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Optimization of Pentadentate Bispidines as Bifunctional Chelators for ^{64}Cu Positron Emission Tomography (PET)

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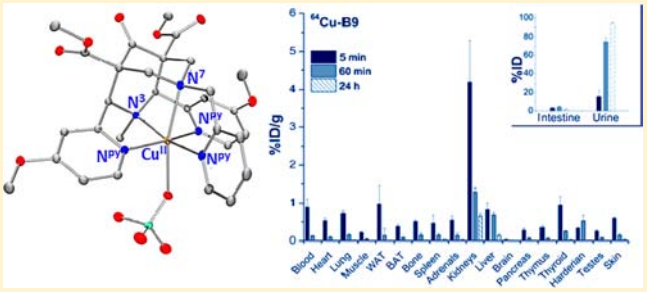
Supporting Information

ABSTRACT: Pentadentate bispidine ligands (3,7-diazabicyclo[3.3.1]nonanes) are optimized for maximum complex stability and facile functionalization with respect to their coupling to biological vector molecules and/or fluorescence markers for PET (positron emission tomography) and multimodal imaging (i.e., PET and optical imaging). The pentadentate ligand with two tertiary amine donors, two *p*-methoxy substituted pyridines, and one unsubsituted pyridine group is shown to best fulfill important conditions for PET applications, i.e., fast complexation with Cu^{II} and high in vivo stability, and this was predicted from the solution chemistry, in particular the $Cu^{II/I}$ redox potentials. Also, solvent partition experiments to model the lipophilicity of the Cu^{II} complexes indicate that the bis *p*-methoxy substituted ligand leads to cationic complexes with an appreciable lipophilicity. This is supported by the biodistribution experiments that show that the complex with the *p*-methoxy substituted ligand is excreted very quickly and primarily via the renal route and therefore is ideally suited for the development of PET tracers with ligands of this type coupled to biomolecules.



INTRODUCTION

In recent years, positron emission tomography (PET) developed into one of the most important diagnostic tools in oncology. A main research focus is on the development of radiolabeled biomolecules, i.e., antibodies, proteins, and peptides that attach to distinct diseased cells. Bifunctional chelators (BFCs), often macrocyclic ligands with further functionalities that enable the coupling to biomolecules, coordinated to radiometal ions, are therefore important and efficient imaging tools. Because of its decay scheme (β^+, 19%; β^-, 40%; electron capture, 40%), the close to ideal half-life of 12.7 h, and the "matched-pair" ^{64}Cu/^{67}Cu (diagnosis and therapy, respectively), copper-64 is an ideal positron emitter for PET in oncology.[1−3]

A large range of ligands with well-defined and stable Cu^{II} complexes, ranging from acyclic systems like diacetyl-bis(*N*4-methylthiosemicarbazone) (ATSM)[4−7] to macrocyclic ligand systems[4,8−17] and cages, specifically Sargeson's sepulchrates and sarcophagine (sar-type) systems[18−21] have been proposed to be useful BFCs (see Chart 1). Aliphatic, mostly cyclic nitrogen donor set based systems (e.g., TETA and DOTA, derived from the 12- and 14-membered macrocycles cyclen and cyclam; see Chart 1) prevail,[11,22−27] because of the high thermodynamic stability of the corresponding Cu^{II} complexes (macrocyclic effect[28,29]) and easy to attach functionalities, such as carboxylic acid groups, for the coupling to biomolecules. However, these Cu^{II} complexes often lack satisfactory in vivo stability (i.e., with respect to transchelation to superoxide dismutase and ceruloplasmin).[11,30,31] Also, many of the commonly used polyazamacrocycles and their bioconjugates are based on elaborate multistep synthetic routes.[25,32−34]

Most of the requirements for BFCs for PET are fulfilled by bispidine-based systems, i.e., (i) fast complexation , (ii) high in vivo stability of the complexes, (iii) appropriate lipophilicity of the chelate and the possibility to reduce the positive charge of the complex by anionic donors substituted to the bispidine scaffold, (iv) availability of diverse functionalities for the coupling to targeting vectors or molecular fragments for other imaging techniques (e.g., fluorescence or magnetic resonance), and (v) an easily accessible, cost-effective synthetic route for a multigram scale.[10,35]

Bispidines (3,7-diazabicyclo[3.3.1]nonane derivatives) with pyridyl groups at C^2 and C^4 are highly preorganized (specifically also for Cu^{II}) and very rigid ligands and therefore form stable Cu^{II} complexes (tetradentate ligand B1, log $K_{Cu(II)L}$ = 16.6 M^{-1}; pentadentate ligand B3, log $K_{Cu(II)L}$ = 18.3 M^{-1}; see Charts 1 and 2 for ligand structures and numbering).[36] In

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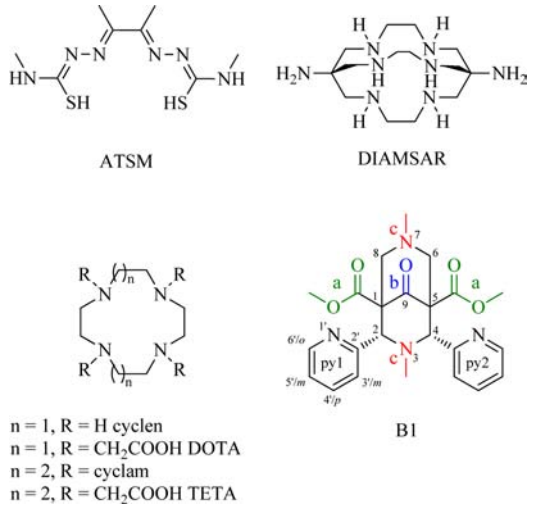
Chart 1. Chelators currently used in ^{64}Cu-based radiopharmaceutical imaging[a]



[a]As a derivative of bispidines the tetradentate ligand B1 is depicted (the colored fragments a - c show possible sites for functionalization, see text).

addition, these bispidines are easily built up by two double Mannich reactions, usually via the isolated piperidone intermediate, and this highly modular route therefore allows for a broad variation of the components and leads to a large diversity of ligands.[37−42] For example, this allows the tuning of the electronic properties of the pyridine groups and consequently the $Cu^{II/I}$ redox potentials and Cu^{II} complex stabilities. The most promising and synthetically available tetradentate ligand is the bis(*p*-MeO) substituted bispidine B2.[43] Importantly, the bispidine-based BFCs also bear various possibilities for their coupling to biomolecules (color coded in Chart 1), i.e., (a) the ester groups at C^1 and C^5 (ester hydrolysis or reduction to the corresponding primary alcohols),[44] (b) the carbonyl function at C^9 (reduction to a secondary alcohol;[45] this modification is also important to avoid an induced retro-Mannich reaction in vivo, resulting in the decomposition of the ligand and its complex), and (c) various functionalities at R^1 and R^2 (see below).

Here, we extend the ligand tuning and functionalization to the pentadentate bispidine derivatives B3−B6 and also evaluate the kinetic stabilities of the $^{64}Cu^{II}$ complexes of the substituted tetra- and pentadentate bispidine ligands with cyclam-based challenge experiments. Possibilities for introducing biofunctionalities into the bispidine backbone, as a prerequisite for pharmaceutical targeting, have also been evaluated (ligands B11−B16). In addition, partition experiments in 1-octanol/water and biodistribution experiments in male Wistar Kyoto rats are used to evaluate the potential of radiocopper−bispidine complexes as PET tracers.

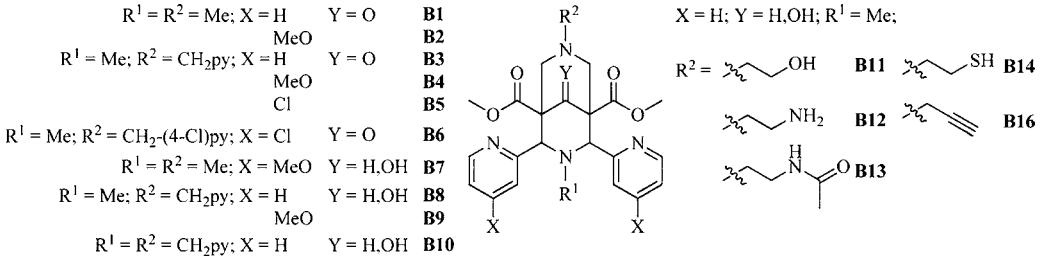
RESULTS AND DISCUSSION

Ligand Synthesis, Solution Chemistry, and Structural Properties. The syntheses of the tetradentate bispidine ligands B1, B2, B8, and B9,[43] the hexadentate bispidine B10,[44,46] and the corresponding piperidone precursors P1−P3 (see Chart 2 and Scheme 1)[43,47] have been described before. The pentadentate bispidines were also obtained via double Mannich reactions from the piperidones P1−P3 (also accessible via a double Mannich reaction), formaldehyde, and the corresponding (substituted) picolylamines **7**−**9**.[37−39,42,48−50] The required substituted picolylamines were obtained in two ways. The pyridinecarbaldehydes **1** and **2** were transformed into the oximes **3** and **4** by condensation with hydroxylamine.[51] Subsequent reduction, mediated by zinc/TFA (TFA = trifluoroacetic acid), yielded the desired substituted picolylamines **7** and **8**.[51] A more efficient approach converts the 4-substituted (pyridine-2-yl)methanols **5** and **6**, accessible in two to three steps,[43] in one-pot Mitsunobu and Staudinger reactions directly to the corresponding amines (Scheme 2).[52] The unsubstituted and *p*-methoxy-substituted bispidones were also reduced at C^9 with sodium borohydride, according to known procedures (Scheme 1).[53,54]

On the basis of the thorough theoretical and experimental analysis of the structural, electronic, and thermodynamic properties of tetradentate bispidine-based Cu^{II} complexes,[43] the bispidine with three tris(*p*-MeO) substituted pyridine groups was assumed to be the most promising ligand in the series of pentadentate bispidines for PET applications. According to literature-known procedures,[49,55] the precursor P2 was refluxed with (4-methoxypyridin-2-yl)methanamine **7** and formalin in THF. Interestingly, instead of the desired *syn*-isomer (see Chart 2; Y = O, X = MeO, R^1 = Me, R^2 = (4-MeO)py), the betainic hemiaminal **10** was obtained. The methyl ester at C^5 was hydrolyzed (aqueous formaldehyde was used in the reaction; we assume that the free carboxylic acid facilitates protonation of the C^9 carbonyl group and may then be attacked by the *exo*-positioned pyridinyl group to yield the stable betainic structure **10** (Figure 1). Unfortunately, the conversion of this *endo*/*exo*-isomer to the desired *endo*/*endo*-isomer failed. The formation of a similar hemiaminal was reported before for the unsubstituted tetradentate bispidine B1.[56,57]

Coordination of the bispidine ligands to Cu^{II} at ambient temperature in acetonitrile is generally a fast process. With the pentadentate bispidine B4 the resulting Cu^{II} complex was

Chart 2. Bispidine ligands discussed, i.e. tetra-, penta-, and hexadentate pyridinyl-substituted bispidines B1−B10, and N^7-functionalized bispidines B11−B14 and B16



