Synthesis and Application of B-N Containing Tryptophan

2021.6.12. Literature Session

M1 Yosuke Nakata

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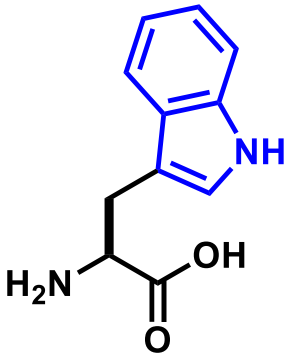
Tryptophan : Characteristic Residue of Protein



L-tryptophan

Natural amino acid possessing indole ring

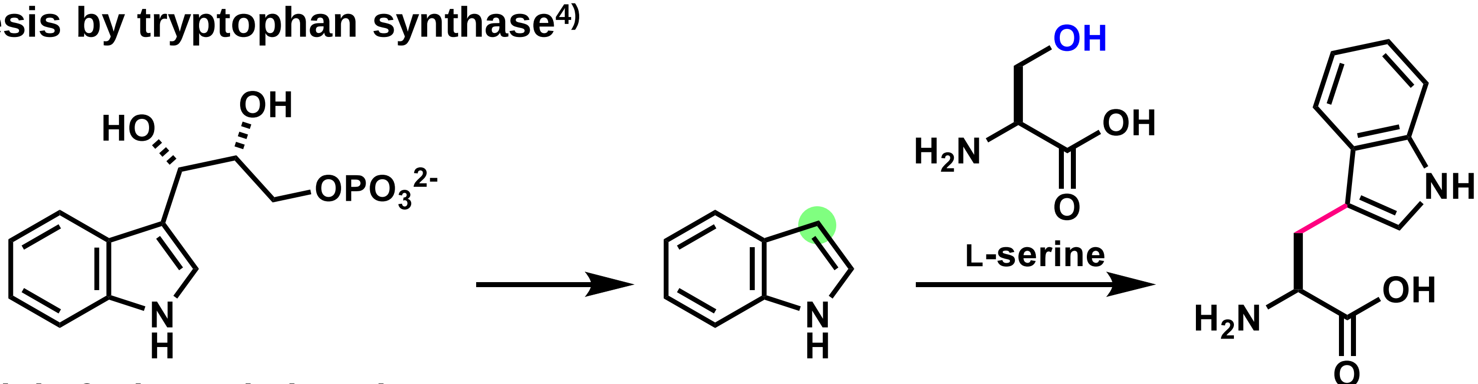
- intrinsic fluorescence[1)]
  λ_{em}=348 nm (Phe : λ_{em}=282 nm, Tyr : λ_{em}=303 nm)

- electron rich aromatic ring[2)]
  π-interaction, high redox potential

Relatively rare amino acid

…1% of amino acids in found protein[3)]

Biosynthesis by tryptophan synthase[4)]



indole-3-glycerol phosphate (from shikimic acid pathway)

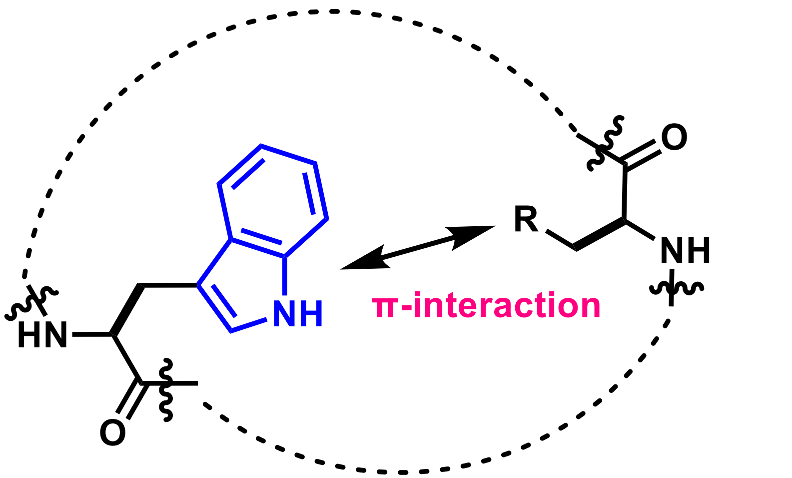
indole

L-tryptophan

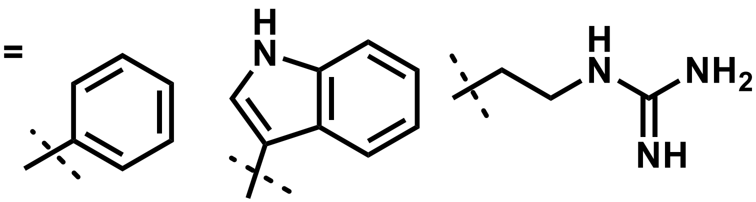
1) Teale, J. W. F.; Weber, G. *Biochme. J.* **1957**, *65*, 476.
2) (*a*) Ruan, C.; Rodgers, T. M. *J. Am. Chem. Soc*. **2004**, *126*, 14600. (*b*) Dougherty, A. D. *J. Nutr.* **2007**, *137*, 1504S. (*c*) Dougherty, A. D. *J. Org. Chem.* ***2008***, *73*, 3667. (*d*) Burley, K. S.; Petsko, A. G. *Science.* **1985**, *229*, 23.
3) McCaul, P. C.; Ludescher, D. R. *Photochem. Photobiol.* **1999**, *70*, 166.
4) Dunn, M. F.; Schlichting, I.; Barends, R. T. *Curr. Opin. Chem. Biol.* **2008**, *12*, 593.

Biological Function and Application of Trp

stabilize peptides by π-interaction[1)]

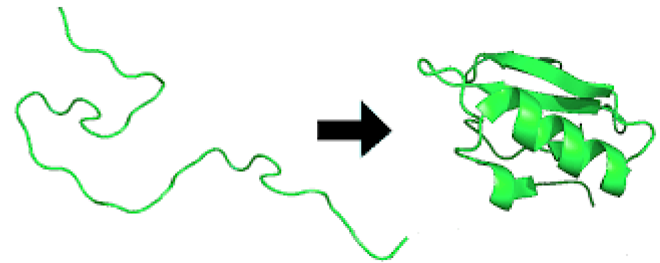


R =



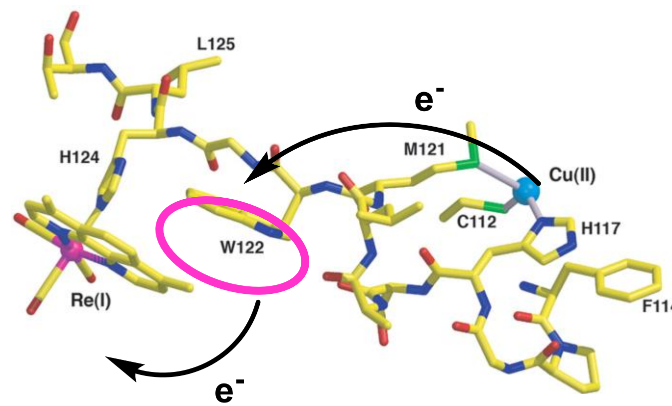
Phe	Trp	Arg	
π-π, NH-π	π-π, NH-π	NH-π	...etc

Probe protein folding[2)]



Wavelength or total emission of fluorescence changes by protein folding.

Accelerate electron transfer[3)]



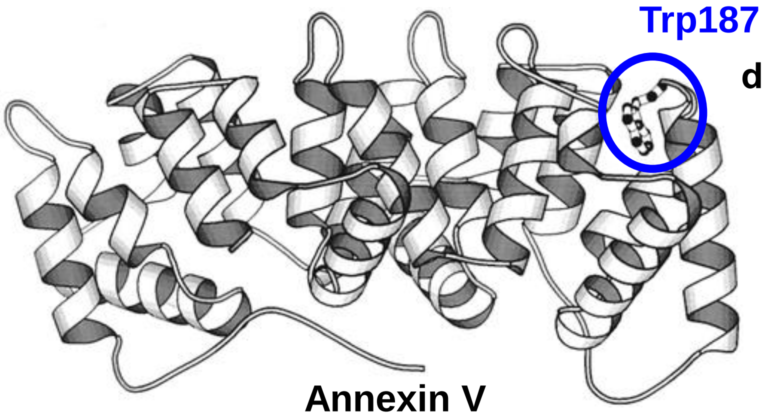
Trp plays a very important role in peptides and proteins despite its rarity.

⇓

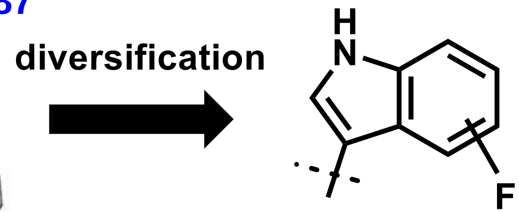
Useful target for protein study using unnatural amino acids (UAAs)

1) Santiveri, M. C.; Jiménez, A. M. *Biopolymers.* **2010**, *94*, 779.
2) Royer, A. C. *Chem, Rev.* **2006**, *106*, 1769.
3) Shih, C.; Museth, K. A.; Abrahamsson, M.; Blanco-Rodriguez, M. A.; DiBilio, J. A.; Sudhamsu, J.; Crane, R. B.; Ronayne, L. K.; Towrie, M.; Vlcek, A. Jr.; Richards, H. J.; Winkler, R. J.; Gray, B. H. *Science.* **2008**, *320*, 1760.

