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Fluorogen-based amino acids with Aggregation-Induced Emission features for bioprobes design

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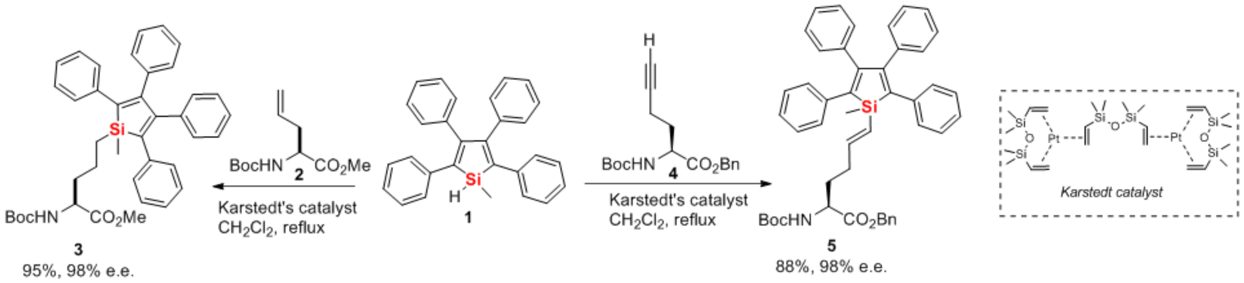
Introduction

Fluorescently labelled peptides are widely used as tools for molecular imaging to provide biological information on living systems. However, most of the conventional organic fluorophores used for such purpose aggregate when dispersed in aqueous media.[1] This aggregation generally implies a self-quenching and thus, a drastic decrease of the fluorescence ("turn-off"), which is a thorny problem for developing efficient bioprobes. Luminophores with Aggregation-Induced Emission (AIE) features are found to be weakly or not fluorescent when molecularly dissolved but highly fluorescent when aggregated.[2] Although fluorophores displaying AIE-effects are increasingly used in sensing and bioimaging studies,[3] they generally suffer from a lack of selectivity. To develop highly specific AIE fluorogen-based bioprobes, we decided to exploit the previous work of our laboratory on silylated amino acids[4] and on hydrosilylation[5, 6] in view of generating silole amino acids and peptides to investigate their fluorescent properties.

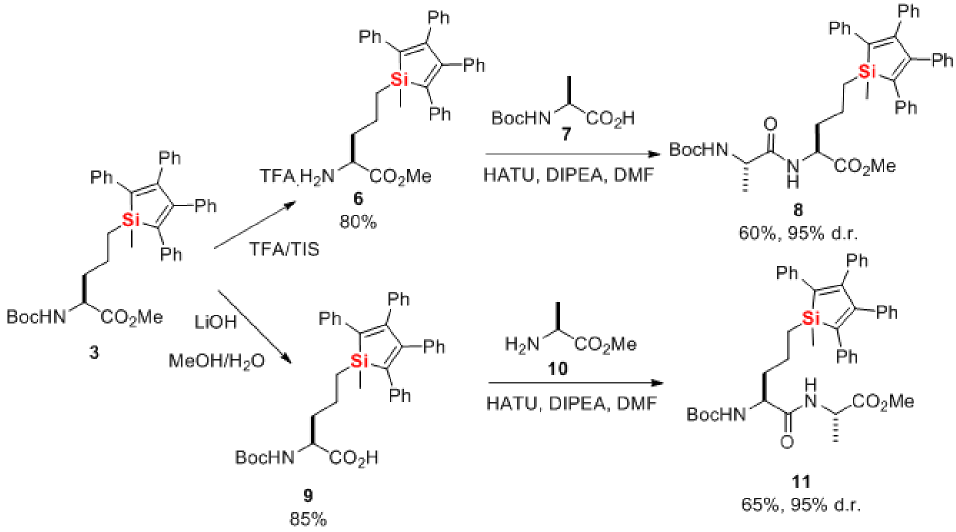
Results and Discussion

The synthesis of silole amino acids was achieved by hydrosilylation of protected amino acids 2 and 4 bearing alkene or alkyne on side chains. Among the different catalysts tested, Karstedt's revealed to be the most efficient and silole amino acids 3 and 5 were isolated with very good yields of 95 and 88%. Completion of the hydrosilylation was inferred from IR spectroscopy since the Si-H band of the silole 1 at $\nu = 2118$ cm^{-1} disappeared in the IR spectrum of 3. The optical purities of 3 and 5 were verified on HPLC using a chiral column by comparison with racemic samples. Formation of silole amino acid 3 was also confirmed by 1H, 13C{1H}, 29Si{1H} NMR spectroscopy in $CDCl_3$. Effectively, a clear difference is observed between the chemical shift of Si-H precursor 3 ($\delta = -11$ ppm) and the products of hydrosilylation 3 ($\delta = -17$ ppm) and 5 ($\delta = -2$ ppm).



