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ScienceDirect
Tetrahedron
Tetrahedron 62 (2006) 12191–12196

Synthesis and peptide-binding properties of a luminescent pyrimidine zinc(II) complex

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Received 12 September 2006; revised 7 October 2006; accepted 10 October 2006
Available online 27 October 2006

Abstract—The synthesis and peptide-binding properties of a Zn(II)nitrilotriacetate complex substituted with pyrimidine hydrazine amides are reported. The metal complex provides millimolar binding affinity in aqueous buffer to peptides bearing N-terminal His. The pyrimidine heterocycles intermolecularly interact with the bound peptide and quench the emission of nearby Trp residues by energy transfer.

1. Introduction

Reversible interactions of ions or molecules by hydrogen bonds, electrostatic or van der Waals interactions are the foundation of molecular recognition processes.[1] However, the strength of hydrogen bonds and electrostatic interactions decreases rapidly as the polarity of the surrounding solvent increases.[2] This hampers the binding of substrates such as peptides, hormones, or carbohydrates under physiological conditions, which is of interest for medicinal applications and the design of biosensors. The use of reversibly coordinating metal complexes as binding sites is a suitable alternative, which may provide high affinity in competitive solvents.[3] Recent examples showed the ability of suitable metal complexes for selective binding to peptides[3c] and protein surface epitopes[4,5] under physiological conditions. Such synthetic receptors find use as bioanalytical probes[6] or markers[7] or can interfere with protein function, e.g., inhibiting enzyme activity[8] or protein–protein interactions.[9] We report here the use of a functionalized zinc(II) nitrilotriacetato (NTA) complex to label small peptides with pyrimidine hydrazino amides. Fluorescence resonance energy transfer (FRET)[10] from nearby Trp residues sensitizes an emission of the heteroaromatic pyrimidine ring.

2. Results and discussion

2.1. Synthesis

Various transition metal ion (e.g., Cu^{2+}, Ni^{2+}, and Zn^{2+}) complexes of NTA or IDA[11] bind to the imidazole side chains of surface exposed histidines of proteins.[12] This coordinative interaction is widely used for protein purification by immobilized metal affinity chromatography (IMAC)[13,14] and two-dimensional protein crystallization.[15] The dependence of the NTA binding constant on the divalent metal in $[M(NTA)]^-$ ($M=Mn^{2+}$, Co^{2+}, Ni^{2+}, Cu^{2+}, and Zn^{2+}) has been intensively studied.[16] Although Ni^{2+} or Cu^{2+} NTA complexes show higher affinities to N-terminal His,[12] a Zn^{2+} complex[17,18] was chosen for peptide binding to obtain a diamagnetic compound, which allows NMR investigations. The synthesis of the peptide-binding Zn(II)–pyrimidine complex **6** is shown in Figure 1. As spacer between the complex and the heteroarene we choose a Gly unit to assist the possible formation of a hydrogen bond to a coordinated peptide. Compound **1**,[19] obtained from lysine methyl ester, is coupled to Boc-Gly-OH. After Boc deprotection, heterocycle **3**, which was reported recently,[3b] was introduced by standard peptide coupling procedures. Cleavage of the methyl ester under basic conditions generates the NTA ligand and complexation with Zn^{2+} leads to the desired functionalized complex **5**. To improve water solubility, the analogous complex **9**, extended by one pyrimidine hydrazine unit, was prepared (Fig. 2).

2.2. Structure

To derive structural information about the binding motif of **5** to the pentapeptide NH_2-His-Leu-Leu-Val-Phe-OMe

Keywords: Metal complex; Peptide binding; Histidine; Luminescence; Pyrimidine.
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doi:10.1016/j.tet.2006.10.019

