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Enhanced Peptide β-Sheet Affinity by Metal to Ligand Coordination

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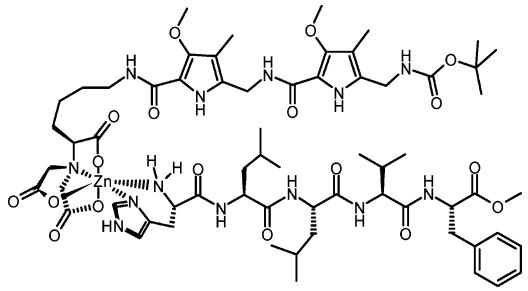

A histidine-coordinating metal complex substituted with methoxypyrrole amino acids (MOPAS) as a peptide β-sheet binder shows high affinity for a H_2N−His-Leu-Leu-Val-Phe-OMe pentapeptide in DMSO solution. Within the complex, a β-sheet conformation is induced into the pentapeptide by weak intra-assembly interactions with the MOPAS units.

Intramolecular processes are typically more favorable than intermolecular processes.[1] This includes the formation of weak reversible interactions such as hydrogen bonds. Recent studies on hydrogen bonding patterns complementary to peptide β-sheet structures[2] by Nowick,[3] Gellman,[4] and others[5] have taken advantage of this.[6] The peptide and its complementary binding sites are covalently linked by a turn structure allowing intramolecular hydrogen bond formation.[7] The selective binding of amino acid side chain functional groups such as carboxyl, ammonium, phosphate, or imidazole groups is an alternative way of peptide binding, which has been thoroughly investigated.[8] Schmuck et al.[9] recently reported the combination of a carboxyl-binding pyrrole guanidinium group with pyrazoles, which provides hydrogen bond donor and acceptor sites complementary to a peptide structure.[10] We report here a compound that combines strong intermolecular coordination with hydrogen bonds to create a synthetic peptide binding site. The concept is illustrated using a histidine-coordinating nitrilotriacetic acid (NTA) complex[11] and methoxy pyrrole amino acids (MOPAS)[5] with peptide β-sheet binding ability. After complex formation, the subsequent process of peptide backbone binding becomes intramolecular, facilitating hydrogen bond formation and control of the small peptide conformation in DMSO.

Results and Discussion

Scheme 1 shows the synthesis of the peptide binding complex **5**. Compound **1**,[12] obtained from lysine methyl ester, is coupled to activated ester **2**, which we have reported recently.[5] After Boc deprotection, a second MOPAS unit was introduced, again using compound **2**. Cleavage of the methyl ester under basic conditions generates the NTA ligand, from which zinc complex **5** was obtained in good yield.

Although nickel(II) or copper(II) NTA complexes show higher affinities to N-terminal His,[11] zinc(II) was chosen as a metal ion for complex formation to obtain a diamag-

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(1) Typical examples are cycloadditions or cyclization reactions and intramolecular folding.

(2) One important motivation to develop synthetic binding sites for peptide β-sheets is the interception of prion protein aggregation. (a) Kirsten, C. N.; Schrader, T. H. *J. Am. Chem. Soc.* **1997**, *119*, 12061−12068. (b) Wehner, M.; Schrader, T. *Angew. Chem., Int. Ed.* **2002**, *41*, 1751−1754. (c) Cernovska, K.; Kemter, M.; Gallmeier, H.-C.; Rzepecki, P.; Schrader, T. H.; König, B. *Org. Biomol. Chem.* **2004**, *2*, 1603−1611.

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(6) Many examples of intermolecular β-sheet binders based on complementary hydrogen bonding patterns have been reported, but their use is mostly restricted to nonpolar solvents, which do not intercept the hydrogen bonds.

(7) Nowick, J. S.; Brower, J. O. *J. Am. Chem. Soc*. **2003**, *125*, 876−877.

