Molecular recognition of a tris(histidine) ligand

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Design and synthesis of a tri-Hg^{2+} complex to selectivity recognize a tris(histidine) ligand is presented.

We are interested in the design and synthesis of transition metal ion based receptors for histidine-containing peptides.[1] Histidine-to-metal ion interactions (Cu^{2+}, Ni^{2+}, Zn^{2+} *etc*.) have been used for various applications, *e.g*. protein purification,[2] cross-linking,[3] targeting proteins to lipid bilayers.[4] It is these strong, directed metal ion-to-histidine interactions that we are using as the basis of the recognition process.[5]

As a model system for peptide recognition, we have chosen the ligand **L** to position three (*S*)-histidines 12 Å apart (Fig. 1). The compound **C**, with one histidine, served as the control for our studies. **L′** and **C′** were tested as histidine-mimetic ligand and control, respectively. Three-dimensional structures for the receptor and ligands were constructed using the molecular modelling software INSIGHT II and DISCOVER (ver. 95.0, BioSym Technologies/MSI, San Diego, CA) and energy-minimized in the gas phase using the consistent valence force field (cvff).

Syntheses of the receptor **R**, ligands **L**, **L′** and the control **C**, **C′** are shown in Scheme 1. Selective protection of three nitrogens of the cyclam (1,4,8,11-tetraazacyclotetradecane) ring **1** was carried out following a literature procedure.[6]

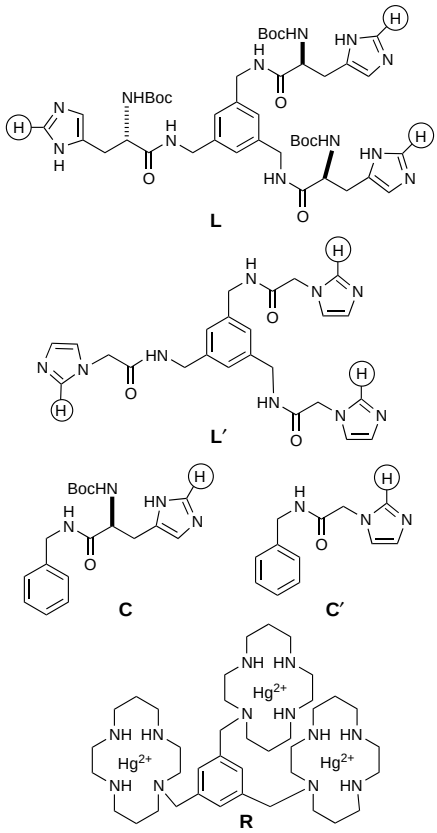
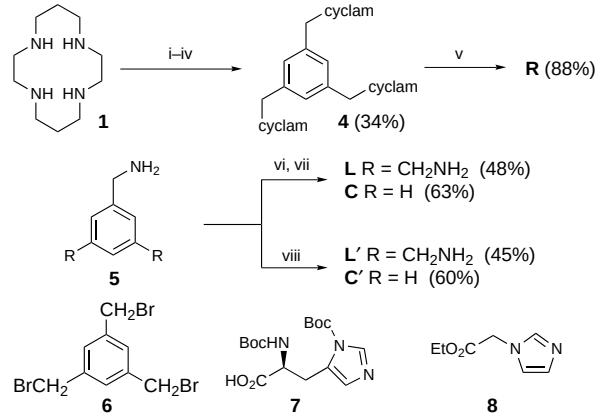


Fig. 1 Structures of the tris(histidine) ligand **L**, histidine mimetic ligand **L′**, controls **C**, **C′** and the designed receptor **R**. Hydrogens monitored in the titration studies are circled.



Scheme 1 *Reagents and conditions*: TsCl, Et_3N, $CHCl_3$; ii, **6**, K_2CO_3, MeCN, sonication; iii, HBr, AcOH, 70 °C; iv, ion exchange; v, $Hg(ClO_4)_2.3H_2O$, MeOH–MeCN; vi, *N*-hydroxysuccinimide, DCC, Et_3N, THF, **7**; vii, NaOH; viii, **8**, EtOH, sonication

Reaction of cyclam tritosylate **2** with 1,3,5-tris(bromomethyl)benzene **6**[7] proceeded smoothly in MeCN (using powdered K_2CO_3 as the base) under sonication (8 h). The crude product was purified by flash chromatography, using 5% MeOH–CH_2Cl_2 as the solvent (R_f 0.3). This reaction was found to yield a complex mixture of products under reflux. Removal of the tosyl groups was carried out in HBr–AcOH at 70 °C (10 h). Free ligand **4** was isolated by ion-exchange chromatography (IRA-400 column, hydroxide form) using water as eluent. Receptor **R** was synthesized by adding a solution of the free ligand **4** in MeCN to a methanolic solution of $Hg(ClO_4)_2.3H_2O$. Receptor **R** was isolated as the air-stable perchlorate salt after addition of Et_2O to the reaction mixture. (**Note**: we did not observe any explosive tendency for this compound.)§

