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Synthetic peptides caged on histidine residues with a bisbipyridyl ruthenium(II) complex that can be photolyzed by visible light†

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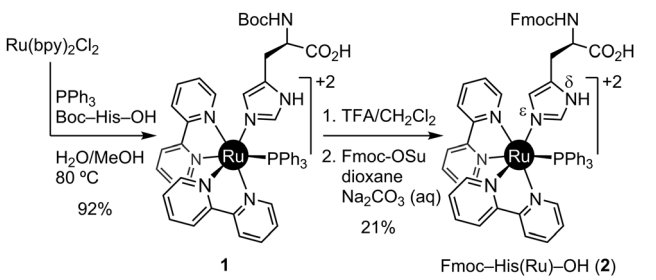
We report a light-sensitive histidine building block for Fmoc/*t*Bu solid-phase peptide synthesis in which the imidazole side chain is coordinated to a ruthenium complex. We have applied this building block for the synthesis of caged-histidine peptides that can be readily deprotected by irradiation with visible light, and demonstrated the application of this approach for the photocontrol of the activity of Ni(II)-dependent peptide nucleases.

Caged peptides are bioactive species that include a photocleavable protecting group masking a key functionality required for their action. Photolysis of the caging group releases the effector peptide,[1] thus providing researchers with spatial and temporal control over biological processes.[2,3] Peptides can be caged by modifications in their backbone,[4] or by introduction of photolabile groups in specific amino acid side chains, including amines and carboxylates in Lys or Asp/Glu residues, thiols in cysteines, or hydroxyl groups in Ser, Thr and Tyr.[5] Oddly enough, the photocontrol of biological processes with caged histidine peptides has not yet been described.[6] This constitutes a significant gap in caging technology because histidine, although relatively uncommon in protein sequences (<2.5%), is a highly versatile amino acid that plays key roles in the activity of many peptides and proteins, acting as an aromatic residue, a hydrogen bond donor or acceptor, or as a coordinating ligand,[7] and can even suffer posttranslational modifications.[8] Therefore, given the functional plasticity and biochemical relevance of this amino acid it would be highly relevant to develop a practical method for the synthesis of caged histidine peptide derivatives.

Most peptide caging approaches developed so far rely on the use of *o*-nitrobenzyl groups as photosensitive cleavable units.[1,9] However, despite their wide range of applications, these caging groups are not particularly suited for biological studies, because they require irradiation with harmful short-wavelength UV light for photolysis (about 365 nm).[10] Therefore, there has been a great interest in the development of substitute long-wavelength sensitive caging groups.[11,12] In this context, photolabile bisbipyridyl ruthenium(II) complexes have been explored as alternative caging groups with promising spectroscopic properties (*i.e.* a long photolysis wavelength and high uncaging quantum yields).[13,14] With these premises, we decided to explore the application of ruthenium(II) bipyridyl complexes as photolabile protecting groups for caging histidine residues, anticipating that the coordination of the imidazole side chain with these complexes should effectively impair any peptide requiring the free imidazole for its activity. In addition to the spectroscopic advantages afforded by the use of Ru(II) complexes as caging groups, relying on the coordination of the pros nitrogen (N^{ε}, Scheme 1) of the imidazole would also avoid potential synthetic problems related with the known tendency of N^{δ} to N^{α} acyl transfer during peptide elongation,[15] as well as effectively block the metal-coordinating nitrogen in the imidazole side-chain.

The caged histidine building block, Fmoc–His(Ru)–OH (**2**), was efficiently synthesized by the two-step process outlined in Scheme 1. In short, the commercially available *cis*-bis(2,2′-bipyridine)dichloro ruthenium(II) complex ($Ru(bpy)_2Cl_2$) was treated with triphenylphosphine and then with Boc–His–OH



Scheme 1 Synthesis of Fmoc–His(Ru)–OH.

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in a one-pot reaction to yield the Boc-protected intermediate **1**. Removal of the Boc protecting group with trifluoroacetic acid followed by installation of the Fmoc group with 9-fluorenylmethyl *N*-succinimidyl carbonate (Fmoc–OSu), afforded the desired Fmoc–His(Ru)–OH building block. The synthesis of the trimethylphosphine analog of **2** was also attempted following the same set of transformations,[16] but deprotection of the Boc intermediate with TFA resulted in partial decomplexation of the histidine building block, which led us to focus our studies on the more stable triphenylphosphine derivative.