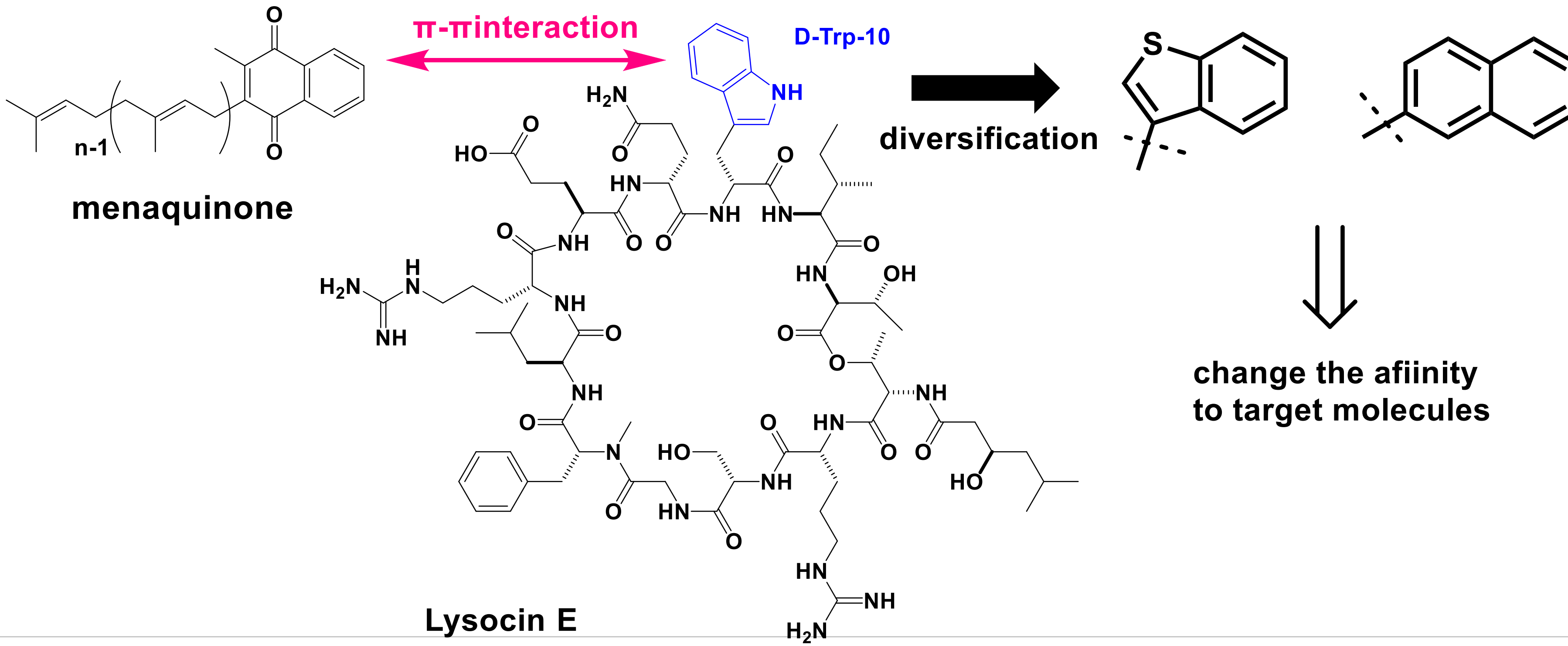
Application of UAAs to Trp residues



Annexin V
(biomarker of apoptosis)



change [1)]
- fluorescence
- biological activity



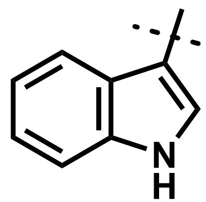
⇓

change the afiinity to target molecules

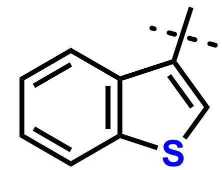
1) Minks, C.; Huber, R.; Moroder, L.; Budisa, N. *Biochemistry*, **1999**, *38*, 10649.

New Chemical Space for UAAs

Trp alteration

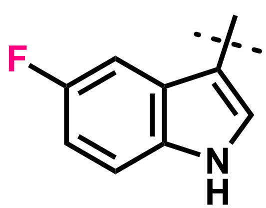


electron rich →



○stronger π-interaction
×blue shift (overlap with Tyr or Phe)

electron deficient ↓



○red shift
×low incorporation efficiency[1)]

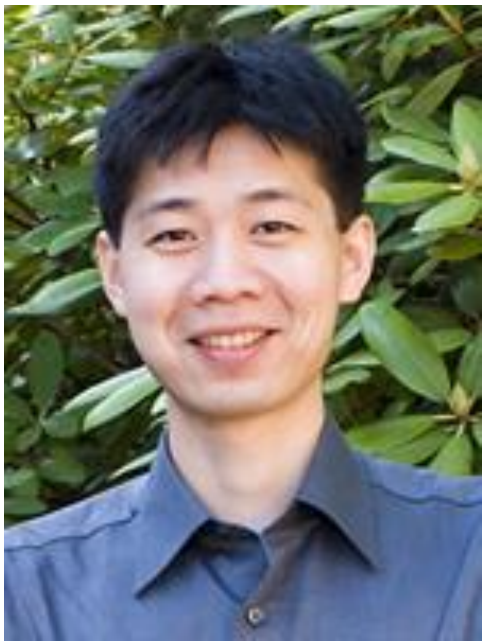
Trp analogues which have
・similar steric profiles
・red shifted fluorescence
Explored these study

1) Pratt, A. E.; Ho, C. *Biochemistry*, **1975**, *14*, 3035.

Contents

Author's Profile[1)]

Prof. Shin-Yuan Liu



Research Career

1995-1997 1st diploma in chemistry @Vienna University of Technology

1998-2003 Ph.D. in organic chemistry @Massachusetts Institute of Technology (Prof. Gregory C. Fu)

2003-2006 Postdoctoral fellow @Massachusetts Institute of Technology (Prof. Daniel G. Nocera)

2006-2012 Assistant Professor @University of Oregon

2012-2013 Associate Professor @University of Oregon

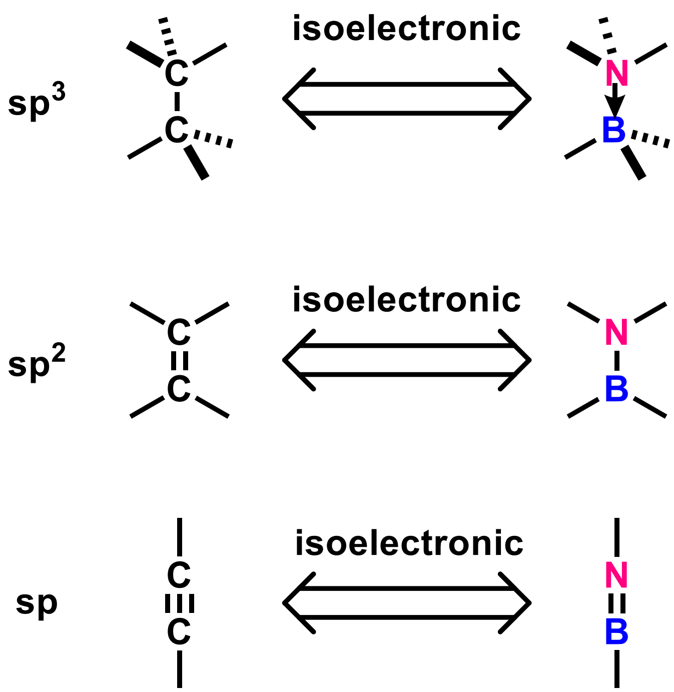
2013- Full Professor @Boston College

Research Interests:

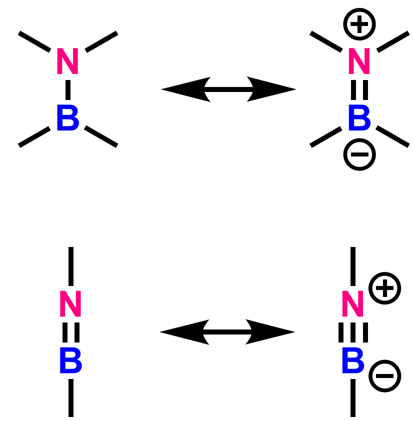
Development of boron(B)-nitrogen(N)-containing heterocycles, specifically azaborines.

1) http://capricorn.bc.edu/lsy/public_html/liu.html

BN/CC Isosterism[1)]



element	B	C	N
valence electron	3	4	5



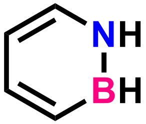
BN bond is highly polarized though total valence electron count was the same as CC bond.

BN/CC isosteres have

- **similar steric profile**
- **different electronic character (dipole moment, reactivity, hydrogen bonding)**

1) Campbell, G. P.; Marwitz, V. J. D.; Liu, S.-Y. *Angew. Chem. Int. Ed*. **2012**, *51*, 6074.

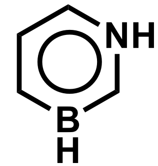
BN-arenes[1)]



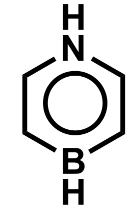
1,2-azaboreine

different properties from natural arenes

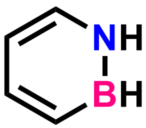
- relatively low aromaticity
- high energy HOMO
- red shifted absorption and emission



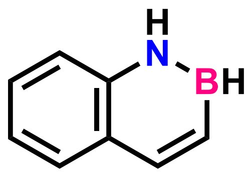
1,3-azaboreine



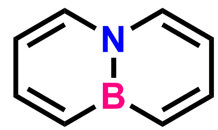
1,4-azaboreine



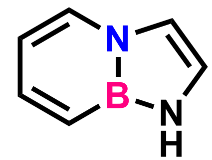
Liu (2013)



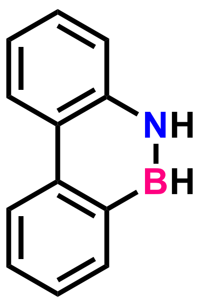
Dewar (1959)



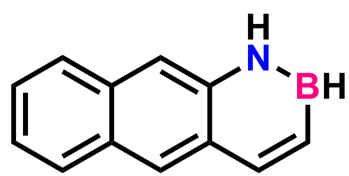
Dewar (1964)



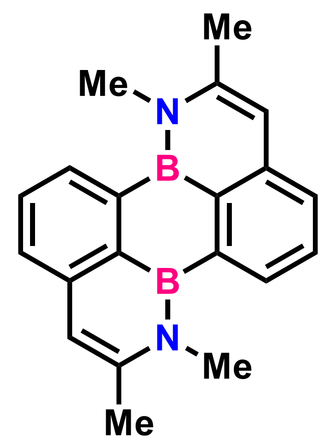
Liu (2011)



Dewar (1958)



Liu (2014)

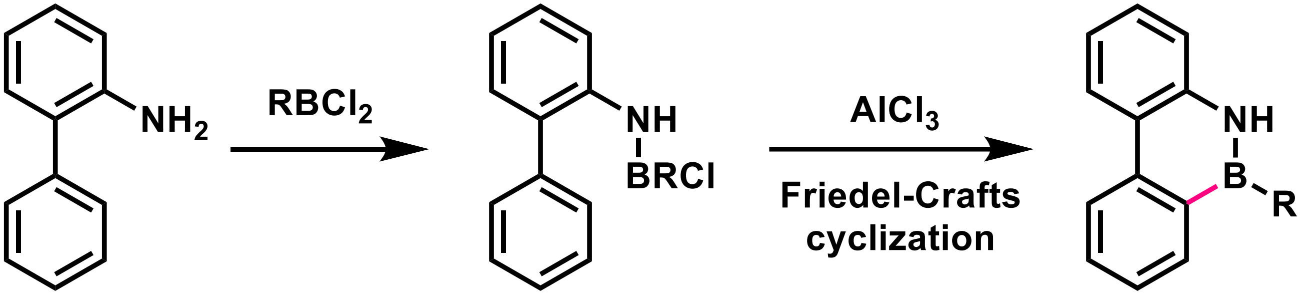


Wagner (2019)

