxane/H₂O (3/1, 12 mL) was added 2,6-lutidine (238 μ L, 2.04 mmol, 2 eq), osmium tetroxide solution in *tert*-butanol (2.5 %wt, 7.0 μ L, 0.0204 mmol, 0.02 eq) and sodium periodate (873 mg, 4.08 mmol, 4.0 eq). The reaction was stirred for 1h under Argon at room temperature (monitored by TLC, PE/EtOAc: 9/1). The residue was diluted in 20 mL of CH₂Cl₂, and then few drops of HCl 1N were added. The mixture was washed with H₂O (3x20 mL) and extracted with in CH₂Cl₂ (3x20 mL). The organics layers were dried over MgSO₄ and concentrated in vacuo. The residue was purified by flash chromatography (PE/EtOAc: 8/2) to give compound **12** as a colorless oil (530 mg, 88%); *R*_f: 0.72 (PE/EtOAc: 8/2); [α]_D: - 6.0 (C 1,5, CH₂Cl₂); ¹H NMR (400MHz, CDCl₃): δ (ppm) = 9.68-9.66 (dd, *J* = 2.1 Hz, *J* = 3.5 Hz, 1H, CHO); 7.39-7.16 (m, 20H, CH-Ar); 4.96-4.89 (dd, *J* = 11.1 Hz, 2H, OCH₂-Ph); 4.80 (d, *J* = 10.9 Hz, 1H, OCH₂-Ph); 4.62-4.50 (m, 3H, OCH₂-Ph); 3.94-3.88 (m, 2H, NCH₂-Ph); 3.76-3.49 (m, 5H, H-2, H-3, H-4, H-5, H-7a); 3.43-3.39 (dd, *J* = 13.3 Hz, *J* = 4.0 Hz, 1H, H-7b); 3.19-3.14 (m, 1H, H-6); 2.83-2.76 (ddd, *J* = 2.1 Hz, 6.3 Hz, *J* = 15.7 Hz, 1H, CH-CHO); 2.61-2.54 (ddd, *J* = 3.5 Hz, 8.1 Hz, *J* = 15.7 Hz, 1H, CH-CHO); ¹³C NMR (100MHz, (CD₃)₂CO): δ (ppm) = 201.2 (C=O); 140.4-139.3 (Cq-Ar); 129.5-127.9 (CH-Ar); 84.6 (C-4); 80.6 (C-3); 77.4 (C-5); 75.7-73.4 (OCH₂Ph); 58.5 (C-6); 53.8 (C-2); 51.7 (NCH₂Ph); 47.4 (C-7); 44.9 (C-8); HRMS (ESI+) *m/z*: [M+Na⁺] calcd for C₃₆H₃₈N₄O₄Na: 613.2790; found: 613.2792.

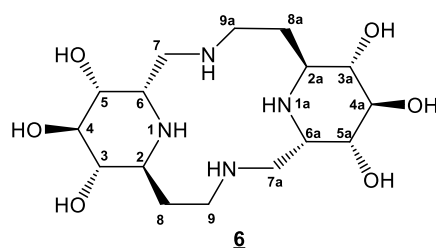
IminoSugar-Aza-Crown **13**



To a solution of compound **12** (500 mg, 0.847 mmol) in a mixture THF/NH₄OH (7.0 / 0.7 mL) was added triphenylphosphine bound polymer (667 mg, 2.54 mmol, 3 eq). The reaction mixture was stirred at 40°C overnight, then filtered through Celite© and concentrated under reduced pressure. The residue was diluted in dry THF (C=5.10⁻² M, 17.0 mL) and NaBH(OAc)₃ (450 mg, 2.12 mmol, 2.5 eq) was added. The reaction mixture was stirred for 15h under Argon at room temperature (monitored by TLC, CH₂Cl₂/MeOH/NH₄OH: 9/1/0.1). Then the crude was washed with H₂O (2 x 50 mL), NaCl sat (50 mL)

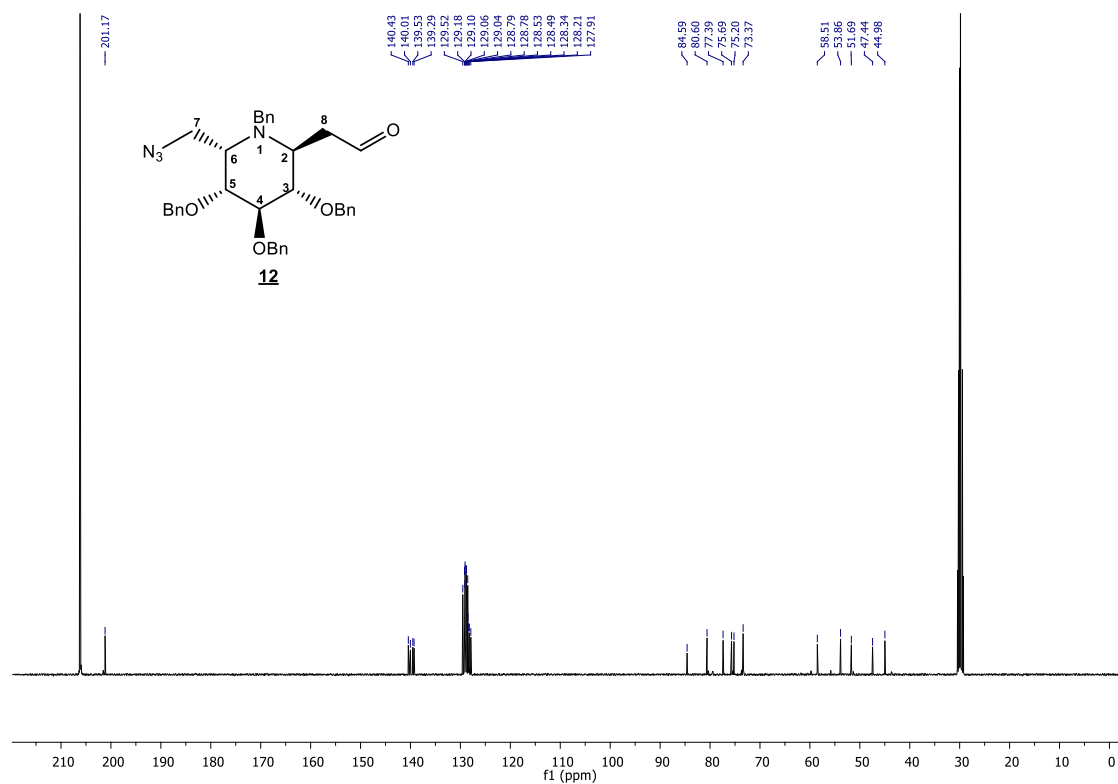
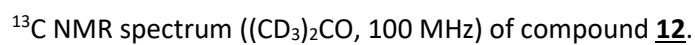
and extracted with CH_2Cl_2 (2 x 50 mL). The organics layers were dried over MgSO_4 and concentrated in vacuo. The residue was purified by reverse flash chromatography (C18 column redisepp gold, 20-40 μm , 100Å, 5.5mg, $\text{H}_2\text{O}/\text{CH}_3\text{CN}$: 0 to 100% CH_3CN) to give compound **13** as a colorless oil (367 mg, 40%, 2 steps); R_f : 0.12 ($\text{CH}_2\text{Cl}_2/\text{MeOH}/\text{NH}_4\text{OH}$: 9/1/0.1); $[\alpha]_D^{25}$: +85.9 (C 0.6, CH_2Cl_2); **Rt**: 12.32 min (**Method 1**); **^1H NMR (400MHz, CDCl_3)**: δ (ppm) = 7.32-7.10 (m, 40H, CH-Ar); 4.86-4.75 (qAB, J = 11.2 Hz, 4H, $\text{OCH}_2\text{-Ph}$); 4.64-4.57 (qAB, J = 10.9 Hz, 4H, $\text{OCH}_2\text{-Ph}$); 4.48-4.40 (qAB, J = 11.7 Hz, 4H, $\text{OCH}_2\text{-Ph}$); 3.90-3.80 (m, 4H, H-5, H-5a, $\text{NCH}_2\text{-Ph}$); 3.73-3.68 (t, J = 8.0 Hz, 2H, H-4, H-4a); 3.60-3.44 (m, 6H, H-3, H-3a, H-6, H-6a, $\text{NCH}_2\text{-Ph}$); 3.30-3.24 (m, 2H, H-2, H-2a); 3.16-3.10 (t, J = 12.0 Hz, 2H, H-7, H-7a); 2.76-2.59 (m, 6H, H-7', H-7a', H-9, H-9', H-9a, H-9a'); 2.25-2.13 (m, 2H, H-8, H-8a); 1.59-1.52 (m, 2H, H-8', H-8a'); **^{13}C NMR (100MHz, CDCl_3)**: δ (ppm) = 138.7-137.9 (Cq-Ar); 129.2-127.7 (CH-Ar); 85.4 (C-4, C-4a); 78.7 (C-3, C-3a); 77.8 (C-5, C-5a); 76.0-72.7 ($\text{OCH}_2\text{-Ph}$); 53.1 (C-2, C-2a); 52.3 (C-6, C-6a); 50.4 ($\text{NCH}_2\text{-Ph}$); 44.5 (C-9, C-9a); 43.5 (C-7, C-7a); 22.03 (C-8, C-8a); **HRMS (ESI+) m/z** : $[\text{M}+\text{H}^+]$ calcd for $\text{C}_{72}\text{H}_{81}\text{N}_4\text{O}_6$: 1097.6150; found: 1097.6151.