(8) Such ionic interactions provide much higher affinity than hydrogen bonds and allow peptide binding in polar solvents. Examples of carboxyl group binding: (a) Peczuh, M. W.; Hamilton, A. D.; Sánchez-Quesada, J.; de Mendoza, J.; Haack, T.; Giralt, E. *J. Am. Chem. Soc.* **1997**, *119*, 9327−9328. Example of phosphate-binding: (b) Ojida, A.; Mito-oka, Y.; Sada, K.; Hamachi, I. *J. Am. Chem. Soc.* **2004**, *126*, 2454−2463. Examples of ammonium group binding: (c) Hossain, M. A.; Schneider, H.-J. *J. Am. Chem. Soc*. **1998**, *120*, 11208−11209. (d) Herm, M.; Molt, O.; Schrader, T. H. *Chem. Eur. J.* **2002**, *8*, 1485−1499. Examples of binding to hydrophobic groups or areas: (e) Breslow, R.; Yang, Z.; Ching, R.; Trojandt, G.; Odobel, F. *J. Am. Chem. Soc.* **1998**, *120*, 3536−3537. (f) Baldini, L.; Wilson, A. J.; Hong, J.; Hamilton, A. D. *J. Am. Chem. Soc.* **2004**, *126*, 5656−5657. (g) Jain, R. K.; Hamilton, A. D. *Org. Lett.* **2000**, *2*, 1721−1723. (h) Ernst, J. T.; Kutzki, O.; Debnath, A. K.; Jiang, S.; Lu, H.; Hamilton, A. D. *Angew. Chem., Int. Ed.* **2002**, *41*, 117−119. (i) Jasper, C.; Schrader, T.; Panitzky, J.; Klärner, F.-G. *Angew. Chem., Int. Ed*. **2002**, *41*, 1355−1358. (j) Malinovski, V.; Tumir, L.; Piantanida, I.; Zinic, M.; Schneider, H.-J. *Eur. J. Org. Chem.* **2002**, 3785−3795.

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(10) Other examples of combined use of different reversible interaction: Ligand to metal coordination and ionic interaction: (a) Wright, A. T.; Anslyn, E. V. *Org. Lett.* **2004**, *6*, 1341−1344. Ligand to metal coordination and hydrogen bonding: (b) Tobey, S. L.; Anslyn, E. V. *J. Am. Chem. Soc.* **2003**, *125*, 14807−14815. Guanidinium ion and boronic acid: (c) Wiskur, S. L.; Lavigne, J. J.; Metzger, A.; Tobey, S. L.; Lynch, V.; Anslyn, E. V. *Chem. Eur. J*. **2004**, *10*, 3792−3804. (d) Tobey, S. L.; Anslyn, E. V. *J. Am. Chem. Soc.* **2003**, *125*, 14807−14815. (e) Shirin, Z.; Hammes, B. S.; Young, V. G.; Borovik, A. S. *J. Am. Chem. Soc.* **2000**, *122*, 1836−1837. (f) MacBeth, E. C.; Hammes, B. S.; Young, V. G.; Borovik, A. S. *Inorg. Chem.* **2001**, *40*, 4733−4741.

(11) (a) Beauchamp, A. L.; Israeli, J.; Saulnier, H. *Can. J. Chem.* **1969**, *47*, 1269−1273. (b) Hopgood, D.; Angelici, R. J. *J. Am. Chem. Soc.* **1968**, *90*, 2508−2513.

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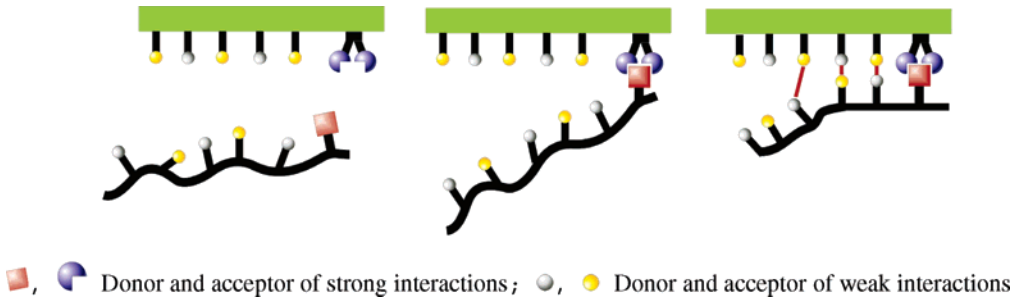
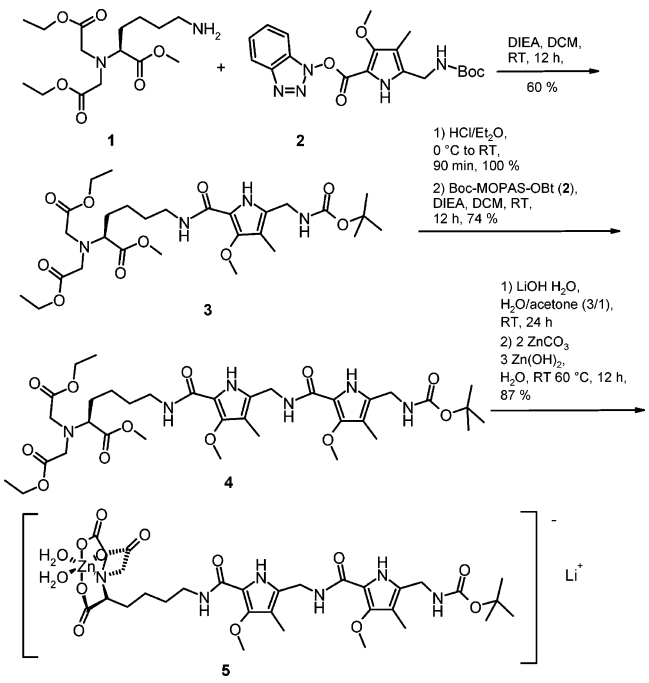