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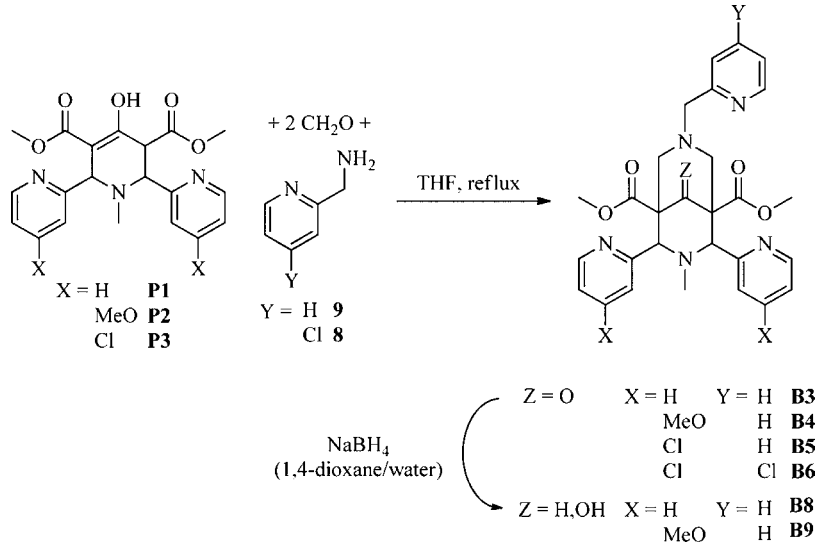
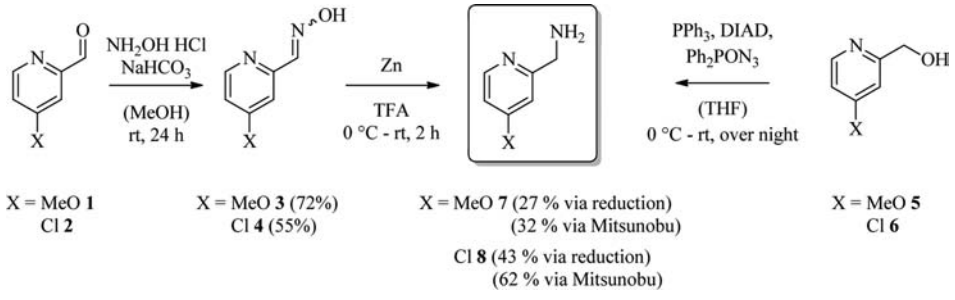
Scheme 1. General Synthetic Route to the Pentadentate Bispidine Ligands



Scheme 2. Syntheses of the 4-Methoxy- and 4-Chloropicolylamines 7 and 8



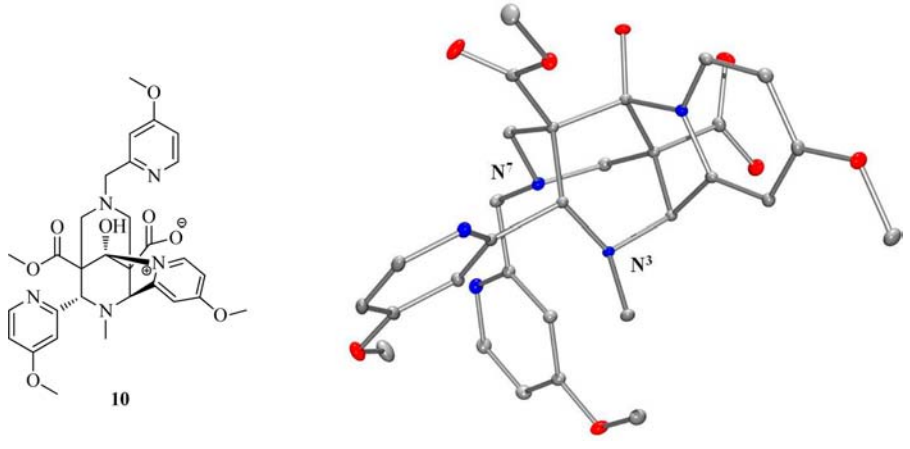


Figure 1. Formula (left) and plot of the crystal structure (right) of the monoester−hemiaminal product of the tris(*p*-MeO) bispidine derivative **10**. Ellipsoids are shown at the 30% probability level. Solvent molecules and hydrogen atoms are omitted for clarity.

isolated by crystallization from acetonitrile (ether diffusion; see Experimental Section). The molecular cation is plotted in Figure 2, and selected structural parameters are presented in Table 1 (also shown in Figure 2 is the structure of the metal-free ligand B5; these structures are as expected from others reported before).[42,48,55,58,59]

The introduction of electron donating groups such as methoxy groups at the para position of the pyridinyl moieties significantly enhances the thermodynamic stability of the Cu^{II} bispidine complexes and therefore their in vivo performance as PET tracers. Another important requirement for the development of BFCs for PET tracers is to incorporate functionalities for their coupling to biological vectors or nanoparticles. First generation bispidine ligands[60] offer various possible sites for linkers, as highlighted in Chart 1. Functionalization via the ester groups at C^1 and C^5 has the disadvantage of rather long reaction sequences and the diastereotopic nature of the ester groups, leading to elaborate purification of the products.[44] The functionalization of the carbonyl group at C^9 requires its reduction; this has been achieved stereoselectively[54] and is

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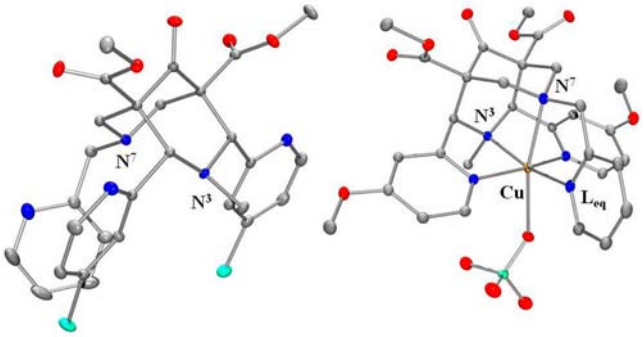


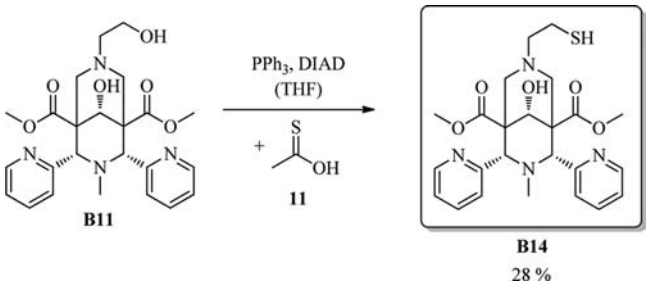
Figure 2. Experimental structures of the metal-free ligand B5 (left) and $[Cu^{II}(B4)(OClO_3)](ClO_4)$ (right). Ellipsoids are shown at the 30% probability level. Hydrogen atoms and noncoordinating perchlorate ion are omitted for clarity.

furthermore required to prevent retro-Mannich activity (see Introduction), but the resulting secondary alcohol function is rather unreactive.

Therefore, we concentrate here on the most facile and straightforward possibility to introduce functionalities at the amine component of the second double Mannich reaction, leading to N^7-functionalized bispidines. The resulting ligands (e.g., B11−B14 and B16 in Chart 2) emerge from a short reaction sequence and are stereochemically pure. Since labile peptides may be employed as targeting vectors, the reactions used need to enable quantitative coupling procedures under mild conditions (low temperature, aqueous solution, moderate pH), e.g., related to "click-type" chemistry.[64−66] Functional groups fulfilling these requirements include (i) primary alcohols for isocyanate coupling,[67−69] (ii) amines for NHS-ester coupling,[70−75] (iii) thiols for maleimide coupling[75−77] and the thiol−ene reaction,[78,79] and (iv) alkynes for sterically induced or the Cu^I-mediated 1,3-dipolar cycloaddition (Huisgen reaction),[67,80−85] i.e., the bispidines B11−B14 and B16 in Chart 2. The synthesis of the amine-functionalized ligand B12 (and its conjugation to fluorescence dyes leading to chemosselective sensors) and the alcohol-bearing ligand B11 are reported elsewhere.[55,86]

A route to a thiol-functionalized tetradentate bispidine ligand was accomplished in one step, using the bispidiol B11 as precursor. This was dissolved in dry THF and subjected to a Mitsunobu-type reaction with thioacetic acid **11**.[87,88] The resultant thioester intermediate is hydrolyzed during workup (Scheme 3).

Scheme 3. Synthetic Approach to the Thiol-Functionalized Bispidine B14



An alkyne-functionalized derivate of B1 was obtained in a similar procedure. The precursor P1, formaldehyde, and the propargylamine **12** were reacted to the bispidone B15,[89] which was reduced at C^9 according to a known procedure, using sodium borohydride in a mixture of 1,4-dioxane/water[53] to afford the functionalized bispidine B16 (Scheme 4; see Supporting Information for the experimental structure of B16).

The bispidine B12 has a pendent primary amine at N^7 and therefore is a pentadentate ligand (see Figure 3 for an experimental structure of the corresponding Cu^{II} complex; selected structural parameters are given in Table 1). Carboxylates are abundant in peptide vectors, and there is a multitude of procedures for their coupling to free amines. The model amide system B13 was prepared in two steps in order to study its coordination mode.[86] Various methods and Cu^{II} salts for complexation were used. Under standard procedures for bispidine-Cu^{II} chemistry (equimolar amounts of $Cu^{II}(ClO_4)_2{\cdot}6H_2O$ and bispidine in MeCN, ambient temperature; see Experimental Section), $[Cu^{II}(B13)](ClO_4)_2$ was isolated, and the X-ray structural analysis shows that its amide oxygen is coordinated to the Cu^{II} center (see Scheme 5).[86] In a similar reaction but using $Cu^{II}(OAc)_2$ in MeOH, an acetate completes the square pyramidal coordination sphere with a dangling amide pendent group.[86] When the complex $[Cu^{II}(B13)](ClO_4)_2$ is deprotonated in acetonitrile, using the noncoordinating bulky base diisopropylethylamine, $[Cu^{II}(B13-H)](ClO_4)$ was isolated, and crystallization from water produced X-ray quality crystals (see Scheme 5, Figure 3, and Table 1). Cu^{II} is known to be able to deprotonate amides (oligopeptides: $pK_a \approx 15$; deprotonation and coordination to Cu^{II} at pH ≈ 4−5),[90−92] and $[Cu^{II}(B13\text{-}H)]^+$ therefore is assumed to be stable in this form in aqueous solution at physiological pH. Importantly, this stable complex has an

Table 1. Selected Bond Lengths (Å) and Angles (deg) of the Molecular Cations of the Cu^{II} Complexes