IminoSugar-Aza-Crown **6**

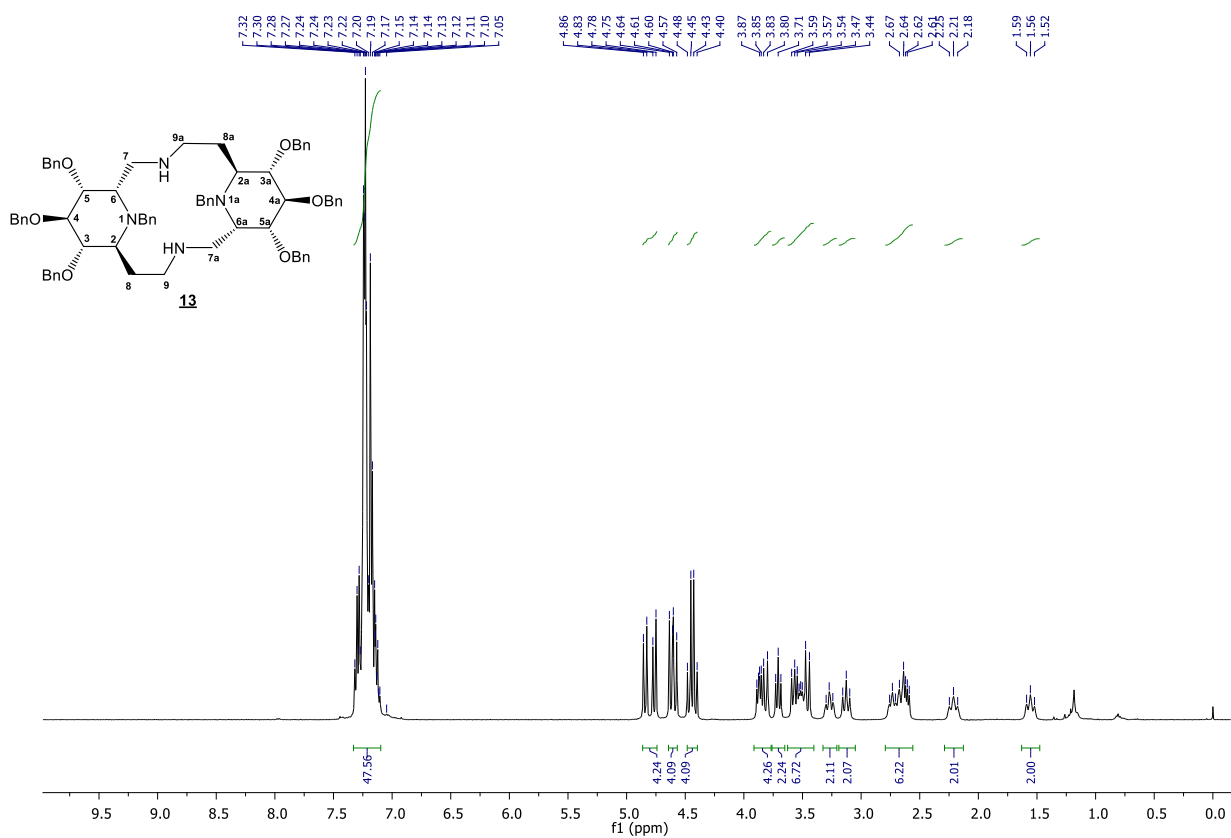


To a solution of compound **13** (50 mg, 0.046 mmol) in a mixture 1,2-dichloroethane/ H_2O (9/1, 5 mL) were added HCl (C= 12 M, 15.3 μL , 0.184 mmol, 4 eq) and Pd black (100 mg, 200% wt). The reaction mixture was then stirred under H_2 atmosphere at 60°C for 15 h (monitored by TLC, $\text{CH}_2\text{Cl}_2/\text{MeOH}/\text{NH}_4\text{OH}$: 8/2/0.3). Then the residue was filtered three times on Celite® and concentrated under reduced pressure to afford compound **6** as a colorless oil (17 mg, 98%); R_f : 0.18 ($\text{CH}_2\text{Cl}_2/\text{MeOH}/\text{NH}_4\text{OH}$: 8/2/0.3); $[\alpha]_D^{25}$: +24.3 (C 0.7, CHCl_3); **^1H NMR (400MHz, D_2O)**: δ (ppm) = 3.75 (dd, J = 6.4 Hz, J = 10.0 Hz, 2H, H-5, H-5a); 3.59-3.55 (m, 2H, H-4, H-4a); 3.46-3.32 (m, 6H, H-3, H-3a, H-6, H-6a, H-9, H-9a); 3.19-3.07 (m, 6H, H-7, H-7a, H-7', H-7a', H-9', H-9a'); 2.88-2.63 (m, 2H, H-2, H-2a); 2.41-2.36 (m, 2H, H-8, H-8a); 1.63-1.54 (m, 2H, H-8', H-8a'); **^{13}C NMR (100MHz, D_2O)**: δ (ppm) = 75.8 (C-3, C-3a); 73.7 (C-4, C-4a); 72.0 (C-5, C-5a); 53.5 (C-2, C-2a, C-6, C-6a); 47.3 (C-9, C-9a); 43.1 (C-7, C-7a); 28.7 (C-8, C-8a); **HRMS (ESI+) m/z** : $[\text{M}+\text{H}^+]$ calcd for $\text{C}_{16}\text{H}_{33}\text{N}_4\text{O}_6$: 377.2371, found: 377.2385.

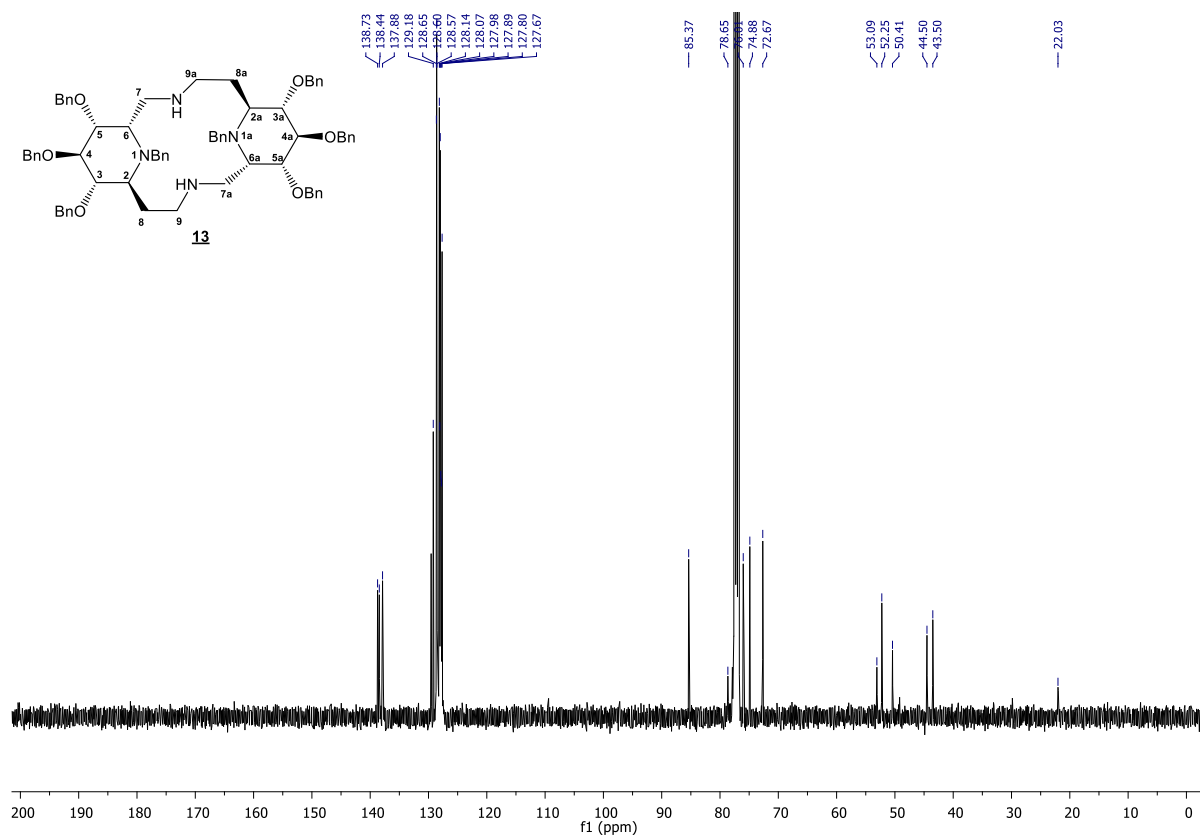
¹H NMR spectrum (CDCl₃, 400 MHz) of compound **12**.



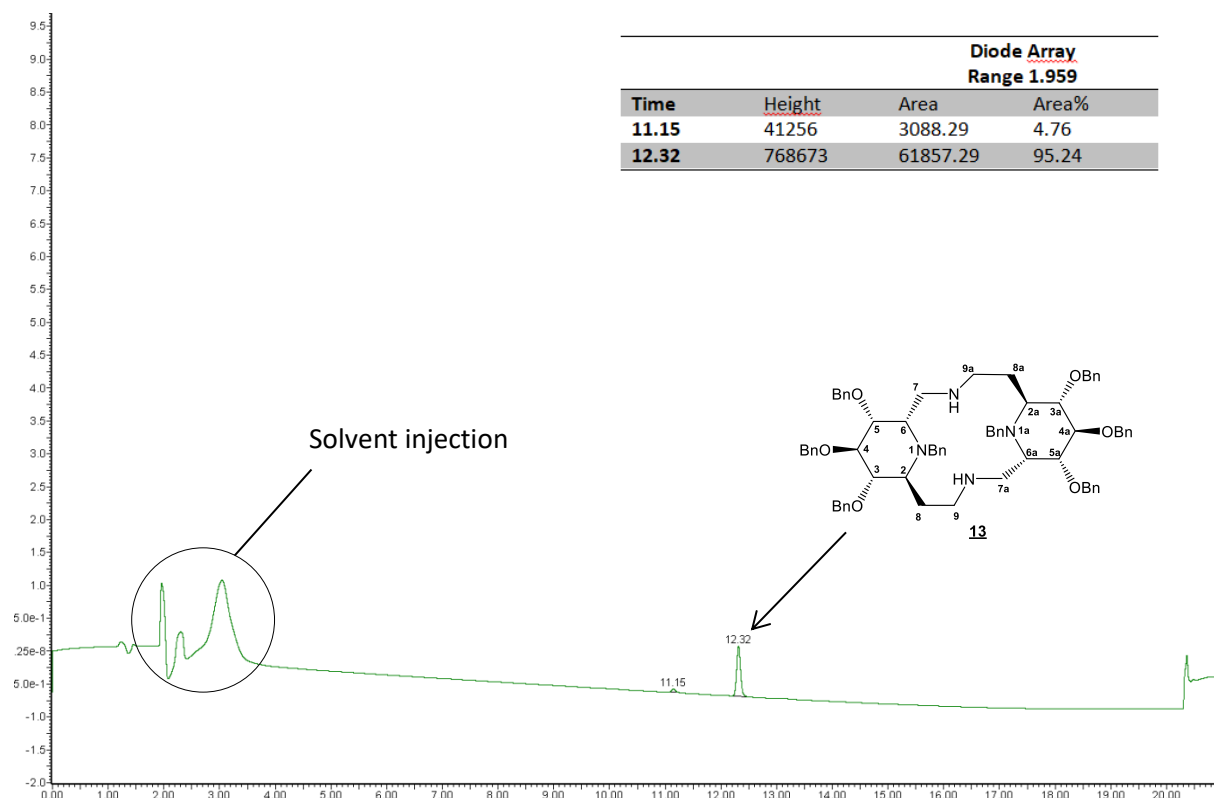
^1H NMR spectrum (CDCl_3 , 400 MHz) of compound **13**.



