Utilization of diversity-oriented transformations of 1,3-dicarbonyl based Mannich substrates towards *N*-containing heterocycles

Thesis of PhD dissertation

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1. INTRODUCTION AND AIMS

In modern clinical practice, novel and efficient treatments are introduced increasing the possibility of recovery from lethal diseases or serious illnesses. In order to support its background with novel clinical candidates, organic and medicinal chemistry research and development are essential for the accomplishment of efficient and industry-compatible multistep synthetic procedures.

Mannich-type precursors incorporating an α-(di)carbonil-β-amino unit are suitable starting materials for construction of more complex structures involving *N*-hetero(poly)cycles via intra- or intermolecular annulation methodologies. During my PhD work, our goal was the investigation of further transformation possibilities of Mannich precursors as well as the design and execution of novel synthetic pathways to access unique *N*-heterocyclic entities with diverse substitution patterns possessing various bioactivities.

The following conceptions have been accomplished by means of the utilisation of Mannich substrates:

1) Preparation of imidazole-fused heterobicycles via oxidative annulation with IBX preferring scores involving imidazo[1,2-*a*]-pyridine, -pyrimidine and -pyrazines; elaboration of an efficient one-pot synthetic procedure as well as demonstration of further synthetic utilities.

2) Preparation of 2-aminoimidazoles (2-AI) with diverse substitution patterns at C-4 and C-5 focusing on the development of a sequential, one-pot procedure involving intramolecular oxidative annulation and reductive ring cleavage sequences; demonstrating further synthetic possibilities towards synthetic marine alkaloid analogues and GBB products and their cytotoxic characterisation.

3) Development of a novel phosphine-induced intramolecular annulation via formation of a new C–C bond; investigation of the reaction outcome in the presence and absence of aldehyde reactants.

2. MATERIALS AND METHODS

In the course of the synthetic work, the majority of reactions were performed in the millimolar scale. Transformations were monitored by either thin-layer chromatography or HPLC-MS analyses. Products were purified by column chromatography (silica gel 40–63 or 60–200 μm), flash chromatography (Teledyne Isco CombiFlash® R_f, Redisept™ silica column) or utilizing simple filtration and/or recrystallisation. Molecular structures of products were determined by

one- and two-dimensional techniques combined with mass spectrometric measurements as well as X-Ray crystallography. Commercially available reagents used in the research were purchased from Sigma-Aldrich, AK Scientific, Fluorochem, Combi-Blocks, Apollo Scientific and Molar, and they were used without further purification. Biological assays were performed by biologist co-workers at Avidin.

