https://doi.org/10.17952/37EPS.2024.P2022

Department of Chemistry - Organic and Bioorganic Chemistry - OCIII

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Fluorescent Amino Acids with large STOKES Shift

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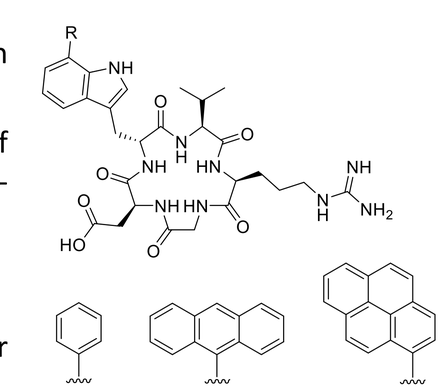
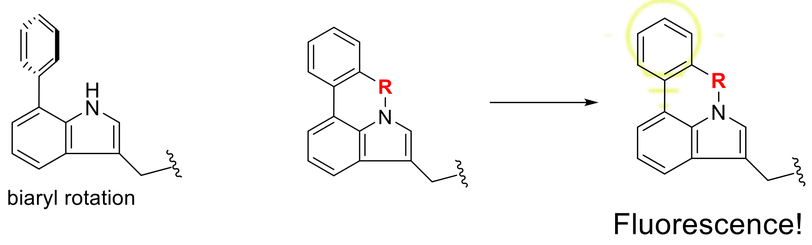
Introduction

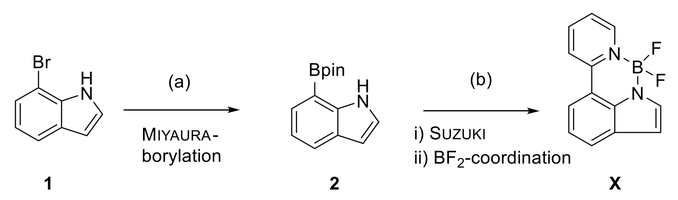
- Classic method for fluorescent peptide modification uses active esters or isothiocyanates
- Recently fluorescent modification of bromotryptophan containing peptides by cross-couplings has been demonstrated[1]
- Biaryl coupling leads to an extension of π-system
- Excitation wavelength does not shift as expected for π-system extension
- This hints to biaryl rotation around formed σ-bond
- For 7-bromotryptophan this can be suppressed by cyclisation with the indole-nitrogen





Dye Synthesis

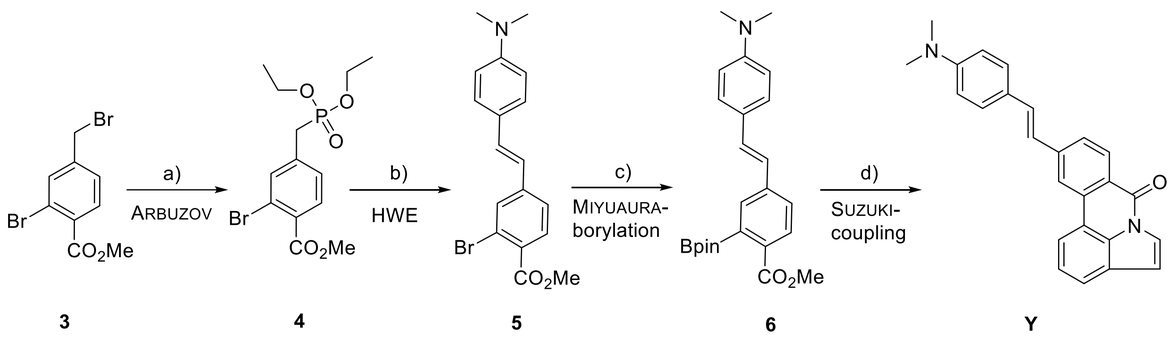
Bor Coordination



λ_{ex}: max 360 nm (DCM)
till 420 nm excitation possible
λ_{em}: max 490/520 nm
Φ < 0.27 (DCM)

Scheme 1: (a) B_2pin_2, KOAc, $PdCl_2(dppf)$, dioxane, 92 °C (b) i) 2-Bromopyridine, $PdCl_2(dppf)$, K_2CO_3, dioxane/H_2O, 90 °C ii) DIPEA, $BF_3{\cdot}OEt_2$, DCM, rt.

Lactam Cyclisation



λ_{ex}: max 415 nm (MeOH)
till 450 nm excitation possible
λ_{em}: max 650 nm
Φ =0.28 (MeOH)

Scheme 2: (a) $P(OEt)_3$, dioxane, 100 °C. (b) *p*-dimethylaminobenzaldehyde, NaH, THF, 0 °C -> rt. (c) $PdCl_2(dppf)$, B_2pin_2, KOAc, dioxane, 90 °C. (d) 7-Br-indole, $PdCl_2(dppf)$, K_2CO_3, DME/MeOH, 90 °C.