FIGURE 1. Schematic illustration of a binding process combining strong metal to ligand coordination and weaker interactions.

SCHEME 1. Synthesis of Complex 5



netic compound, which allows spectroscopic investigation of the supramolecular structure with a bound peptide. The affinity of the zinc(II)−NTA complex for N-terminal His in DMSO[13] is still sufficiently high ($K > 10^4$ L/mol) to ensure in a millimolar solution, which is necessary for NMR investigations, complete complex formation. Pentapeptide H_2N−His-Leu-Leu-Val-Phe-OMe[14] was used to investigate the binding properties of **5**. The NMR spectra of **5**·H_2N−His-Leu-Leu-Val-Phe-OMe ($c = 2.22 \times 10^{-2}$ mol/L), obtained by dissolving equimolar amounts of the receptor and the pentapeptide in DMSO [D_6], shows a single set of resonances, which were structurally assigned. The temperature dependence of the chemical shifts (see Supporting Information for data) confirmed the expected hydrogen bond interaction of the MOPAS amide NH to methoxy groups, which should keep the structure planar.[15]

A small ppb/K value for one of the central peptide amide protons indicates a possible hydrogen bond to a

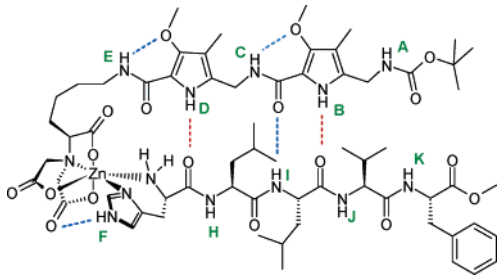


FIGURE 2. Hydrogen bonds as suggested from temperature dependence of chemical shifts (blue dotted lines). Red hydrogen bonds are expected, but without a reference value for the temperature dependence of the chemical shift of hydrogen-bound pyrrole NH, no support can be derived from the experiment.

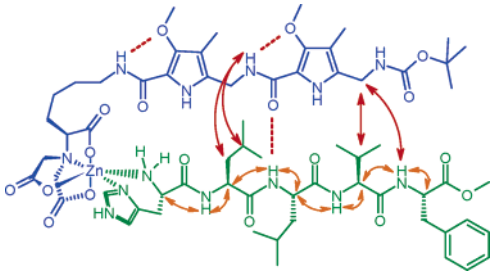


FIGURE 3. Spectroscopically detected interactions of **5** and H_2N−His-Leu-Leu-Val-Phe-OMe in DMSO solution. Red dotted lines: Hydrogen bonding suggested from the temperature dependence of the chemical shift. Red arrows: Intrastrand NOE contacts. Orange arrows: Intramolecular distances within the pentapeptide used for the determination of its conformation.

MOPAS carbonyl group. Two-dimensional NOESY spectra (see Supporting Information) revealed four intrastrand contacts, which are indicated in Figure 3.[16] This proves the existence of strong interactions between the bound pentapeptide and the MOPAS units.

The interactions of the conformationally restricted MOPAS units with the bound pentapeptide induce a conformational preference of the peptide. To show this, intramolecular distances of the bound pentapeptide were derived from integrated NOESY spectra and used as constraints to calculate the solution structure of the pentapeptide bound to MOPAS complex **5** (see Supporting Information for data). Figure 4 shows the resulting pentapeptide structures, which have a β-sheet-like con-

(13) In neutral aqueous solution, the affinity of **5** for the pentapeptide is too small to be accurately determined by microcalorimetry, which has its limit at about 10^3L/mol with our apparatus.