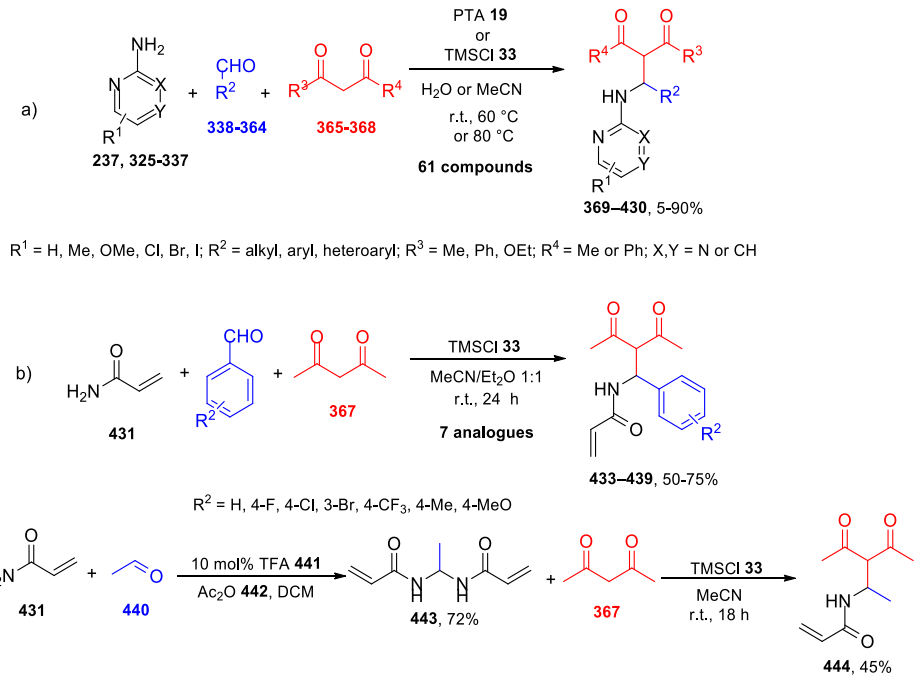
3. NOVEL SCIENTIFIC RESULTS

3.1. Preparation of a 69-membered Mannich library

Following a slightly modified Mannich-3CR (M-3CR) protocol, the assemblies of 2-amino-substituted primary heteroaromatic amines **237** and **325–337**, aliphatic and aromatic aldehydes **338**–**364** as well as 1,3-diketones **365**–**368** in the presence of TMSCl or PTA led to the formation of the desired Mannich-type precursors (**369**–**430**) in isolated yields of 5–90% (*Scheme 1*, *a*).

For Mannich precursors **433**–**439** bearing a reactive acrylamide unit, a synthetic procedure involving TMSCl, MeCN/Et_2O mixture, ambient temperature, 24 hours, was accomplished to give the corresponding analogues in yields of 50–75% (*Scheme 1*, *b*).

In the case of aliphatic variants (R^2 = Me), the utilised M-3CR procedure did not afford the desired analogue **444**; therefore, an alternative method through the formation and transformation of *N*,*N*-aminal was carried out (*Scheme 1*, *c*).



Scheme 1.

3.2. The development of an oxidative, intramolecular annulation procedure

In a preliminary experiment, substrate **370** was treated with 2.5 equivalents of PIDA and 1.5 equivalents of NIS in MeCN at 45 °C. After 2.5 h stirring, 100% conversion was observed and the main product could be isolated; its structure as **445** was determined via 1D- and 2D-NMR analysis.

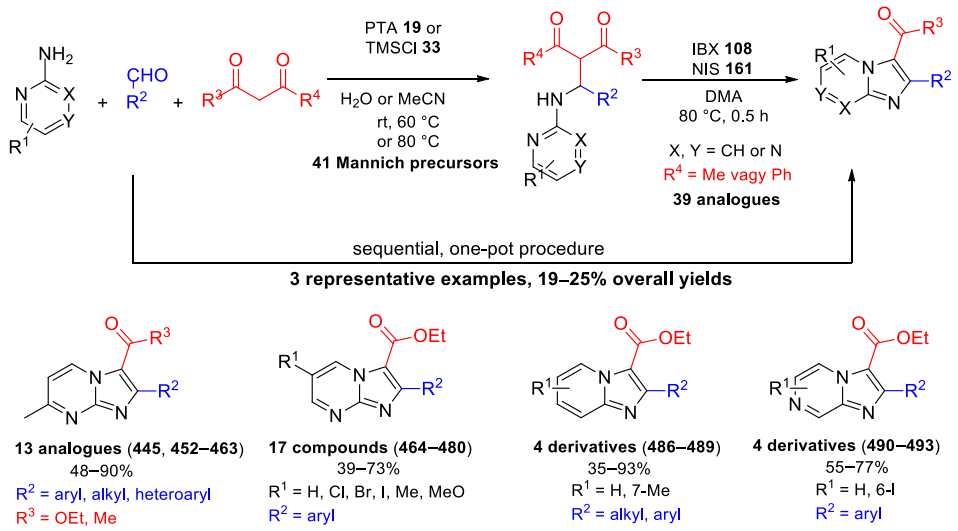
Afterwards, solvent, temperature and concentration ranges was explored during the first stage of optimisation. Probe reactions revealed that DMA as solvent and a temperature of 80°C are optimal for further studies. Subsequently, several hypervalent iodine reagents and halogen-containing additives were tested. It was found that the combination of 1.1 equivalents of IBX and 1.5 equivalents of NIS provides a superior yield of 80% in DMA at 80 °C after 30 minutes of stirring.