	$[Cu^{II}(B4)(OClO_3)]^+$	$[Cu^{II}(B12)(OClO_3)]^+$	$[Cu^{II}(B13\text{-}H)]^+$
$Cu-N^3$	2.025(2)	2.014(1)	1.993(2)
$Cu-N^7$	2.347(2)	2.259(1)	2.202(2)
Cu−N(py)	1.990(2)/2.010(2)	2.049(2)/2.050(2)	2.045(3)/2.053(3)
$Cu-L_{eq}$	1.983(2)	1.994(2)	1.912(2)
$Cu-O(ClO_3)_{ax}$	2.522(2)	2.626(1)	
$N^3\cdots N^7$	2.928(2)	2.905(2)	2.884(3)
N(py1)···N(py2)	3.953(2)	4.001(2)	4.009(4)
N^3-Cu-N^7	83.75(6)	85.45(5)	86.71(9)
$N^3-Cu-N(py)$	82.52(6)/82.29(6)	81.35(6)/79.65(6)	80.97(9)/81.40(9)
N^7-Cu-L_{eq}	82.23(7)	84.10(6)	86.96(10)
$N^3-Cu-O(ClO_3)_{ax}$	100.49(6)	103.04(5)	
N(py1)−Cu−N(py2)	162.39(7)	155.00(6)	156.01(10)

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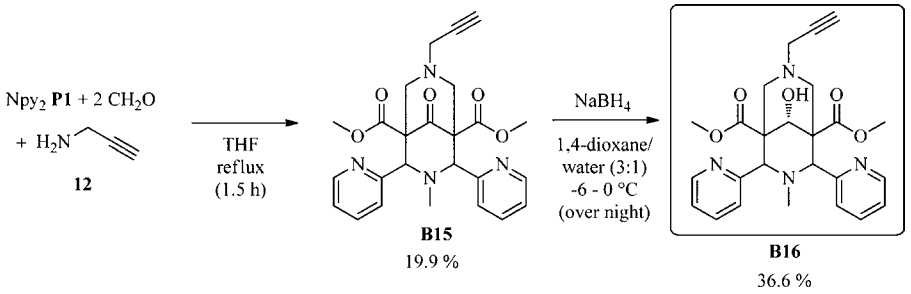
Scheme 4. Synthetic Approach to the Alkyne-Functionalized Tetradentate Bispidine B16



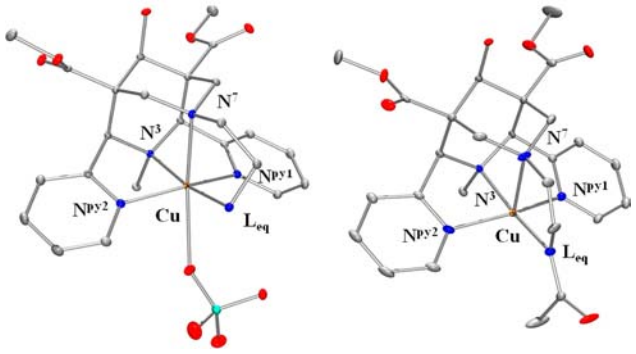


Figure 3. Experimental structures of the molecular cations of the Cu^{II} complexes with B12 (left) and B13-H (right). Ellipsoids are shown at the 30% probability level. Hydrogen atoms and noncoordinating counterions are omitted for clarity. The N^7-pendent arm of $[Cu^{II}(B13\text{-}H)](ClO_4)$ is disordered, and only one position is shown in the plot.

overall charge of +1. It is well-recognized that the nature and charge of metal complexes significantly influence the biological profile of metal complexes and bioconjugates,[93−96] and less positively charged complexes might lead to a reduced accumulation in nontargeted organs. Note that tumor cells are hypoxic and therefore more acidic; that is, in tumor cells the amide might be protonated and uncoordinated and the complex might be +2 charged.

The complex stabilities of the Cu^{II} complexes with the (*p*-MeO)-substituted pentadentate bispidine B4 and the functionalized bispidines B12 and B13-H are estimated from their half-wave potentials (in order to compare the potentials with others reported before, these were also measured at 25 °C in MeCN). It is known that $Cu^{II/I}$ redox couples are correlated to the thermodynamic stability of the corresponding Cu^{II} complexes.[36,97] On the basis of such a correlation, the thermodynamic stability of a series of new tetradentate bispidine ligands, including B1 and B2, was predicted,[43] and the (*p*-MeO)-substituted derivative B2 turned out to be the most promising of the synthetically available bispidines for PET applications, with a predicted log *K* value of 16.0 (vs a predicted log *K* of 15.0 for the unsubstituted parent ligand B1). The substitution of the pentadentate bispidines with electron donating methoxy groups is predicted to result in even more stable Cu^{II} complexes: the half-wave potential of the corresponding pentadentate ligand B4 ($E_{1/2}$ = −760.5 mV vs Fc/Fc^+; see Table 2) is significantly more negative than that of the unsubstituted derivative B3 ($E_{1/2}$ = −690.0 mV vs Fc/Fc^+),[36] and a complex stability of log *K* = 19.4 for $[Cu^{II}(B4)]^{2+}$ is expected (predicted log *K* of 18.2 for B3).[98]

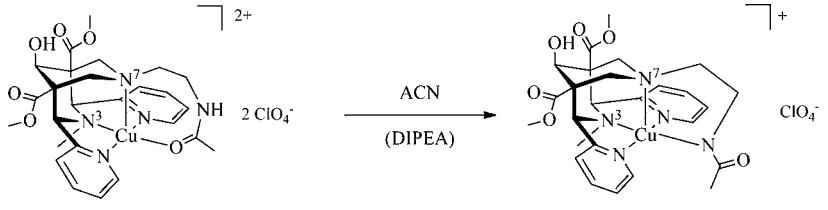
Table 2. dd Transitions and Half-Wave Potentials ($E_{1/2}$) of the Cu^{II} Complexes with the Pentadentate Bispidine Ligands B4−B6 and with the Functionalized Bispidine Ligands B12 and B13 (the Latter in Its Deprotonated Form)

bispidine Cu^{II} complex	dd transitions [nm]	$E_{1/2}$ [mV][b]
$[Cu^{II}(B4)(OClO_3)](ClO_4)$	629 (λ_{max}), 941	−760.5
$[Cu^{II}(B5)](ClO_4)_2$[a]	639 (λ_{max}), 988	−544.5
$[Cu^{II}(B6)](ClO_4)_2$[a]	639 (λ_{max}), 997	−498.0
$[Cu^{II}(B12)(OClO_3)](ClO_4)$	627 (λ_{max}), 983	−807.0
$[Cu^{II}(B13\text{-}H)](ClO_4)$	564, 698 (λ_{max})	−1287.0

[a]Complexes not isolated prior to measurements. [b]Measurements at 25 °C in MeCN, 0.1 M $(Bu_4N)(BF_4)$, applying a slow stream of Ar above the solution. The values are referenced against Fc/Fc^+ (+86 mV).

Similarly, $[Cu^{II}(B12)]^{2+}$ has a reversible redox potential with $E_{1/2}$ = −807.0 mV (vs Fc/Fc^+) and therefore has an estimated log *K* of 20.2 That is, as expected, the pentadentate ligand leads to a more stable complex than that of the corresponding tetradentate bispidine B1 (predicted log *K* = 15.0), and the predicted value is even larger than that with the corresponding pyridine analogue B3 (predicted log *K* = 19.4).[36] The reversible redox potential of the complex with the coordinated deprotonated amide $[Cu^{II}(B13\text{-}H)]^+$ is significantly more negative ($E_{1/2}$ = −1.287 V vs Fc/Fc^+; see Table 2), and this leads to a very high predicted complex stability (log *K* = 28.5). Note that care should be taken with these predictions; there is some uncertainty in these correlations, specifically when different types of ligands are correlated.[36] However, a high thermodynamic stability of $[Cu^{II}(B13\text{-}H)](ClO_4)$ also emerges from comparison of its structural parameters with those of

Scheme 5. Base-Mediated Formation of $[Cu^{II}(B13\text{-}H)](ClO_4)$ from $[Cu^{II}(B13)](ClO_4)_2$



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$[Cu^{II}(B12)(OClO_3](ClO_4)$ (see Table 1): the bonds of the Cu^{II} center to the pyridinyl groups are almost identical (2.049 and 2.050 Å for the amine species in comparison to 2.045 and 2.053 Å for the amide complex), but the distances to the N^7-based pendent nitrogen donor is significantly shortened in the amide complex (1.912 Å) in comparison with the amine species (1.994 Å). That is, the Cu^{II} center in the amide complex is more efficiently encapsulated by an obviously more rigid ligand scaffold, and the increased electron density on the deprotonated amide significantly stabilizes the divalent copper center.

Radiolabeling, in Vitro Stability, Lipophilicity, and Biodistribution. The bispidine ligands B1–B4, B7, and B9 were labeled with ^{64}Cu in MES/NaOH buffer at ambient temperature. The complexation kinetics were monitored by radio-TLC, using neutral alumina plates that were developed in 2 M NH_4OAc/MeOH (1/1). $^{64}CuCl_2$ remains at the origin, and in general, $^{64}Cu^{II}$-bispidine complexes move with R_f values of 0.6–0.7. The complexation reaction was completed within 1 min under the experimental conditions used (ambient temperature, 150 μg bispidine/mL aqueous buffer). In order to estimate the stability of the $^{64}Cu^{II}$-labeled bispidine complexes, the ligand exchange reaction in presence of cyclam as competing ligand was studied (cyclam-challenge experiment; addition of 80 equiv of cyclam).[99] The radio-HPLC protocol applied gives well-separated t_R values (^{64}Cu–cyclam, 3.8 min, ^{64}Cu–bispidine, 8.5–11.2 min; see Figure 4 for ^{64}Cu–cyclam/^{64}Cu–B2 and Table 3 for a summary of the data). It emerges that, as expected, the stability increases from tetra- to pentadentate, with *p*-MeO substitution and with reduction of the C^9 keto group (prevention of retro-Mannich-type ligand and complex decay). The pentadentate ligands B4 and B9 form the most stable $^{64}Cu^{II}$ complexes, and these show no transchelation in the presence of cyclam.[100]

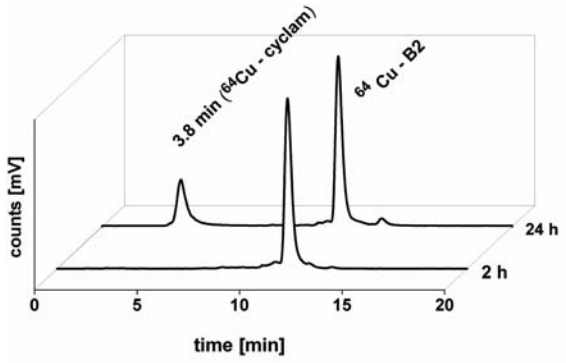

Figure 4. Radio-HPLC traces of ^{64}Cu–B2 in the presence of cyclam (80 equiv) after 2 and 24 h of incubation.

