UNIVERSITY OF PANNONIA

Doctoral School in Chemistry and Environmental Science

Organic Synthesis and Catalysis Research Group

APPLICATION OF IONIC LIQUID CATALYSTS IN STEROID SYNTHESIS

THESIS OF THE PhD DISSERTATION

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I. PRELIMINARIES AND AIMS OF THE WORK

A common characteristic of ionic liquids is their relatively low melting point, below 100°C, due to the large size of their constituent ions and weak electrostatic interactions. Among the first ionic liquids is ethylammonium nitrate, discovered by Paul Walden in 1914. However, interest in ionic liquids only surged after Thomas Welton's 1999 literature review. A specific type of ionic liquids, known as reversible ionic liquids or switchable polarity solvents, can be switched from an initial molecular state to an ionic state with an external trigger, such as interaction with CO_2 or SO_2 gas. Removal of the external trigger reverts the system to its original non-ionic state.

One significant filed of application of ionic liquids is their use in organic reactions. Their solubility properties enable the selective separation of low-volatility products and other components from the ionic liquid phase. Unlike traditional solvents and catalysts, ionic liquids can be reused, reducing the waste generated in organic reactions. Additionally, they can achieve high catalytic efficiency and enhance reaction selectivity. Despite the widespread research concerning the use of ionic liquids in organic chemistry, there is only a few examples for their application in steroid chemistry.

Several nitrogen- and sulfur-containing pregnenolone derivatives have anticancer properties. The biological activity of structures modified at the C-17 position of the steroid nucleus has been extensively studied. In contrast, compounds substituted at the C-16 position are less common, but some of them also exhibit beneficial biological effects. Accordingly, this experimental work focused on synthesizing biologically active nitrogen- and sulfur-containing pregnenolone derivatives and analogues using aza- and thia-Michael addition.

The primary goal of the work was to develop reaction conditions that allow for the recycling of catalysts, making them advantageous from a green chemistry perspective. Therefore, investigation of the use of ionic liquids both as solvents and catalysts to ensure the separation of steroid products and unreacted reagents as well as the recovery of the catalyst/solvent was planned.

II. EXPERIMENTAL METHODS

During the synthetic work a Schlenk technique was applied.

The progress of the reactions was monitored by thin layer chromatography. The invisible spots were made visible using a UV lamp or sulfuric acid. The products were purified by column chromatography. The structur of the steroidal products and ionic liquids were proved by various NMR spectroscopic measurements and by HRMS and IR spectroscopy. In one case X-ray diffraction analyses were also performed to prove the structure of one of the products.