3.3. Utilisation of an oxidative intramolecular annulation towards the preparation of di- and trisubstituted imidazo[1,2-*a*]-pyrimidines, -pyridines and -pyrazines; presentation of a sequential, one-pot procedure and further transformation attempts

Having established an optimal protocol (1.1 equiv. of IBX, 1.5 equiv. of NIS, DMA as solvent, heating at 80 °C and 30 min reaction time), a 30-membered highly diverse imidazo[1,2-*a*]-pyrimidine chemical library has been constructed (*Scheme 2*), and the cytotoxic characterisation of the compounds has been performed. For the analogues tested first (**445** and **452**–**463)**, the R^1 7-Me function was fixed. For imidazo[1,2-*a*]-pyrimidine derivatives **464**–**480** prepared subsequently, the R^1 and R^2 functions were varied; hence, the R^3 = OEt was locked. Although an optimal diversity was accessed, weak to moderate biological activities were assessed. In addition, no significant dependency on the substitution pattern was found after investigation of the yields obtained.

Following the aforementioned procedure, other heterobicyclic structures have been constructed including imidazo[1,2-*a*]-pyridine and -pyrazine moieties. Products **486**–**493** were isolated in moderate to good yields (35–93%, *Scheme 2*).

Next, the efficiency of the improved sequential one-pot procedure was demonstrated via synthesis of three representative examples (*Scheme 2*). Upon reacting of the selected 2-amino-4-methylpyrimidine, 2-amino-4-methylpyridine and 2-aminopyrazine reagents with 4-trifluoro-methylbenzaldehyde and ethyl benzoyl acetate, the corresponding Mannich adducts were formed *in situ* through an intramolecular oxidative annulation to give the desired heterobicycles in overall yields of 19–25%.



Scheme 2.

To demonstrate further synthetic possibilities, four carboxylic acids and their corresponding carboxamides were synthesised via simple hydrolysis and subsequent peptide coupling.

3.4. Mechanistic studies for exploring the background of the intramolecular oxidative annulation

As a next step, the background of the proposed reaction mechanism has been investigated. The presence of water as potential nucleophile did not influence the outcome of the reactions, but it was found that the presence of a high amount of iodonium ion blocks conversion delivering only a trace amount of the target compound. The fact that similar yields were obtained with or without TEMPO demonstrated the ionic nature of the transformation.

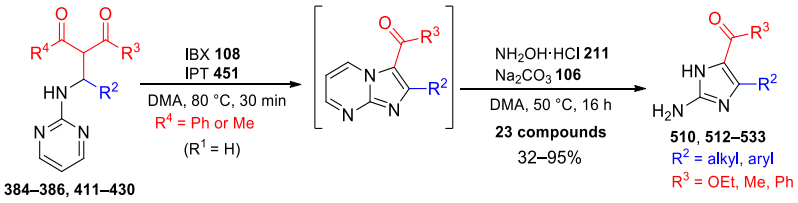
In case the assembly was tried in the absence of either the oxidant or the additive, a Knoevenagel-type product was furnished presumably via thermal decomposition. Correlating with this observation, the assembly of the Knoevenagel adduct and 2-amino-substituted aromatic amines under oxidative conditions did not result in the expected bicyclic compound. Consequently, the Knoevenagel adduct did not play any role as intermediate.

On the basis of this information, we suggested a multi-step mechanism. The first step involves the iodination of the α-carbon followed by an IBX mediated amino-imination step, which is followed by the attack of an *N*-nucleophile and a retro-Claisen–Schmidt reaction affording the desired heterobicyclic compound.

3.5. The development of a sequential, one-pot, synthetic procedure involving intramolecular oxidative annulation then reductive ring cleavage sequences; Preparation of 4,5-disubstituted 2-aminoimidazoles

After slight modification of the intramolecular annulation methodology exploiting IPT instead of NIS as halogenation reactants and equimolar IBX oxidants, the desired heterobicyclic compound was observed in a yield of 94%. In accordance with this result, the investigation was focused onto the second step as reductive cleavage without isolation of the bicyclic intermediate. For optimisation, several secondary amines, hydrazines as well as hydroxylamine hydrochlorides were tested in the presence or absence of (in)organic bases and the combination of $NH_2OH.HCl/Na_2CO_3$ (10 equivalents of each) were found the most efficient for reductive ring cleavage after 16 h of stirring at 50 ˚C.