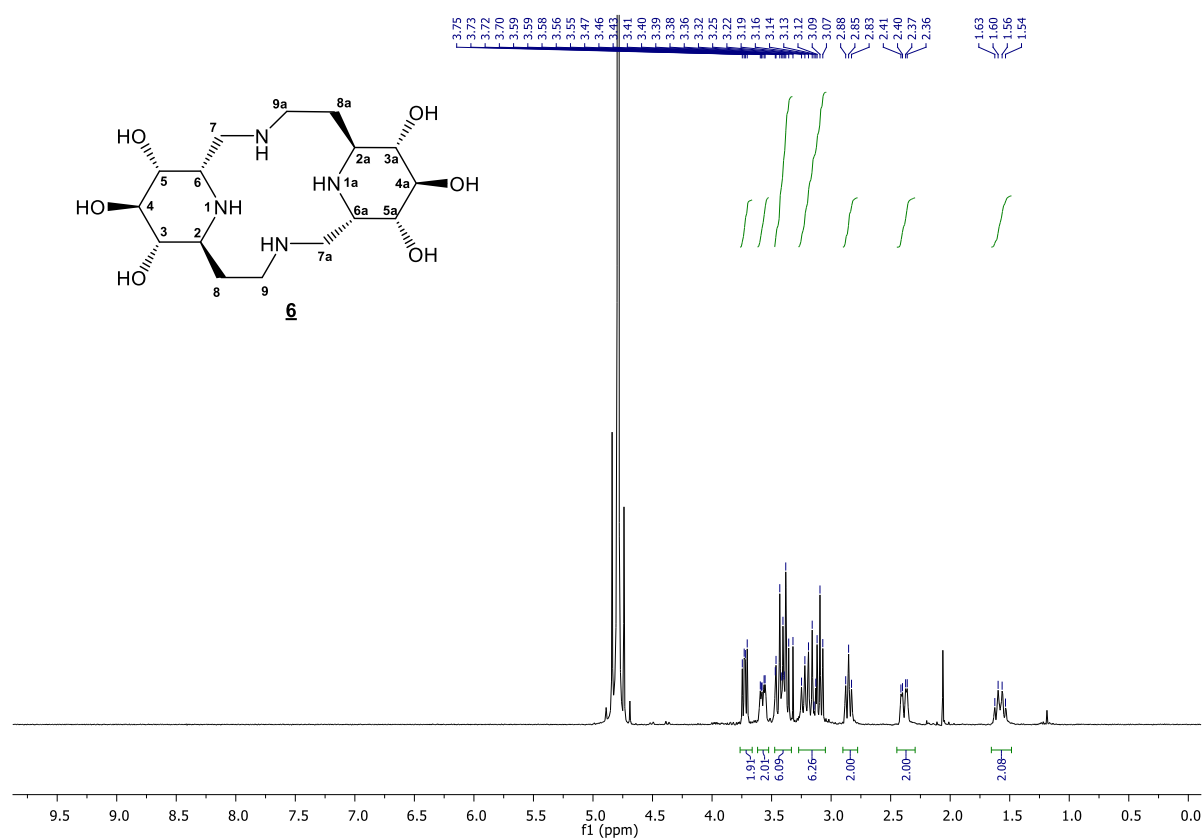
^{13}C NMR spectrum (CDCl_3 , 100 MHz) of compound **13**.



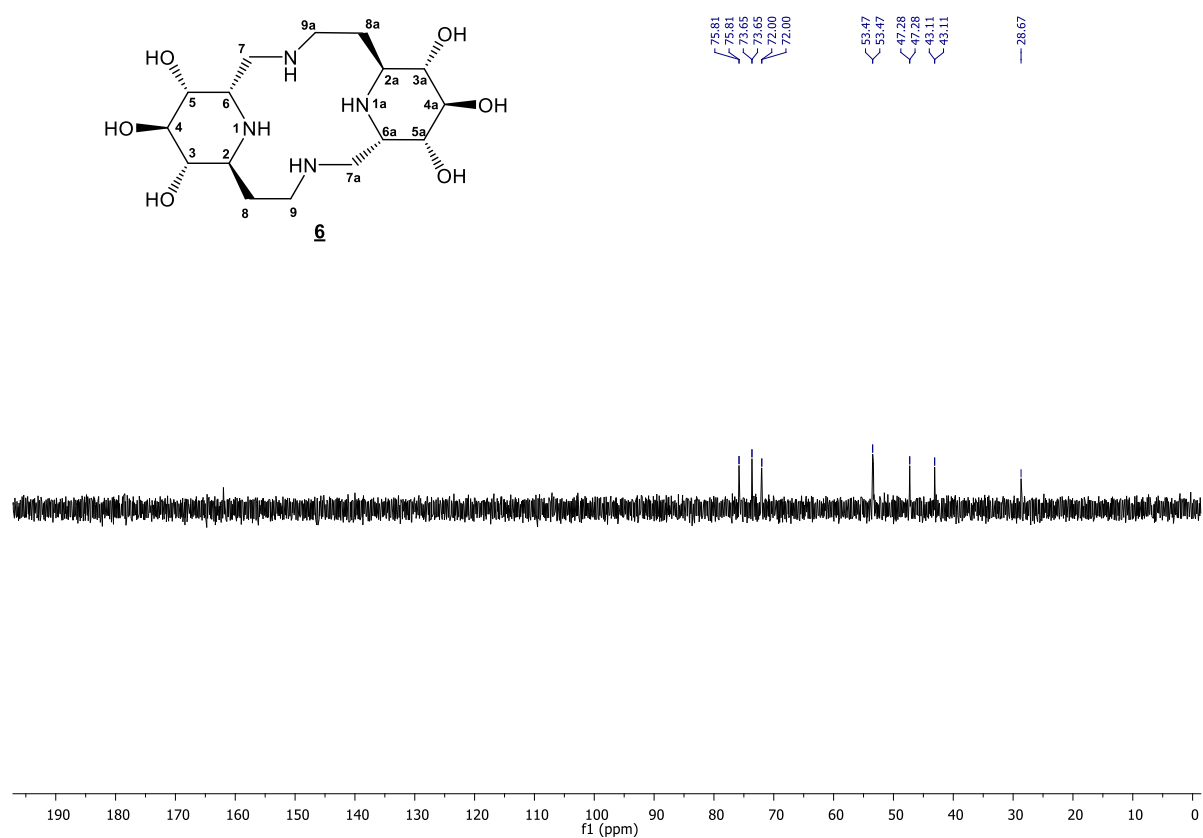
HPLC chromatogram of compound **13**.



^1H NMR spectrum (D_2O , 400 MHz) of compound **6**.



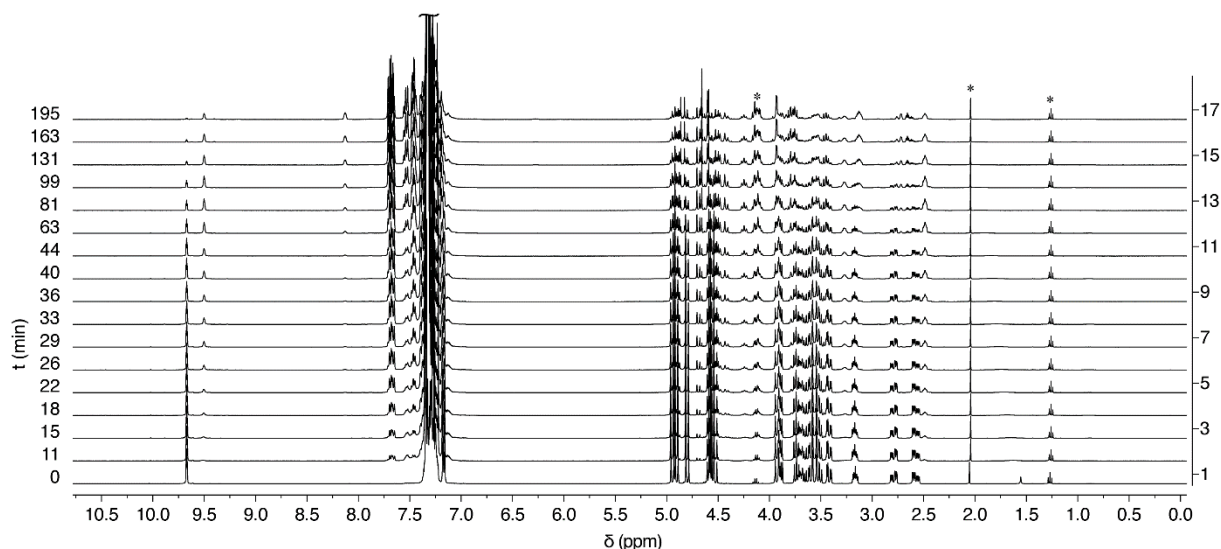
^{13}C NMR spectrum (D_2O , 100 MHz) of compound **6**.



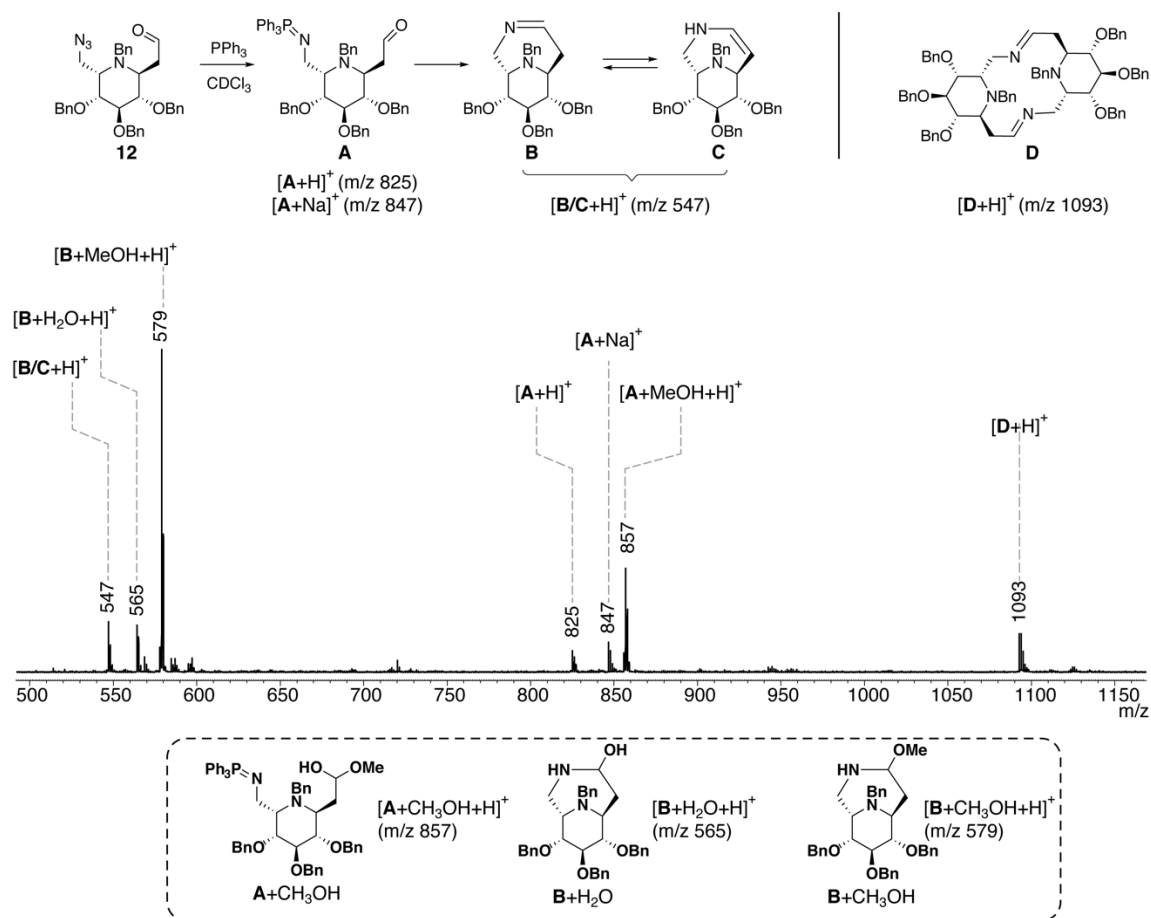
Monitoring of the Staudinger-aza-Wittig reaction for **12**

^1H NMR spectra (CDCl_3 , 400 MHz, 300 K).

Compound **12** was recorded in CDCl_3 (spectrum 1), then PPh_3 (ca. 3 equiv.) was added to the solution and the evolution was monitored for a 3h35 period of time (spectra 2 to 17). (*: residual solvents)



ESIMS spectrum (in MeOH) of the crude mixture after 195 min reaction time. Species in the dashed box are produced during the ESIMS analysis.