Table 3. Radiochallenge Experiments of the Bispidine Ligands B1–B4, B7, and B9 with Cyclam: Amount of ^{64}Cu–Cyclam (%) Formed As a Function of Time at 25 °C, with 80 equiv of Cyclam Added

	bispidine ligand	^{64}Cu–cyclam, 2 h [%]	^{64}Cu–cyclam, 24 h [%]
B1	tetradentate	19	70
B2	tetradentate, *p*-MeO	<1	24
B7	tetradentate, *p*-MeO, {C^9-OH}	<1	5
B3	pentadentate	1	2
B4	pentadentate, *p*-MeO	<1	<1
B9	pentadentate, *p*-MeO, {C^9-OH}	<1	<1

The hexadentate bis-amine-tetrakis-pyridine bispidine ligand B10 is known to form relatively hydrophilic ^{64}Cu complexes, and this emerges from the 1-octanol/water partition coefficient log $D_{o/w} < -2.7$.[44] For comparison and to predict the biodistribution of the radiotracers studied here, partition experiments were also performed in a two-phase 1-octanol/water system (see Experimental Section). The radiotracer technique used here allows a precise determination of lipophilicity data.[101] The log $D_{o/w}$ values of the $^{64}Cu^{II}$-labeled tetradentate and pentadentate bispidine ligands are in the same range as that of the Cu^{II} complex with B10 (Table 4).[44] As expected, *p*-methoxy substitution of the pyridinyl groups increases the lipophilicity of the corresponding complexes. A slight increase of hydrophilicity is observed when the bispidones B2 and B4 are reduced to the alcohols B7 and B9. Altogether, the ^{64}Cu-labeled bispidine ligands B1–B4, B7, and B9 are relatively hydrophilic and are predicted to be rapidly excreted via the renal pathway. Note that, based on an efficient charge model[102] we have developed a QSAR method for the prediction of lipophilicities/hydrophilicities,[103] and this will in future allow efficient prediction of this type of data.

Table 4. Partition Coefficients (log $D_{o/w}$) of the ^{64}Cu–Bispidine Complexes at Different pH[a]

	bispidine ligand	pH 7.2	pH 7.4	pH 7.6
B1	tetradentate	−2.43	−2.45	−2.33
B2	tetradentate, *p*-MeO	−2.08	−2.05	−2.04
B7	tetradentate, *p*-MeO, {C^9-OH}	−2.13	−2.11	−2.10
B3	pentadentate	−2.64	−2.68	−2.67
B4	pentadentate, *p*-MeO	−2.36	−2.31	−2.28
B9	pentadentate, *p*-MeO, {C^9-OH}	−2.49	−2.44	−2.42

[a]The solubility of the ^{64}Cu complexes of the halide derivatives B5 and B6 was too low to perform adequate experiments.

The biodistribution data in rats demonstrate the ^{64}Cu–B9 complex (pentadentate ligand) to be excreted more quickly from the organism than the ^{64}Cu–B7 complex (tetradentate ligand; see Figure 5). ^{64}Cu–B9 is nearly exclusively eliminated via the renal route, while for ^{64}Cu–B7 the liver contributes a substantial pathway for elimination via the hepatobiliary route (see inlets of Figure 5 for the radioactivity of urine and intestine as indicators for renal and hepatobiliary elimination routes, respectively). At 24 h pi (after injection) of ^{64}Cu–B7 most organs still show a substantial activity uptake but predominantly in kidneys, liver, and thyroid gland. At 24 h pi the activity uptake of ^{64}Cu–B9 was lower than that of ^{64}Cu–B7 in all organs. The pentadentate $^{64}Cu^{II}$-labeled bispidine ligand B9 shows a very rapid blood and normal-tissue clearance (%ID/g < 0.03 for most organs and tissues: liver, 0.14 ± 0.04; kidneys, 0.66 ± 0.07, 24 h pi; see Supporting Information), and this supports the very high complex stability. It is worth mentioning that in particular the liver accumulation is remarkably reduced for ^{64}Cu–B9 in comparison to the hexadentate bis-amine-tetrakis-pyridine bispidine B10.[44] Accordingly, the bis(*p*-MeO)-substituted pentadentate bispidine ligand B9 is ideally suited for the development of novel bifunctional chelating agents for PET imaging.

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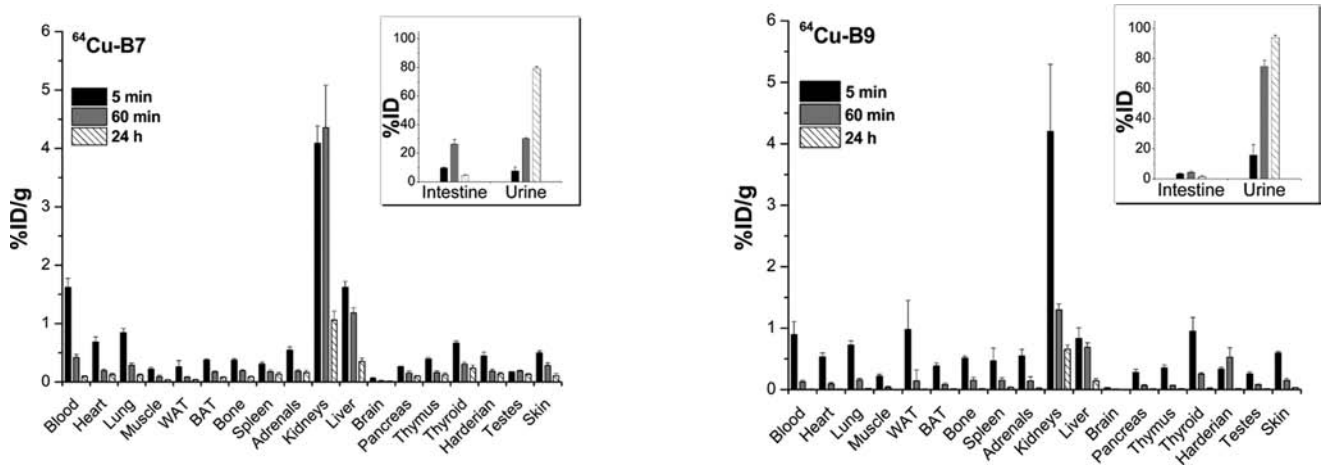

Figure 5. Biodistribution pattern of the ^{64}Cu complexes with the tetradentate {C^9-OH}(*p*-MeO) ligand B7 (left) and the pentadentate {C^9-OH}(*p*-MeO) ligand B9 (right) at 5 min, 60 min, and 24 h pi (four Wistar Kyoto rats per time point and compound, mean ± standard deviation).

CONCLUSIONS

As expected, and this also emerges to some extent from potentiometric titrations,[36] the stabilities of Cu^{II} complexes with the pentadentate bispidines discussed here are higher than those of the corresponding tetradentate ligands. Substitution of the pyridine donor groups allows for further tuning of the stability, and this, based on the electronic properties of the substituents and the basicity of the corresponding pyridine groups, is predictable.[43] Therefore, the bis(*p*-MeO)-substituted pentadentate bispidine ligand B9, which also has fast ^{64}Cu complex formation kinetics, is predicted to lead to an optimal chelator for PET applications. Importantly, the biodistribution studies of ^{64}Cu−B9 reveal a very rapid blood and normal tissue clearance and therefore indicate that it is an ideally suited platform for the development of ^{64}Cu-based PET tracers. In terms of the biofunctionalization of bispidine ligands in general, the derivative with an amide pendent group (ligand B13-H) to model the bioconjugation of the amine substituted bispidine B12 to a vector molecule is of special interest, specifically because of the predicted high complex stability of the corresponding Cu^{II} complex. Importantly, these systems have an overall charge of +1 and the resulting tracers may therefore have a reduced accumulation in nontargeted organs.

EXPERIMENTAL SECTION

Materials and Methods. All reactions in dry solvents were carried out under an inert atmosphere of argon or nitrogen applying standard Schlenk techniques. All glassware was heated and dried under vacuum prior use. Chemicals were purchased from Sigma-Aldrich Chemie GmbH (Taufkirchen, Germany), ABCR GmbH & Co. KG (Karlsruhe, Germany), and Merck KGaA (Darmstadt, Germany) and were of the highest available purity. Dry solvents were used as delivered without further purification. The syntheses of 4-methoxy/4-chloropicolinaldehyde **1**/**2**, the piperidones P1−P3, and the bispidines B1, B2, and B7,[43] B3, B8,[55] and B10[44] are reported elsewhere.

NMR spectra were recorded on a Bruker DRX 200, Bruker Avance II 400, or Bruker Avance III 600 spectrometer. The last spectrometer was equipped with a direct detection cryoprobe for maximum sensitivity in the detection of ^{13}C. ^{1}H and ^{13}C NMR chemical shifts are referenced to the signals of the solvent ($CDCl_3$, DMSO-d_6).

UV−vis−NIR spectra were recorded on a Jasco V-570 UV−vis−near-IR spectrophotometer. Solution spectra were measured from in situ prepared complexes at room temperature in acetonitrile as solvent.

EPR measurements were performed on a Bruker ELEXSYS-E-500 instrument at 110 K using methanol as solvent. The spin Hamiltonian parameters were obtained by simulation of the spectra with XSophe.[104,105]

Mass spectra were recorded on a Finnigan MAT8230 and a Joel JMS-700 spectrometer.

Elemental analyses were performed on a CHN-O-vario EL by the "Mikroanalytische Labor", Department of Organic Chemistry, University of Heidelberg, Germany, and by the laboratory of Prof. Dr. Andres Jäschke, Institute of Pharmacy and Molecular Biotechnology, University of Heidelberg.

Electrochemical measurements were performed on a CH Instruments CHI660D electrochemical workstation equipped with a CH Instruments picoamp booster and Faraday cage, with a three-electrode setup consisting of a glassy-carbon working electrode, a Pt wire as the auxiliary electrode, and an $Ag/AgNO_3$ reference electrode (0.01 M Ag^+, 0.1 M $(Bu_4N)(BF_4)$ in MeCN). The solutions were thoroughly degassed, and a slight argon stream was set above the solution during the measurement. A scan rate of 100 mV s^{-1} was used.

X-ray Crystallography. Data were collected on a Bruker AXS Smart 1000 CCD diffractometer (Mo Kα radiation, sealed tube, graphite monochromator) or an Agilent Technologies Supernova-E CCD diffractometer (Mo or Cu Kα radiation, microfocus tube, multilayer mirror optics); see Suporting Information for details on the crystal structure determination.

Production of ^{64}Cu. The production of ^{64}Cu was performed at a PET cyclotron, similar to those described for the ^{64}Cu−^{61}Co production process.[106−108] For the ^{64}Ni(p,n)^{64}Cu nuclear reaction, 15 MeV protons of a Cyclone 18/9 with a beam current of 12 μA for 150 min were used. The complete separation of ^{64}Cu and ^{61}Co was confirmed by γ-ray spectroscopy. Nickel targets were prepared by electrodeposition of enriched ^{64}Ni (99.6%) on gold disks at amounts of 95−120 mg. The plated diameter was 7 mm, matching more than 80% intensity of the cyclotron proton beam, as measured by autoradiography of a ^{nat}Ni disk irradiated with 15 MeV protons to induce the ^{nat}Ni(p,x)^{57}Ni reaction. The yields of the nuclear reaction ^{64}Ni(p,n)^{64}Cu were between 3.6 and 5.2 GBq (EOB) with specific activities of 150−250 GBq/μmol Cu. For the determination of the ^{64}Cu specific activities, titration with TETA (l,4,8,11-tetraazacyclotetradecane-*N*,*N*′,*N*″,*N*‴-tetraacetic acid) in combination with radio-TLC was applied.[109]

Radio-TLC chromatograms were scanned using a radioisotope thin layer analyzer (Rita Star, raytest). Analytical HPLC of bispidine derivatives and ^{64}Cu-labeled bispidines was performed on a Knauer Smartline system, consisting of a pump 1000, a DAD detector 2600, an activity detector Raytest Ramona Star, software Chromgate 2.8, and a manager 5000 with a Gemini C18 column (Phenomenex, 250 mm × 4.6 mm, 110 Å, 5 μm) using a gradient eluent of water + 0.1% TFA (A) and acetonitrile + 0.1% TFA (B); gradient of 10−70% of eluent A in 240 min, 1 mL/min.