Under the optimal reaction conditions, a 23-membered 4,5-disubstituted 2-aminoimidazole (2-AI) chemical library has been prepared (*Scheme 3*). For the analogues synthesised with R^2 = phenyl decorated with electron-withdrawing group(s), significantly higher yields were found than with aliphatic substituents or R^2 = phenyl groups decorated with electron-donating substituent(s). Decreased yield also occurred in case when R^3 = phenyl.

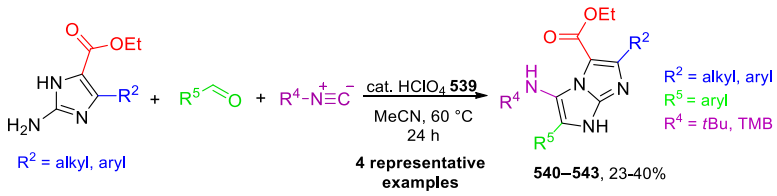


Scheme 3.

3.6. Transformation of 4,5-disubstituted 2-aminoimidazoles for cytotoxic studies

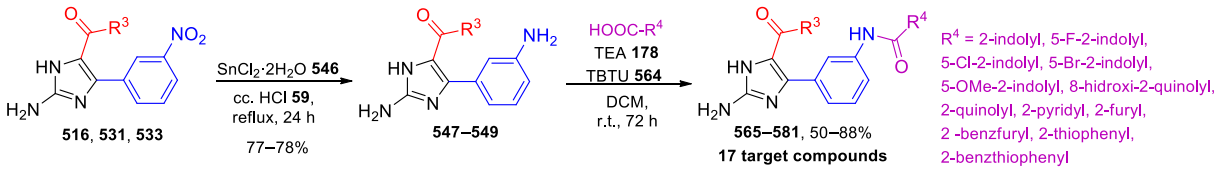
For cytotoxic studies, C-5 amino and 5:5 condensed bicycles with diverse substituent patterns have been constructed by means of Groebke–Blackburn–Bienaymé three-component transformations (GBB-3CR). Reaction of the selected 2-AI derivatives with isocyanides and aromatic aldehydes led to the desired tetrasubstituted compounds **540–543** in yields of 23–40% (*Scheme 4*).

The cytotoxic characterisation was accomplished by biologists at Avidin Ltd. The tested compounds proved to be inactive in the range of 1–30 μM.



Scheme 4.

Synthetic marine alkaloid analogues were also prepared starting from selected 2-AI compounds **516**, **531** and **533** (*Scheme 5*). The corresponding C-4 3-nitrophenyl/C-5 carbonyl-substituted 2-AI variants were reduced and the resulting amino function was functionalised via peptide coupling with several heterocyclic carboxylic acids, such as (substituted) indole, quinoline, pyridine, furan, benzfuran, thiophene or benzthiophene derivatives. The prepared carboxamides were tested against A549, HepG2, HL60, 3T3 and 4T1 tumour cell lines (Avidin Ltd.), but all 17 derivatives showed only weak to moderate IC_{50} values. The introduction of the C-5 carbonyl function involving carbetoxy, acyl or benzoyl units and the inserted heterocycles at R^4 did not have any significant influence on the cytotoxic activity.



Scheme 5.

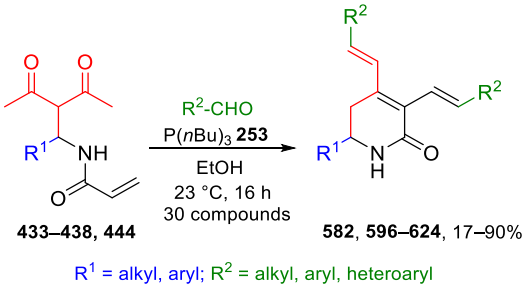
3.7. Formation of new C–C bonds via phosphine-triggered intramolecular transformation

Finally, a novel phosphine-triggered transformation was introduced by assemblies of acrylamide-based Mannich precursors and aldehydes in the presence of a phoshine catalyst. In our first effort, selected Mannich precursors were reacted with 2.5 equivalents of benzaldehyde in the presence of *n*-butylphosphine as catalyst in THF and MeCN without any detectable transformation. Surprisingly, upon using EtOH as solvent, the formation of a unique architecture was detected instead of the expected Stetter, MBH or IMBH products. This compound was identified as 5,6-dihydropyridine-2(1*H*)-one decorated with aromatic moieties at the C-3, C-4 and C-6 positions.