(14) Methyl ester was used to avoid any additional ionic interactions of the carboxylate group.

(15) This concept of using intramolecular hydrogen bonds to planarize a β-sheet mimic was extensively used by Nowick in methoxy-substituted hydrazine benzoic acids.

(16) Overlap of resonance signals restricts the number of detectable cross-peaks.

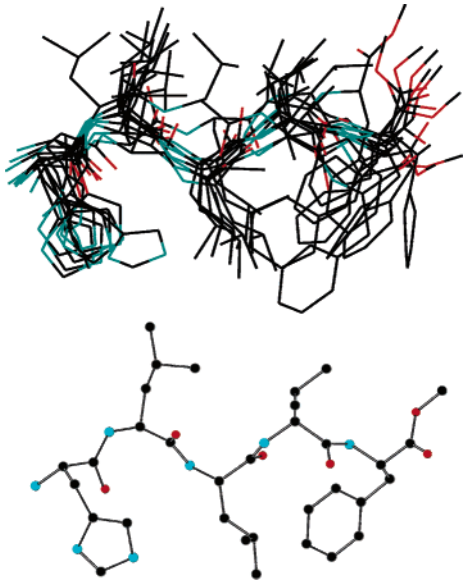


FIGURE 4. Structure of H_2N−His-Leu-Leu-Val-Phe-OMe bound to **5**. Top: Overlaid structures from MD simulation. Bottom: Minimized average structure.

TABLE 1. Temperature Dependence of NH Signal Shifts

	ppb/K		ppb/K
NH A	−4.1	NH G	−5.4
NH B	−4.6[a]	NH H	−4.0
NH C	−2.3	NH I	−2.8
NH D	−5.4[a]	NH J	−4.1
NH E	−2.3	NH K	−5.6
NH F	−1.1		

[a] For NH B and NH D pyrrole hydrogen atoms, which are expected to form intrastrand hydrogen bonds to the pentapeptide, and for the NH F imidazole hydrogen atom, no reference values are known. Therefore, no conclusions can be derived from the temperature dependence.

TABLE 2. Distances within the Assembly of H_2N−His-Leu-Leu-Val-Phe-OMe and 5

interacting protons	distance from NOE [Å]	distance of average structure [Å}
NH G−24′	2.8	2.5
NH H−24′	2.1	2.3
NH H−19′	2.8	2.9
NH I−19′	2.1	2.2
NH I−14′	2.8	2.9
NH J−14′	2.0	2.2
NH J−10′	2.9	3.0
NH K−10′	2.1	2.2
NH K−3′	2.8	3.0

formation.[17] Distances of the derived structure correspond well with the experimental NOE values (see Table 2).[18] As control experiments, equimolar mixtures of the ligand of **5** without the NTA unit and the pentapeptide were investigated under identical conditions (see Supporting Information for data). No cross-peaks indicating close interactions between the peptide and the MOPAS units could be detected. Intramolecular NOEs within the peptide were of low intensity, again showing a flexible conformation.[19]

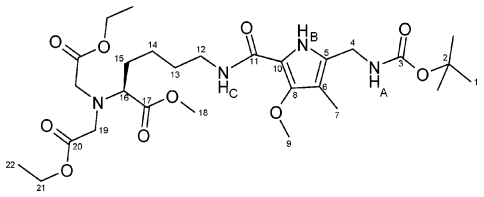
Conclusion

Mallik and Srivastava[20] recently reported the significantly enhanced binding affinity of an inhibitor of carbonic anhydrase II by attaching a metal complex tether that interacts with surface-exposed histidine residues. Our experiments now have shown that by combining a Zn−NTA tether with a heterocyclic peptide β-sheet binder, it is possible to restrict the conformation of a small flexible pentapeptide in DMSO solution. Intermolecular interactions of the MOPAS units with the pentapeptide in DMSO would be too weak to induce a conformational preference. The principle of "two-prong" binders is applicable to other peptide receptors that suffer from competing solvent interactions. Exchanging zinc in **5** with copper(II) or nickel(II) leads to peptide-binding compounds with an affinity for H_2N−His-Leu-Leu-Val-Phe-OMe in the lower micromolar range[21] under aqueous physiological conditions. Other amino acid residues than imidazole can be targeted using other transition metal ion conjugates.[22] This allows selective recognition and conformation control of small peptides in biological environment.