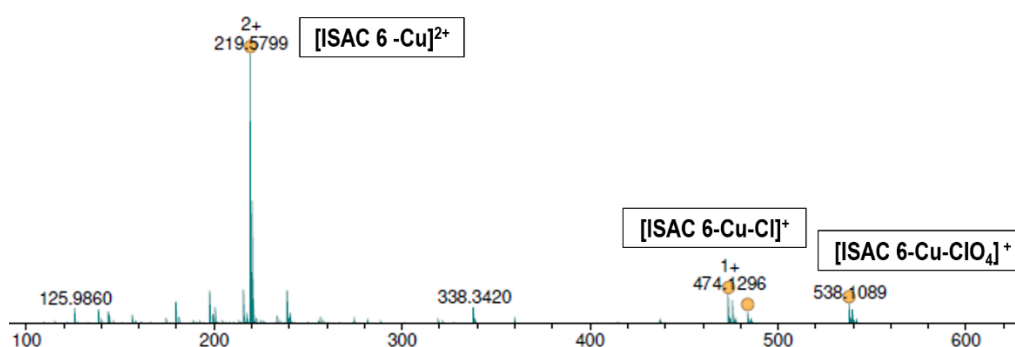
ISAC 6-Cu²⁺ complex

Synthesis

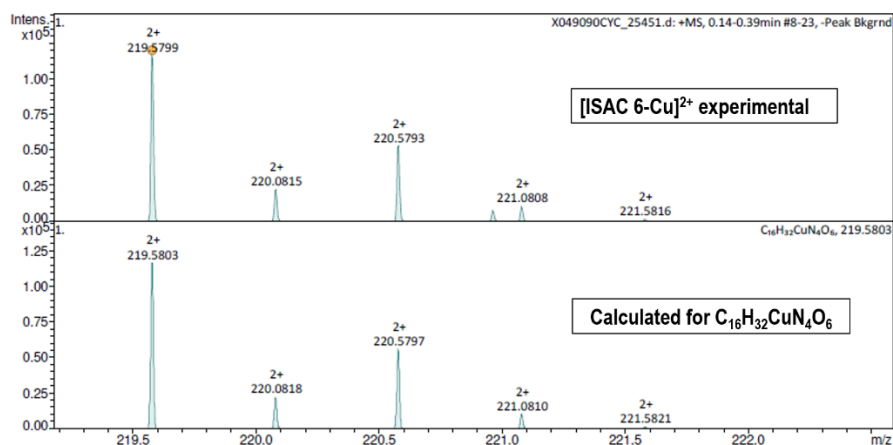
6 (10 mg, 0.026 mmol) was placed in 3 mL of distilled water, and the pH was adjusted from 2.8 to 6.5 with dilute 0.5 M NaOH. Upon addition of Cu(ClO₄)₂·6H₂O (9.8 mg, 0.026 mmol), a strong dark blue color was observed, showing a fast complexation of copper cation. pH was readjusted from 3.1 to 6.5 with 0.5 M NaOH and the mixture placed at 50°C for 2h to ensure full coordination. Volatiles were removed and the product was isolated as a dark blue solid through precipitation in a water/acetone mixture (14.3 mg, 86%).

High Resolution Mass Spectrometry

HRMS were recorded at the Institute of Organic and Analytic Chemistry - ICOA (Orléans, France) using a HRMS Q-ToF MaXis spectrometer equipped with ESI, APCI, APPI and nano-ESI sources.



(a) Full spectrum ($m/z = 100-640$)

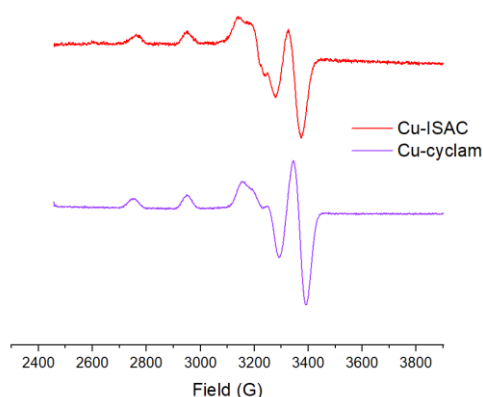


(b) Main signal ($m/z = 219$) and corresponding simulated isotopic pattern

Electron Paramagnetic Resonance

EPR spectra were recorded at "Service Commun de RMN-RPE" at UBO (Brest, France) on a Bruker Elexsys 500 instrument, in a 0.5 mm capillary within a quartz tube, at 9.34 GHz (band X) and with C = 4 mM in complex.

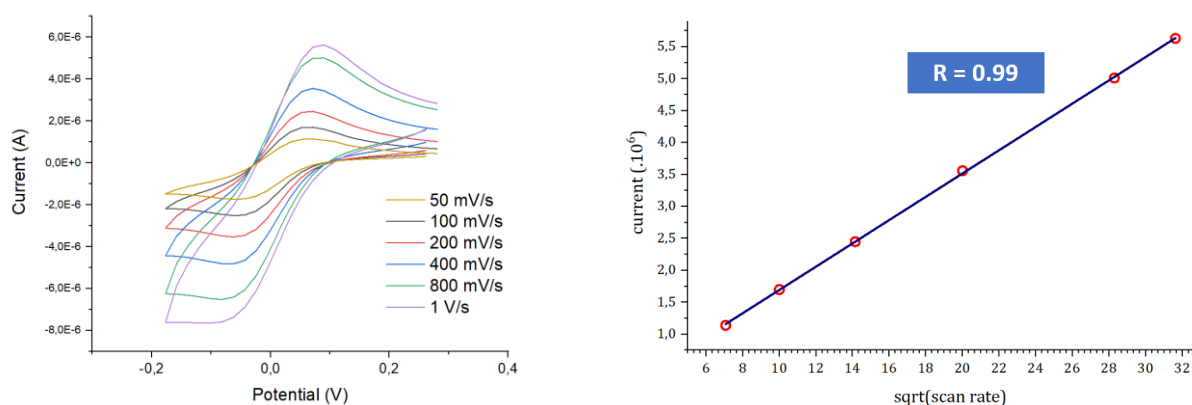
The simulation of the spectra (Simfonia software), indicates three different principal values of the g parameter for the complex, with $g_{\parallel} > g_{\perp}$, while the lowest g value is ≥ 2.04 , which is characteristic of mononuclear copper(II) complexes in dx^2-y^2 ground state.⁸



(c) overlap of Cu-ISAC **6** and Cu-cyclam at 143K in DMF/water (1:1)

Cyclic Voltammetry

Measurements were performed on a Autolab PGstat12 instrument, with a 3 electrode-setup: Ag/AgCl (3M NaCl) as reference electrode ($E = + 0.197$ vs. NHE), glassy carbon as the working electrode and platinum wire as the counter-electrode. Cyclic voltammetry was recorded under nitrogen at room temperature, pH=7.0, $C = 2.5$ mM in complex, with 0.1 M $Li(ClO_4)$ as the supporting electrolyte.



(d) quasi-reversible reduction wave at different scan rates (e) and linear regression of current vs. \sqrt{v} (scan rate)

A large scan from 0.5 to - 0.5 V at 100 mV.s^{-1} shows the consecutive reductions of Cu^{2+} to Cu^+ and Cu^+ to Cu^0 with anodic waves at $E_{pa} = - 0.05$ V and $E_{pa} = - 0.24$ V respectively vs. Ag/AgCl. This leads to metallic copper deposition of the electrode and results in a very high intensity of the cathodic wave corresponding to the copper(0) oxidation on the return scan ($E_{pc} = 0.10$ V).

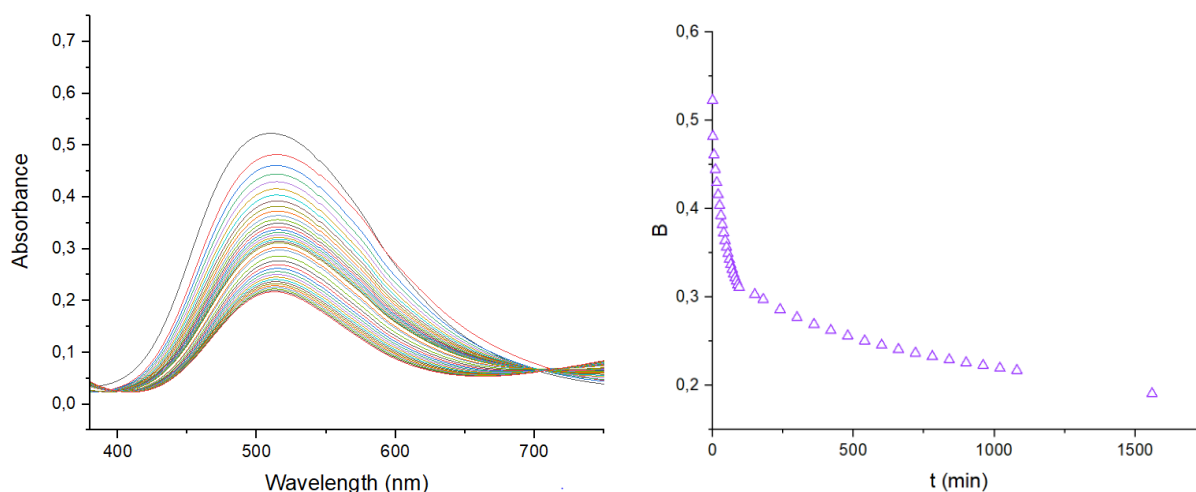
Upon scanning from 0.3 to - 0.180 V at different scan rates, a diffusion-controlled process is evidenced, with a linear correlation between the scan rate square-root and current intensity.

⁸ Hataway, B. J.; Billing, D. E., *Coord. Chem. Rev.* **1970**, 5, 143-207.

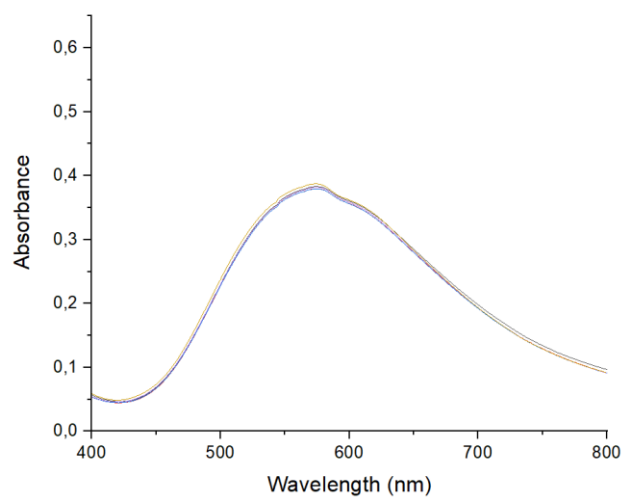
UV-Vis Spectroscopy

UV-Vis spectra were recorded at 20°C on a Agilent Cary 5000 UV-Vis-NIR in 700 microliter cuvettes with 1 cm optical path, and baseline correction was applied.

Kinetic inertness of the complexes was assessed through acid-assisted dissociation, at 20°C in 1.5 M HCl, with recording of the absorbance decrease of Cu d-d transition.



(f) Cu-cyclam in 1.5M HCl at 20°C and Absorbance plot at 514 nm (λ_{max}) as a function of time



(g) Cu-ISAC in 1.5M HCl at 20°C (spectra shown at $t = 1 \text{ min}, 30 \text{ min}, 1 \text{ h}, 3 \text{ h}, 7 \text{ days}, 14 \text{ days}$)

NMR conformational study of ISAC 6 and Cd²⁺ complex

NMR experimental details

NMR spectra were acquired on either Bruker AV-500 equipped with a TXI probehead with Z-gradient, Bruker AVIII-600 equipped with TBI [¹H, ¹³C, X-broad band] or TXI [¹H, ¹³C, ¹⁵N] probeheads, or Bruker AVIII-800 equipped with a Cryo TCI probehead.