Fluorescence Spectra

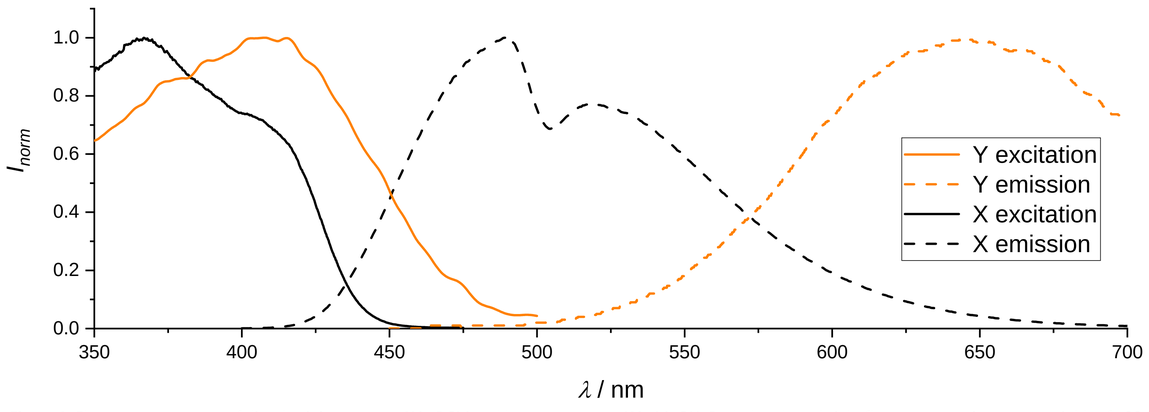
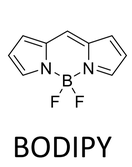


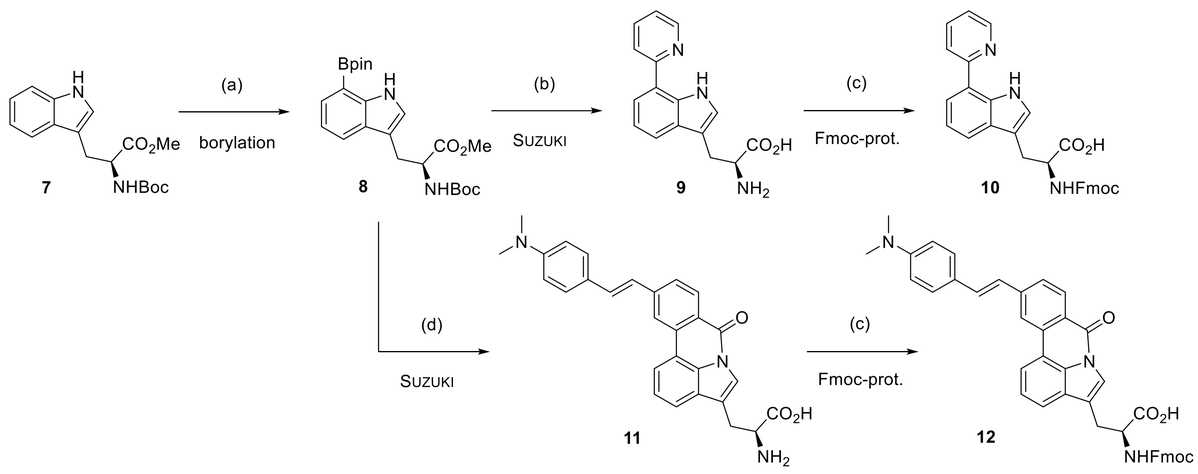
Figure 1: Fluorescence spectra of diazaborinin-structure **X** in DCM and lactam-structure **Y** in MeOH. Both dyes have bright fluorescence mit quantum yields of ~28 % with STOKES-shifts of 130 and 220 nm.

Amino Acid Modification

- 7-borylated Trp is accessable via Ir-catalysed C-H borylation[2]
- Since other diazaborinin-structures (BODPIY) are known to be unstable during peptide synthesis, bor coordination is the last step

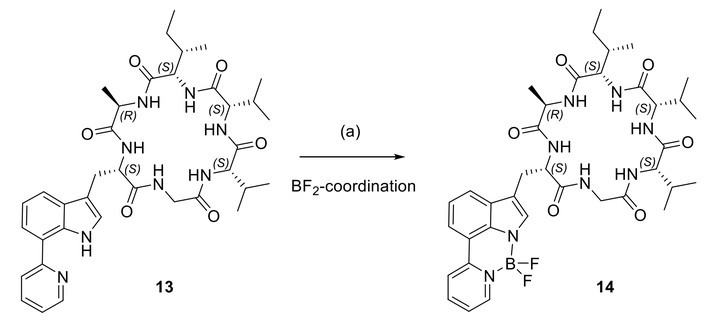


BODIPY



Scheme 3: (a) i) $[Ir(cod)OMe]_2$, dtbpy, HBpin, THF, 60 °C ii) $Pd(OAc)_2$, AcOH, 70 °C. (b) i) 2-Bromopyridine, $PdCl_2(dppf)$, K_2CO_3, dioxane/H_2O, 90 °C. ii) TFA/TIS/H_2O. (c) Fmoc-OSu, Na_2CO_3, ACN/H_2O. (d) **5**, $PdCl_2(dppf)$, K_2CO_3, DME/MeOH, 90 °C.