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Before 1-octanol/water distribution, challenge, and biodistribution experiments were conducted, bispidine ligands were purified by preparative RP-HPLC on a Knauer Smartline system consisting of a pump 1000, UV detector Smartline 2500 (220 nm), software Chromgate 2.8, and manager 5000 with a Gemini C18 column (Phenomenex, 250 mm × 10 mm, 110 Å, 5 μm) using a gradient eluent of water + 0.1% TFA (A) and acetonitrile + 0.1 TFA (B); gradient of 10% A for 1 min, 10−70% of eluent A in 19 min, 4 mL/min.

Radiolabeling Experiments. To 15 μL of a ligand stock solution (1 mg bispidine/mL acetonitrile/0.05 M 2-(*N*-morpholino)ethansulfonic acid (MES)−NaOH buffer = 1/1) were added 15 μL of an aqueous solution of [^{64}Cu]$CuCl_2$ (10 MBq) and 70 μL of MES/NaOH buffer (pH 5.5). Formation of the radiocopper complexes was verified by radio-TLC on neutral alumina plates (Merck, F254) using a mobile phase methanol/2 M ammonium acetate (1/1): $^{64}CuCl_2$, R_f = 0; ^{64}Cu−bispidine complexes, R_f = 0.6 - 0.7.

Ligand Exchange "Challenge" Studies. After formation of the ^{64}Cu−bispidine complexes, an amount of 80 equiv of cyclam was added. The solution was incubated for 24 h. After 2 and 24 h, radio-HPLC analysis was done to determine the stability of the radio copper−bispidine complexes (^{64}Cu−cyclam, t_R = 3.8 min; ^{64}Cu−B1, t_R = 9.5 min; ^{64}Cu−B2, t_R = 11.2 min; ^{64}Cu−B3, t_R = 8.5 min; ^{64}Cu−B4, t_R = 10.7 min; ^{64}Cu−B7, t_R = 11.1 min; ^{64}Cu−B9, t_R = 9.5 min).

Determination of Distribution Ratio log $D_{o/w}$ at 25 ± 1 °C. Information about the lipophilicity of the ^{64}Cu-labeled bispidine ligands B1−B4, B7, and B9 was obtained using water/1-octanol mixtures. The experiments were performed with 100 μM solutions of bispidine ligands dissolved in 1-octanol. Aqueous phases consist of 440 μL of 0.05 M 4-(2-hydroxyethyl)-1-piperazine ethanesulfonic acid (HEPES)−NaOH buffer (pH of 7.2, 7.4, 7.6), 10 μL of [^{64}Cu]$CuCl_2$ solution (500 kBq), and 50 μL of a 100 μM $Cu(NO_3)_2$ solution. The distribution experiments were carried out at 25 ± 1 °C in microcentrifuge tubes (2 cm^3) with mechanical shaking for 30 min. The phase ratio $V_{(1\text{-octanol})}$:$V_{(aq)}$ was 1:1 (0.5 mL each). Full complexation was checked by radio-HPLC, which gave no evidence of free copper(II) in the aqueous phase. All samples were centrifuged and the phases then separated. The copper complex concentration in both phases was determined radiometrically using γ-radiation [^{64}Cu, NaI(Tl) scintillation counter automatic γ counter 1480, Wizard 3″, PerkinElmer]. The results are the average values of three independent experiments.

Biodistribution Experiments. All animal experiments were carried out in male Wistar rats (Wistar Kyoto strain; aged 7−8 weeks, 140−195 g; Harlan Winkelmann GmbH, Borchen, Germany) according to the guidelines of the German Regulations for Animal Welfare. The protocol was approved by the local Ethical Committee for Animal Experiments. Animals were kept under a 12 h light−dark cycle and fed with commercial animal diet and water ad libitum. For biodistribution studies, injection was into the tail vein of the animal under anesthesia (inhalation of 9% (v/v) desflurane (Suprane, Baxter, Germany) in 40% oxygen/air (gas flow 0.5 L/min). The injection volume of either ^{64}Cu−B7 or ^{64}Cu−B9 (0.4−2.0 MBq; radiochemical purity of >99%; specific activity, 50 MBq/μg (^{64}Cu−B7); 27 MBq/μg (^{64}Cu−B9); dissolved in electrolyte solution E-153 (Serumwerk Bernburg, Germany), pH 7.2) was 0.5 mL. Biodistribution was determined in groups of four rats (per time point) sacrificed by heart puncture under ether anesthesia at 5 min, 60 min, and 24 h after injection (pi). Organs and tissues of interest were rapidly excised, weighed, and the radioactivity was determined in a Wallac WIZARD automatic γ counter (PerkinElmer, Germany) and a dose calibrator (dose calibrator ISOMED 2000, MED Nuklear-Medizintechnik Dresden GmbH, Germany), respectively. The activity of ^{64}Cu in the tissue samples was decay-corrected and calibrated by comparing the counts in tissue with the counts in aliquots of injected radiotracer that had been measured in the γ counter and the dose calibrator and at the same time. The accumulated radioactivity in organs and tissues was calculated as either the percentage of the injected dose per gram tissue (%ID/g tissue) or, for both intestine and urine, the percentage of the injected dose (%ID).

Syntheses. ***Caution!*** *Although we did not encounter any difficulties with the perchlorate salts employed in the syntheses described, these are potentially explosive and need to be handled with care. Heating, especially when dry, must be avoided.*

*4-Methoxypicolinaldoxime **3** ($C_7H_8N_2O_2$, M_W = 152.15 g/mol).* 4-Methoxypicolinaldehyde **1** (4.36 g, 31.79 mmol, 1.0 equiv) was dissolved in 25 mL of a 1:4 mixture of ethanol/water, and hydroxylamine hydrochloride (3.31 g, 47.69 mmol, 1.5 equiv) was added. The mixture was cooled in a water bath. Then sodium hydroxide (6.36 g, 158.95 mmol, 5.0 equiv) was slowly added. The formation of a slurry was observed. Afterward the mixture was heated to 80 °C for 30 min. After the mixture was cooled to ambient temperature, the pH was adjusted to 7 by adding concentrated hydrochloric acid. A white solid formed which was filtered and thoroughly washed with water (300 mL) and dried in air. The pure product was obtained in a yield of 72.2% (3.49 g, 22.9 mmol). ^{1}H NMR (200 MHz, CD_3OD): δ = 3.89 (s, 3H, OCH_3), 6.94 (dd, $^3J_{H,H}$ = 5.87 Hz, $^4J_{H,H}$ = 2.59 Hz, 1H, **H**^{5}), 7.37 (d, $^4J_{H,H}$ = 2.53 Hz, 1H, **H**^{3}), 8.04 (s, 1H, C**H**N), 8.30 (d, $^3J_{H,H}$ = 5.94 Hz, 1H, **H**^{6}). ^{13}C NMR (50 MHz, CD_3OD): δ = 56.23, 107.21, 112.11, 149.46, 151.35, 155.28, 168.31. HR-EI (pos): $[M]^+$ calcd 152.0586, obsd 152.0577. Elemental analysis: [M] calcd C, 55.26; H, 5.30; N, 18.41; obsd C, 55.26; H, 5.35; N, 18.35.

*4-Chloropicolinaldoxime **4** ($C_6H_5ClN_2O$, M_W = 156.57 g/mol).* 4-Chloropicolinaldehyde **2** (5.28 g, 37.3 mmol, 1.0 equiv) was dissolved in 50 mL of methanol. To this solution were added sodium hydrogen carbonate (3.13 g, 37.3 mmol, 1.0 equiv) and hydroxylamine hydrochloride (5.18 g, 74.60 mmol, 2.0 equiv). A gas formation was observed. This suspension was stirred at ambient temperature for 24 h. To this suspension was added 100 mL of ethyl acetate, and the solid was filtered. The solid was suspended in 120 mL of ethyl acetate, and this phase was washed with 80 mL of saturated sodium hydrogen carbonate solution, 150 mL of aqua dest, and finally 100 mL of brine. The organic phase was dried over Na_2SO_4, filtered, and concentrated in vacuo. The crude product was recrystallized from acetone to afford a yellow crystalline solid in a yield of 55.3% (3.23 g, 20.63 mmol). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 7.50 (dd, $^3J_{H,H}$ = 5.14 Hz, $^4J_{H,H}$ = 1.88 Hz, 1H, **H**^{5}), 7.77 (br s, 1H, **H**^{3}/C**H**NOH), 8.08 (s, 1H, **H**^{3}/C**H**NOH), 8.54 (d, $^3J_{H,H}$ = 5.40 Hz, 1H, **H**^{6}), 11.92 (s, 1H, CHNO**H**). ^{13}C NMR (100 MHz, DMSO-d_6): δ = 119.55, 123.90, 143.36, 147.90, 150.94 (5C, C_{ar}), 153.84 (**C**HNOH). HR-EI: $[M]^+$ calcd 156.0090, obsd 156.0091; $[M - Cl]^+$ calcd 122.0480, obsd 122.0456.

*4-Methoxypicolylamine **7** ($C_7H_{10}N_2O$, M_W = 138.17 g/mol). Method A (Reduction of the Oxime Precursor).* Oxime 3 (3.37 g, 22.15 mmol, 1.0 equiv) was dissolved in trifluoroacetic acid (30 mL) and cooled to 0 °C. At this temperature zinc dust was added portionswise to the reaction solution; a strong delayed reaction was observed. The mixture was stirred for 2 h at ambient temperature and afterward added to 400 mL of an ice-cold 1/1 mixture of DCM/2 M NaOH and stirred for another 3 h at 0 °C to ambient temperature. Gas formation was observed. The solution was filtered. The phases were separated. The aqueous phase was extracted with DCM, and the combined organic phases were washed with aqua dest (2 × 200 mL) and brine (1 × 200 mL), dried over Na_2SO_4, and concentrated in vacuo. A yellow oil was obtained as the pure product in a yield of 26.5% (0.81 g, 5.86 mmol).

