Synthesis of Substrates for the Investigations of Monoamine Neurotransmitter Transporters

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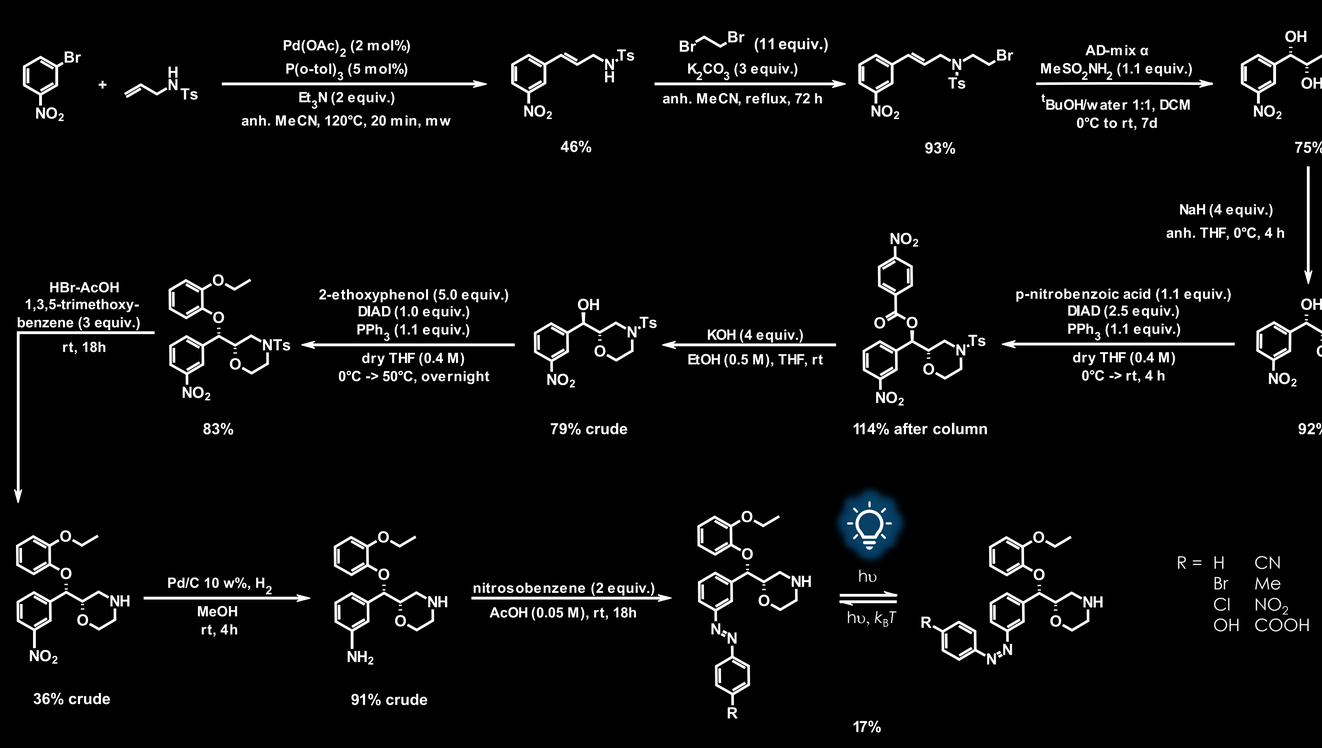
Introduction

The monoamine neurotransmitters serotonin, norepinephrine and dopamine play a major role in our bodies' everyday functions. Reuptake of these neurotransmitters from the synaptic cleft is controlled by the transporters DAT (dopamine transporter), NET (norepinephrine transporter) and SERT (serotonin transporter). Malfunctions of these transporters are associated with diseases like depression, epilepsy, anxiety, ADHD and Parkinson's disease. This makes in-depth understanding of DAT, NET and SERT indispensable for the design of novel drugs addressing these illnesses.[1] Building upon earlier work[2], we are currently exploring two approaches to control and investigate DAT, NET and SERT.

Photoswitchable Monoamine Transporter Ligands

Photopharmacology has received a lot of attention lately for enabling the synthesis of compounds that allow light-induced, highly precise temporal and spatial control of ion channels and enzymes. However, photoswitchable ligands for transporters and pumps are still rare and underdeveloped.[3] In this work, transporter-selective inhibitors like Reboxetine or Escitalopram were used as starting points for implementation of photoswitchable handles, eg. azobenzenes, into their scaffolds. Thereby, libraries of photoswitchable monoamine transporter ligands were synthesized (Scheme 1).
Typically, *E*- and *Z*-isomers of such photoswitchable monoamine transporter ligands show different biological affinity towards their respective transporter DAT, NET or SERT. This allows switching the transporters on and off by illuminating them with ligand-specific wavelengths in the presence of photoswitchable inhibitors.