All data were acquired at 298 K, unless other temperature is specified. ¹H chemical shifts were internally referenced to the methyl singlet of TSP (3-trimethylsilylpropionate-*d*4) at 0 ppm; ¹³C chemical shifts were referenced externally also using TSP; ¹¹³Cd chemical shifts are referred to CdMe₂. All NMR experiments were performed in Na₂CO₃/NaHCO₃ buffer solution, in 10% D₂O/90% H₂O or net D₂O, at pH=11 unless otherwise stated. Concentration of ISAC 6 was 0.5–3.5 mM.

1D ¹H NMR data were acquired over a ¹H width of 10–12 ppm into 32K data points. The water resonance was suppressed by presaturation using 1D-noesy pulse sequence provided by Bruker 2D ¹H-¹³C HSQC NMR data were acquired over a ¹H frequency width of 10 ppm and a ¹³C width of 60–80 ppm centered at 50 ppm. Data were acquired with 16–96 transients into 2–4K complex data points for each of 256–512 *t*₁ increments.

Using the same ¹H window, other heteronuclear correlations were performed. For 2D ¹H-¹⁵N HSQC NMR data a ¹⁵N frequency width of 1.2 kHz (15 ppm) centered at 39.5 ppm was used. Data were acquired with 32 transients into 4K complex data points for each of 512 *t*₁ increments. This experiment was run in a Bruker AVIII-800 equipped with a Cryo TCI probehead.

For 2D ¹H-¹¹³Cd HSQC NMR data a ¹¹³Cd frequency width of 2.7 kHz (20 ppm) centered at -390 ppm (related to CdMe₂). The evolution delay was adjusted for a ²*J*(¹HN-¹¹³Cd) coupling of 30 Hz. Data were acquired with 16 transients into 2K complex data points for each of 64 *t*₁ increments.

Computational methods

Structural models for non-complexed ISAC 6 were calculated with the MacroModel application within the Maestro software (Maestro, A powerful, all-purpose molecular modelling environment, version 8.5, Schrödinger, LLC, New York, NY 2008). An initial structure was minimized using conjugate gradients with the MM3* force field. A maximum number of 5000 iterations were employed with the PRCG scheme, until the convergence energy threshold was 0.05. The minimized structure was submitted to a conformational search protocol, using a Monte Carlo torsional sampling method (MCMC) with automatic setup during the calculation, energy window of 50 kJ mol⁻¹, 1000 maximum number of steps, and 100 steps per torsion of the bond to be rotated.

All DFT calculations were performed by means of Gaussian 16 program⁹. Geometry optimizations were carried out using the hybrid PBE0 functional¹⁰ and the Ahlrichs def2-TZVP basis set¹¹ was selected for this purpose. In addition, D3 version of Grimme's empirical dispersion correction was also applied¹². In all cases, our model present +2 charge since Cd was considered as cation. In order to faithfully reproduced the NMR data, the isotopic mass of Cd atom was selected in the molecule specifications

⁹ Gaussian 16, Revision B.01, Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Scalmani, G.; Barone, V.; Petersson, G. A.; Nakatsuji, H.; Li, X.; Caricato, M.; Marenich, A. V.; Bloino, J.; Janesko, B. G.; Gomperts, R.; Mennucci, B.; Hratchian, H. P.; Ortiz, J. V.; Izmaylov, A. F.; Sonnenberg, J. L.; Williams-Young, D.; Ding, F.; Lipparini, F.; Egidi, F.; Goings, J.; Peng, B.; Petrone, A.; Henderson, T.; Ranasinghe, D.; Zakrzewski, V. G.; Gao, J.; Rega, N.; Zheng, G.; Liang, W.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Vreven, T.; Throssell, K.; Montgomery, J. A., Jr.; Peralta, J. E.; Ogliaro, F.; Bearpark, M. J.; Heyd, J. J.; Brothers, E. N.; Kudin, K. N.; Staroverov, V. N.; Keith, T. A.; Kobayashi, R.; Normand, J.; Raghavachari, K.; Rendell, A. P.; Burant, J. C.; Iyengar, S. S.; Tomasi, J.; Cossi, M.; Millam, J. M.; Klene, M.; Adamo, C.; Cammi, R.; Ochterski, J. W.; Martin, R. L.; Morokuma, K.; Farkas, O.; Foresman, J. B.; Fox, D. J. Gaussian, Inc., Wallingford CT, 2016.

¹⁰ Adamo, C. Toward reliable density functional methods without adjustable parameters: The PBE0 model. *J. Chem. Phys.* **1999**, *110*, 6158–6170.

¹¹ Weigend, F.; Ahlrichs, R. Balanced basis sets of split valence, triple zeta valence and quadruple zeta valence quality for H to Rn: Design and assessment of accuracy. *Phys. Chem. Chem. Phys.* **2005**, *7*, 3297–3305.

¹² Grimme, S.; Antony, J.; Ehrlich, S.; Krieg, H. A consistent and accurate ab initio parametrization of density functional dispersion correction (DFT-D) for the 94 elements H-Pu. *J. Chem. Phys.* **2010**, *132*, 154104.

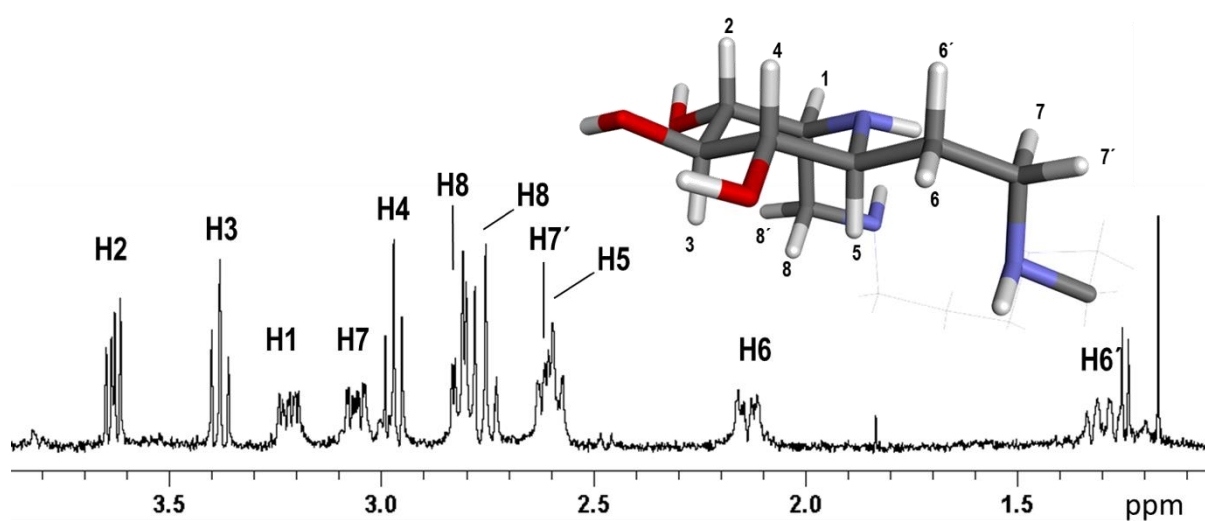
(iso=113, spin=1/2). The nature (minima or transition state) of obtained structures was characterized by analysis of frequencies. In this case, the absence of imaginary frequencies (Nimag = 0) was indicative that we found minima after optimization step.

The calculations of NMR properties were calculated using DFT/GIAO (Gauge-Independent Atomic Orbital)¹³ approach as implemented in Gaussian 16 to estimate theoretical spin-spin coupling constants¹⁴. The same level of theory was used on the previously optimized molecular systems. The consideration of implicit solvent effect (H₂O) using C-PCM polarizable conductor calculation model^{15,16} did not provide any improvement on spin-spin coupling constant values in comparison with gas phase calculations.

1. Free ISAC 6 study

1.1. Assignment, chemical shifts and J values

[1mM in Na₂CO₃/NaHCO₃ buffer in D₂O, 298K, 500MHz]



¹³ Wolinski, K.; Hilton, J. F.; Pulay, P. Efficient Implementation of the Gauge-Independent Atomic Orbital Method for NMR Chemical Shift Calculations. *J. Am. Chem. Soc.* **1990**, *112*, 8251-8260.

¹⁴ Deng, W.; Cheeseman, J. R.; Frisch, M. J. Calculation of Nuclear Spin-Spin Coupling Constants of Molecules with First and Second Row Atoms in Study of Basis Set Dependence. *J. Chem. Theory Comput.* **2006**, *2*, 1028-1037.

¹⁵ Barone, V.; Cossi, M. Quantum calculation of molecular energies and energy gradients in solution by a conductor solvent model. *J. Phys. Chem. A* **1998**, *102*, 1995-2001.

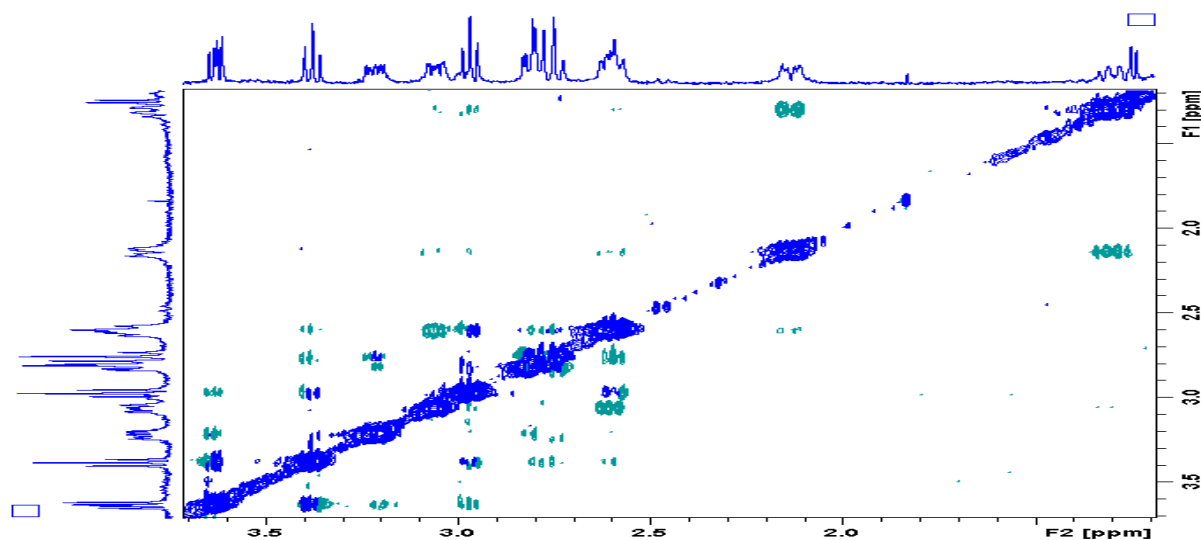
¹⁶ Cossi, M.; Rega, N.; Scalmani, G.; Barone, V. Energies, structures, and electronic properties of molecules in solution with the C-PCM solvation model. *J. Comp. Chem.* **2003**, *24*, 669-681.