Before its incorporation into peptides, we determined the uncaging quantum yield of the Boc–His(Ru)–OH building block, which was obtained by comparing its photolysis rate with that of $[Ru(bpy)_2PPh_3\text{–}GABA]^+$ upon irradiation using a 455 nm LED source (see the ESI†).[13c,17] The resulting uncaging quantum yield ($\Phi_{unc} \approx 0.06$) is comparable to the photolysis efficiency reported for other Ru(II)-photolabile compounds,[13c,18] and of most organic cages.[1d] In addition to the expected uncaging of the histidine side chain and release of the side chain-deprotected Boc–His–OH, the HPLC analysis also showed the competitive cleavage of the PPh_3 ligand as a minor side reaction (≈5%), as well as peaks indicating the formation of $[Ru(bpy)_2PPh_3(MeCN)]^{+2}$ and $[Ru(bpy)_2PPh_3(TFA)]^+$ complexes, possibly resulting from the reaction of the $[Ru(bpy)_2PPh_3(H_2O)]^{+2}$ photolysis byproduct with the HPLC solvent system (see ESI†).[19] The stability of the ruthenium cage in the presence of various potentially reactive species under physiological conditions (*e.g.* H_2O_2, histidine, glutathione), or competitive ions, such as nickel(II), was confirmed by HPLC after 24 h incubation (see the ESI†).

With the desired building block in hand and having successfully demonstrated its photolabile properties, we tested its integration in standard solid phase peptide synthesis (SPPS) protocols by synthesizing a series of test peptides. Coupling of the caged histidine building block was conducted in all cases using 5 equivalents of the Fmoc–His(Ru)–OH building block, and a mixture of HATU/HOAt (5 eq.) and DIEA (6 eq.) as base in DMF. The stereochemical integrity of the caged residue is maintained under these conditions, optimized to avoid epimerization of the Cα stereocenter (see ESI†). Cleavage of the resulting peptides with a standard acidic TFA cocktail (TFA : CH_2Cl_2 : triisopropylsilane : H_2O: 90 : 5 : 2.5 : 2.5),[20] afforded in all cases the expected caged His peptides as major components in the crude samples (Fig. 1, left); only the synthesis of the longer peptide was problematic after the 15th coupling, resulting in the appearance of significant secondary products, although the desired peptide was obtained as the major product of the synthesis (Fig. 1, left, trace c).

Irradiation of the purified peptides with visible light resulted in all cases in complete uncaging and liberation of the unprotected parent peptides, as well as formation of the ruthenium photobyproducts (Fig. 1 right, and ESI†). Furthermore, in contrast with the preliminary studies with the Boc–His(Ru)–OH building block, no photodissociation of the PPh_3 group was observed in the uncaging of the peptides.

As a simple model system in which the newly developed photolabile histidine building block is applied, we focused our attention on the Arg–Gly–His tripeptide (RGH), which has been described as an efficient metal-chelating sequence with DNA binding and endonuclease properties in the presence of Ni(II) ions and oxidizing agents.[21] Considering that the imidazole group in the histidine side chain is required for chelation of the Ni(II) ion, we reasoned that a caged histidine analog (©RGH) should be unable to coordinate the metal ion and form the catalytic metallopeptide. Furthermore, its nuclease activity should be recovered upon irradiation and uncaging of the histidine residue (Fig. 2).

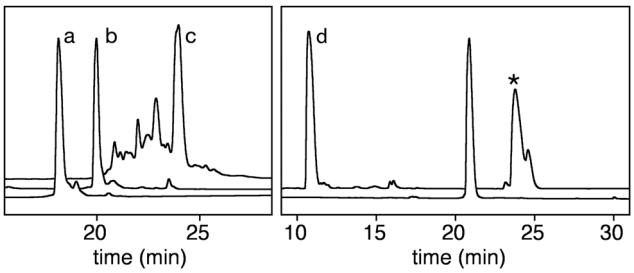

Fig. 1 Left: normalized HPLC traces at 220 nm of crude mixtures resulting from the automated synthesis of test peptides. Purity (in brackets) was estimated from the area of the HPLC peaks. (a) HAKAEAEAKAK (86%); (b) WLAHKYLQGGC (92%); (c) LFQFLGKIIHHVGNFVHGFSHVF (46%). Right: representative peptide uncaging; bottom trace: crude peptide YEGKH-SAEWG upper trace: HPLC after irradiation of the purified peptide, showing the uncaged peptide (d) and the ruthenium photolysis byproducts (*). H represents the caged histidine.

