Supporting Information

Synthesis, $^{64}$Cu-labeling and PET imaging of 1,4,7-triazacyclononane derived chelators with pendant azaheterocyclic arms

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**NMR Measurements**

**$^1$H NMR NOTI**

![$^1$H NMR spectrum](image1)

**$^{13}$C NMR NOTI**

![$^{13}$C NMR spectrum](image2)
\(^1\)H NMR NOTThia

\(^{13}\)C NMR NOTThia
NOTI

NODI
MS Data Complexes

[Cu(NOTI)H][ClO₄]₃·1.3 H₂O (Cu-1)

[Cu(NOTI-Me)₂[ClO₄]₃·2TFA · 4H₂O (Cu-2)
[Cu(NOThia)](NO₃)₂ (Cu-S)
HPLC Data Complexes

Cu nat. NOTI

64Cu NOTI
Cu nat. NOTThia

64Cu NOTThia
Electronic spectra Cu nat. complexes

Cu nat. NOTI

Cu nat. NOTI-Me

Cu nat. NOTThia

Cu NOTThia 5M HCl challenge
UV/vis titration experiments

NOTI

![Graph showing NOTI titration experiment results.]

NOTI-Me

![Graph showing NOTI-Me titration experiment results.]

Radiochemical yield

$^{64}$Cu labeling experiments of NOTI, NOTI-Me, NOTThia, NOTA and DOTA.[a]

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<tr>
<th>Ligand</th>
<th>pH 4[b]</th>
<th>pH 5.5[c]</th>
<th>pH 7.4[d]</th>
<th>pH 8.0[e]</th>
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<tr>
<td>NOTI</td>
<td>96.0 ± 0.4%</td>
<td>92.9 ± 0.2%</td>
<td>95.4 ± 1.1%</td>
<td>99.9 ± 0.6%</td>
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<tr>
<td>NOTI-Me</td>
<td>98.2 ± 0.7%</td>
<td>93.4 ± 4.4%</td>
<td>96.3 ± 0.7%</td>
<td>98.6 ± 0.3%</td>
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<tr>
<td>NOTThia</td>
<td>95.8 ± 1.2%</td>
<td>87.4 ± 0.1%</td>
<td>98.0 ± 0.7%</td>
<td>97.0 ± 0.5%</td>
</tr>
<tr>
<td>NOTA</td>
<td>97.2 ± 1.0%</td>
<td>12.0 ± 0.2%</td>
<td>54.4 ± 3.6%</td>
<td>-</td>
</tr>
<tr>
<td>DOTA</td>
<td>45.8 ± 2.8%</td>
<td>5.9 ± 0.1%</td>
<td>81.5 ± 6.1%</td>
<td>93.7 ± 1.6%</td>
</tr>
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[a] Unless otherwise stated c(L) = 1 µM, r.t., 1 min; all buffers c = 0.1M. [b] Na-acetate. [c] MES. [d] HEPES. [e] NH$_4$OAc. [f] c(L) = 5 µM, 95°C, 30 min.

Percentage $^{64}$Cu complex activity associated with serum proteins

Time-activity curves of PET scans in nude mice

A = kidneys, B = liver
Auxiliary information on X-ray structures

CCDC 1043399 (Cu-1), CCDC 1043400 (Cu-22), CCDC 1043398 (Cu-3), CCDC 1043401 (Cu-4) and CCDC 1043403 (Cu-5) contain the supplementary crystallographic data for this paper, including coordinates, experimental details, refinement instructions and structure factors. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44(1223)-336-033; e-mail: deposit@ccdc.cam.ac.uk), or online via www.ccdc.cam.ac.uk/data_request/cif

Refinement of [Cu(NOTI)H](ClO$_4$)$_3$·1.3 H$_2$O (Cu-1)

The structure exhibits isolated hydrogen-bond networks running through a perchlorate anion, the protonated imidazole group, a water molecule and a second anion. 70:30 disorder in the imidazolium group consequently also affects the hydrogen-bonded moieties (Fig. S 1). Only the minor domain provides enough space for a second water molecule, hence the stoichiometry of 1.3. Accordingly, the calculated solvent accessible void vanished after assigning the highest remaining residual peak to oxygen, with a site occupation factor tied to the minor part of the disorder. Hydrogen atom of the 30% occupied water site were not found on the difference Fourier map and have not been assigned in the model.