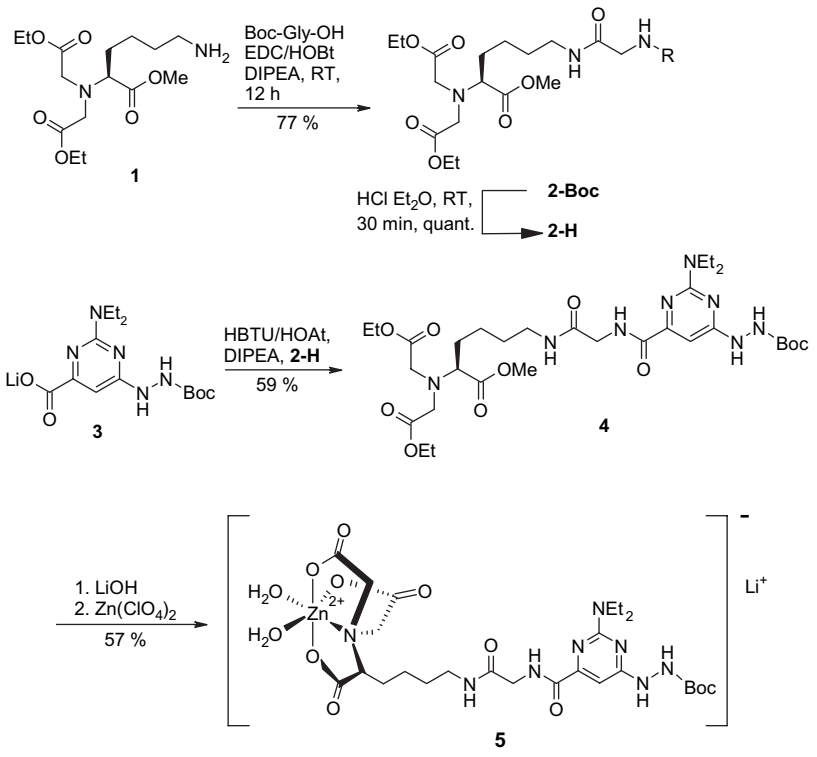

Figure 1. Synthesis of zinc(II)-NTA pyrimidine complex **5**.

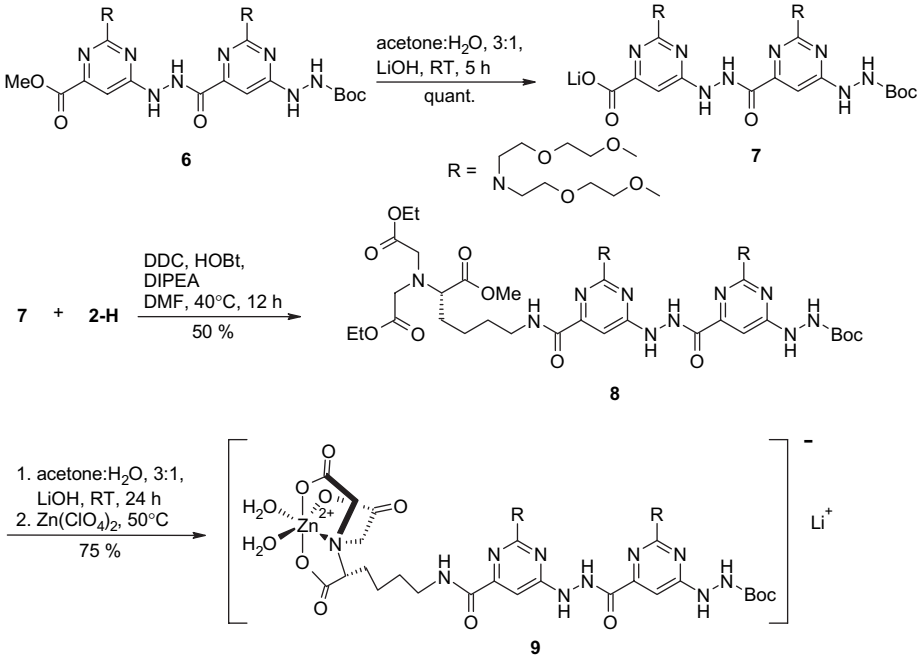

Figure 2. Synthesis of water-soluble zinc(II)-NTA pyrimidine complex **9**.

(Fig. 3) NMR experiments in DMSO-d_6 were performed.[20] Resonance signals of the NMR spectra of **5**—H-His-Leu-Leu-Val-Phe-OMe (c=3.3×10^{-2} M) were assigned (see Supplementary data, Fig. S-1 for details) and temperature-induced shift was used to identify hydrogen bonding of NH groups (see Supplementary data, Tables S-1, S-2 and Fig. S-2).[21] Shifts larger than −2 ppb/K typically indicate a strong interaction, while values smaller than −4 ppb/K show solvent exposed atoms.[22] The smallest ppb/K value (−2.57 ppb/K) in the aggregates spectrum was obtained for NH-**C**. This proton is most likely hydrogen bound to both the lone pair of the oxygen atom of the amide bond and the lone pair of the nitrogen atom in the pyrimidine ring. The temperature dependent shift of −2.95 ppb/K of NH-**G** indicates a hydrogen bond between peptide and complex. All other temperature dependent shifts of