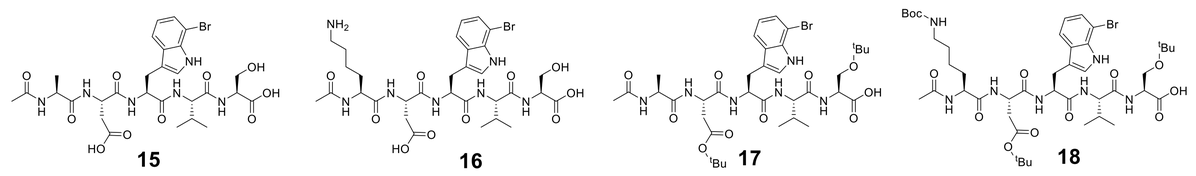
Amyloid-affine Peptide Modified with new Dye



Scheme 4: (a) $BF_3{\cdot}OEt_2$, DIPEA, 1,4-dioxane, rt.

Late Stage Peptide Modification: Lactam

- Late stage SUZUKI-couplings with peptides are well known in the literature[1,3]
- To prevent saponification of ester **6** water-free conditions are necessary
- Lactam cyclisation reaction needs temperature of min. 90°C
- Due to peptide solubility, solvent was changed to DMF/MeOH
- Based on DACHWITZ *et al.,* pentapeptides for test reactions have been synthesized[3]



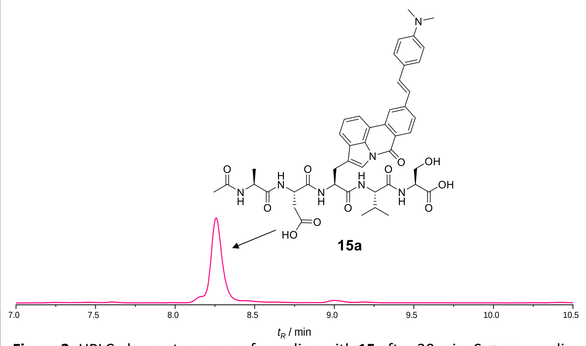


Figure 2: HPLC-chromatogramm of coupling with **15** after 30 min: SUZUKI-coupling with free aspartic acid leads to desired product

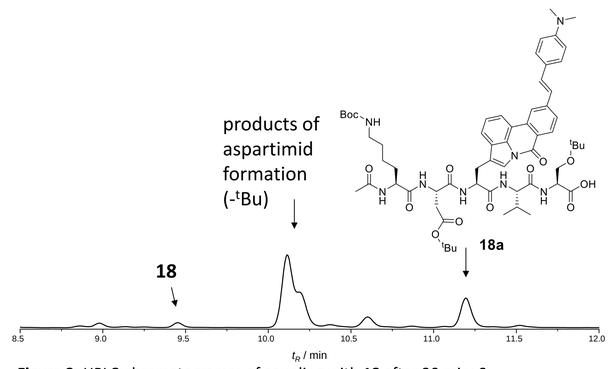


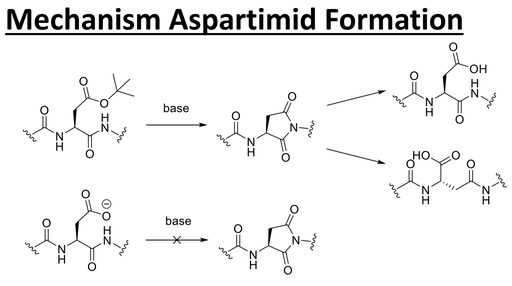
Figure 3: HPLC-chromatogramm of coupling with **18** after 30 min: SUZUKI-coupling protected aspartic acid leads to aspartimid formation, proven by a shouldered peak with am mass $[M-{}^tBu+H]^+$

- Coupling with free amine (peptide **16**) showed bad conversion

Conclusion:

- Coupling of amines needs Boc-protection
- Deprotection of Asp prevents aspartimid formation

Mechanism Aspartimid Formation



References

[1] I. Kemker, D. C. Schröder, R. C. Feiner, K. M. Müller, A. Marion, N. Sewald, *J. Med. Chem.* **2021**, *64*, 586.
[2] R. P. Loach, O. S. Fenton, K. Amaike, D. S. Siegel, E. Ozkal, M. Movassaghi, *J. Org. Chem.* **2014**, *79*, 11254.
[3] S. Dachwitz, D. H. Duwe, Y. H. Wang, H. Gruß, Y. Hannappel, T. Hellweg, N. Sewald, *Chem. Eur. J.* **2020**, *26,* 16357.