[3.5mM in Na₂CO₃/NaHCO₃ buffer in H₂O/D₂O (9:1), 600MHz]

	ISAC 6		
	$\delta^1\text{H}$ (ppm)	Coupling constants	$\delta^{13}\text{C}$ (ppm)
NH(b)	nd		-
H7' ●	2.68		49.7
H7 ●	3.11		49.7
H6' ●	1.37	$^3J(\text{H6}', \text{H7}') = 10 \text{ Hz}$ $^3J(\text{H6}', \text{H7}) = 2 \text{ Hz}$	31.9
H6 ●	2.24	$^3J(\text{H6}, \text{H7}') = 6 \text{ Hz}$ $^3J(\text{H6}, \text{H7}) = 1.5 \text{ Hz}$	31.9
NH(a)	nd		-
H5 ●	2.69	$^3J(\text{H5}, \text{H6}) = 2 \text{ Hz}$ $^3J(\text{H5}, \text{H6}') = 12 \text{ Hz}$	55.8
H4	3.07	$^3J(\text{H4}, \text{H5}) = 9.5 \text{ Hz}$	78.7
H3	3.49	$^3J(\text{H3}, \text{H4}) = 9.5 \text{ Hz}$	76.6
H2	3.73	$^3J(\text{H2}, \text{H3}) = 9.5 \text{ Hz}$	75.0
H1 ●	3.31	$^3J(\text{H1}, \text{H8}) = 12 \text{ Hz}$ $^3J(\text{H1}, \text{H8}') = 4.1 \text{ Hz}$ $^3J(\text{H1}, \text{H2}) = 6.4 \text{ Hz}$	56.2
H8 <i>ax</i> ●	2.86		45.7
H8 <i>ec</i> ●	2.86		45.7

1.2. NOESY

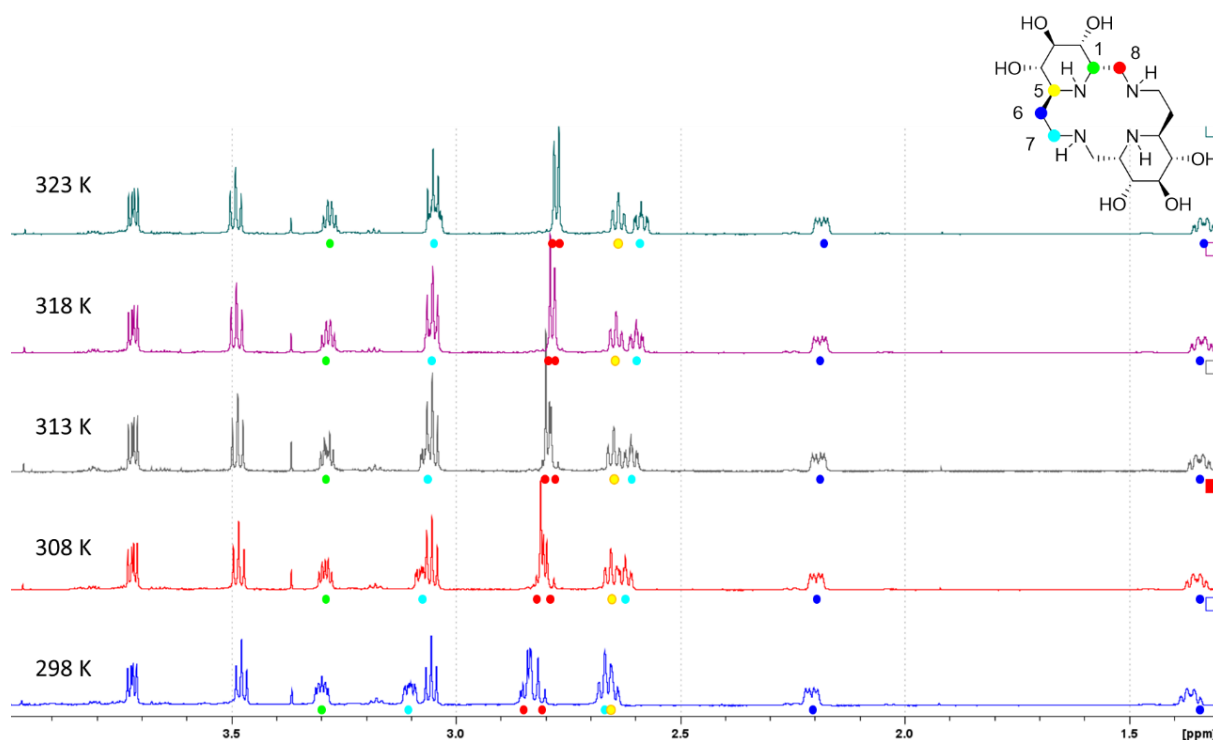
[1mM in Na₂CO₃/NaHCO₃ buffer in D₂O, 298K, 500 MHz, mixing time 500ms]



Typical NOEs from ⁴C₁-chair like conformation can be found: H1-H2, H2-H4, H3-H5

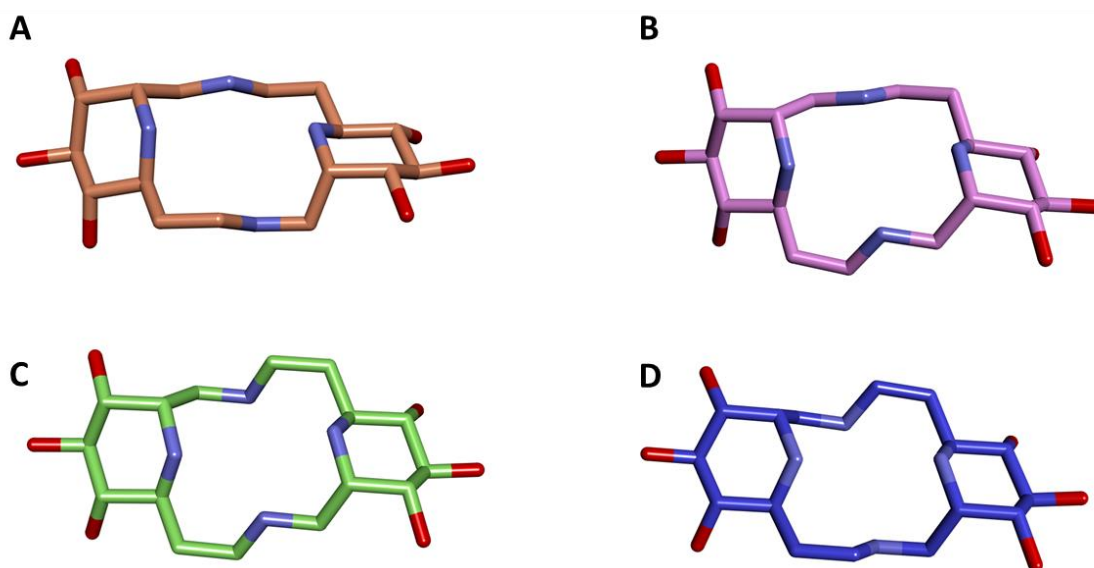
1.3. Temperature study

[3.5mM in Na₂CO₃/NaHCO₃ buffer in H₂O/D₂O (9:1), 600MHz]



¹H NMR spectra obtained at different temperatures for the free ISAC 6 (600 MHz). Greater mobility of the signals corresponding to positions 7 and 8 is observed, while the rest of the signals are barely altered. These signals correspond to the methylenes of the macrocycle, which has greater conformational flexibility. The NH protons are not observed.

1.4. Proposed models according to MM3* conformational search (Schrodinger Macromodel software)

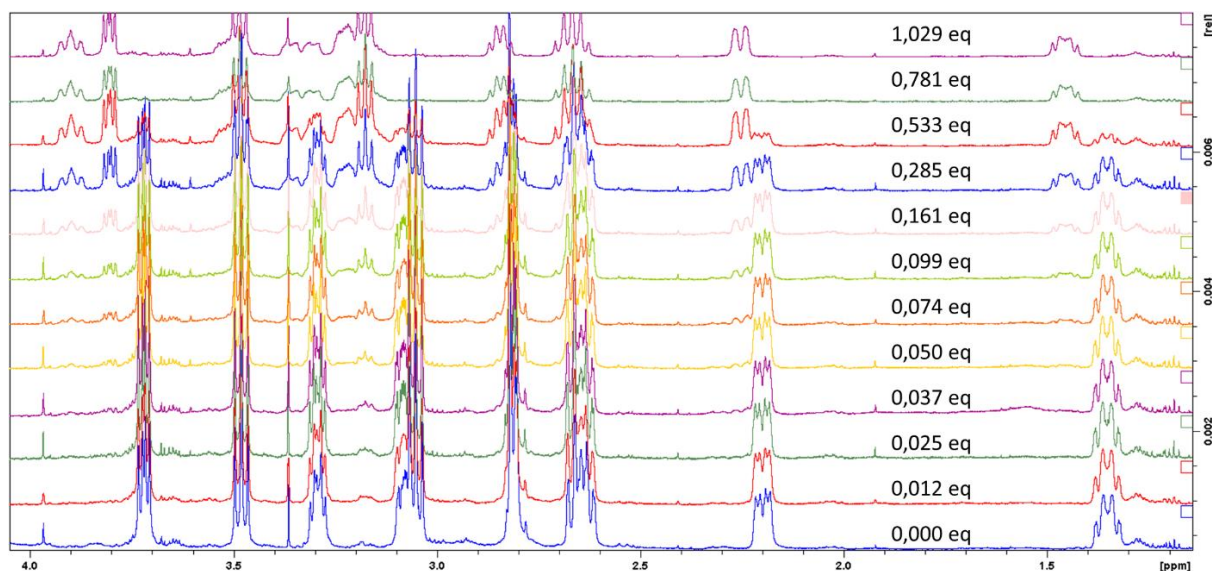


The structures can be clustered in 4 families. **A** is the energy minimum

2. ISAC 6 - Cd complex

2.1. Formation

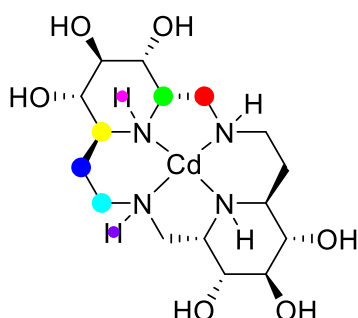
[3.5mM in Na₂CO₃/NaHCO₃ buffer in H₂O/D₂O (9:1), 298K, 600MHz]