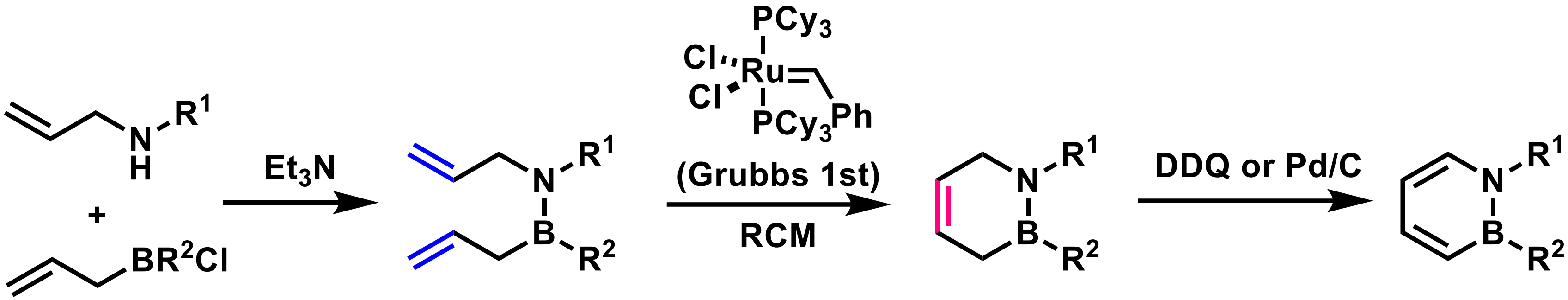
1) Abengózar, A.; G-García, P.; F-Rodríguez, A. M.; Sucunza, D.; Vaquero, J. J. *Advances in Heterocyclic Chemistry*. **2021**.

Synthesis of BN-arenes

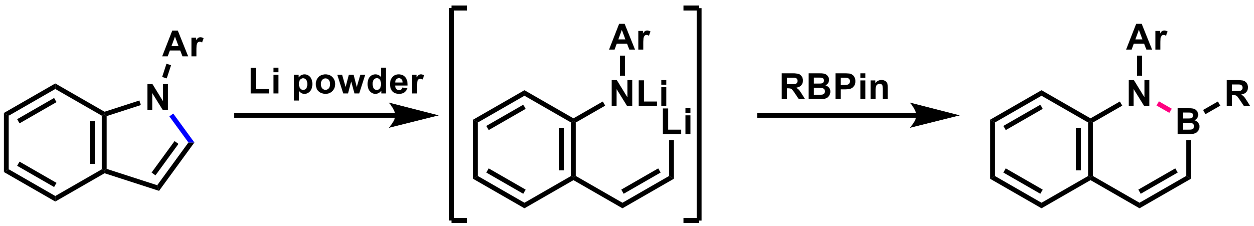
1. Borylative cyclization[1)]



2. Ring-closing metathesis (RCM)[2)]



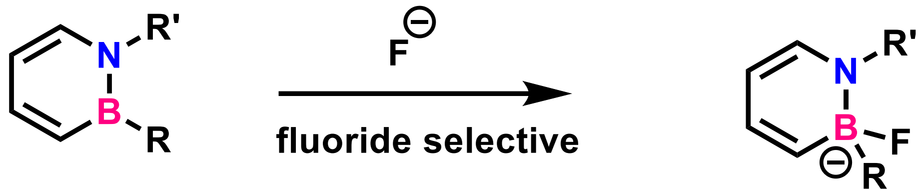
3. Ring expansion[3)]



1) Dewar, S. J. M.; Kubba, P. V.; Pettit, R. *J. Chem. Soc*. **1958**, 3073.
2) Ashe, S. A. III.; Fang, X. *Org. Lett*. **2000**, *2*, 2089.
3) Tsuchiya, S.; Saito, H.; Nogi, K.; Yorimitsu, H. *Org. Lett.* **2019**, *21*, 3855.

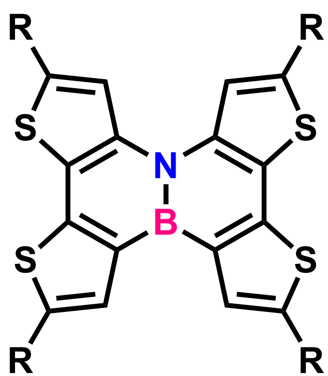
Application of BN-arenes

Fluoride sensor[1)]

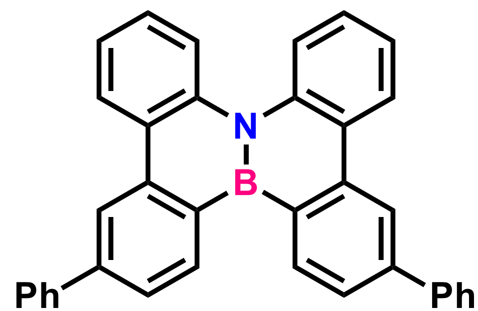


red-shigted absorebance and emission

Optoelectronic materials[2), 3)]



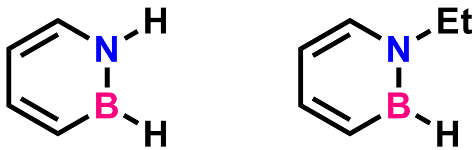
organic field-effect transitor (OFET)



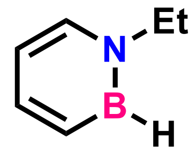
organic light-emmiting diode (OLED)

1) Agou, T.; Sekine, M.; Kobayashi, J.; Kawashima, T. *Chem. A Eur. J*. **2009**, *15*, 5056.
2) Wang, X.-Y.; Lin, H.-R.; Lei, T.; Yang, D.-C.; Zhuang, F.-D.; Wang, J.-Y.; Yuan, S.-C.; Pei, J. *Angew. Chem. Int. Ed*. **2013**, *52*, 3117.
3) Hashimoto, S.; Ikuta, T.; Shiren, K.; Nakatsuka, S.; Ni, J.; Nakamura, M.; Hatakeyama, T. *Chem. Mater*. **2014**, *26*, 6265.

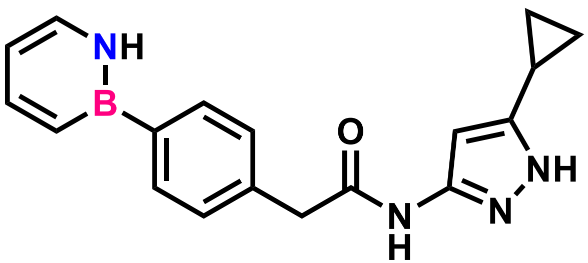
Biological Application of BN-arenes



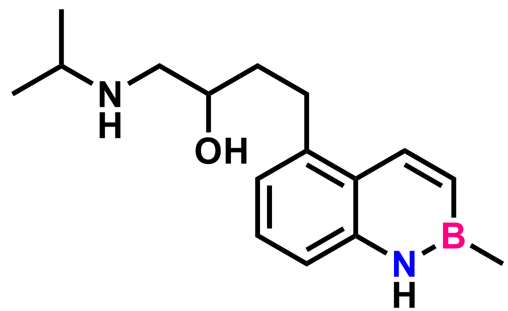
better ligand to T4 lysozome L99A[1)]



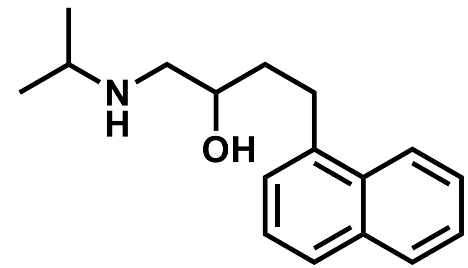
EbDH inhibitor[2)]
IC_{50} = 2.8 μM



CDK2 inhibitor[3)]
IC_{50} = 87 nM
(all carbon analogus = 320 nm)



***β*-blocker[4)]**
different ADMET profile
from propranolol



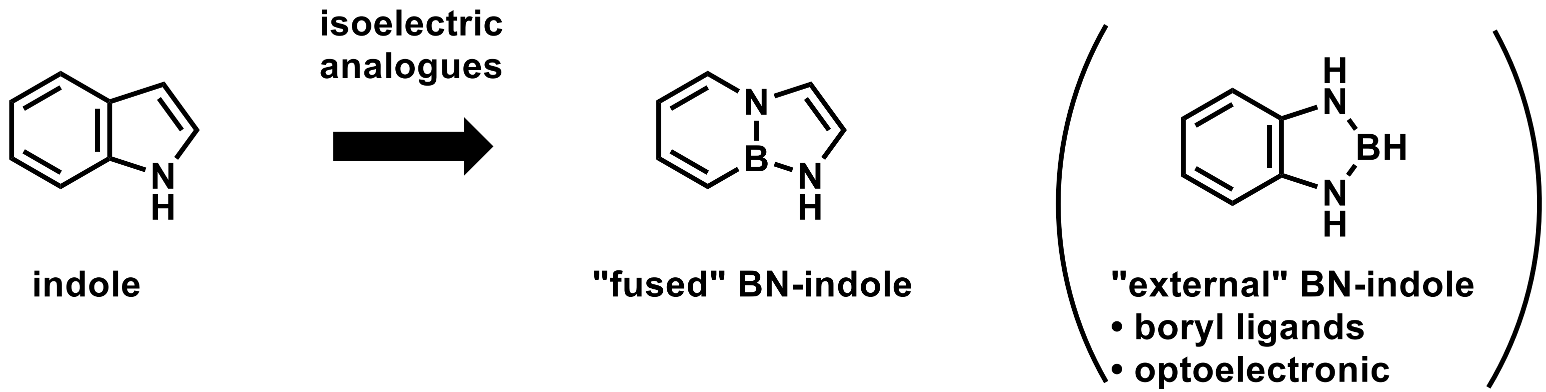
propranolol

Biological application of BN arenes showed effectiveness especially in medicinal chemistry.