Method B (Azide Mitsunobu Reaction Starting from the Alcohol Precursor). (4-Methoxypyridin-2-yl)methanol **5** (1.50 g, 10.78 mmol, 1.0 equiv) and triphenylphosphine (8.48 g, 32.34 mmol, 3.0 equiv) were suspended in 40 mL of dry THF at 0 °C under an atmosphere of argon, and DIAD (3.27 g, 3.21 mL, 16.17 mmol, 1.5 equiv) and diphenylphosphorylazide (3.56 g, 2.79 mL, 12.94 mmol, 1.2 equiv) were added dropwise consecutively. The reaction mixture was stirred overnight allowing warming to ambient temperature very slowly (Dewar ice bath). The mixture was heated to 55 °C for 1 h. Then 50 mL of aqua dest was added and stirring was continued for another hour at 55 °C. After the mixture was cooled to ambient temperature, the solvent was removed in vacuo and the residue was taken up in 30 mL of aqua dest. The pH was adjusted to 3 using concentrated sulfuric acid. This aqueous phase was washed with DCM (1 × 30 mL), and the

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pH was adjusted to 12 using an aqueous solution of sodium hydroxide (20%). The aqueous phase was extracted with DCM (4 × 30 mL). The combined organic phases were dried over Na_2SO_4, filtered, and concentrated. The resulting red oil was purified by distillation (170 °C, 2.3 mbar), affording a colorless oil as a reasonably pure product in a yield of 32.2% (0.48 g, 3.47 mmol). ^{1}H NMR ($CDCl_3$, 200 MHz): δ = 1.99 (br s, 2H, NH_2), 3.78 (s, 3H, OCH_3), 3.86 (s, 2H, CH_2NH_2), 6.62 (dd, $^3J_{H,H}$ = 5.81 Hz, $^4J_{H,H}$ = 2.53 Hz, 1H, **H**^{5}), 6.76 (d, $^4J_{H,H}$ = 2.27 Hz, 1H, **H**^{3}), 8.29 (d, $^3J_{H,H}$ = 5.68 Hz, 1H, **H**^{6}). ^{13}C NMR ($CDCl_3$, 50 MHz): δ = 47.71, 54.91, 106.74, 108.05, 150.29, 163.62, 166.15. HR-EI (pos): $[M]^+$ calcd 138.0793, obsd 138.0787.

4-Chloropicolylamine ***8*** *($C_6H_7ClN_2$, M_W = 142.59 g/mol). Method A (Reduction of the Oxime Precursor).* Oxime **4** (3.04 g, 19.42 mmol, 1.0 equiv) was dissolved in TFA (44.24 g, 29.71 mL, 0.388 mol, 20.0 equiv) at 0 °C, and zinc dust (7.65 g, 0.12 mol, 6.0 equiv) was slowly added to the mixture. A strongly exothermic reaction was observed. After complete addition the mixture was stirred at 25−30 °C for 1 h. Then the suspension was slowly poured onto an ice-cold mixture of 300 mL of a 2 M aqueous solution of NaOH and 200 mL of DCM and stirred for 1 h. The suspension was freed from insoluble material by filtration, and the organic phase was separated, washed with aqua dest (2 × 150 mL) and brine (1 × 150 mL), dried over Na_2SO_4, and concentrated in vacuo to afford the crude product as red-brown oil in a yield of 43.4% (1.20 g, 8.42 mmol).

Method B (Azide Mitsunobu Reaction Starting from the Alcohol Precursor). (4-Chloropyridin-2-yl)methanol **6** (1.00 g, 6.97 mmol, 1.0 equiv) and triphenylphosphine (5.48 g, 20.91 mmol, 3.0 equiv) were dissolved in 40 mL of dry THF at 0 °C under an atmosphere of argon. Then DIAD (2.12 g, 2.08 mL, 10.46 mmol, 1.5 equiv) and diphenylphosphorylazide (2.30 g, 1.80 mL, 8.36 mmol, 1.2 equiv) were added dropwise to the mixture. Within an hour a white precipitate formed. The suspension was stirred overnight, allowing it to warm up to ambient temperature (Dewar ice bath). Afterward the mixture was stirred at 55 °C for 1 h, whereupon 5 mL of aqua dest was added. The resulting mixture was stirred at 55 °C for another hour. The orange-red solution was concentrated in vacuo. The residue was taken up in a mixture of DCM/aqua dest (30 mL each). The pH of the aqueous phase was adjusted to 3 with concentrated sulfuric acid. The aqueous phase was separated and washed with DCM (1 × 30 mL). Then the pH of the aqueous phase was adjusted to 12 with an aqueous solution of NaOH (20 wt %) and extracted with DCM (3 × 50 mL). The combined organic phases were dried over Na_2SO_4 and concentrated. The product was obtained as a red-brown oil in a yield of 62.4% (0.62 g, 4.35 mmol). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.75 (s, 2H, CH_2NH_2), 3.98 (s, 2H, CH_2NH_2), 7.18 (dd, $^3J_{H,H}$ = 5.14 Hz, $^4J_{H,H}$ = 1.10 Hz, 1H, **H**^{5}), 7.34−7.36 (m, 1H, **H**^{3}), 8.46 (d, $^3J_{H,H}$ = 5.40 Hz, 1H, **H**^{6}). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 47.49, 121.56, 122.18, 144.58, 150.14, 163.75. HR-EI (pos): $[M]^+$ calcd 142.0298, obsd 142.0283; $[M - NH]^+$ calcd 127.0189, obsd 127.0190.

Bispidine B4 ($C_{30}H_{33}N_5O_7$, M_W = 575.61 g/mol). (*p*-MeO)Npy$_2$ P2 (3.00 g, 6.77 mmol, 1.0 equiv) was suspended in 40 mL of methanol and heated to 50 °C. Then 2-aminomethylpyridine **9** (0.73 g, 0.70 mL, 6.77 mmol, 1.0 equiv) and an aqueous solution of formaldehyde (37%, 1.10 g, 1.01 mL, 13.53 mmol, 2.0 equiv) were added to the suspension and heated to reflux for 3 h. After cooling to ambient temperature, the solution was concentrated to half of its volume and stored at −6 °C for several days. The resulting solid was filtered and washed with cold methanol to afford a white solid as the pure product in a yield of 7.4% (0.29 g, 0.50 mmol). t_R (HPLC): 9.7 min. ^{1}H NMR (200 MHz, $CDCl_3$): δ = 2.06 (s, 3H, N^3CH_3), 2.74 (d, $^2J_{H,H}$ = 12.00 Hz, 2H, $N^7CH_{ax}H_{eq}$), 3.27 (d, $^2J_{H,H}$ = 12.76 Hz, 2H, $N^7CH_{ax}H_{eq}$), 3.69 (s, 2H, N^7CH_2py), 3.80 (s, 6H, $COOCH_3$/$pyOCH_3$), 3.90 (s, 6H, $COOCH_3$/$pyOCH_3$), 4.64 (s, 2H, N^7CH), 6.70 (dd, $^3J_{H,H}$ = 5.81 Hz, $^4J_{H,H}$ = 2.65 Hz, 2H, **H**$^{5'}$), 7.17 (ddd, $^3J_{H,H}$ = 7.48 Hz, $^4J_{H,H}$ = 4.83 Hz, $^5J_{H,H}$ = 1.20 Hz, 1H, **H**^{pyN7}), 7.43 (br. d, $^3J_{H,H}$ = 7.71 Hz, 1H, **H**^{pyN7}), 7.58 (d, $^4J_{H,H}$ = 2.53 Hz, 2H, **H**$^{3'}$), 7.65 (dt, $^3J_{H,H}$ = 7.58 Hz, $^4J_{H,H}$ = 1.77 Hz, 1H, **H**^{pyN7}), 8.30 (d, $^3J_{H,H}$ = 5.68 Hz, 2H, **H**$^{6'}$), 8.55 (ddd, $^3J_{H,H}$ = 4.89 Hz, $^4J_{H,H}$ = 1.74 Hz, $^5J_{H,H}$ = 0.82 Hz, 1H, **H**^{pyN7}). ^{13}C NMR (50 MHz, $CDCl_3$): δ = 43.34, 52.42, 55.13, 58.54, 62.42, 63.58, 73.69, 108.47, 110.37, 122.01, 123.97, 136.24, 149.47, 150.43, 156.51, 160.12, 166.19, 168.62, 203.30. HR-ESI(pos): $[M + H]^+$ calcd 576.245 82, obsd 576.245 46. Elemental analysis: $[M + H_2O]$ calcd C, 60.70; H, 5.94; N, 11.80; obsd C, 60.95; H, 6.01; N, 11.72.

Bispidine B5 ($C_{28}H_{27}Cl_2N_5O_5$, M_W = 584.45 g/mol). (*p*-Cl)Npy$_2$ P3 (2.00 g, 4.42 mmol, 1.0 equiv) was suspended in 30 mL of methanol and heated to 50 °C. Then 2-aminomethylpyridine 9 (0.48 g, 0.46 mL, 4.42 mmol, 1.0 equiv) and an aqueous solution of formaldehyde (37%, 0.72 g, 0.66 mL, 8.84 mmol, 2.0 equiv) were added to the suspension and heated to reflux for 3 h. After the mixture was cooled to ambient temperature the fine black precipitate formed was removed by filtration and the solution was concentrated to approximately 15 mL and stored at −6 °C overnight. The solid was filtered and washed with cold methanol. A white solid was obtained as the pure product in a yield of 19.5% (0.50 g, 0.86 mmol). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.05 (s, 3H, N^3CH_3), 2.78 (d, $^2J_{H,H}$ = 12.17 Hz, 2H, $CH_{ax}H_{eq}$), 3.16 (d, $^2J_{H,H}$ = 11.92 Hz, 2H, $CH_{ax}H_{eq}$), 3.66 (s, 2H, N^7CH_2py), 3.80 (s, 6H, $COOCH_3$), 4.73 (s, 2H, CH), 7.21 (dd, $^3J_{H,H}$ = 5.33 Hz, $^4J_{H,H}$ = 1.95 Hz, 2H, **H**$^{5'}$), 7.23 (dd, $^3J_{H,H}$ = 7.10 Hz, $^4J_{H,H}$ = 4.80 Hz, 1H, **H**^{PyN7}), 7.47 (d, $^3J_{H,H}$ = 7.40 Hz, 1H, **H**^{PyN7}), 7.76 (t, $^3J_{H,H}$ = 7.22 Hz, 1H, **H**^{PyN7}), 8.02 (br s, 2H, **H**$^{3'}$), 8.37 (d, $^3J_{H,H}$ = 5.27 Hz, 2H, **H**$^{6'}$), 8.60 (d, $^4J_{H,H}$ = 4.77 Hz, 1H, **H**^{PyN7}). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 43.54, 52.57, 58.60, 62.16, 63.47, 73.33, 77.20, 122.65, 123.53, 123.82, 136.75, 144.81, 149.46, 150.05, 156.27, 160.21, 168.12, 202.54. HR-ESI (pos): $[M + H]^+$ calcd 584.146 75, obsd 584.146 93; $[M + Na]^+$ calcd 606.128 69, obsd 606.128 92. Elemental analysis: $[M + {}^1/_2H_2O]$ calcd C, 56.67; H, 4.76; N, 11.80; obsd C, 56.42; H, 4.80; N, 11.59.