Ligand **L** is known in the literature[8] and control **C** was synthesized by an analogous procedure. Reactions for the synthesis of **L′** and **C′** gave higher yields under sonication compared to refluxing conditions. Control **C′** was purified by recrystallization from $CHCl_3$–hexane and **L′** was purified by flash chromatography using MeOH as the eluent (R_f = 0.3).§ In the binding studies, a diamagnetic metal ion (Hg^{2+}) with strong affinity for histidine ($> 10^3$ M^{-1}) was used so that the binding could be monitored by ^{1}H NMR spectroscopy. Since the receptor **R** contains the embedded distance information, cyclam was used to hold the metal ions. Cyclam has very high affinities ($> 10^{20}$ M^{-1}) for transition metal ions. This ensures that the metal ions will not get displaced from **R** at high histidine concentrations. Cyclam also gives us the flexibility to synthesize the receptor with a variety of transition metal ions and to optimize the recognition properties.

Recognition studies were conducted in highly polar [$^2H_6$]DMSO, and were followed by ^{1}H NMR spectroscopy. The C-2-H of the imidazole moieties (indicated in Fig. 1) of **C** and **C′** were found to be shifted downfield (0.80 ppm for **C**; 0.87 ppm for **C′**) upon complexation with the Hg^{2+} ions of the receptor **R** (10 mM in **R**, 0–60 mM in **C** or **C′**). Both of these controls were in fast exchange with the receptor and an average signal was observed in each case. Resultant titration curves are

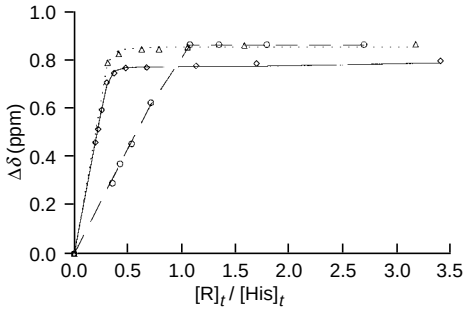


Fig. 2 Titration curves for (◇)C, (△)**C′** and (○) **L′**. The curves indicate the calculated titration curves with the reported binding constants.

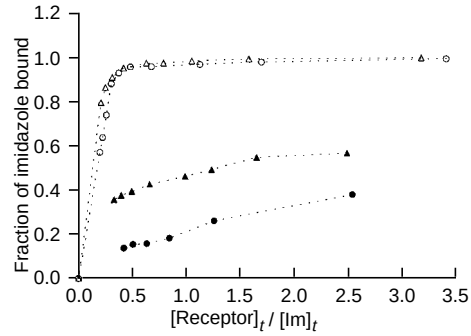


Fig. 3 Fraction of bound (●) **C** and (▲) **C′** in the competition experiments. The corresponding fractions bound in the titration experiments for (○) **C** and (△) **C'** are also plotted.

shown in Fig. 2. The turning of the titration curves at a 3 : 1 ratio of $[R]_t/[His]_t$ indicated a 3 : 1 stoichiometry of binding between **R** and **C** (or **C′**).[9]

For data analysis, the three metal ions were taken as interacting independently. Also shown in Fig. 2 are the calculated titration curves with the best-fit estimates of the binding constants. Non-linear regression analysis of the binding data following a previously-developed procedure[5] (SIGMA PLOT 4.0 for Windows, Jandel Scientific Inc.) provided the value of the binding constants (K_{RC} = 1.1 × 10^4 M^{-1}, $[R]_t$ = 10.25–6.5 mM; $[C]_t$ = 0–32 mM; $K_{RC'}$ = 10^4 M^{-1}, $[R]_t$ = 9.6–6.6 mM; $[C']_t$ = 0–32 mM; in both regressions, error: < 10%). The regression analysis converged to these numbers starting from either a smaller or a larger value as the initial estimate.

Ligand **L′** was also in fast exchange with the receptor (Fig. 2). The sharp turning of the titration curve at [**R**]/[**L′**] = 1 indicated a 1 : 1 stoichiometry of the complex and a high affinity. Due to the high affinity, only a lower limit of *K* can be estimated from the binding data ($K_{RL'} > 10^5$ M^{-1}).