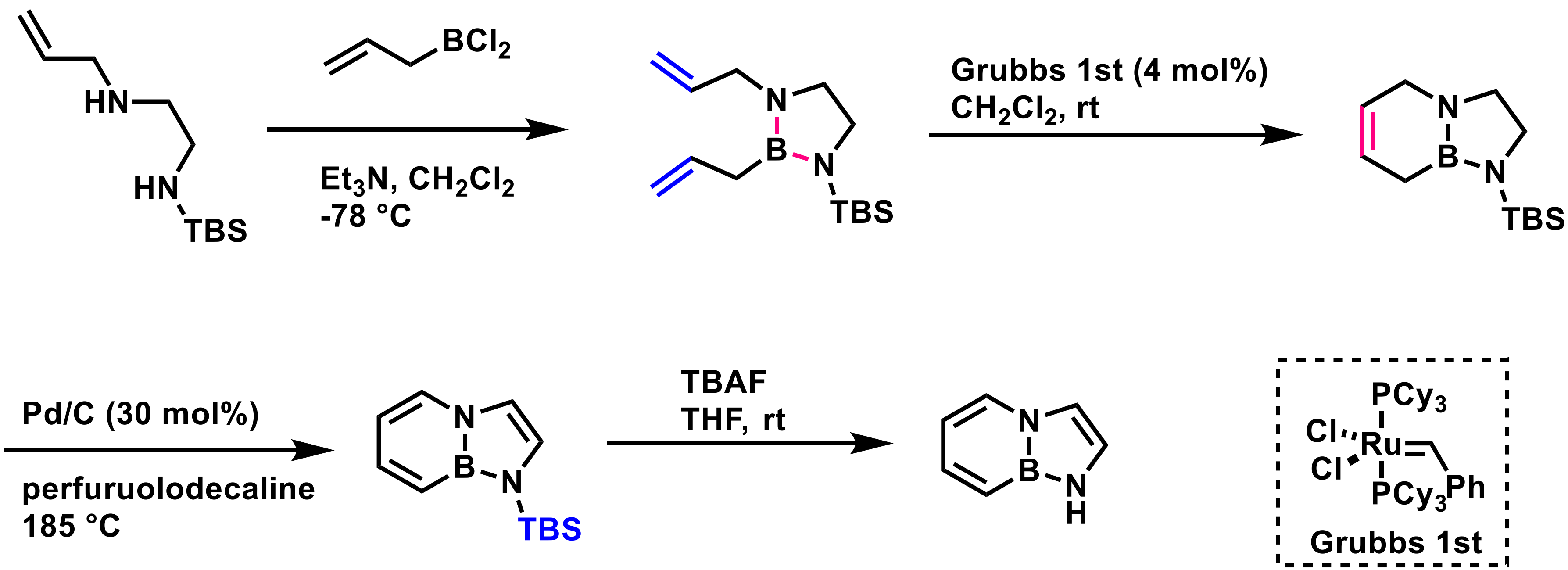
→Installing BN bond into biomolecules will lead to broader application.

1) Liu, L.; Marwitz, V. J. A.; Matthews, W. B.; Liu, S.-Y. *Angew. Chem. Int. Ed.* **2009**, *48*, 6817.
2) Knack, H. D.; Marshall, L. J.; Harlow, P. G.; Dudzik, A.; Szaleniec, M.; Liu, S.-Y.; Heider, J. *Angew. Chem. Int. Ed*. **2013**, *52*, 2599.
3) Zhao, P.; Nettleton, O. D.; Karki, G. R.; Zecri, J. F.; Liu, S.-Y. *ChemMedChem*, **2017**, *5*, 358.
4) Rombouts, R. J. F.; Tovar, F.; Austin, N.; Tresadern, G.; Trabanco, A. A. *J. Med. Chem*, **2015**, *58*, 9287.

BN-indoles[1)]



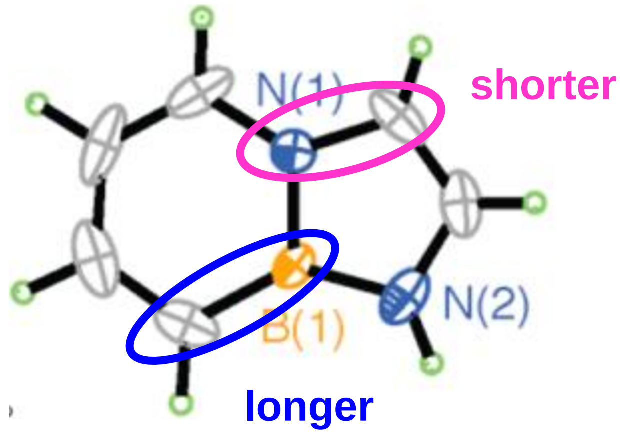
synthesis of “fused” BN-indole[2)]



1) Abbey, R. E.; Liu, S.-Y. *Org. Biomol. Chem*. **2013**, *11*, 2060.
2) Abbey, R. E.; Zakharov, N. L.; Liu, S.-Y. *J. Am. Chem. Soc*. **2011**, *133*, 11508.

Properties of BN-indole (1)

X-ray diffraction structure (crystal of π-complex with electron deficient arene)



Highly planner and aromatic

Compared with natural indole

- **$N-C_2$ bond (pink circled) was shorter**
- **$B-C_7$ bond (blue circled) was longer**

→larger/shorter Atom radius of B/N

acidity of NH

lower acidity

→ inductive effects exerted by the neighboring boron atom

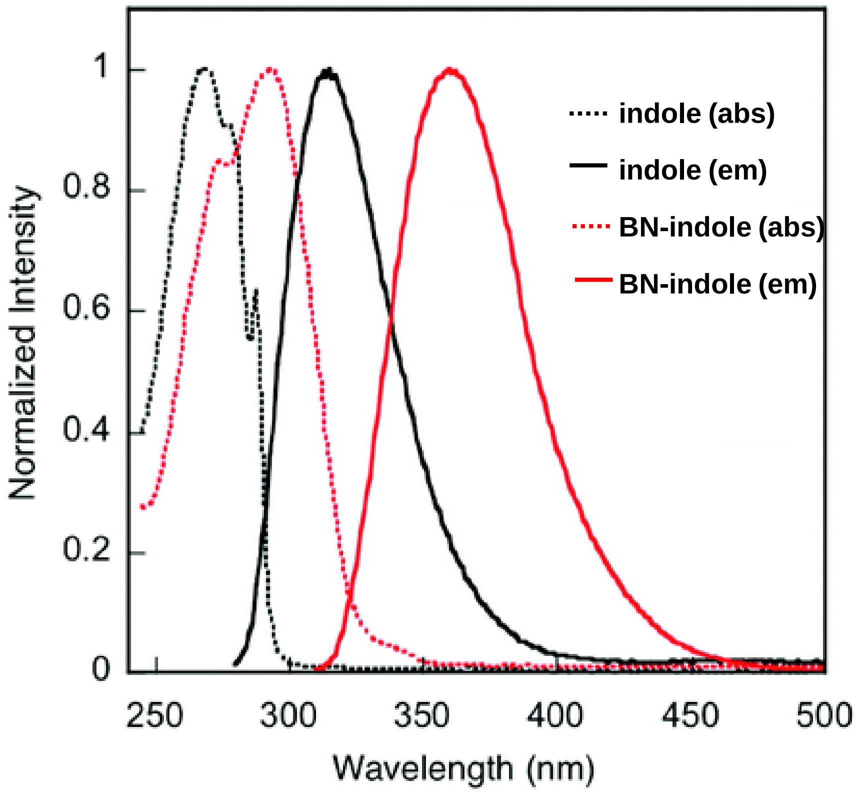
pK_a	**~30**	**20.95**

electronegativity
B : 2.04 C : 2.55

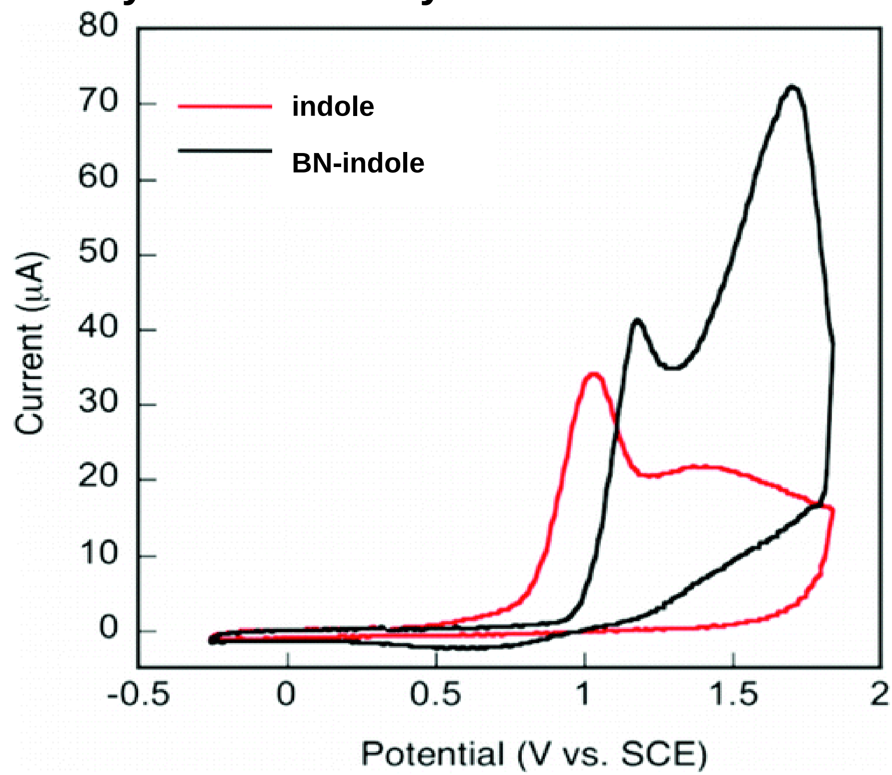
1) Abbey, R. E.; Zakharov, N. L.; Liu, S.-Y. *J. Am. Chem. Soc*. **2011**, *133*, 11508.

Properties of BN-indole (2)

Fluorescence



Cyclic voltametry



	λ_{abs} (nm)	λ_{em} (nm)	Stokes Shift (cm^{-1})	*E* (V)
indole	268	315	5570	1.18
BN-indole	293	360	6350	1.04

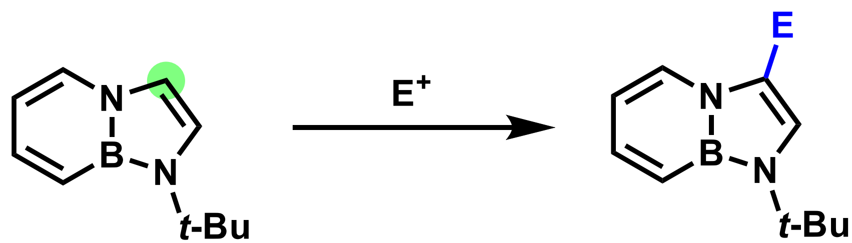
The wavelengths were <u>red shifted</u> and the oxidation potential was lower.