To determine the optimal conditions, various amounts of tri-*n*-butylphosphine were tested and solvents as well as phosphine/phosphite screening were carried out. After a preliminary study, the optimal conditions were determined as 2.5 equivalents of aldehyde and 1.2 equivalents of tri-*n*-butylphosphine with 16 h of stirring at ambient temperature.

3.8. The synthesis of 5,6-dihydropyridine-2(1*H*)-ones

Exploiting this protocol, a 30-membered chemical library of 5,6-dihydropyridine-2(1*H*)-ones symmetrically decorated at C-3 and C-4 was constructed in yields of 17–90% (*Scheme 6*). For diversification, R^1 and R^2 were varied by utilisation of aliphatic and (hetero)aromatic units including (un)substituted phenyl groups decorated with EWG or ED groups as well as 3-furyl, 3-thiophenyl, 3-benzothiophenyl moieties as heteroaromatic part of the structure.



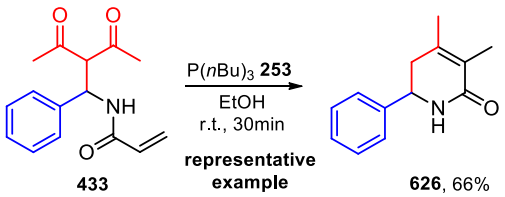
Scheme 6.

3.9. Suggested reaction mechanism of the domino transformations

According to our assumption, the annulation process is a domino cascade transformation involving a retro-Claisen–Schmidt reaction in EtOH, trialkylphoshine-mediated IMBH followed by Wittig coupling, then lactam–lactim tautomerisation and vinyl-aldol condensation terminated by stabilisation/tautomerism sequences to afford the desired multisubstituted 5,6-dihidropyridin-2(1*H*)-one products.

3.10. Synthetic strategy towards the formation of pyridin-2(1*H*)-one

The treatment of substrate **433** with 1.2 equivalents of P(*n*Bu)$_3$ in the absence of aldehyde component in EtOH afforded the formation of 3,4-dimethyl-6-phenylpyridine-2(1*H*)-one **626** (*Scheme 7*). The structure was identified by means of 1D- and 2D-NMR as well as by HRMS measurements. Presumably, the product was formed in an alternative synthetic route involving retro-Claisen/IMBH/phosphine hydrolysis/auto-oxidation sequences.

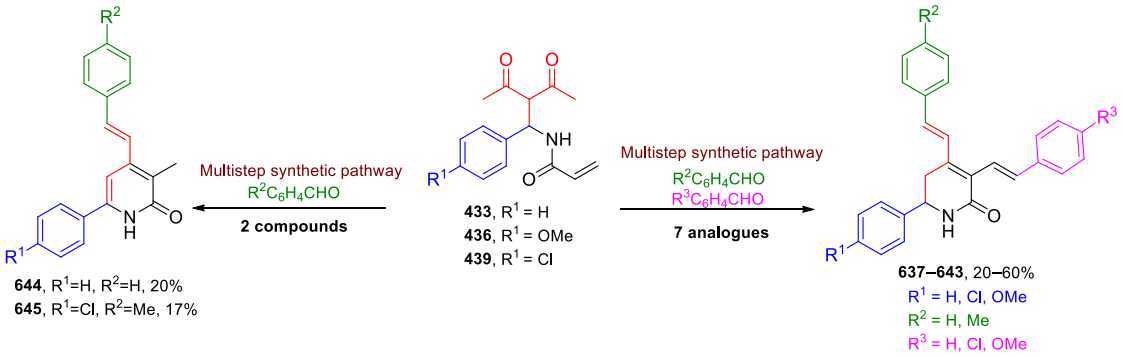


Scheme 7.