Bispidine B6 ($C_{28}H_{26}Cl_3N_5O_5$, M_W = 618.90 g/mol). (*p*-Cl)Npy$_2$ P3 (1.27 g, 2.81 mmol, 1.0 equiv) was suspended in 30 mL of methanol and heated to 50 °C. Then an aqueous solution of formaldehyde (37 wt %, 0.46 g, 0.42 mL, 5.62 mmol, 2.0 equiv) and (4-chloropyridin-2-yl)methanamine **8** (0.40 g, 2.81 mmol, 1.0 equiv) were added to the suspension and stirred to reflux for 3 h. After the mixture was cooled to ambient temperature the solvent was removed in vacuo and the resulting residue was taken up in a little amount of methanol and stored at −6 °C for several days. A white precipitate formed which was filtered and recrystallized from hot methanol. The colorless crystals were filtered and washed with a little amount of cold methanol to afford the pure product in a yield of 4.6% (80.0 mg, 0.13 mmol). ^{1}H NMR (600 MHz, $CDCl_3$): δ = 2.04 (s, 3H, N^3CH_3), 2.85 (d, $^2J_{H,H}$ = 12.20 Hz, 2H, $CH_{ax}H_{eq}$), 3.25 (d, $^2J_{H,H}$ = 11.83 Hz, 2H, $CH_{ax}H_{eq}$), 3.63 (s, 2H, N^7CH_2py), 3.80 (s, 6H, $COOCH_3$), 4.69 (s, 2H, N^3CH), 7.21−7.23 (m, 2H, **H**^{ar}), 7.25−7.28 (m, 2H, **H**^{ar}), 7.62 (s, 1H, **H**^{ar}), 7.96 (s, 2H, **H**^{ar}), 8.40 (d, $^3J_{H,H}$ = 5.14 Hz, 2H, **H**^{ar}), 8.52 (d, $^3J_{H,H}$ = 5.23 Hz, 1H, **H**^{ar}). ^{13}C NMR (150 MHz, $CDCl_3$): δ = 43.47, 52.60, 58.62, 62.26, 62.98, 73.39, 123.09, 123.54, 123.56, 123.95, 124.07, 144.79, 150.15, 150.37, 158.53, 160.10, 168.12, 202.37. HR-ESI (pos): $[M + H]^+$ calcd 618.107 78, obsd 618.107 97; $[M + Na]^+$ calcd 640.089 72, obsd 640.088 76. Elemental analysis: $[M]$ calcd C, 54.34; H, 4.23; N, 11.32; obsd C, 54.17; H, 4.35; N, 11.13.

Bispidone B9 ($C_{30}H_{35}N_5O_7$, M_W = 577.63 g/mol). The bispidine B4 (0.61 g, 1.06 mmol, 1.0 equiv) was suspended in 45 mL of a 2:1 mixture 1,4-dioxane/water and cooled to −6 °C. At this temperature sodium borohydride (20.0 mg, 0.53 mmol, 0.5 equiv) dissolved in further 15 mL of 2:1 mixture 1,4-dioxane/water was slowly added dropwise to the suspension. The reaction mixture was stirred for 1 h at −6 °C and at 0 °C overnight. Then the pH was adjusted to 1−2 by slowly adding concentrated sulfuric acid and stirred for 1 h at ambient temperature. Afterward the pH of the mixture was adjusted to 12 by slowly adding an aqueous solution of sodium hydroxide (20 wt %) and stirred for another 2 h at ambient temperature. The solvent was removed in vacuo, and the resulting white residue was taken up in 50 mL of water. The aqueous phase was extracted with DCM (4 × 50 mL), and the combined organic phases were dried over Na_2SO_4, filtered, and concentrated. A white to pale yellow solid was obtained as crude product (0.45 g, 0.78 mmol, 73.6%). This was purified by preparative RP-HPLC to afford pure product after lyophilization in approximately 85% yield. t_R (HPLC): 8.7 min. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.99 (s, 3H, N^3CH_3), 2.59 (d, $^2J_{H,H}$ = 12.72 Hz, 2H, N^7CH_2), 2.69 (d, $^2J_{H,H}$ = 12.7 Hz, 2H, N^7CH_2), 3.75 (s, 6H,

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COOCH_3), 4.07 (s, 1H, N^{3}CH), 4.10 (s, 6H, pyOCH_3), 4.42 (s, 2H, N^{7}CH_2py), 4.84 (s, 1H, C^{9}H), 4.99 (s, 1H, N^{3}CH), 7.00 (br, 2H, H^{ar}), 7.50 (s, 1H, H^{ar}), 7.67 (m, 1H, H^{ar}), 7.80 (m, 2H, H^{ar}), 8.29 (m, 2H, H^{ar}), 8.86 (m, 1H, H^{ar}), 9.00 (m, 1H, H^{ar}). HR-ESI (pos): $[M + H]^+$ calcd 578.261 47, obsd 578.261 57.

Hemiaminal ***10*** *($C_{30}H_{33}N_5O_8$, M_W = 591.61 g/mol).* (*p*-MeO)Npy$_2$ P2 (1.27 g, 2.86 mmol, 1.0 equiv) was suspended in 20 mL of THF and heated to 50 °C. At this temperature were added (4-methoxypyridin-2-yl)methanamine **7** (0.47 g, 3.43 mmol, 1.2 equiv) and an aqueous solution of formaldehyde (37 wt %, 0.56 g, 0.51 mL, 6.86 mmol, 2.4 equiv) to the mixture, and the mixture was heated to reflux for 1 h. After the mixture was cooled to ambient temperature, the solvent was removed in vacuo. The residue was taken up in methanol. The solvent was allowed to slowly evaporate at ambient temperature. After several days a white precipitate formed. The monoesterificated hemiaminal was obtained as the pure product in a yield of 13.3% (0.23 g, 0.38 mmol). ^{1}H NMR (200 MHz, $CDCl_3$): δ = 2.30 (s, 3H, N^{3}CH_3), 2.77 (d, $^2J_{H,H}$ = 12.38 Hz, 1H, N^{7}CH), 2.91 (d, $^2J_{H,H}$ = 12.25 Hz, 1H, N^{7}CH), 3.19 (d, $^2J_{H,H}$ = 12.00 Hz, 1H, N^{7}CH), 3.19 (s, 1H, N^{3}CH), 3.60 (d, $^2J_{H,H}$ = 12.38 Hz, 1H, N^{7}CH), 3.61 (s, 3H, COOCH_3/pyOCH_3), 3.65 (s, 3H, COOCH_3/pyOCH_3), 3.87 (d, $^2J_{H,H}$ = 15.00 Hz, 2H, N^{7}CH_2py), 3.93 (s, 3H, COOCH_3/pyOCH_3), 4.10 (s, 3H, COOCH_3/pyOCH_3), 5.07 (s, 1H, N^{3}CH), 6.71 (dd, $^3J_{H,H}$ = 5.37 Hz, $^5J_{H,H}$ = 1.96 Hz, 1H, H$^{5'}$), 6.81 (d, $^3J_{H,H}$ = 5.00 Hz, 1H, H$^{6'}$), 7.13 (d, $^5J_{H,H}$ = 2.10 Hz, 1H, H$^{3'}$), 7.21 (dd, $^3J_{H,H}$ = 7.07 Hz, $^5J_{H,H}$ = 2.27 Hz, 1H, H$^{5'}$), 7.41 (d, $^5J_{H,H}$ = 2.40 Hz, 1H, H$^{3'}$), 7.48 (br. s, 1H, H$^{3'}$), 8.18 (d, $^3J_{H,H}$ = 5.68 Hz, 1H, H$^{6'}$), 8.43 (br. d, $^3J_{H,H}$ = 5.56 Hz, 1H, H$^{5'}$), 8.68 (d, $^3J_{H,H}$ = 7.07 Hz, 1H, H$^{6'}$). ^{13}C NMR (50 MHz, $CDCl_3$): δ = 41.01, 52.34, 52.53, 52.71, 54.66, 55.05, 55.16, 56.74, 57.59, 63.26, 69.61, 72.53, 101.88, 108.01, 108.28, 108.54, 108.94, 110.05, 110.46, 141.82, 149.58, 150.71, 152.59, 158.51, 159.44, 166.42, 166.52, 170.41, 171.38, 173.09.

Bispidine B14 ($C_{24}H_{30}N_4O_5S$, M_W = 486.58 g/mol). Bispidine B11 (289.3 mg, 0.61 mmol, 1.0 equiv) was dissolved in 10 mL of dry THF under an atmosphere of argon at 0 °C. Then triphenylphosphine (0.24 g, 0.92 mmol, 1.5 equiv), DIAD (0.186 g, 0.182 mL, 0.92 mmol, 1.5 equiv), and thioacetic acid **11** (0.056 g, 0.05 mL, 0.73 mmol, 1.2 equiv) were subsequently added. The suspension was stirred at 0 °C overnight while slowly allowing warming to ambient temperature (ice bath using Dewar). Then 50 mL of aqua dest was added to the suspension, and it was acidified with concentrated sulfuric acid to pH 2. The mixture was extracted with DCM (2 × 50 mL). The aqueous phase was basified with an aqueous solution of sodium hydroxide (20 wt %) to pH 14 and then extracted with DCM (3 × 50 mL). The organic phase was dried over Na_2SO_4, filtered, and concentrated. The orange oil was recrystallized from acetonitrile overnight to afford a white solid as the pure product in a yield of 27.9% (83.1 mg, 0.17 mmol). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.96 (s, 3H, NCH_3), 2.49 (d, 2H, $^2J_{H,H}$ = 12.05 Hz, CH_{ax}H$_{eq}$), 2.63 (d, 2H, $^2J_{H,H}$ = 12.05 Hz, CH$_{ax}$H$_{eq}$), 2.70 (t, $^3J_{H,H}$ = 6.02 Hz, 2H, CH_2), 2.83 (t, $^3J_{H,H}$ = 6.02 Hz, 2H, CH_2), 3.72 (s, 6H, COOCH_3), 4.16 (s, 2H, CH), 4.87 (d, $^3J_{H,H}$ = 5.52 Hz, 1H, C^{9}HOH), 7.16 (dd, $^3J_{H,H}$ = 6.96 Hz, $^3J_{H,H}$ = 5.46 Hz, 2H, H^{ar}), 7.76 (td, $^3J_{H,H}$ = 7.69 Hz, $^4J_{H,H}$ = 1.57 Hz, 2H, H^{ar}), 8.15 (d, $^3J_{H,H}$ = 8.16 Hz, 2H, H^{ar}), 8.48 (d, $^3J_{H,H}$ = 4.27 Hz, 2H, H^{ar}). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 35.79, 43.70, 48.97, 52.20, 52.80, 57.58, 72.63, 74.69, 122.74, 123.37, 136.26, 148.63, 159.82, 172.41. HR-ESI (pos): $[M]^+$ calcd 486.193 69, obsd 486.193 22. Elemental analysis: [M + H_2O] calcd C, 57.13; H, 6.39; N, 11.10; obsd C, 56.96; H, 6.25; N, 10.98.