Once we synthesized the ©RGH peptide following the procedures described before, we studied its uncaging: irradiation of a 10 μM solution of ©RGH in Na-cacodylate buffer at pH 7.5 for 1 min using a 455 nm LED source results in quantitative photolysis of the caged ©RGH peptide as shown by HPLC (Fig. 3 left, top trace); in addition to the peak corresponding to the uncaged peptide (RGH) that is eluted with the injection peak, we also observed the ruthenium complexes arising from the reaction of the photolyzed $[Ru(bpy)_2PPh_3(H_2O)]^{+2}$ with the HPLC solvent system (Fig. 3 left, top trace, peaks labeled with an asterisk). We next examined whether the uncaging event

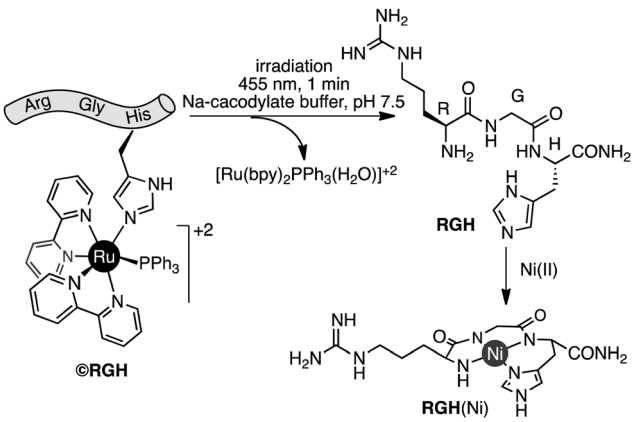

Fig. 2 Uncaging of the ©RGH peptide yields the metal-chelating RGH tripeptide, which displays nuclease activity in the form of a Ni(II) complex, RGH(Ni).

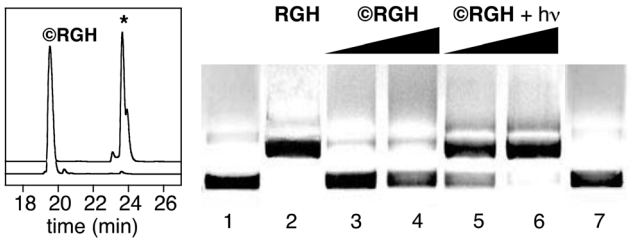

Fig. 3 Left: uncaging of ©RGH monitored by HPLC. Bottom trace: caged peptide before photolysis; top trace: the photolyzed mixture showing the complete disappearance of the caged peptide, and the formation of the ruthenium byproducts (*); the uncaged peptide is eluted with the injection peak (not shown). Right: nuclease activity of ©RGH is monitored by 1% agarose gel electrophoresis (see main text for reaction conditions). Lanes 1–7: 27.6 μg mL^{-1} of pcDNA 3.1 Neo plasmid; lane 2: 10 μM of RGH and $Ni(ClO_4)_2$; lanes 3 and 4: 7.5 and 10 μM of ©RGH and $Ni(ClO_4)_2$; lanes 5 and 6: 7.5 and 10 μM of ©RGH and $Ni(ClO_4)_2$ after photolysis; lane 7: Fmoc–His(Ru)–OH (10 μM) and $Ni(ClO_4)_2$ after photolysis. Photolysis was carried out before addition of $KHSO_5$ by irradiation at 455 nm for 1 min in the presence of the plasmid.

could trigger the nuclease activity of the ©RGH–RGH(Ni) system. Towards this end we incubated the pcDNA 3.1 Neo plasmid (as DNA substrate) with a mixture of 10 μM RGH and $Ni(ClO_4)_2$ and 100 μM $KHSO_5$ in Na-cacodylate buffer at pH 7.5 and 20 °C for 15 min, and analyzed the resulting mixture by agarose electrophoresis. As expected, the band corresponding to the supercoiled DNA (Fig. 3, lane 1) is completely converted to a slower-migrating band, consistent with the formation of the nicked-circular form of the DNA (Fig. 3, lane 2). In contrast, the caged version of the peptide (©RGH) does not display nuclease activity under the same conditions (Fig. 3, lanes 3 and 4). However, irradiation of ©RGH in the presence of the plasmid with visible light for just 1 min allowed the recovery of the nuclease activity, and the degradation of the DNA (Fig. 3, lanes 5 and 6). No degradation of the DNA band is observed in the control experiment in which the amino acid Fmoc–His(Ru)–OH is irradiated under the same conditions (Fig. 3, lane 7), which confirms that the nuclease activity arises from the tripeptide Ni(II) complex, and not from the ruthenium complex or its photobyproducts (see the ESI†).

In summary, we describe the first effective caged histidine building block and its incorporation into peptides using standard Fmoc/*t*Bu SPPS protocols. In contrast with common UV-sensitive *o*-nitrobenzyl groups, the photolabile Ru(II) bisbipyridyl complex can be efficiently removed using visible light. The potential of this approach was illustrated by controlling a metallopeptide nuclease, but it could be readily extended to other histidine-mediated interactions.

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