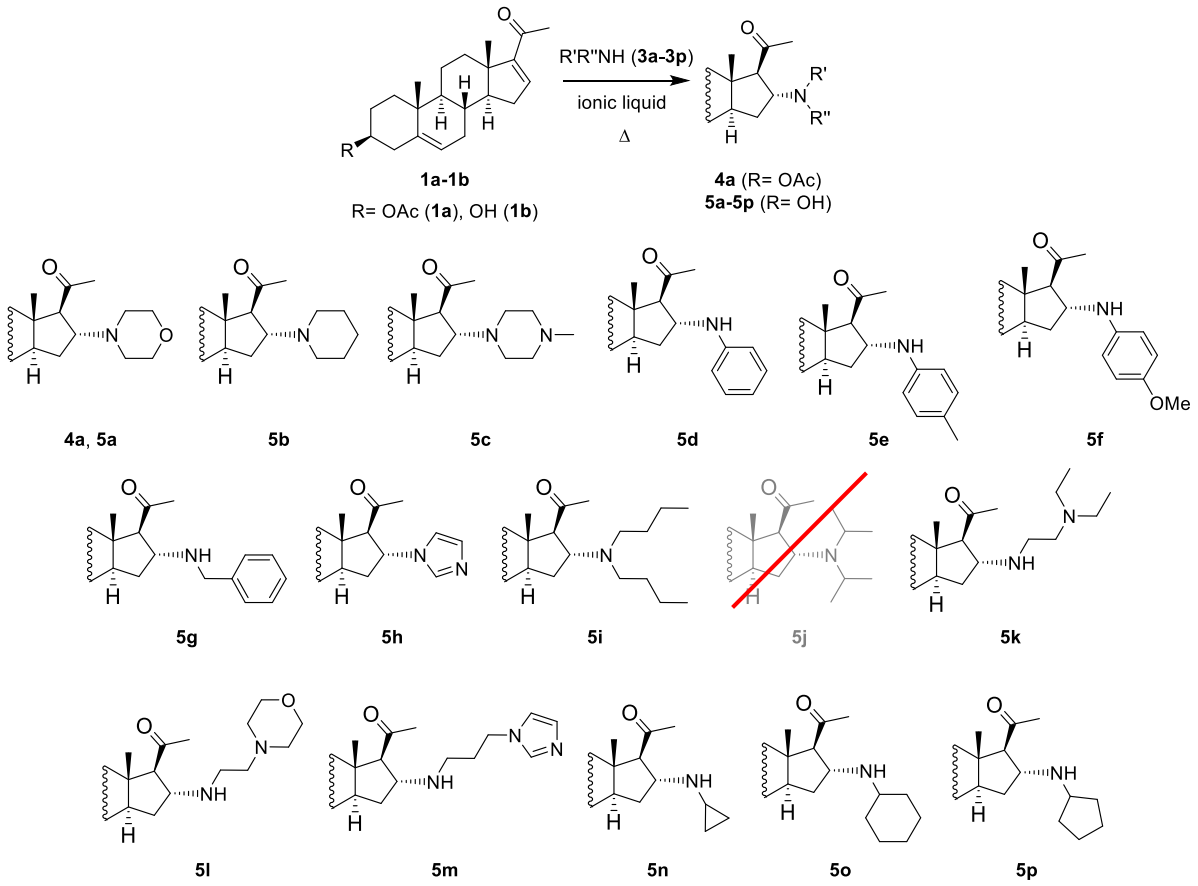
The process of converting reversible ionic liquids back and forth was verified through the acquisition of ^{1}H NMR and ^{13}C NMR spectra.

III. NEW SCIENTIFIC RESULTS

1. 16α-Amino-pregnenolone derivatives were produced by the aza-Michael addition reaction of different amines on 3β-hydroxypregna-5,16-dien-20-one (**1b**) in the presence of basic ionic liquids. Among the 16 compounds synthesized, 14 steroids were novel compounds. No by-products were formed in the reactions of steroid **1b**, while the acetyl group of the the 3β-acetoxy derivatives **1a** was partially cleaved under the reaction conditions, leading to the formation of a product mixture.

The products were characterized by different spectroscopic methods (^{1}H NMR, ^{13}C NMR, HRMS, and IR). The stereochemistry of compound **5a** was determined based on HSQC and ROESY spectra. The 16α position of the amino group was also confirmed by X-ray diffraction measurements of compound **5d**.

The products were investigated for the inhibition of in vitro $C_{17,20}$-lyase activity and were found to display moderate inhibitory effect.



2. Based on the results of the aza-Michael additions, the following conclusions can be drawn:

a) The lower yield achieved in the absence of [HDBU][OAc] (**2a**) demonstrates the necessity of a base catalyst.

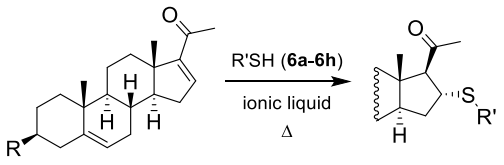
b) [HDBU][OAc] (**2a**) was proved to be a reusable catalyst and solvent through five cycles in the reaction of **1b** and **3a**.

c) [HDBU][Lac] (**2b**) showed nearly identical catalytic activity compared to **2a**. Successful extraction of products **5f**, **5g**, **5h**, and **5m** could only be achieved when [HDBU][Lac] (**2b**) was used as the solvent and catalyst.