Bispidine B15 ($C_{25}H_{26}N_4O_5$, M_W = 462.50 g/mol). Npy$_2$ P1 (3.50 g, 9.13 mmol, 1.0 equiv) was suspended in 50 mL of THF and heated to 60 °C. At this temperature were added propargylamine **12** (0.50 g, 0.58 mL, 9.13 mmol, 1.0 equiv) and an aqueous solution of formaldehyde (37%, 1.48 g, 1.36 mL, 18.26 mmol, 2.0 equiv). The mixture was heated to reflux for 1.5 h. After the mixture was cooled to ambient temperature, the solvent was removed in vacuo and the resulting residue was taken up in methanol. A tan-colored precipitate formed which was filtered and washed with cold methanol. The pure product was obtained in a yield of 19.9% (0.84 g, 1.82 mmol). ^{1}H NMR (200 MHz, $CDCl_3$): δ = 2.04 (s, 3H, N^{3}CH_3), 2.23 (t, $^4J_{H,H}$ = 2.50 Hz, 1H, CCH), 2.72 (d, $^2J_{H,H}$ = 12.25 Hz, 2H, CH_{ax}H$_{eq}$), 2.98 (d, $^2J_{H,H}$ = 12.63 Hz, 2H, CH$_{ax}$H$_{eq}$), 3.29 (d, $^4J_{H,H}$ = 2.15 Hz, 2H, N^{7}CH_2CCH), 3.83 (s, 6H, COOCH_3), 4.76 (s, 2H, N^{3}CH), 7.22 (dd, $^3J_{H,H}$ = 7.33 Hz, $^3J_{H,H}$ = 4.93 Hz, 2H, H^{ar}), 7.78 (td, $^3J_{H,H}$ = 7.67 Hz, $^4J_{H,H}$ = 1.58 Hz, 2H, H^{ar}), 8.10 (d, $^3J_{H,H}$ = 7.83 Hz, 2H, H^{ar}), 8.50 (d, $^3J_{H,H}$ = 4.80 Hz, 2H, H^{ar}). ^{13}C NMR (50 MHz, $CDCl_3$): δ = 43.28, 45.57, 50.71, 52.47, 57.61, 62.11, 73.70, 74.06, 122.98, 123.57, 136.41, 149.21, 158.79, 168.32, 203.01. HR-ESI (pos): $[M + H]^+$ calcd 463.198 14, obsd 463.197 36; $[M + Na]^+$ calcd 485.180 09, obsd 485.179 46; $[M + K]^+$ calcd 501.154 03, obsd 501.153 41. Elemental analysis: [M + $^1/_2H_2O$] calcd C, 63.68; H, 5.77; N, 11.88; obsd C, 63.26; H, 6.10; N, 11.54.

Bispidine B16 ($C_{25}H_{28}N_4O_5$, M_W = 464.51 g/mol). Bispidone B15 (0.43 g, 0.93 mmol, 1.0 equiv) was suspended in 30 mL of a 3:1 mixture of 1,4-dioxane/aqua dest at −6 °C, and sodium borohydride (17.8 mg, 0.47 mmol, 0.5 equiv) dissolved in an additional 10 mL of a 3:1 mixture of 1,4-dioxane/aqua dest was added over 15 min. The reaction mixture was stirred for 2.5 h at −6 °C and another 20 h at 0 °C. Then concentrated sulfuric acid was added to adjust the pH to 1. After the mixture was stirred for 3.5 h at ambient temperature, the pH was adjusted to 12 by adding an aqueous solution of sodium hydroxide (20 wt %) and the mixture was stirred for another 3 h. 1,4-Dioxane was evaporated from the reaction mixture in vacuo. The aqueous solution was decanted from solid material and diluted with further 50 mL of aqua dest and extracted with DCM (6 × 50 mL). The combined organic phases were dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was recrystallized from acetonitrile, filtered, and washed with a little amount of acetonitrile to afford the pure product in a yield of 36.6% (0.16 g, 0.34 mmol). ^{1}H NMR (200 MHz, $CDCl_3$): δ = 2.01 (s, 3H, N^{3}CH_3), 2.23 (t, $^4J_{H,H}$ = 2.40 Hz, 1H, CCH), 2.46 (d, $^3J_{H,H}$ = 5.56 Hz, 1H, C^{9}OH), 2.58 (s, 4H, N^{7}CH_2), 3.25 (d, $^4J_{H,H}$ = 2.53 Hz, 2H, N^{7}CH_2CCH), 3.68 (s, 6H, COOCH_3), 4.14 (s, 2H, N^{3}CH), 4.85 (d, $^3J_{H,H}$ = 5.56 Hz, 1H, C^{9}H), 7.19 (ddd, $^3J_{H,H}$ = 7.48 Hz, $^3J_{H,H}$ = 4.95 Hz, $^4J_{H,H}$ = 1.26 Hz, 2H, H^{ar}), 7.74 (td, $^3J_{H,H}$ = 7.64 Hz, $^4J_{H,H}$ = 1.77 Hz, 2H, H^{ar}), 7.98 (br d, $^3J_{H,H}$ = 7.96 Hz, 2H, H^{ar}), 8.47 (ddd, $^3J_{H,H}$ = 4.89 Hz, $^4J_{H,H}$ = 1.74 Hz, $^4J_{H,H}$ = 0.82 Hz, 2H, H^{ar}). ^{13}C NMR (50 MHz, $CDCl_3$): δ = 43.76, 46.74, 47.58, 52.10, 52.84, 72.12, 73.16, 74.77, 78.96, 122.64, 123.20, 136.23, 148.51, 160.03, 172.34. HR-ESI (pos): $[M + H]^+$ calcd 465.213 80, obsd 465.213 21; $[M + Na]^+$ calcd 487.195 74, obsd 487.195 48. Elemental analysis: [M + CH_3CN] calcd C, 64.14; H, 6.18; N, 13.85; obsd C, 64.00; H, 6.22; N, 13.84.

$[Cu^{II}(B4)](ClO_4)_2$ ($C_{30}H_{33}Cl_2CuN_5O_{15}$, M_W = 838.06 g/mol). A solution of copper(II) perchlorate hexahydrate (74.11 mg, 0.2 mmol, 1.0 equiv) in 2.5 mL of acetonitrile was poured into a suspension of ligand B4 (115.12 mg, 0.2 mmol, 1.0 equiv) in 2.5 mL of acetonitrile. The resultant blue solution was stirred at ambient temperature overnight and afterward subjected to ether diffusion. Dark blue/violet crystals were obtained in a yield of 84.0% (147.4 mg, 0.168 mmol). HR-FAB (pos): $[B4 + H_2O + Cu^I]^+$ calcd 656.1782, obsd 656.1790. Elemental analysis: [B4 + Cu(ClO$_4$)$_2$ + 3H_2O] calcd C, 40.39; H, 4.41; N, 7.85; obsd C, 40.89; H, 4.35; N, 7.95. UV−vis (CH_3CN, rt), λ [nm]: 629 (λ_{max}), 941. CV (CH_3CN, rt): $E_{1/2}$ = −760.5 mV (vs Fc/Fc$^+$).

$[Cu^{II}(B12)](ClO_4)_2$ ($C_{24}H_{31}Cl_2CuN_5O_{13}$, M_W = 731.98 g/mol). The ligand B12 (46.9 mg, 0.1 mmol, 1.0 equiv) was dissolved in 2.0 mL of acetonitrile, and copper(II) perchlorate hexahydrate (37.1 mg, 0.1 mmol, 1.0 equiv) dissolved in further 2.0 mL of acetonitrile was added to the solution. This blue solution was stirred at ambient temperature overnight and afterward subjected to an ether diffusion. The product was obtained as blue crystals in a yield of 69.4% (50.8 mg, 69.4 μmol). HR-ESI (pos): $[B12 + Cu^{II} + ClO_4^-]^+$ calcd 631.110 63, obsd 631.109 40. Elemental analysis: [B12 + Cu(ClO$_4$)$_2$] calcd C, 39.38; H, 4.27; N, 9.57; obsd C, 39.35; H, 4.48; N, 9.52. UV (CH_3CN, rt), λ [nm]: 627 (λ_{max}), 983. CV (CH_3CN, rt): $E_{1/2}$ = −807.0 mV (vs Fc/Fc$^+$).

$[Cu^{II}(B13\text{-}H)](ClO_4)$ ($C_{26}H_{32}ClCuN_5O_{10}$, M_W = 673.56 g/mol). The Cu^{II} complex $[Cu^{II}(B13)](ClO_4)_2$ (23.0 mg, 30.8 μmol) was dissolved in 1.0 mL of acetonitrile, and 0.1 mL of DIPEA was added to the blue solution. Immediately, a purple precipitate formed. The suspension

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was concentrated, and the precipitate was allowed to crystallize from water. Fine purple needles were obtained as the product in a yield of 91.56% (19.0 mg, 28.2 μmol). HR-FAB(pos): $[Cu^{II} + B13 - H]^+$ calcd 573.1649, obsd 573.1660. UV (H_2O, rt), λ [nm]: 564, 698 (λ_{max}). CV (CH_3CN, rt): $E_{1/2} = -1.287$ V (vs Fc/Fc^+).

General in Situ Preparation of the Cu^{II} Complexes with Pentadentate Bispidines. Commercially available $Cu(ClO_4)_2 \cdot 6\ H_2O$ was dried in vacuo for 1 day and was considered dry. A stock solution of Cu^{II} was prepared by dissolving 131.23 mg (0.5 mmol) of $Cu(ClO_4)_2$ in 100 mL of absolute acetonitrile. Then 1 mL of the Cu^{II} stock solution (5 μmol) was added to 6 μmol of the (substituted) N_2py_2 ligand (approximately 3−4 mg, depending on the substituent), i.e., Cu^{II} and ligand are dissolved in a ratio of 5/6. An immediate dissolution of the ligand and a pale blue color of the solution indicate complex formation. The solution was stirred for 30 min at ambient temperature. Then UV−vis spectra and CV measurements were subsequently undertaken using the same solution for both experiments.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

UV−vis−NIR spectra of the Cu^{II} complexes with the pentadentate bispidine ligands B4−B6 and the functionalized bispidine B13, the EPR spectrum of $[Cu^{II}(B4)](ClO_4)_2$, the cyclovoltammograms of $[Cu^{II}(B4)(OClO_3)](ClO_4)$, $[Cu^{II}(B12)](ClO_4)_2$, and $[Cu^{II}(B13\text{-}H)](ClO_4)$, the biodistribution data of ^{64}Cu−B7 and ^{64}Cu−B9 in rats, presented as % ID/g, a plot of the experimental structure of B16, details of the crystallographic experiments, ^1H and ^{13}C NMR spectra of all new organic compounds, and crystallographic data in CIF format. This material is available free of charge via the Internet at http://pubs.acs.org. Also, CCDC 926343-926348 contains the supplementary crystallographic data that can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

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Notes

The authors declare no competing financial interest.

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(60) In contrast to second generation bispidines which do not have pendent donor groups at C^2 and C^4,[61−63] these are derived from dimethyl acetonedicarboxylate as the CH-acidic component (see Chart 1).
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(99) Note that with the hexadentate bispidine ligand, cyclam challenge experiments have been shown to lead, in terms of transchelation, to conclusions similar to those with competition reactions with the endogenous tripeptide glutathione.[44]
(100) Preliminary experiments with an SOD test system, which we are currently developing to model in vivo transchelation, indicate, in agreement with the cyclam challenge experiments, that the most promising system based on B9 has a stability that is similar to that with the corresponding hexadentate ligand,[44] and this is also confirmed in blood serum experiments. Both the hexadentate bispidine and B9

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