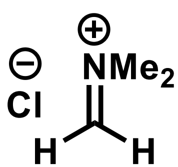
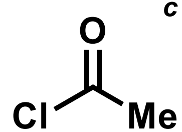
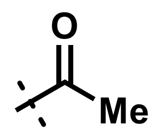
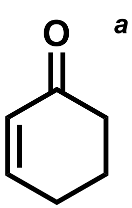
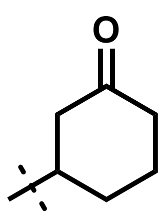
→indicate higher energy HOMO and smaller HOMO-LUMO gap.

1) Abbey, R. E.; Zakharov, N. L.; Liu, S.-Y. *J. Am. Chem. Soc*. **2011**, *133*, 11508.

Reactivity of BN-indole (1)

electrophilic aromatic substitution (EAS) at 3-position

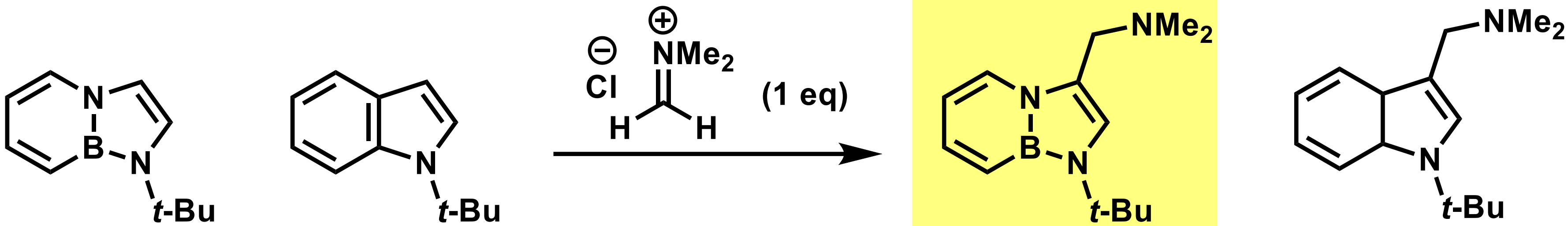


E^+	E		E^+	E	yield
Br_2	Br	39	CD_3OD/D_2O	D	39[b]
		53	 [c]		23
 [a]		57			

[a] $ZrCl_4$ was used as catalyst. [b] ~80% D enrichment [c] Et_2AlCl was used as catalyst.

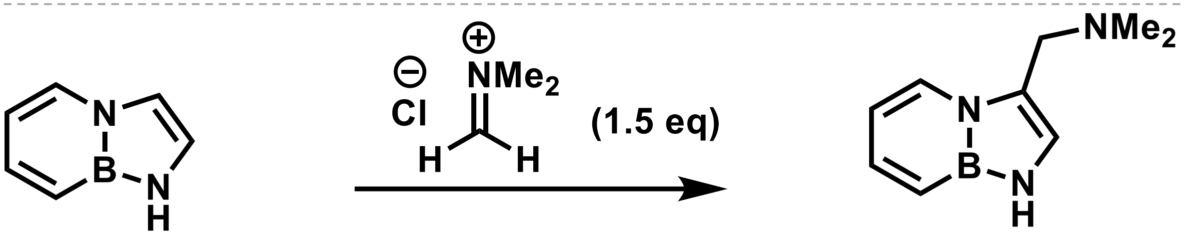
1) Abbey, R. E.; Zakharov, N, L.; Liu, S.-Y. *J. Am. Chem. Soc*. **2010**, *132*, 16340.

Reactivity of BN-indole (2)



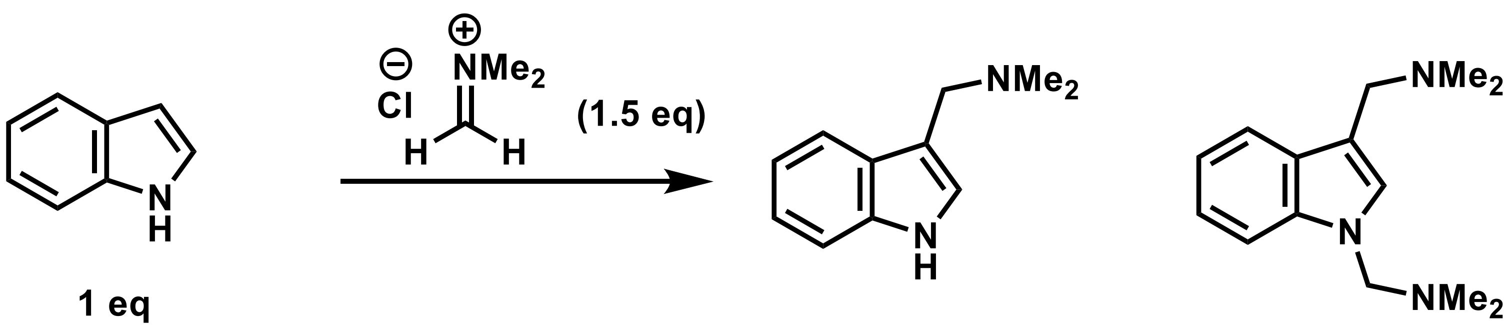
1 eq 1 eq **1.9** 1

3-position of BN indole is more nucleophilic.



1 eq

only obtained

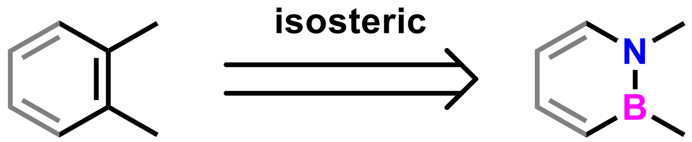


1 eq **2.1 : 1**

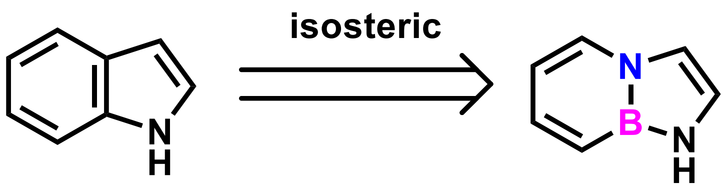
1-position of BN-indole is less nucleophilic.

1) Chrostowska, A.; Xu, S.; Mazière, A.; Boknevitz, K.; Li, B.; Abbey, E. R.; Dargelos, A.; Graciaa, A.; Liu, S.-Y. *J. Am. Chem. Soc*. **2014**, 136, 11813.

Short Summary



BN isosterism is effective method for altering electronical character without altering steric profiles.

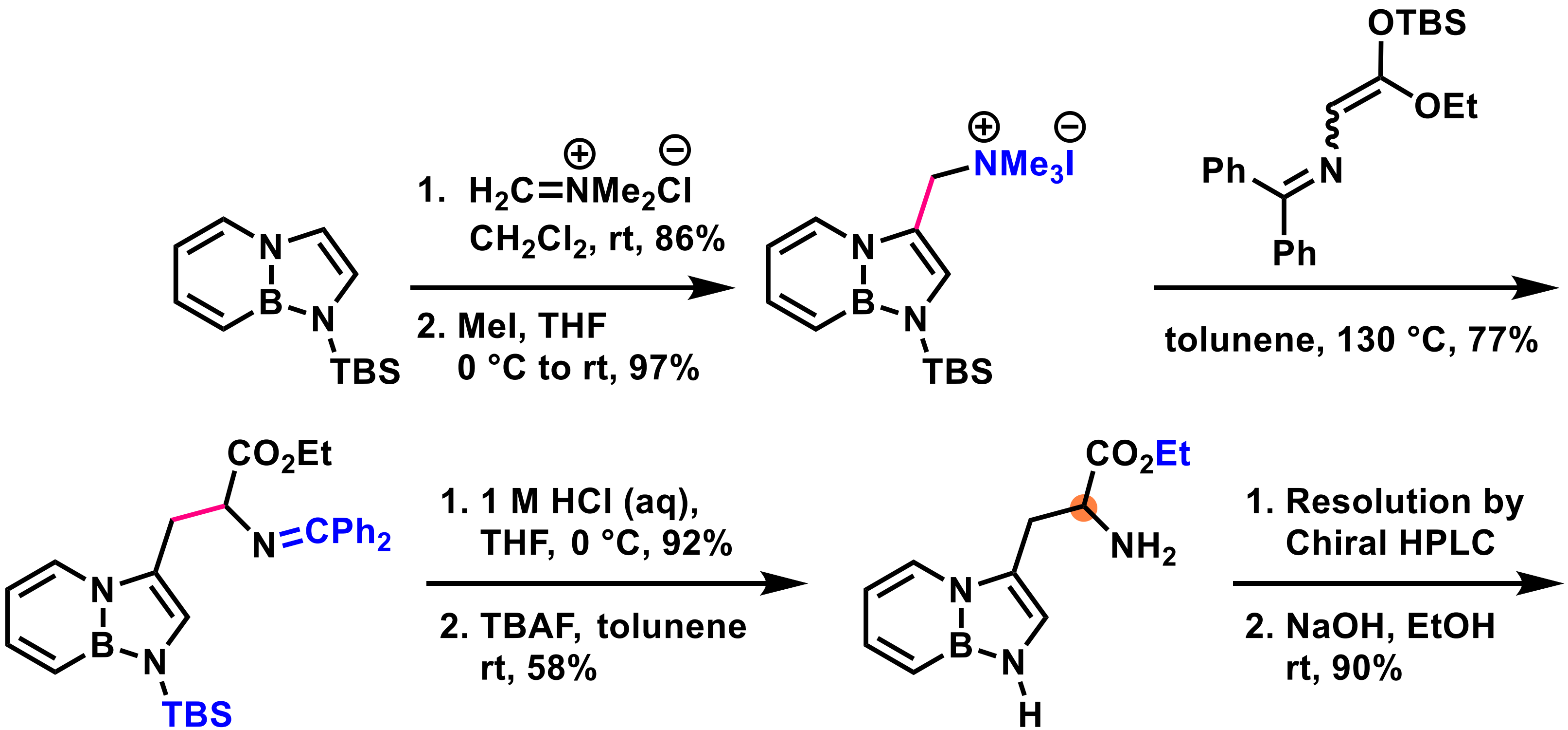


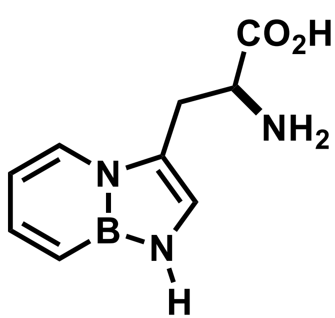
BN-indole showed different properties from natural indole

- **red shifted UV absorption and fluorescence**
- **higher redox potential**
- **higher and more selective reactivity with electrophile**

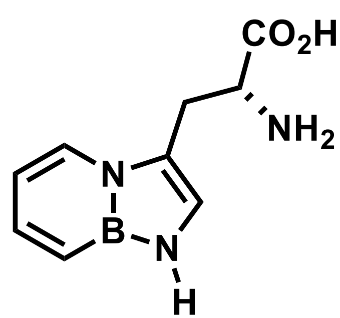
Contents

Synthesis of BN-tryotophan





L-BN-tryptophan



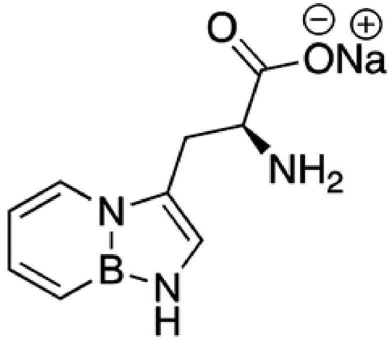
D-BN-tryptophan

Racemic BN-tryptophan ethyl ester was separated by Chiral HPLC.