Experimental Section

Compounds **1**[12] and Boc-MOPAS−OBt (**2**)[5] were prepared as previously reported.

Et/Me-NTA-MOPAS−Boc (3-Boc). Boc-Mopas-OBt (**2**, 72 mg, 0.18 mmol) was dissolved in DCM (15 mL). After the solution was cooled to 0 °C, **1** (55 mg, 0.15 mmol) and DIEA (0.07 mL, 0.4 mmol) were added. The ice bath was removed, and the reaction mixture was stirred for 24 h. The resulting product was extracted twice with 5% aqueous $KHSO_4$ (25 mL), once with water (25 mL), and twice with 0.5 M aqueous $NaHCO_3$ (25 mL) solution. After drying over Na_2SO_4, evaporation of the solvent and subsequent column chromatography (EE, $R_f = 0.64$) gave **3** (54 mg, 60%), as a yellow oil.



^{1}H NMR (400 MHz, $CDCl_3$, 25 °C, COSY): δ = 1.18 (t, ^3J (H,H) = 7.2 Hz, 6 H; H(22)), 1.29−1.50 (m, 11 H; H(1) + H(14)), 1.51−1.60 (m, 2 H; H(13)), 1.62−1.74 (m, 2 H; H(15)), 2.01 (s, 3

(17) Due to signal broadening and overlap with receptor resonance signals, it was not possible to determine $^3J_{NHH\alpha}$ coupling constants of the aggregate. The $^3J_{NHH\alpha}$ coupling constants of the nonbound pentapeptide are given in Supporting Information.

(18) Importance of comparing modeling results with primary derived data was recently described. Glättli, A.; van Gunsteren, W. F. *Angew. Chem.* **2004**, *116*, 6472−6476.

(19) Absence of intermolecular contacts shows that no aggregates are formed between peptides or peptide and MOPAS under the experimental conditions.

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(21) Affinity was determined by microcalorimetry. Paramagnetism of the copper complex and substantial line broadening in the nickel complex so far have prevented a detailed investigation of the structure-inducing effect of the receptor on the pentapeptide in water.

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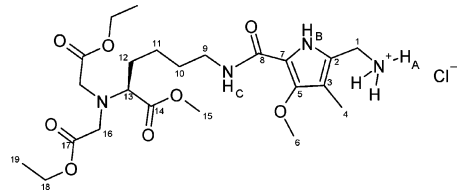
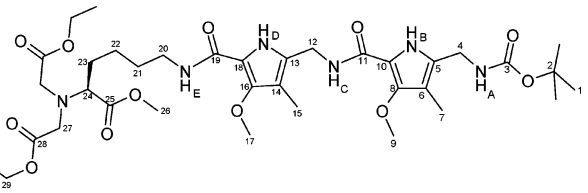
H; H(7)), 3.30−3.38 (m, 2 H; H(12)), 3.37 (t, ^3J (H,H) = 7.4 Hz, 1 H; H(16)), 3.54−3.59 (m, 4 H; H(19)), 3.61 (s, 3 H; H(18)), 3.79 (s, 3 H; H(9)), 4.07 (q, ^3J (H,H) = 7.2 Hz, 4 H; H(21)), 4.11−4.20 (m, 2 H; H(4)), 5.74−5.84 (m, 1 H; H_A), 6.91−7.01 (m, 1 H; H_C), 10.25 (bs, 1 H; H_B). ^{13}C NMR (150 MHz, $CDCl_3$, 25 °C, HSQC, HMBC): δ = 8.1 (+, C(7)), 14.2 (+, 2 C, C(22)), 23.4 (−, C(14)), 28.4 (+, C(1)), 29.6 (−, C(3)), 30.2 (−, C(15)), 35.4 (−, C(4)), 38.8 (−, C(11)), 51.4 (+, C(18)), 52.6 (−, 2 C, C(19)), 60.5 (−, 2 C, C(21)), 61.4 (−, C(9)), 64.7 (+, C(16)), 79.2 (C_{quat}, C(2)), 107.9 (C_{quat}, C(6)), 112.4 (C_{quat}, C(10)), 128.7 (C_{quat}, C(5)), 147.0 (C_{quat}, C(8)), 155.9 (C_{quat}, C(3)), 161.5 (C_{quat}, C(11)), 171.3 (C_{quat}, 2 C, C(20)), 173.0 (C_{quat}, C(17)). IR (KBr): $\tilde{\nu}$ = 3426 cm^{-1}, 3074, 2951, 1744, 1680, 1534, 1218, 1025. MS (ESI, H_2O/MeOH + 10 mmol/L NH_4Ac): *m/z* (%) = 637 (12) [MK^+], 621 (22) [MNa^+], 599 (100) [MH^+]. Anal. Calcd (%) for $C_{28}H_{46}N_4O_{10}$ (598.7): C, 56.17; H, 7.74; N, 9.36. Found: C, 55.68; H, 7.66; N, 9.27.