Scheme 1: Hydrosilylation of alkene 2 and alkyne 5 on amino acids side chains

The fully protected silole amino acid 3 was selectively N- and C- deprotected in order to prove the coupling feasibility (Scheme 2). The amine function was deprotected under acidic conditions (TFA) to afford the corresponding ammonium 6 in 80% yield. The methyl ester was saponified with lithium hydroxide to afford the free carboxylic acid 9 in 85% yield. The peptide coupling of 6 and 9 was achieved under classical conditions (HATU, DIPEA, DMF) with alanine moiety 7 and 10 as model. A tripeptide with two alanine residues was also synthesized by SPPS on Wang resin starting using the corresponding N-Fmoc protected silole amino acid.



Scheme 2: Synthesis of silole dipeptides

As shown in Figure 1 for compound 3, when fw varies from 0 to 60 vol %, the fluorescence remains very low. A dramatic increase in the fluorescence was noted after the water fraction reached 70 and 80 vol %, typical from AIE fluorogens leading to I_{agg}/I_{THF} ~6 (except for 5, I_{agg}/I_{THF} ~44).

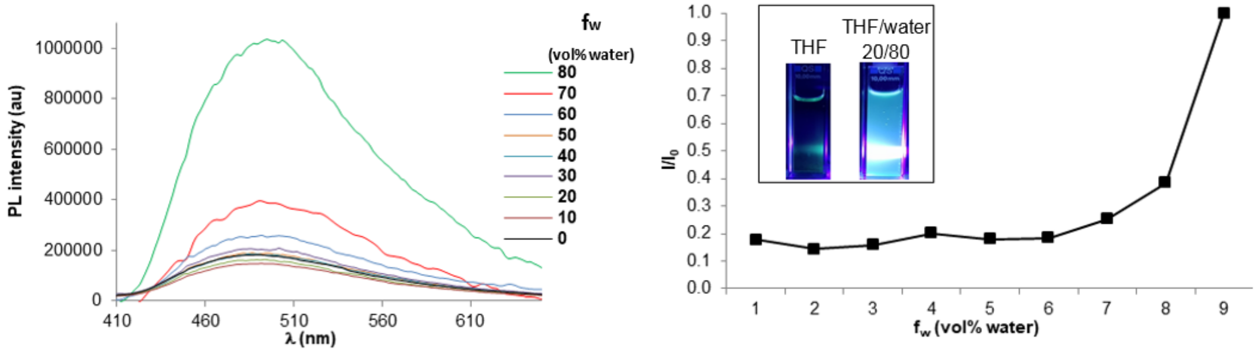


Figure 1: Emission spectra of 3 in THF/water mixtures with different water fractions (fw) (Concentration: 5.10-4M, Excitation wavelength: 360 nm) (left). Plot of I/I0 values vs. water fractions. I0 is the PL intensity for the highest fw. Inset: photos of silole 4 under the illumination of a UV lamp at 405 nm in THF/water mixture (fw = 0 and 80%) (right).

New silole amino acids were synthesized by hydrosilylation of alkyne or alkene amino acids, catalyzed by Karstedt platinum complex. After selective deprotection of carboxylic function or amine, C- or N- peptide coupling in solution and on SPPS proved the possible incorporation into peptides. Such silole amino acids and peptides exhibit AIE properties with δem around 500 nm and $\Delta\delta$ ~100 nm. The quantum yield for the aggregated state is 5 to 10 times higher than in solution. They constitute a new class of promising fluorophores for the development of labelled peptides.

Acknowledgements

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References

[1] Birks J., Photophysics of Aromatic Molecules, Wiley, London 1970.

[2] Mei J., Legung N., Kwok R., La, J., Tang B., Chem. Rev., 2015, 115, 11718.

[3] Ding D., Li K., Liu B., Tang B., Acc. Chem. Res., 2013, 46, 2441.

[4] Rémond, E., Martin, C., Martinez, J., Cavelier, F. Chem. Rev., 2016, 116, 11654.

[5] a) Marchand D., Martinez J., Cavelier F., EurJOC, 2008, 3107. b) Cavelier, F.; Marchand, D.; Martinez, J. Chem. Biodiversity, 2008, 5, 1279.

[6] Fanelli R., Berthomieu D., Didierjean C., Doudouh A., Lebrun, A., Martinez J., Cavelier F., Org. Lett., 2017, 19, 2937.