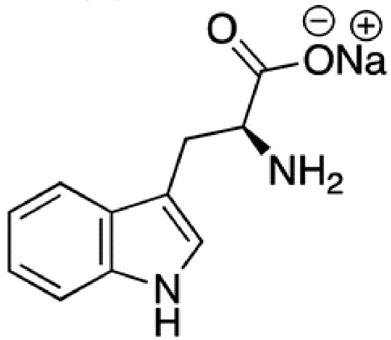
Asymmetric synthesis will be needed for future study.

1) Boknevitz, K.; Italia, S. J.; Li, B.; Chatterjee, A.; Liu, S.-Y. *Chem, Sci*. **2019**, *10*, 4994.

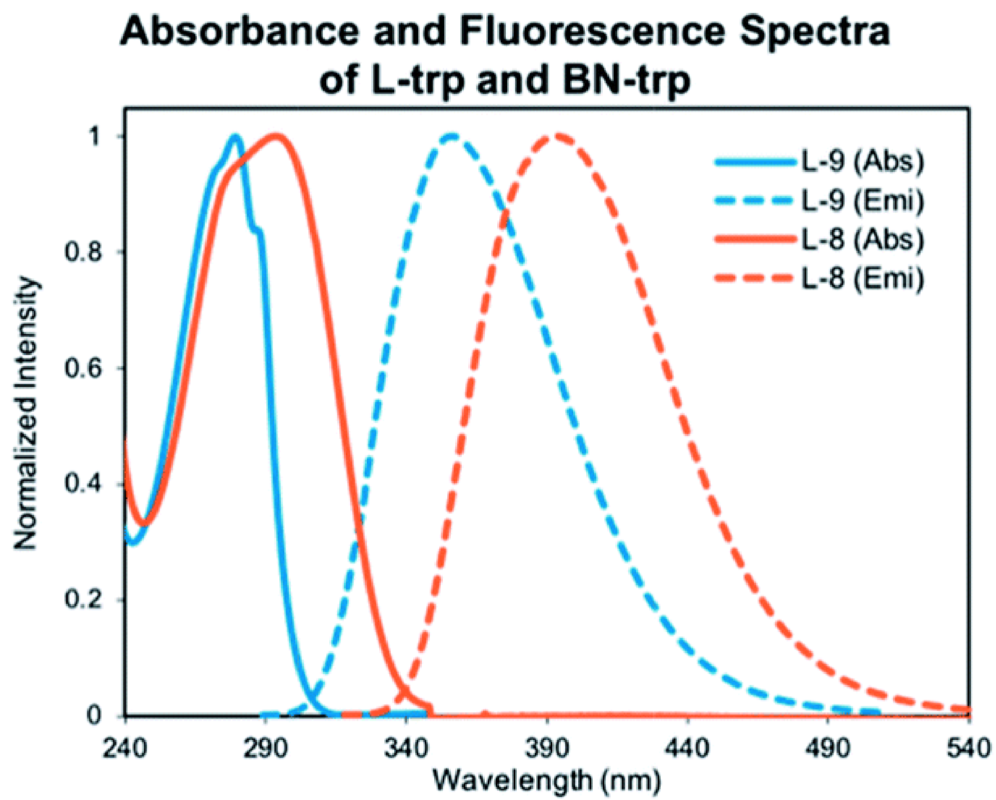
Photophysical Properties of BN-Trp



(L)-8



(L)-9



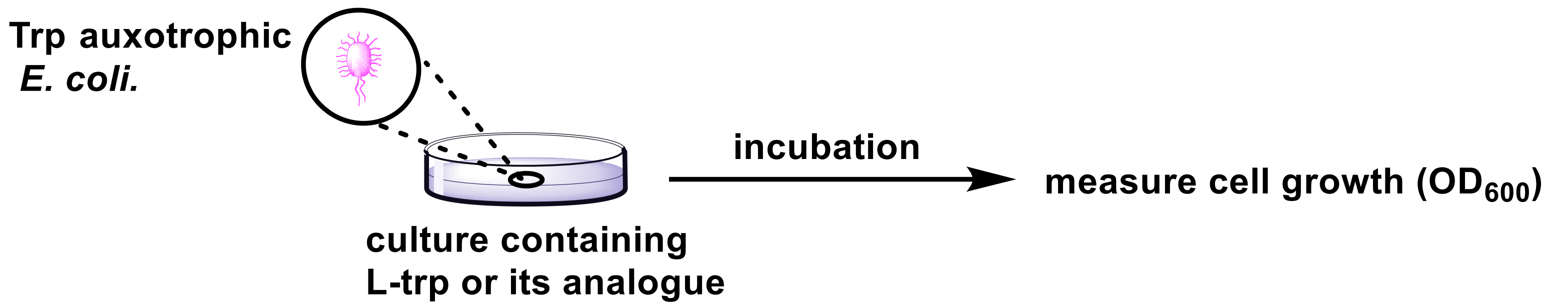
	λ_{abs} (nm)	λ_{em} (nm)	Stokes Shift (cm^{-1})
Trp (L)-9	279	356	7752
BN-Trp (L)-8	295	394	8633

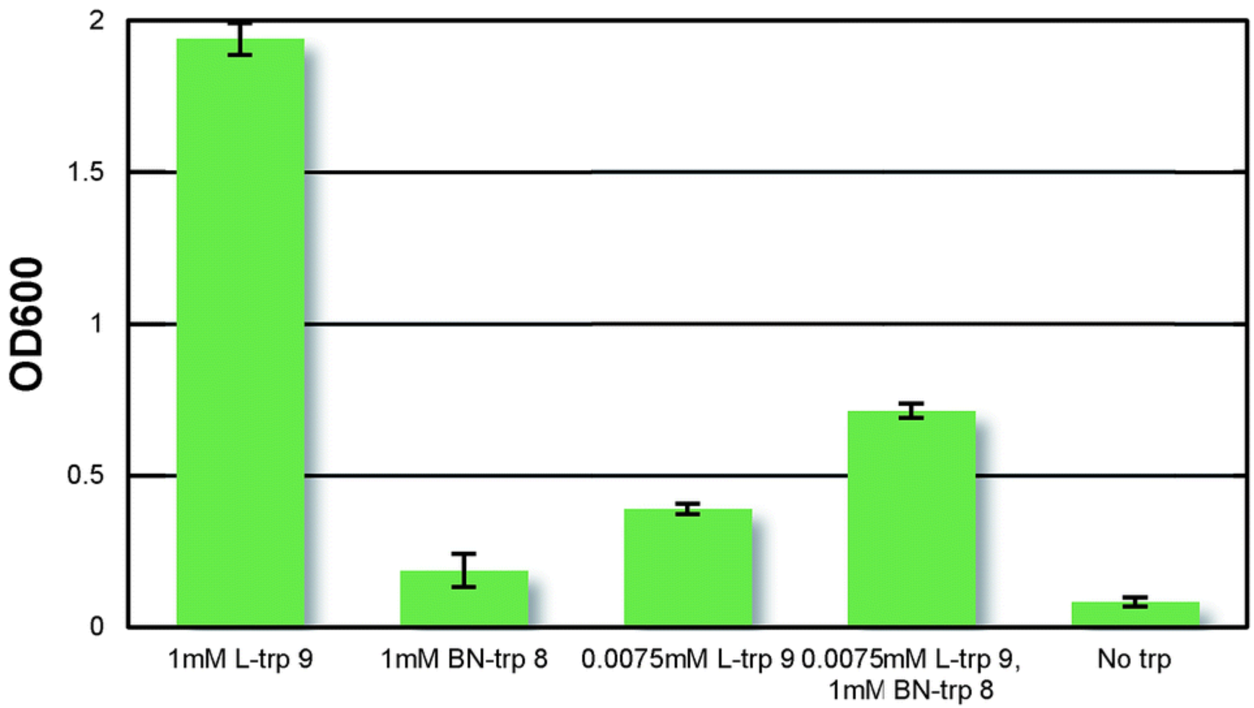
in water, pH 10

The wavelengths of absorption and emission were red shifted.

1) Boknevitz, K.; Italia, S. J.; Li, B.; Chatterjee, A.; Liu, S.-Y. *Chem, Sci*. **2019**, *10*, 4994.

Incorporation as a Surrogate of Trp





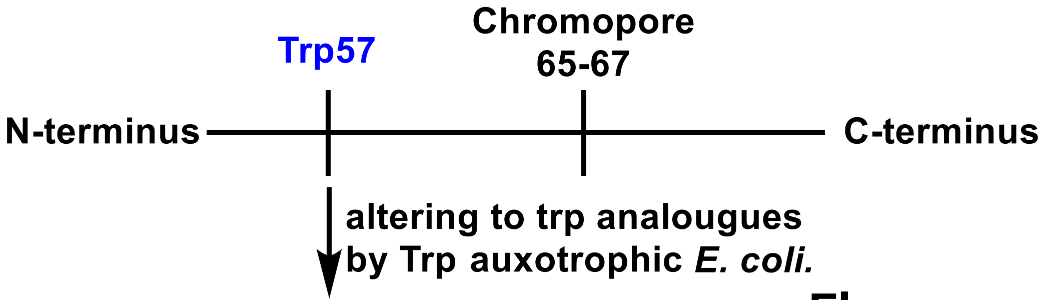
BN-trp has quite lower but sure cell growth ability.