Et/Me-NTA-MOPAS−NH_2·HCl (3-H). Compound **3-Boc** (81 g, 0.14 mmol) was dissolved in Et_2O (5 mL) and cooled to 0 °C in ice. To deprotect the Boc group, HCl saturated Et_2O was added dropwise until the product started to precipitate. The ice bath was removed and the reaction progress monitored by TLC (EE). After 90 min, the solvent was evaporated. Compound **3-H** (75 mg, 100%) was isolated as a very hygroscopic, colorless solid, mp 81−83 °C.



^{1}H NMR (400 MHz, [D_6]DMSO, 25 °C, COSY, HMBC, HSQC): δ = 1.17 (t, ^3J (H,H) = 7.1 Hz, 6 H; H(19)), 1.21−1.41 (m, 2 H; H(11)), 1.49 (m, 2 H; H(10)), 1.60 (m, 2 H; H(12)), 2.01 (s, 3 H; H(4)), 3.23 (m, 2 H; H(9)), 3.39 (m, 1 H; H(13)), 3.50−3.65 (m, 7 H; H(16), H(15)), 3.75 (s, 3 H; H(6)), 3.88 (m, 2 H; H(1)), 4.04 (q, ^3J (H,H) = 7.1 Hz, 4 H; H(18)), 6.35 (bs, 6 H), 7.14 (m, 1 H; H_C), 8.29 (m, 3 H; H_A), 10.93 (s, 1 H; H_B). ^{13}C NMR (100 MHz, [D_6]DMSO, 25 °C, HMBC, HSQC): δ = 7.7 (+, C(4)), 13.9 (+, 2 C, C(19)), 22.6 (−, C(11)), 29.1 (−, C(10)), 29.3 (−, C(12)), 33.1 (−, C(1)), 37.9 (−, C(9)), 51.0 (+, C(15)), 52.1 (−, 2 C, C(16)), 59.8 (−, 2 C, C(18)), 61.3 (+, C(6)), 63.7 (+, C(13)), 110.1 (C_{quat}, C(3)), 113.8 (C_{quat}, C(7)), 121.8 (C_{quat}, C(2)), 145.7 (C_{quat}, C(5)), 159.5 (C_{quat}, C(8)), 170.7 (C_{quat}, C(8)), 170.7 (C_{quat}, 2 C, C(17)), 172.3 (C_{quat}, C(14)); IR (KBr): $\tilde{\nu}$ = 3429, 3010, 2928, 1744, 1631, 1547, 1227 cm^{-1}. MS (ESI, CH_2Cl_2/MeOH + 10 mmol/L NH_4Ac): *m/z* (%) = 521 (64) [MNa^+], 499 (100) [MH^+]. Anal. Calcd (%) for $C_{23}H_{39}N_4O_8Cl + H_2O$ (535.04): C, 49.98; H, 7.48; N, 10.14. Found: C, 49.92; H, 7.37; N, 10.08.

Et/Me-NTA-MOPAS-MOPAS−Boc (4). Compound **3-H** (144 mg, 0.27 mmol) was dissolved in DCM (15 mL). After the solution was cooled to 0 °C, Boc-MOPAS-OBt (159 mg, 0.40 mmol) and DIEA (0.12 mL, 0.7 mmol) were added. The ice bath was removed, and the reaction mixture was stirred for 24 h. The resulting product was extracted twice with 5% aqueous $KHSO_4$ (25 mL), once with water (25 mL), and twice with 0.5 M aqueous $NaHCO_3$ (25 mL) solution. After drying over Na_2SO_4, the solvent was evaporated in a vacuum, and column chromatography (PE: EE = 1:1, R_f (EE) = 0.60) yielded **4** (153 mg, 74%) as a slightly yellow oil.