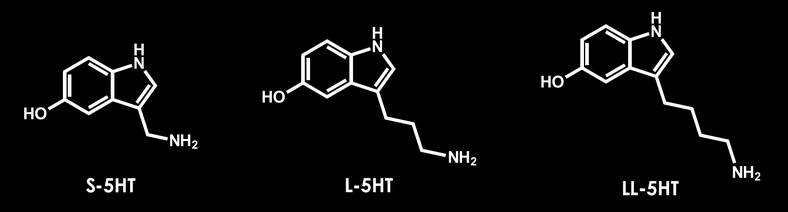


Scheme 1: Optimized ten step synthesis towards photoswitchable meta-azo-Reboxetine derivatives

First biological measurements of the synthesized compounds including uptake assays on the respective transporters showed promising results. The unsubstituted (R=H) meta-azo-Reboxetine derivative showed an IC_{50} of 2.44 µM for the *E*-conformer at NET whereas an IC_{50} of 0.76 µM for the *Z*-conformer was found.

Serotonin Derivatives

Our investigations of the SERT transport-cycle were based on new hypotheses postulated following simulation results. In order to proof them, serotonin (5HT) derivatives with different sidechain lengths were synthesized (Scheme 2). By investigating their interactions with SERT, we strove to further illuminate the SERT transport-cycle, its single steps and conformations.
Biological results showed that the compounds' IC_{50} values decrease with growing sidechain length. Steady state current measurements revealed that only 5HT is a full SERT substrate. S-5HT acts as inhibitor by occluding the transporter, whereas both L-5HT and LL-5HT are partial substrates (Figure 1). These results are in accordance with our hypotheses, proofing that 5HT is too small to perfectly fit SERT's binding site S1, concurrently making it the best substrate.



Scheme 2: Serotonin derivatives: 1C (S-5HT), 3C (L-5HT), 4C (LL-5HT)

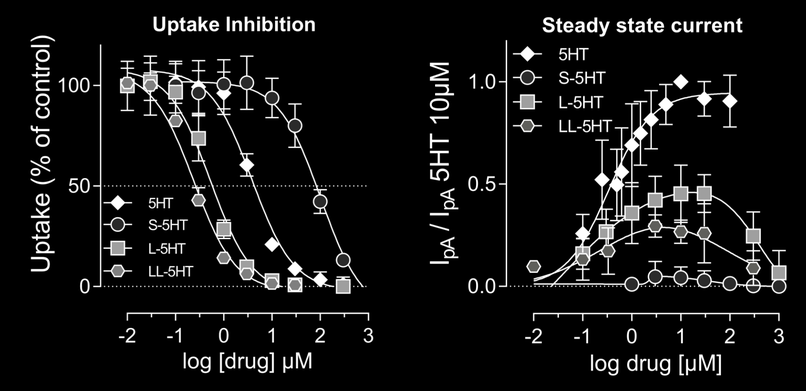


Figure 1: **left**: uptake inhibition measurements of 5HT (IC_{50} 3.9 µM), S-5HT (IC_{50} 102.3 µM), L-5HT (IC_{50} 0.53 µM) and LL-5HT (IC_{50} 0.24 µM); **right**: steady state currents of 5HT, S-5HT, L-5HT and LL-5HT at varying substrate concentrations normalized to I_{pA} of 10 µM 5HT

Conclusion

Several photoswitchable monoamine transporter ligands showing a higher biological activity in their Z- than their *E*-isomer have been synthesized and biological evaluation has started. Thereby, we developed tools to turn NET on and off through targeted illumination with ligand-specific wavelengths. Several serotonin derivatives have been synthesized and characterized in biological measurements. Results led to new findings regarding the SERT transport-cycle like proof of the detain-and-pull mechanism associated mechanism of occlusion.

References
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[3] Hüll, K.; Morstein, J.; Trauner, D., In Vivo Photopharmacology. *Chemical Reviews* **2018**, *118* (21), 10710-10747

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IAS Institute of Applied Synthetic Chemistry
FWF Der Wissenschaftsfonds.
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