- **accepted as a substrate for the endogenous tryptophanyl-tRNA synthetase**
- **not tolerated for proteome-wide replacement**

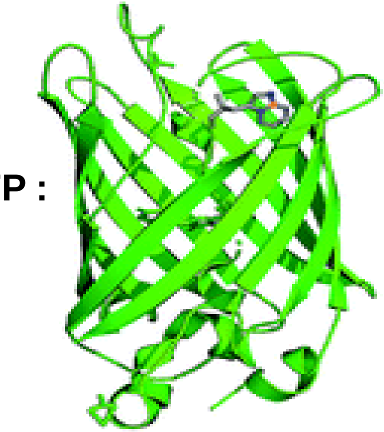
1) Boknevitz, K.; Italia, S. J.; Li, B.; Chatterjee, A.; Liu, S.-Y. *Chem, Sci*. **2019**, *10*, 4994.

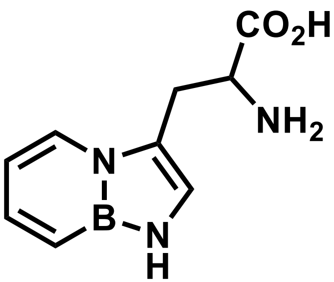
Comparison with Other Trp Analogues

super folding green fluorescent protein (sfGFP)

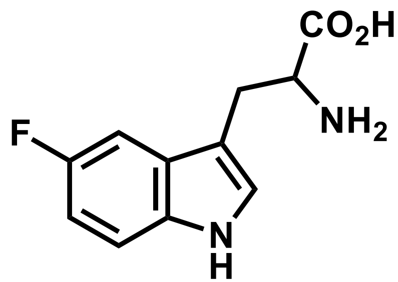


sfGFP :

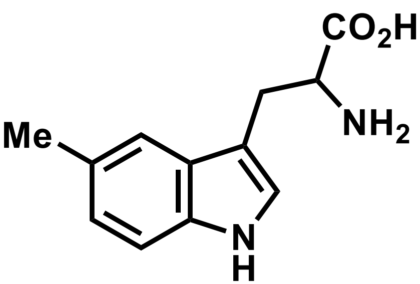




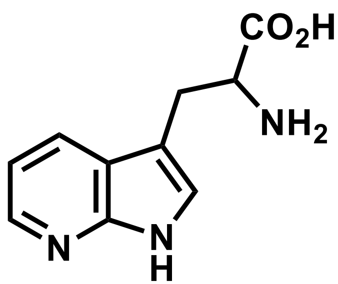
D/L-BN-trp



D/L-5-F-trp

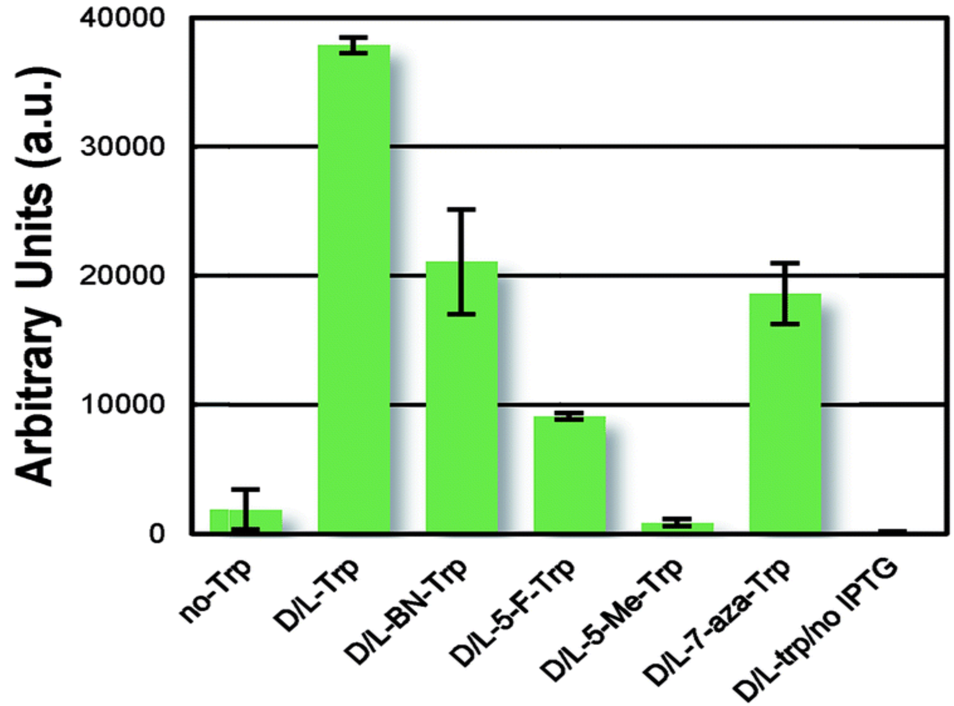


D/L-5-Me-trp



D/L-7-aza-trp

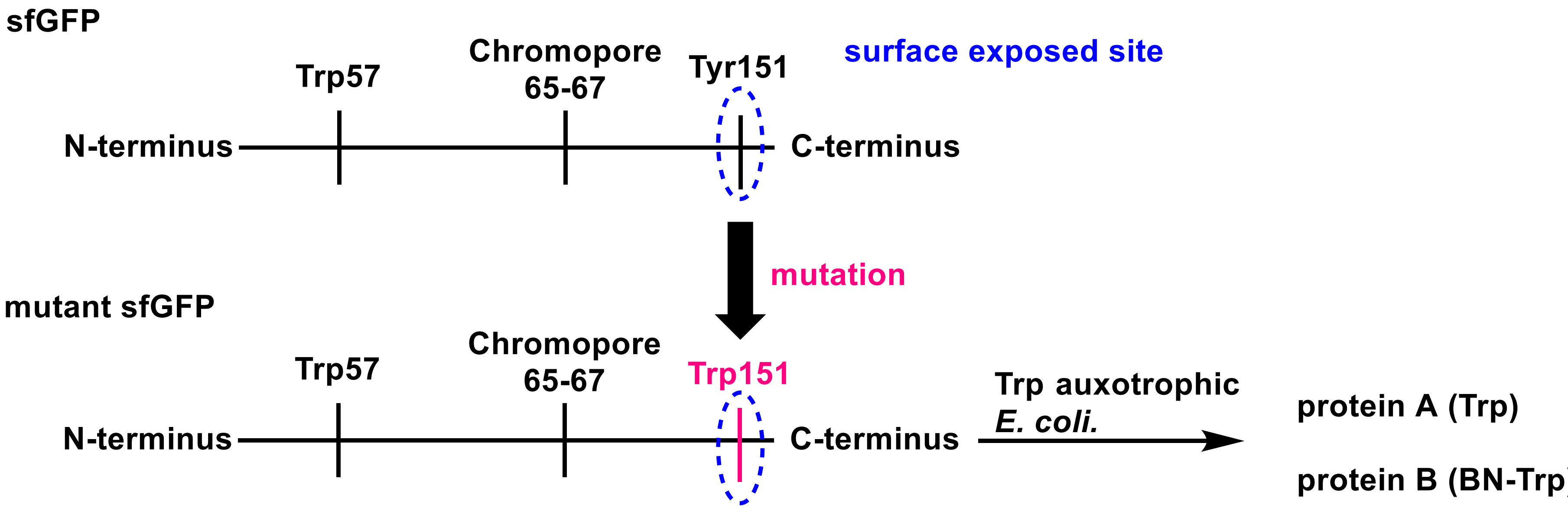
Fluorescence/OD600



BN-Trp shows similar or improved incorporation efficiency relative to other Trp analogues.

1) Boknevitz, K.; Italia, S. J.; Li, B.; Chatterjee, A.; Liu, S.-Y. *Chem, Sci*. **2019**, *10*, 4994.

Change of Reactivity



protein A (Trp)
MS: 27620 [M_A]

protein B (BN-Trp)
MS: 27621 [M_B]

1 mM H_2O_2, 5 min →

protein A (Trp):
[M_A] was mainly detected. [M+O] was also detected.

protein B (BN-Trp):
[M_B] was almost lost. [M+O] was mainly detected.

Although protein A and B only differ by the presence of BN-trp, the reactivity against oxidant was quite different.

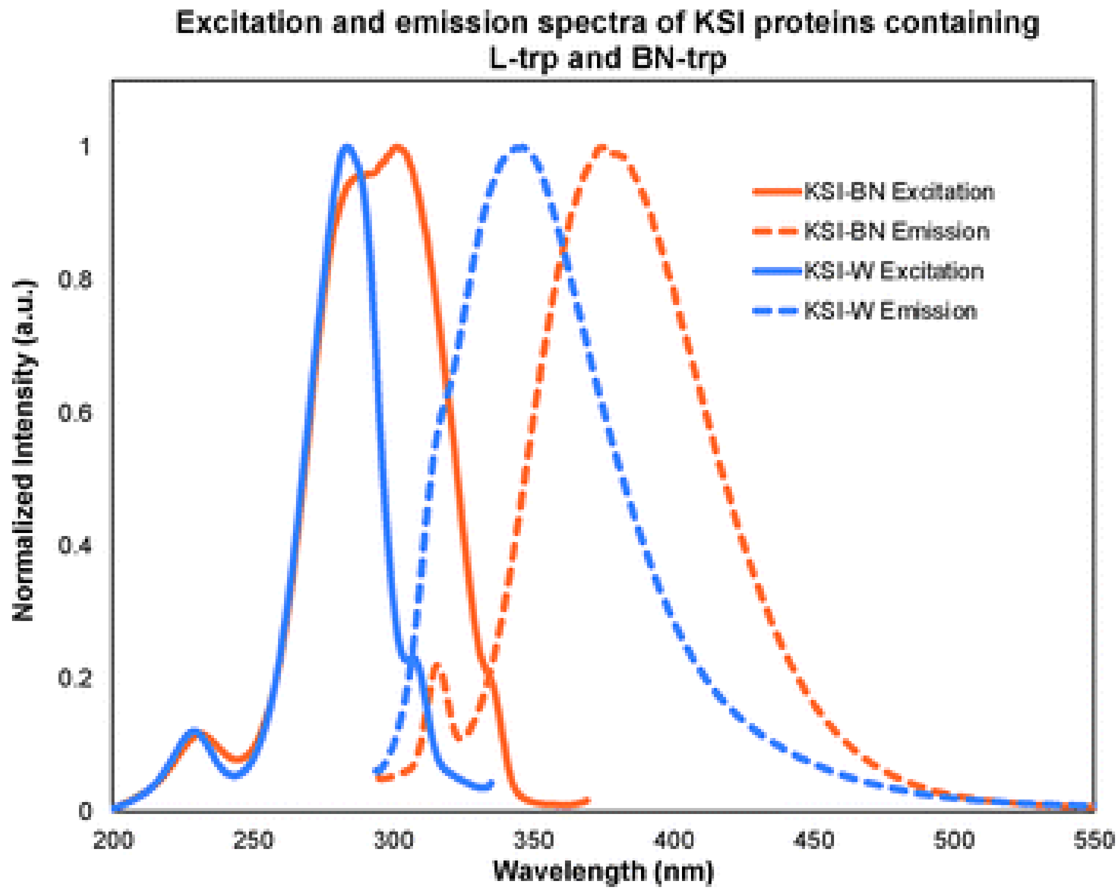
1) Boknevitz, K.; Italia, S. J.; Li, B.; Chatterjee, A.; Liu, S.-Y. *Chem, Sci*. **2019**, *10*, 4994.