Similar titration experiments (10 mM in **R**, 3–30 mM in **L**) showed that **R** interacts differently with **L** compared to **C**. The ligand **L** was found to be in slow exchange with **R**.[10] Two different C-2-H signals were observed for the free (δ 7.67) and bound (δ 8.605) ligand. The amounts of free **L** (measured by the integration of bound and free C-2-H resonances) were very small up to 1 : 1 stoichiometry and then the amount of free **L** increased rapidly. The aromatic hydrogens of **L** were shifted upfield by 0.8 ppm in the presence of the receptor **R**. These observations indicated that **R** is forming a 1 : 1 complex with **L** and that the benzene rings of **R** and **L** are stacking. This was corroborated by the observance of a cross-peak between the aromatic ring hydrogens of the two benzene rings of **R** and **L** in a NOESY spectra and by molecular modeling. The binding constant was estimated from the integration of bound and free signals of **L**.[11] Owing to inherent errors in the integration of very small peaks in ^{1}H NMR spectra, only a lower limit of K_{RL} can be obtained ($K_{RL} > 10^5$ M^{-1}).

In order to determine the binding selectivity of **R** and **L** (or **L′**) over **C** (or **C′**), competitive titration experiments were conducted (Fig. 3).[5] A 10 mM solution of **R**.**L** (or **R**.**L′**) was titrated with **C** or **C′** (1–30 mM). The C-2–H chemical shift of **C** (or **C′**) was followed to measure the concentration of **C** (or **C′**) bound to **R**. The fraction of **R** bound to **L** (or **L′**) compared to the fraction of **R** bound to **C** (or **C′**) was taken as the measure of selectivity. **R** was found to be selective for **L** compared to **C** by a factor of 20; its selectivity for **L′** over **C′** was 10. This difference in selectivity may be due to the difference in inter-imidazole distances of **L** and **L′** and the resultant strain in the complexes arising from non-optimal distance matching between the receptor and the ligand. We are currently probing this by synthesizing tris(histidines) with varying inter-imidazole distances.

Thus, the designed receptor **R** is indeed selective for pattern matched tris(histidine) ligand **L** compared to the control **C**. Studies are currently underway to optimize the selectivity by changing the spacer of the tris(histidine) ligand **L**.

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Notes and References

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§ All new compounds gave satisfactory characterization data. *Selected data* for **4** (*J* values in Hz): glassy solid (80%); mp 88–90 °C; $\delta_H(D_2O)$ 7.15 (s, 3H), 3.47 (s, 6H), 2.71–2.41 (m, 22H), 1.76–1.61 (m, 4H), 1.55–1.42 (m, 4H). HRMS (M^+) Calc. for $C_{39}H_{78}N_{12}$: 714.6472. Found: 714.6471. For **R**: white solid (88%); mp 165–167 °C; $\delta_H([^2H_6]$ DMSO): 7.09 (s, 3H), 5.36 (br s, 2H), 5.25 (br s, 2H), 4.58 (br s, 2H), 4.19 (d, 2H, *J* 15.0), 3.85 (d, 2H, *J* 15.0); the rest of the hydrogens appear as multiplets between 3.05–2.99, 2.88–2.83, 2.47–2.20, 1.90–1.77 and 1.72–1.67. Calc. for $C_{39}H_{78}N_{12}Hg_3(ClO_4)_6.3H_2O$: C, 23.80; H, 4.30; N, 8.54. Found: C, 24.01, H, 4.29; N, 8.51%. For **L**: white foamy solid; $\delta_H([^2H_6]$DMSO) 7.55 (s, 3H, Im-C_2H), 6.99 (s, 3H, Im-C_5H), 6.87 (s, 3H, Ar-H), 4.21 (3H, C_α-H), 4.17 (6H, $ArCH_2$), 2.80 (m, 6H, His- β-CH_2), 1.35(s, 27H, But); $\delta_C([^2H_6]$DMSO) 171.5, 155.2, 139.2, 134.7, 124.3, 78.1, 54.6, 42.1, 33.4, 28.2. HRMS M^+ Calc. for $C_{42}H_{60}N_{12}O_9$: 876.4605. Found: 876.4610. For **C**: white solid (85%); TLC (R_f 0.24, 3% MeOH–CH_2Cl_2); mp 150–152 °C; $[\alpha]_D^{21}$ +58 (MeOH, *c* 7.6); $\delta_H([^2H_6]$DMSO) 7.53 (s, 1H, Im-C_2H), 7.20 (m, 5H, Ar), 6.75 (s, 1H, Im-C_5H), 4.24 (s, 2H, $ArCH_2N$), 4.18 (m, 1H, C_α-H), 2.82 (m, 2H, His-β-CH_2), 1.35 (s, 9H, But); $\delta_C([^2H_6$DMSO) 171.6, 155.1, 139.4, 134.6, 128.1, 126.8, 126.5, 78.1, 54.6, 41.9, 28.1; HRMS (MH^+) Calc. for $C_{18}H_{24}N_4O_3$: 345.1926. Found 345.1911.

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