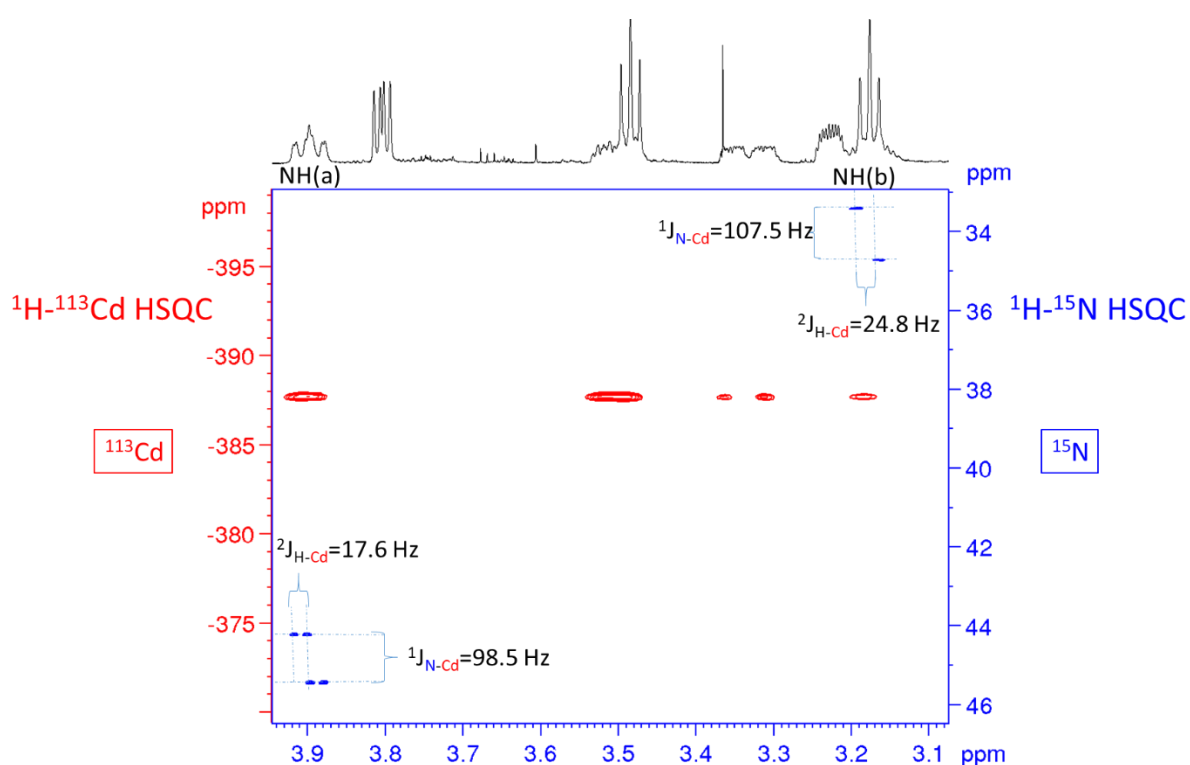
Formation of ISAC 6 - Cd²⁺ complex by addition of a solution of ¹¹³CdCl₂ (¹¹³Cd, 93.35% enrichment) in D₂O to a Na₂CO₃/NaHCO₃ buffer solution of ISAC 6, at pH=11 in H₂O/D₂O (9:1) and 298K (600 MHz). As complex is formed NH signals are visible in the spectra (3.9 ppm) because the nitrogen inversion is blocked.

2.2. Assignment: ¹H, ¹³C, ¹¹³Cd, ¹⁵N NMR chemical shifts

[3.5mM + 1eq ¹¹³CdCl₂, in Na₂CO₃/NaHCO₃ buffer in H₂O/D₂O (9:1), 298K, 600MHz]



ISAC 6 – Cd				
	$\delta^1\text{H}$, 323K (ppm)	$\delta^{13}\text{C}$ (ppm)	$\delta^{15}\text{N}$ (ppm)	$\delta^{113}\text{Cd}$ (ppm)
NH(b)	3.13	-	34.0	-
H7 <i>ax</i>	2.67	55.6	-	-
H7 <i>ec</i>	3.51		-	-
H6 <i>ax</i>	1.46	32,2	-	-
H6 <i>ec</i>	2.26		-	-
NH(a)	3.75	-	44.8	-
H5	2.82	59.7	-	-
H4	3.18	77.0	-	-
H3	3.49	76.0	-	-
H2	3.81	73.8	-	-
H1	3.24	58.7	-	-
H8 <i>ax</i>	2.63	46.3	-	-
H8 <i>ec</i>	3.34		-	-
Cd	-	-	-	-387.7



Superposition of the ^1H - ^{15}N (blue) and ^1H - ^{113}Cd (red) HSQC spectra for the ISAC **6**-Cd complex. The ^1H - ^{15}N HSQC was acquired in a AVIII 800 spectrometer at 298K with a delay optimized for 90Hz. [^1H (800.13 MHz), ^{15}N (81.07 MHz)]. From this experiment ^1H - ^{113}Cd and ^{15}N - ^{113}Cd coupling constants can be obtained. The ^1H - ^{113}Cd HSQC was acquired in an AVIII-600 spectrometer, equipped with a TBI probe, at 298K and a delay optimized for $J=30\text{Hz}$. [^1H (600.27 MHz), ^{113}Cd (133.22 MHz)]

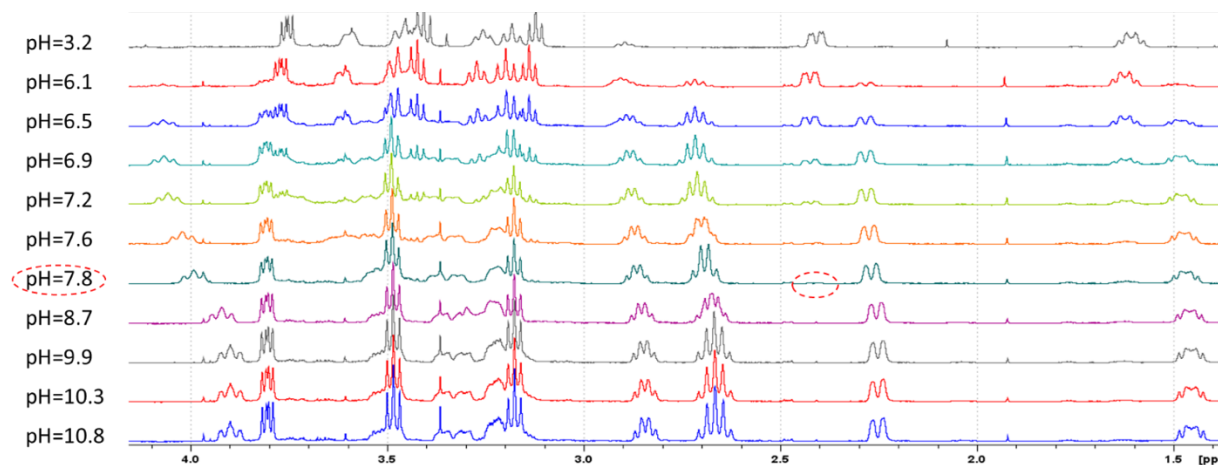
2.3. Coupling constants

	NH(b)	H8 <i>ax</i>	H8 <i>eq</i>	H1	H2	H3	H4	H5	NH(a)	H6 <i>ax</i>	H6 <i>eq</i>	H7 <i>ax</i>	H7 <i>eq</i>
NH(b)	-	12.5 ^a	5.5	-	-	-	-	-	-	-	-	12.5 ^a	5.6 ^b
H8 <i>ax</i>	11.3	-	13.2	13.1	-	-	-	-	-	-	-	-	-
H8 <i>eq</i>	5.6 ^b	12.5 ^a	-	3.4	-	-	-	-	-	-	-	-	-
H1	-	12.5 ^a	3.1	-	6.3	-	-	-	3.6	-	-	-	-
H2	-	-	-	6.5	-	9.6 ^b	-	-	-	-	-	-	-
H3	-	-	-	-	10.1	-	9.0	-	-	-	-	-	-
H4	-	-	-	-	-	9.6 ^b	-	12.5 ^a	-	-	-	-	-
H5	-	-	-	-	-	-	10.1	-	12.5 ^a	11.9	<2Hz	-	-
NH(a)	-	-	-	3.4	-	-	-	12.5 ^a	-	-	-	-	-
H6 <i>ax</i>	-	-	-	-	-	-	-	12.5 ^a	-	-	16.1	12.5 ^a	<2Hz ^c
H6 <i>eq</i>	-	-	-	-	-	-	-	<2Hz	-	16.0	-	<2Hz ^c	5.6 ^b
H7 <i>ax</i>	11.3	-	-	-	-	-	-	-	-	9.7	<2Hz ^c	-	12.7
H7 <i>eq</i>	5.6 ^b	-	-	-	-	-	-	-	-	<2Hz ^c	4.3	12.5 ^a	-
¹ H- ¹¹³ Cd	23.1 (² J)	-	31.9 (³ J)	-	-	-	-	-	15.8 (² J)	-	-	-	26.8 (³ J)
¹³ C- ¹¹³ Cd	-	-	-	-	16.0 (³ J)	-	16.1 (³ J)	7.5 (² J)	-	7.5 (³ J)	-	3.6 (² J)	-
¹⁵ N- ¹¹³ Cd	106.8 (¹ J)	-	-	-	-	-	-	-	99.2 (¹ J)	-	-	-	-

J couplings (Hz) derived for every proton signal. They are not reciprocal due to experimental error or to the presence of averaged signals from apparent quartets^a or other multiplets^b. Couplings below 2Hz were not detected but the signals width would indicate their presence^c.

2.4. pH stability study

[3.5mM + 1eq ¹¹³CdCl₂ in Na₂CO₃/NaHCO₃ buffer in H₂O/D₂O (9:1), 298K, 600MHz]



¹H NMR spectra (600 MHz) obtained at different pH values, adding incrementing amounts of diluted deuterated hydrochloric acid, for the free ISAC 6-Cd complex. The free compound started to appear around pH=7.8. This value match with the calculated pK value for NH(a) obtained in the same experiment.

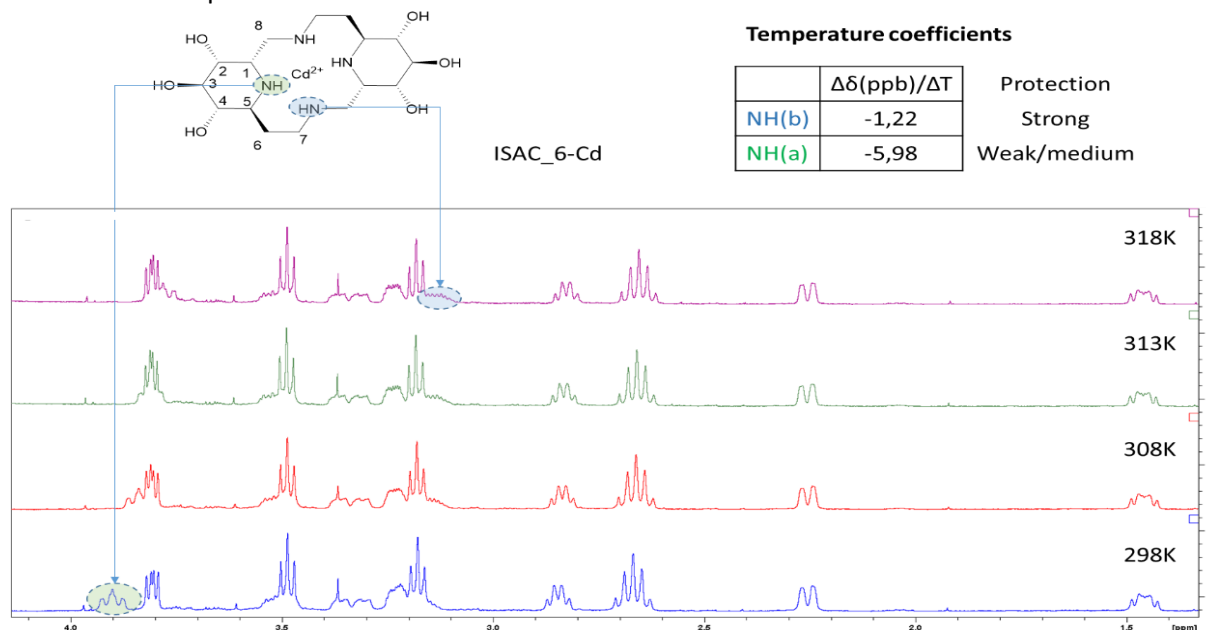
2.5. Temperature study

[3.5mM + 1eq $^{113}\text{CdCl}_2$ in $\text{Na}_2\text{CO}_3/\text{NaHCO}_3$ buffer in $\text{H}_2\text{O}/\text{D}_2\text{O}$ (9:1), 600MHz]



^1H NMR spectra obtained at different temperatures, for the free ISAC 6-Cd complex (600 MHz). Changes in the chemical shift of the macrocycle methylenes are no longer observed indicating that it is a more rigid structure.