The remaining two imidazole groups bound to copper both participate in additional hydrogen bonds with a ClO$_4^-$ on each side (N..O distances: 2.955(4) and 2.897(4) Å), forming infinite zig-zag chains alternating between perchlorate anion and complex cations (not shown). At the unoccupied coordination site of the Cu center, only a van-der-Waals contact with a ClO$_4^-$ is found (2.924(2) Å).

Fig. S 1: Disorder and hydrogen bonding motif in 1. Ellipsoids at 50% probability. Black: 70% occupation, red: 30% occupation. Hydrogens for the 30% occupied second water site could not be assigned. Length of marked Cu-O van-der-Waals contact: 2.924(2) Å (cf. sum of vDW-radii: 292 Å).
Refinement of [Cu(NOTI-Me)H][ClO₄]₂·2TFA·4H₂O (Cu-2)

The asymmetric unit of this structure contains two symmetry-independent complex cations. In one of these cations, copper is additionally coordinated by one water molecule, forming a distorted octahedral coordination geometry. In the other cation, copper is only coordinated by the protonated NOTI-Me ligand with no further contacts shorter than the sum of van-der-Waals radii (distorted tetragonal pyramid). Additionally the asymmetric unit contains three more water molecules, one trifluoroacetic acid molecule with rotational disorder in the CF₃ group (~80:20), one trifluoroacetate and five perchlorate anions, one of which is disordered over two orientations (~80:20). All moieties but one perchlorate anion are interconnected through a complex hydrogen bonding pattern. The hydrogen positions at the copper-coordinated water molecule, the imidazolium group and the trifluoroacetic acid molecule could be clearly identified by analysis of the difference Fourier map, and were then refined with idealized distances in terms of a riding model (water molecules were refined as rigid bodies). All other hydrogen positions were chosen to form a physical meaningful model, where every hydrogen donor is paired with a suitable acceptor.

Fig. S 2: Packing motif of 2. Ellipsoids at 50% probability, hydrogen atoms omitted for clarity, CPK color scheme. Disordered moieties are indicated by dashed bonds.
Refinement of [Cu(NOTI-Me)](BF₄)₂ (Cu-3)

The asymmetric unit of this structure contains one third of complex cation, with the central copper atom resting on a 3-fold axis. The triazonane backbone exhibits disorder in two ethylene subunits with a ratio of ~60:40. Both tetrafluoroborate anions also rest on special positions and are disordered: one on a 6_3 axis (disorder about a virtual mirror plane modeled with ~70:30 ratio), the other on the same 3-fold axis as the cation (disorder could only be described by slightly larger thermal displacement parameters). Overall the structure suffers from slightly substandard resolution, but structure proof can still be claimed with confidence. Absolute structure was determined with a Flack x parameter of 0.035(8).

Fig. S 3: Packing motif of 3, top-down view on the a-b plane (along the rotation axes). Ellipsoids at 50% probability, hydrogen atoms and disorder omitted for clarity, CPK color scheme.
Refinement of $[\text{Cu(NOTThia)}]([\text{ClO}_4])_2$ (Cu-4)

The asymmetric unit of this structure contains two Cu complex cations and four perchlorate anions, one of which is disorder by rotation about one of the Cl-O bonds (~50:50 ratio). The shortest intermolecular contacts are formed between sulphur atoms and oxygen atoms of the anions (2.935 – 3.225 Å).

Refinement of $[\text{Cu(NOTThia)}][\text{NO}_3]_2$ (Cu-5)

The asymmetric unit of this structure contains the Cu complex cation and two nitrate anions. The shortest intermolecular contacts are formed between sulphur atoms and oxygen atoms of the anions (2.919 – 3.246 Å).

Fig. S 4: Packing motif of 6, top-down view on the b-c plane. Ellipsoids at 50% probability, hydrogen atoms and disorder omitted for clarity, CPK color scheme.