Change of Fluorescence

Ketosteroid isomerase (KSI)
- homo-dimeric protein
- two Trp residues in each monomer

KSI-W : wild type

KSI-BN : BN-trp analogue



The fluorescence of KSI-BN was red shifted.

→The Tyr fluorescence peak (316 nm) could be distinguished from the BN-Trp peak.

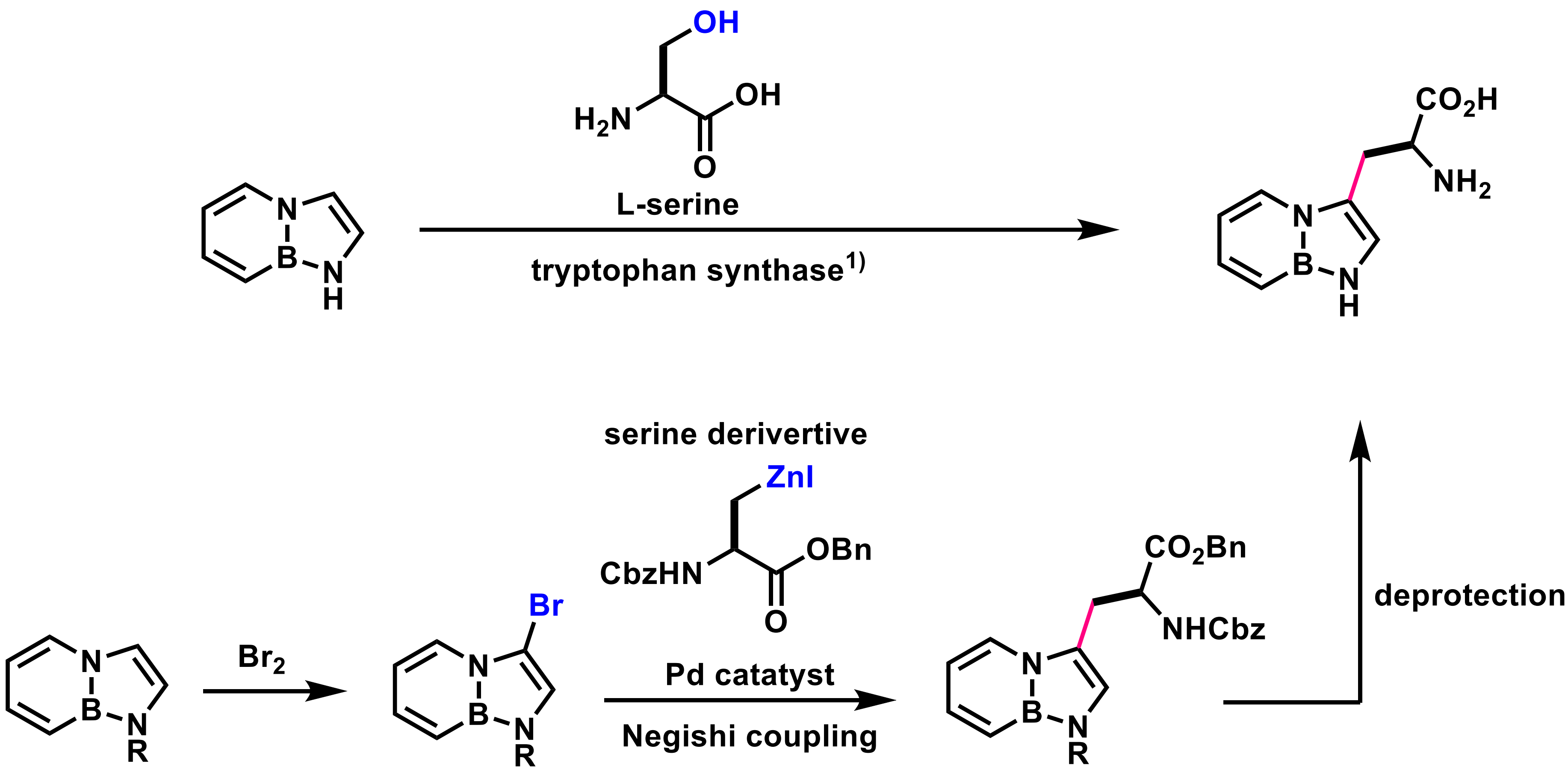
	λ_{ex} (nm)	λ_{em} (nm)
KSI-W	284	342
KSI-BN	285	372

1) Boknevitz, K.; Italia, S. J.; Li, B.; Chatterjee, A.; Liu, S.-Y. *Chem, Sci*. **2019**, *10*, 4994.

Future Study (1)

For further study of BN-tryptophan, methods for asymmetric synthesis of BN-trp will be needed.

My proposal methods are shown below.



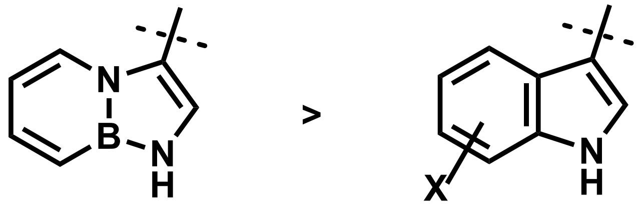
1) Sloan, J. M.; Phillips, S. R. *Bioorg. Med. Chem. Lett*. **1992**, *2*, 1053.

Future Study (2)

Application of BN-tryptophan will

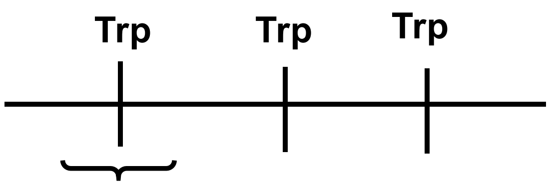
1. Probing proteins possessing multi Trps or Trp at sterically hindered position

incorporation efficiency



red shifted fluorescence

N-terminus

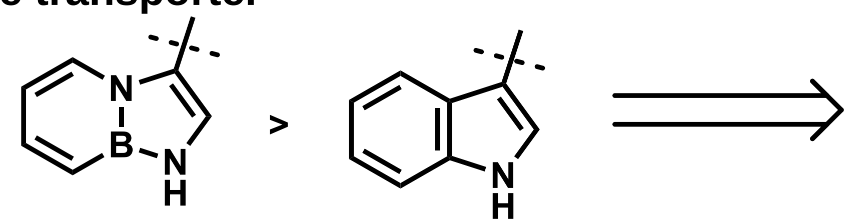


C-terminus → **BN-trp incorporation** → **higher yield of protein**

sterically congeted after folding

2. efficient charge transporter[1)]

redox potential

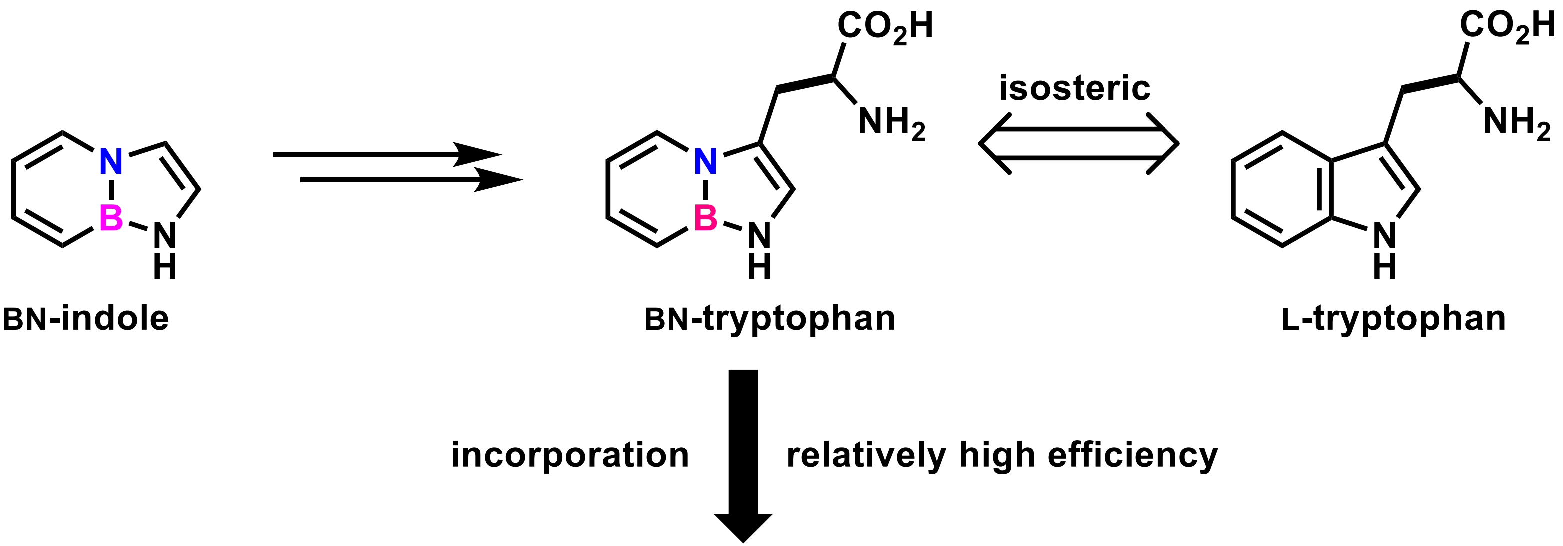


faster charage transport ability

1.04 V **1.18 V**

1) Shih, C.; Museth, K. A.; Abrahamsson, M.; Blanco-Rodriguez, M. A.; DiBilio, J. A.; Sudhamsu, J.; Crane, R. B.; Ronayne, L. K.; Towrie, M.; Vlcek, A. Jr.; Richards, H. J.; Winkler, R. J.; Gray, B. H. *Science.* **2008**, *320*, 1760.

Summary



protein BN-analogues
• red shifted fluorescence
• more reductive

BN-Tryptophan was synthesized and incorporated into proteins.

Protein BN-analogues showed distinct photophysical property and reactivity.

BN-tryptophan will be expected to explore new chemical space for UAAs.