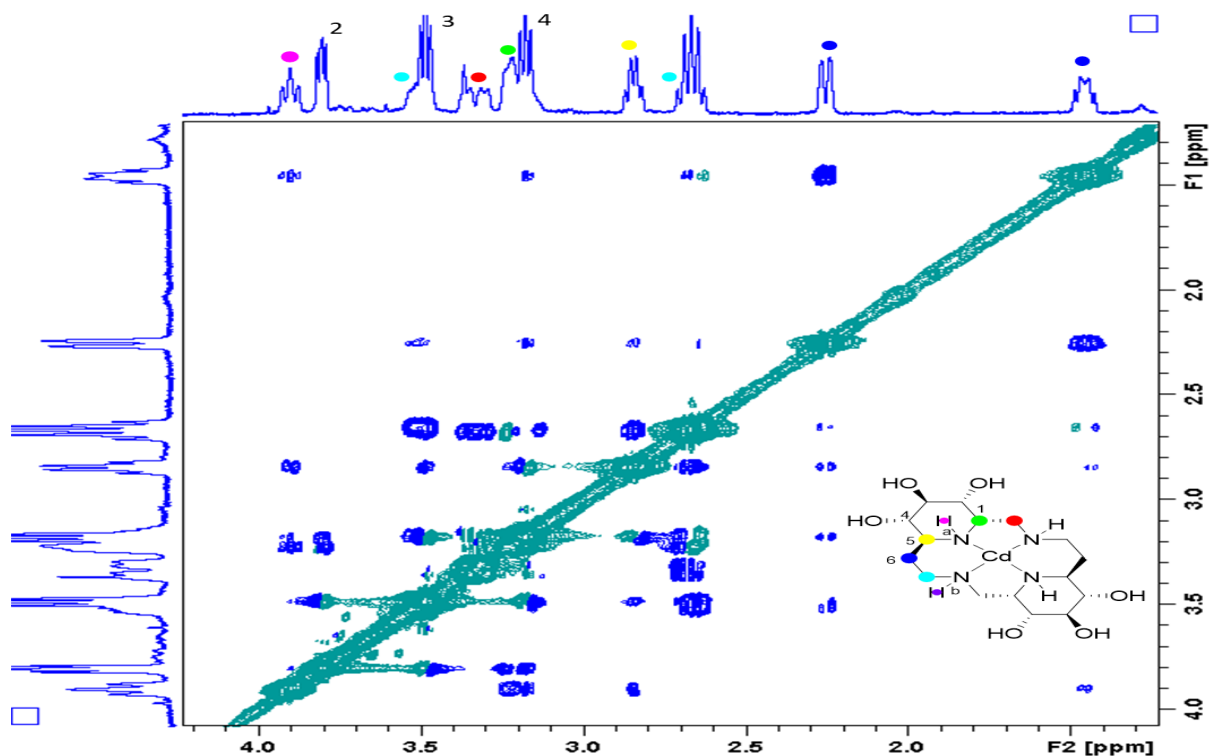
2.6. NH temperature coefficients



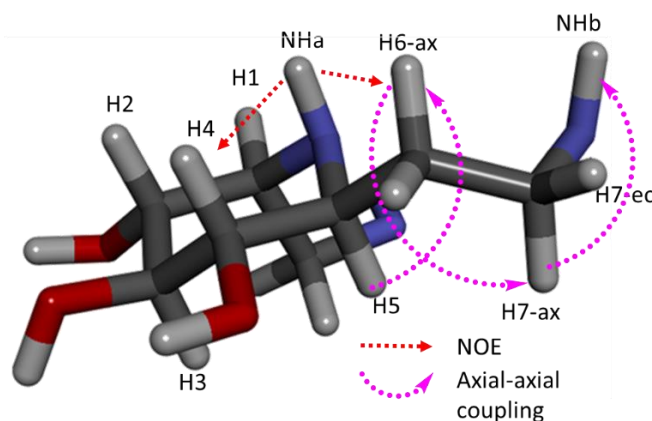
When the ISAC 6-Cd complex is formed, the signals of the NH groups appear, since the exchange with the solvent is slowed down and the nitrogen inversion is blocked. These protons are those that experience a greater variation of chemical shift with temperature. These variations are different for both protons, indicating that their exposure to the solvent is different. Temperature coefficients are calculated indicating that NH(a) is more exposed to solvent.

2.7. NOESY

[3.5mM + 1eq $^{113}\text{CdCl}_2$, in $\text{Na}_2\text{CO}_3/\text{NaHCO}_3$ buffer in $\text{H}_2\text{O}/\text{D}_2\text{O}$ (9:1), 298K, 600MHz]



2.8. Conformational analysis



When Cd complex is formed, the nitrogen inversion gets blocked and NHs orientation are fixed.

NOE data for the NH(a) proton with H6-ax and H4 (also in axial orientation) confirms the axial disposition of this NH. The ^3J coupling constants with H5 (12.6Hz, ax-ax) and H1 (3.4 Hz, ax-eq) also agree with this orientation.

For NH(b), the ax-ax coupling pattern is transmitted through H5-H6ax-H7ax-NH(b), supporting this axial orientation for this NH.

The NHs point to the same face of the molecule as is expected for conformation Trans-I type.

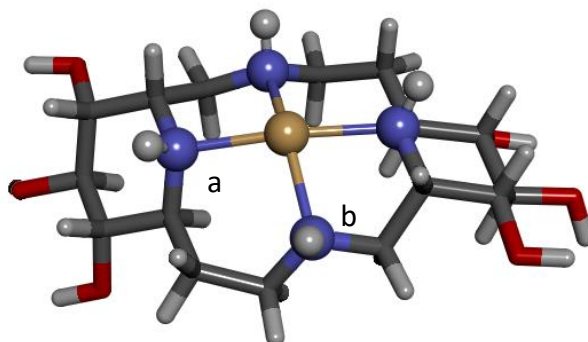
2.9. DFT Calculated data

2.9.1.NMR coupling constants

	NH(b)	H8 ax	H8 eq	H1	H2	H3	H4	H5	NH(a)	H6 ax	H6 eq	H7 ax	H7 eq
NH(b)	-	12.5	5.5	-	-	-	-	-	-	-	-	12.5	5.6
H8 ax	11.3 10.9 10.4	-	13.2	13.1	-	-	-	-	-	-	-	-	-
H8 eq	5.6 5.5 5.2	12.5 13.5 12.9	-	3.4	-	-	-	-	-	-	-	-	-
H1	-	12.5 11.1 11.3	3.1 3.3 3.3	-	6.3	-	-	-	3.6	-	-	-	-
H2	-	-	-	6.5 6. 6.4	-	9.6 ^b	-	-	-	-	-	-	-
H3	-	-	-	-	10.1 8.1 7.9	-	9.0	-	-	-	-	-	-
H4	-	-	-	-	-	9.6 7.3 7.1	-	12.5	-	-	-	-	-
H5	-	-	-	-	-	-	10.1 8.4 8.6	-	12.5	11.9	<2Hz	-	-
NH(a)	-	-	-	3.4 4.2 4.2	-	-	-	12.5 12.2 11.6	-	-	-	-	-
H6 ax	-	-	-	-	-	-	-	12.5 7.1 7.4	-	-	16.1	12.5 ^a	<2Hz
H6 eq	-	-	-	-	-	-	-	<2Hz 0.7 0.7	-	16.0 16.4 15.6	-	<2Hz	5.6
H7ax	11.3 10.6 10.2	-	-	-	-	-	-	-	-	9.7 10.4 10.7	<2Hz 1.7 1.7	-	12.7
H7eq	5.6 5.4 5.1	-	-	-	-	-	-	-	-	<2Hz 1.9 1.9	4.3 4.5 4.6	12.5 13.2 12.6	-

Experimental (black) and calculated data in gas phase (blue) or considering explicit solvent effect H₂O (red).

2.9.2.Calculated structure Optimized geometry by DFT calculations



Trans-I (R,S,R,S)

Cartesian Coordinates

ISAC-Cd complex

Energy (PBE0) = -1465.691650 hartree

Imaginary Frequencies: 0

Charge: +2

C	-1.23164900	2.75535900	0.79662000
C	-2.30591300	3.75982500	0.37584700
C	-3.04801000	3.30280100	-0.87253500
C	-3.62821900	1.90939300	-0.67692300
C	-2.53183000	0.91416300	-0.30358000
N	-1.79233700	1.38545700	0.90042700
C	0.00000000	2.78639200	-0.10924300
O	-1.66634700	4.99177800	0.20010500
O	-4.21762200	1.43533300	-1.85063500
O	-4.12090800	4.17604400	-1.12972500
C	-3.08270300	-0.50287100	-0.12132200
N	1.05615100	1.89546300	0.40815500
C	-2.16418700	-1.64077500	-0.54369600
N	-1.05615100	-1.89546300	0.40815500
C	1.23164900	-2.75535900	0.79662000
C	2.30591300	-3.75982500	0.37584700
C	3.04801000	-3.30280100	-0.87253500
C	3.62821900	-1.90939300	-0.67692300
C	2.53183000	-0.91416300	-0.30358000
N	1.79233700	-1.38545700	0.90042700
C	0.00000000	-2.78639200	-0.10924300
O	1.66634700	-4.99177800	0.20010500
O	4.21762200	-1.43533300	-1.85063500
O	4.12090800	-4.17604400	-1.12972500
C	3.08270300	0.50287100	-0.12132200
C	2.16418700	1.64077500	-0.54369600
H	-0.90439200	3.03210100	1.80504100
H	-3.04514100	3.81674800	1.18904500
H	-2.35777500	3.27297500	-1.72567400
H	-4.36284300	1.95543000	0.14577500
H	-1.82127200	0.90262100	-1.14037700
H	-2.42871000	1.35640400	1.69306900
H	-0.24423800	2.45290600	-1.11986200
H	0.34441700	3.81946700	-0.18558500
H	-2.32685800	5.69186900	0.18237800
H	-4.90870000	2.05376700	-2.11508800
H	-3.92107800	4.74918400	-1.87405000
H	-3.43422200	-0.64735000	0.90794100
H	-3.97228500	-0.57144200	-0.75099100
H	1.46045800	2.33475400	1.23293400
H	0.90439200	-3.03210100	1.80504100
H	3.04514100	-3.81674800	1.18904500
H	2.35777500	-3.27297500	-1.72567400
H	4.36284300	-1.95543000	0.14577500
H	1.82127200	-0.90262100	-1.14037700

H	2.42871000	-1.35640400	1.69306900
H	0.24423800	-2.45290600	-1.11986200
H	-0.34441700	-3.81946700	-0.18558500
H	2.32685800	-5.69186900	0.18237800
H	4.90870000	-2.05376700	-2.11508800
H	3.92107800	-4.74918400	-1.87405000
H	3.43422200	0.64735000	0.90794100
H	3.97228500	0.57144200	-0.75099100
H	-1.71640800	-1.40816100	-1.51377900
H	-2.74683800	-2.55715500	-0.67739900
H	2.74683800	2.55715500	-0.67739900
H	1.71640800	1.40816100	-1.51377900
H	-1.46045800	-2.33475400	1.23293400
Cd(Iso=113)	0.00000000	0.00000000	1.07323300