^{1}H NMR (400 MHz, $CDCl_3$, 25 °C, COSY): δ = 1.23 (t, ^3J (H,H) = 7.0 Hz, 6 H; H(30)), 1.34−1.55 (m, 11 H; H(1) + H(22)), 1.57−1.67 (m, 2 H; H(21)), 1.72−1.86 (m, 2 H; H(23)), 2.00 (s, 3 H; H(7)), 2.13 (s, 3 H; H(15)), 3.38−3.47 (m, 3 H; H(24) + H(20)), 3.61 (s, 3 H; H(17)), 3.62 (s, 4 H; H(27)), 3.65 (s, 3 H; H(26)), 3.88 (s, 3 H; H(9)), 4.12 (q, ^3J (H,H) = 7.0 Hz, 4 H; H(29)), 4.19 (d, ^3J (H,H) = 5.6 Hz, 2 H; H(12)), 4.72 (d, ^3J (H,H) = 4.8 Hz, 2 H; H(4)), 6.14−6.21 (m, 1 H; H_C), 6.96 (t, ^3J (H,H) = 4.9 Hz, 1 H; H_A), 7.12 (t, ^3J (H,H) = 5.7 Hz, 1 H; H_E), 11.1 (bs, 1 H; H_B), 11.46 (bs, 1 H; H_D). ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C, HSQC + HMBC): δ = 7.9 (+, C(7)), 8.2 (+, C(15)), 14.2 (+, 2 C, C(30)), 23.4 (−, C(22)), 28.4 (+, C(1)), 29.5 (−, C(21)), 30.3 (−, C(23)), 34.3 (−, C(4)), 35.3 (−, C(12)), 38.8 (−, C(20)), 51.4 (+, C(26)), 52.6 (−, 2 C, C(27)), 60.5 (−, 2 C, C(29)), 61.2 (+, C(17)), 61.4 (+, C(9)), 64.7 (+, C(24)), 78.9 (C_{quat}, C(2)), 107.6 (C_{quat}, C(6)), 108.3 (C_{quat}, C(14)), 112.2 (C_{quat}, C(10) + C(18)), 128.0 (C_{quat}, C(13)), 129.1 (C_{quat}, C(5)), 147.1 (C_{quat}, C(16)), 147.5 (C_{quat}, C(8)), 155.8 (C_{quat}, C(11)), 160.8 (C_{quat}, C(3)), 161.9 (C_{quat}, C(19)), 171.3 (C_{quat}, 2 C, C(28)), 173.0 (C_{quat}, C(25)); IR (KBr): $\tilde{\nu}$ = 3425 cm^{-1}, 3032, 2960, 1731, 1679, 1580, 1012. MS (ESI, CH_2Cl_2/MeOH + 10 mmol/L NH_4Ac): *m/z* (%) = 803 (15) [MK^+], 787 (43) [MNa^+], 766 (100) [MH^+].

[Zn−NTA-MOPAS-MOPAS−Boc]Li (5). To a solution of **4** (116 mg, 0.15 mmol) in 25% water/acetone (20 mL) is added LiOH·H_2O (19 mg, 0.45 mmol). The reaction mixture was stirred for 12 h at rt; acetone was removed in a vacuum, and the remaining solvent was lyophilized. The completion of the ester cleavage reaction was monitored by NMR.

The crude carboxylate and basic zinc carbonate (27 mg, 0.05 mmol) were dissolved in water (20 mL). After stirring for 30 min at rt, the suspension was heated to 60 °C for 1 day. Insoluble particles were filtered off, and the filtrate was lyophilized. The raw product was suspended in ethanol followed by the addition of ether. The precipitated material was separated from solution by centrifugation to give **5** (103 mg, 86%) as a colorless solid. Mp > 200 °C. MS (ESI, H_2O/MeOH + 10 mmol/L NH_4Ac): *m/z* (%) = 755 (100) [M − $2H_2O$]$^-$, 757 (100) [MH_2 − $2H_2O$]$^+$; IR (KBr): $\tilde{\nu}$ = 3384, 2972, 2932, 1701, 1624, 1537 cm^{-1}. FAB NI-LSIMS (MeOH/glycerine) HRMS ($C_{31}H_{45}N_6O_{12}Zn$ [A^- + $2H^+$]$^+$): calcd 757.2387; found 757.2381 ± 0.0015.

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Supporting Information Available: Details of structure determination by NMR. This material is available free of charge via the Internet at http://pubs.acs.org.

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