3. In thio-Michael addition reactions of **1a** and **1b** steroids, 15 thioether derivatives were produced in the presence of basic ionic liquids, resulting in 14 new compounds beside the two known derivatives.

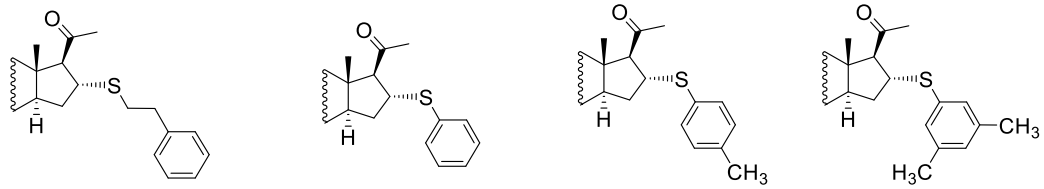
The structures of products were confirmed by ^{1}H NMR, ^{13}C NMR, HRMS, and IR spectroscopy. The α configuration of the 16-thioether functionality was proved with the help of NOESY spectra in the case of **7a**.

The toxicity of the 16α-thio steroid derivatives was tested on MDA-MB-231 and MCF-7 cell lines. Generally, the compounds affected the cells significantly only at high concentrations (≥50 μM).

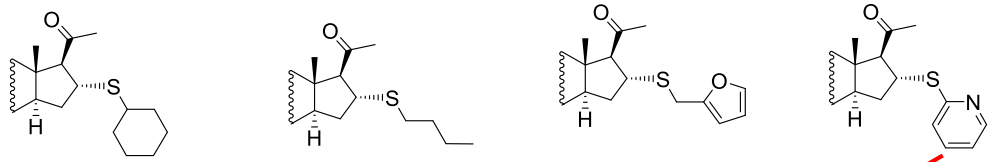


1a-1b
R= OAc (**1a**), OH (**1b**)

7a-7h (R= OAc)
8a-8h (R= OH)



7a **8a** **7b** **8b** **7c** **8c** **7d** **8d**



7e **8e** **7f** **8f** **7g** **8g** **8h**

4. Based on the results of the thio-Michael additions, the following conclusions can be made:

a) The catalytic effect of [HDBU][OAc] (**2a**) was proved.

b) An efficient recycling of [HDBU][OAc] (**2a**) as solvent and catalyst was achieved through five cycles.

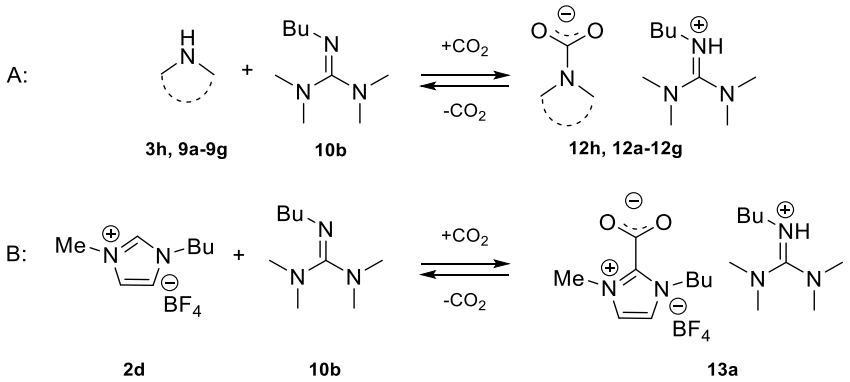
c) Under thio-Michael addition conditions, no cleavage of the acetyl group of the starting steroid **1a** occurred. The production of **8a** was realized through two reaction pathways. The favorable synthetic route was determined based on the overall isolated yields.

d) No thio-Michael addition occurred in the presence of 2-HEAF (**2g**) ionic liquid. Due to uncertainty in the composition of TEAA (**2f**), its catalytic activity was not tested.