3.11. Extension of the diversity

To access the highest diversity, C-3, C-4, C-6 unsymmetrically trisubstituted 5,6-dihydropyridine-2(1*H*)-ones (different R^1, R^2, R^3 substituents) and pyridine-2(1*H*)-ones were also synthesised utilizing multistep synthetic procedures. To achieve ring-closed structures **637**–**643, 433**, **436** and **439**, Mannich precursors were treated under retro-Claisen–Schmidt reaction and the formed intermediate became a suitable substrate for the insertion of a benzaldehyde unit through Claisen–Schmidt condensation. The introduction of an additional benzaldehyde in the presence of tri-*n*-butylphosphine gave the desired target compounds **637–643** in yields of 20–60% (*Scheme 8*). The improved multistep synthetic pathway provided full regio- and chemoselectivity control to yield the desired products.

Finally, two unsymmetrically substituted pyridin-2(1*H*)-ones **644** and **645** have also been synthesised via phoshine-triggered annulation starting from the Claisen–Schmidt adduct in the absence of aldehyde.



Scheme 8.

Scientific publications forming the basis of the thesis

1. **Zs. Makra**, L. G. Puskás, I. Kanizsai, A convenient approach for the preparation of imidazo[1,2-*a*]-fused bicyclic frameworks via IBX/NIS promoted oxidative annulation
Org. Biomol. Chem. **2019**, *17*, 9001–9007.
IF: 3.890

2. **Zs. Makra**, A. Bényei, L. G. Puskás, I. Kanizsai, One-pot access towards 4,5-disubstituted 2-amino-1*H*-imidazoles starting from Mannich substrates and their transformation utilities
Eur. J. Org. Chem. **2020**, *2020*, 7184–7196.
IF: 3.261

3. **Zs. Makra**, R. Madácsi, T. A. Martinek, A. Bényei, L. G. Puskás, M. Gyuris, I. Kanizsai, Phosphine(III)-triggered one-pot domino sequences towards 5,6-dihydropyridine-2-(1*H*)-one and pyridine-2(1*H*)-one scaffolds
Adv. Synth. Catal. **2022**, *364*, 1–11.
IF: 5.981

4. N. Gémes, **Zs. Makra**, P. Neuperger, E. Szabó, J. Á. Balog, L. B. Flink, B. Kari, L. Hackler Jr, L. G. Puskás, I. Kanizsai, G. J. Szebeni, A cytotoxic survey on 2-amino-1*H*-imidazole based synthetic marine sponge alkaloid analogues
Drug. Dev. Res. **2022**, 1-17.
IF: 5.004

Summary IF: 18.136

Conference-Presentations

1. **Zs. Makra**, Imidazo[1,2-*a*]piridinek, pirimidinek és pirazinok előállítása IBX/NIS indukálta oxidatív gyűrűzárással; Heterociklusos és Elemorganikus Kémia Munkabizottság Ülése, Balatonszemes, **2019**.

2. **Zs. Makra**, Trialkil-foszfin indukálta dominó reakciók: 5,6-dihidropiridin-2(1*H*)-on és piridin-2(1*H*)-on származékok szintézise; Heterociklusos és Elemorganikus Kémia Munkabizottság Ülése, Balatonszemes, **2022**.

Conference-Posters

1. **Zs. Makra**, L. G. Puskás, I. Kanizsai, IBX/NIS mediálta oxidatív intramolekuláris gyűrűzárások; MKE Vegyészkonferencia, Hajdúszoboszló, **2017**.

2. **Zs. Makra**, L. G. Puskás, I. Kanizsai, IBX/NIS induced oxidative intramolecular annulation; ESOC, Vienna, **2019**.

3. **Zs. Makra**, L. G. Puskás, I. Kanizsai, One-pot synthesis of 4,5-disubstituted 2-amino-1*H*-imidazoles from Mannich precursors; IBSC, Novi Sad, **2021**.

4. **Zs. Makra**, R. Madácsi, T. Martinek, A. Bényei, L. G. Puskás, M. Gyuris, I. Kanizsai, Phosphine triggered domino transformations: synthesis of unique 5,6-dihydropyridine-2-(1*H*)-one and pyridine-2(1*H*)-one frameworks; BOSS, Namur, **2022**.

5. **Zs. Makra**, R. Madácsi, T. Martinek, A. Bényei, L. G. Puskás, M. Gyuris, I. Kanizsai, One-pot, multistep domino synthesis of trisubstituted 5,6-dihydropyridine-2-(1*H*)-one and pyridine-2(1*H*)-one scaffolds; Austrian Chemistry Days, Vienna, **2022**.