5. To facilitate the separation of 16α-azolyl steroids formed in the aza-Michael addition and to solve the catalyst recycling problem, a switchable polarity system was developed, which also enabled the reuse of the excess of the reagents.

The conditions for reversible transformation of *N*-heterocycle + guanidine base and base + ionic liquid systems were determined. Solubility tests were conducted to select the appropriate

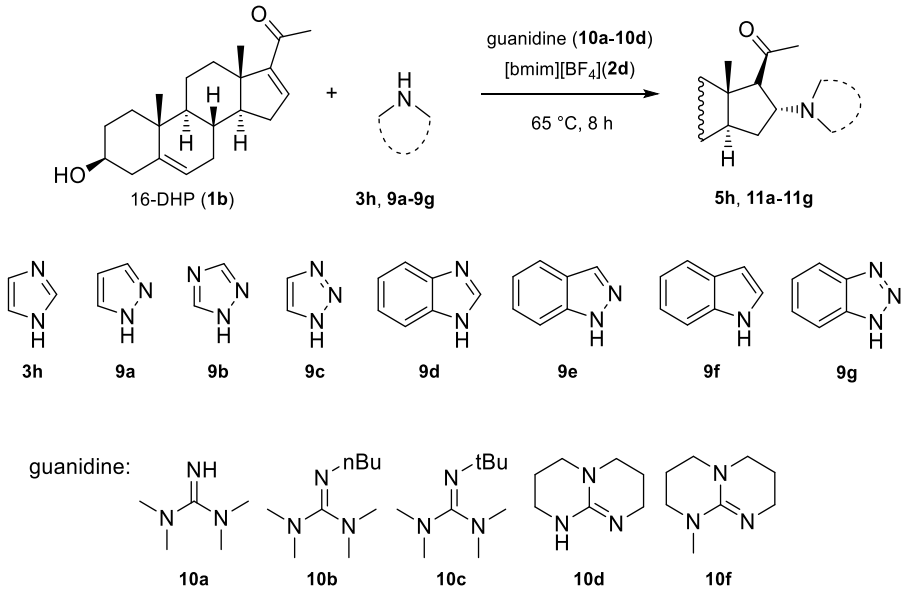
solvent for extraction. The use of TMG (**10a**) catalyst failed due to side reactions occurring under the influence of CO_2. The reversible chemisorption of CO_2 in reactions A and B was confirmed using ^{1}H- and ^{13}C NMR spectra.



6. The production of seven 16α-azolyl-pregnenolone derivatives was achieved in the aza-Michael addition of steroidal substrate **1b** and various *N*-heterocyclic compounds (**3h**, **9a**-**9g**) in the presence of guanidine bases (**10b**, **10d**) as catalysts and [bmim][BF_4] (**2d**) as solvent. No conversion of the substrate could be detected with **9g** as the reaction partner. The products were characterized using ^{1}H NMR, ^{13}C NMR, HRMS, and IR spectroscopy. The configuration of positions C-17 and C-16 in adduct **11a** was determined using NOESY spectra.

7. The synergistic effect of *n*Bu-TMG (**10b**) and [bmim][BF_4] (**2d**) in the aza-Michael addition was proved. Other base catalysts showed lower activity under identical reaction conditions.

After CO_2 chemisorption, products **5h**, **11a**-**11f** were separated by extraction. Then, ionic liquids **12h**, **12a**-**12f** were heated under vacuum to switch them back to the molecular state. The reuse of reversible ionic liquids was achieved over 2-5 cycles without a loss of their components during the extraction of the products except for the reactions of indazole (**9e**) and indole (**9f**). In the latter reactions significant leaching of reagent and catalyst was detected. Replacement of **10b** by MTBD (**10f**) resulted in a reusable reversible system.



I. SIGNIFICANCE OF THE SCIENTIFIC RESULTS

During the doctoral work, the applicability of basic ionic liquids and switchable polarity solvents in the aza- and thia-Michael addition reactions of steroids was investigated. Following the development of optimal reaction conditions, the desired steroid derivatives were successfully produced with good yields.

The basic ionic liquids used as catalysts and solvents could be reused multiple times without significant decrease in the yield. Additionally, with the use of reversible ionic liquids, the recycling of excess reagents was achieved. A total of 37 steroid derivatives were synthesized during the work, 33 of which are new compounds not previously reported in the literature. The use of basic and reversible ionic liquids in steroid synthesis is a less explored research area. Now it was proved that these methodologies can be used efficiently not only for simple model compounds but also for more elaborated structures with biological activity.

In biological activity tests, the 16-aza steroid derivatives showed moderate $C_{17,20}$-lyase enzyme activity. The toxicity of the 16-thia steroid derivatives was tested on MDA-MB-231 and MCF-7 cell lines, where the compounds generally only significantly affected the cells at high concentrations (≥50 μM). The results ensure a good basis for the synthesis of further derivatives with enhanced biological activity.

Scientific publications and presentations related to the dissertation

Publications:

1. Eszter Szánti-Pintér, **Lilla Maksó**, Ágnes Gömöry, Johan Wouters, Bianka Edina Herman, Mihály Szécsi, Gábor Mikle, László Kollár, Rita Skoda-Földes, *Steroids*, **2017**, *123*, 61-66. https://doi.org/10.1016/j.steroids.2017.05.006 IF: 2,58 (2017)

2. **Lilla Maksó**, Krisztina Kovács, Kitti Andreidesz, Ágnes Gömöry, Sándor Mahó, Rita Skoda-Földes; *ChemistrySelect*, **2022**, *7*, e202200967. https://doi.org/10.1002/slct.202200967 IF: 2,23 (2022)

3. **Lilla Maksó**, Boglárka Szele, Dávid Ispán, Ágnes Gömöry, Sándor Mahó, Rita Skoda-Földes, *Organic & Biomolecular Chemistry*, **2024**, *22*, 2465-2473. https://doi.org/10.1039/D3OB02073H IF: 3,2 (2024)

Presentations:

Lilla Maksó, Rita Skoda-Földes, Synthesis of pregnenolone derivatives via ionic liquid-catalyzed aza-Michael and thio-Michael addition, 6th International Conference on New Trends in Chemistry, Kyrenia (Ciprus), 2020 október 16-18. (poszter, online)

Lilla Maksó, Rita Skoda-Földes, Synthesis of 16α-substituted pregnenolone derivatives via ionic liquid-catalyzed aza-Michael and thio-Michael addition, Symposium on Synthesis and Catalysis, Évora (Portugália), 2021.aug.31-szept.3. (poszter, online)

Lilla Maksó, Rita Skoda-Földes, Synthesis of pregnenolone derivatives via ionic liquid-catalyzed aza-Michael and thio-Michael addition, XXXVIII. Biennial Meeting - Spanish Royal Society of Chemistry (RSEQ), Granada (Spanyolország), 2022. június 27-30. (poszter)

Scientific publications not related to the dissertation:

1. Anita Horváth, Kristóf Bolla, Alexandra Wachtler, **Lilla Maksó**, Máté Papp, Sándor Mahó Zsófia Dubrovay, János Kóti, Rita Skoda-Földes, *ACS Omega*, **2021**, 6, 26846–26856. https://doi.org/10.1021/acsomega.1c02470

2. Nagy Enikő, **Maksó Lilla**, Ispán Dávid, Hancsók Jenő, Skoda-Földes Rita, *MKF-Kémiai Közlemények (1997-)*, **2021**, 127(3-4), 103-109. http://doi.org/10.24100/MKF.2021.03-4.103