Total Synthesis via Biomimetic Late-stage Heterocyclization, Assignment of the Relative Configuration and Biological Evaluation of the Nitraria Alkaloid (±)-Nitrabirine

Mervic D. Kagho,[‡,§,*] Helen Hintersatz,[§] Andreas Ihle,[§] Haoxuan Zeng,[¥] Hedda Schrey,[¥] Wera Colisi,[¥] Philipp Klahn,[‡,*] Marc Stadler,[¥,∥] Clemens Bruhn,[#] Tobias Rüffer,[†] Heinrich Lang,[†] Klaus Banert[§,*]

[‡]Institute of Organic Chemistry, Technische Universität Braunschweig, Hagenring 30, D-38106 Braunschweig, Germany.

[§]Organic Chemistry, Technische Universität Chemnitz, Straße der Nationen 62, 09111 Chemnitz, Germany.

[¥]Department of Microbial Drugs, Helmholtz Centre for Infection Research and German Centre for Infection Research (DZIF), Partner Site Hannover-Braunschweig, Inhoffenstraße 7, 38124 Braunschweig, Germany.

[∥]Institute of Microbiology, Technische Universität Braunschweig, Spielmannstraße 7, 38106 Braunschweig, Germany.

[†]Inorganic Chemistry, Technische Universität Chemnitz, Faculty of Natural Sciences, Institute of Chemistry, 09107 Chemnitz, Germany.

[#]Institut für Chemie und CINSaT, Universität Kassel, Heinrich-Plett-Strasse 40, 34132 Kassel, Germany.

In memoriam of Prof. Dr. Klaus Banert.

KEYWORDS: *Nitraria* Spiranic Alkaloids, Nitrabirine, Total Synthesis, Biomimetic Late-stage Heterocyclization, anti-biofilm activity

ABSTRACT: The racemic total synthesis of nitrabirine (**5**) together with its previously undescribed epimer 2-*epi* nitrabirine (**5'**) is accomplished via a six-step route based on a biomimetic late-stage heterocyclization. This allowed the assignment of the relative configuration of nitrabirine by the lanthanide-induced shifts (LIS) experiment, later on confirmed by X-ray diffraction of obtained single crystals. Furthermore, oxidation studies demonstrated that the direct *N*-oxidation of nitrabirine does not yield nitrabirine *N*-oxide as reported earlier. In contrast, the reaction of hydrogen peroxide with nitrabirine (**5**) yields the salt **24'** whereas, under the same conditions, 2-*epi* nitrabirine (**5'**) surprisingly leads to a beforetime uncharacterized product **22**. Finally, a Fischer-Indole reaction gave access to novel tetracyclic nitrabirine derivatives **26a-d**. A comprehensive biological evaluation of nitrabirine (**5**), 2-*epi* nitrabirine (**5'**), and all derivatives synthesized in this study revealed general biofilm dispersal effects against *Candida albicans*. Moreover, specific compounds showed moderate antibacterial as well as potent cytotoxic activities.

INTRODUCTION

The *Nitraria* alkaloids are divided into three main groups: spiranic alkaloids, tripiperidine alkaloids, and indole alkaloids.[1] The simple *Nitraria* spiranic alkaloids include (+)-nitramine (**1**), (+)-isonitramine (**2**), (–)-sibirine (**3**), (–)-sibirinine (**4**), (±)-nitrabirine (**5**) and (±)-nitrabirine-*N*-oxide (**6**) (Figure 1), which have been isolated from two species of the *Nitraria* genus, namely *Nitraria schoberi* (for **1**)[2] and *Nitraria sibirica* (for **2**, **3**, **4**, **5**, and **6**).[1–6] Structurally, the simple *Nitraria* spiranic alkaloids comprise a 2-azaspiro[5.5]undecan-7-ol framework with a central chiral quaternary carbon centre. Additionally, aminal formation bridging the secondary amine in the 2-position with the hydroxy moiety in the 7-position leads to the tricyclic core of (-)sibirinine (**4**). The structures of nitrabirine (**5**) and nitrabirine-*N*-oxide (**6**) contain an additional annulated imidazole ring in their tricyclic tetrahydro-5'H-spiro[cyclohexane-1,8'-imidazo[1,2-a]pyridin]-2-ol skeleton (Figure 1).

The biological activities of *Nitraria* alkaloids in general and simple *Nitraria* spiranic alkaloids, in particular, are significantly under investigated.[1] Aside from (+)-isonitramine (**2**) which displayed significant effects in the protection of pancreatic *β*-cells and the attenuation of postprandial hyperglycemia,[7] there is only one further study reporting on antimicrobial activity *of* the extracts of *Nitraria sibirica* against *Candida albicans* and *Staphylococcus aureus* and their antiproliferative activity against A549, MCF7, and HeLa tumor cells.[8]

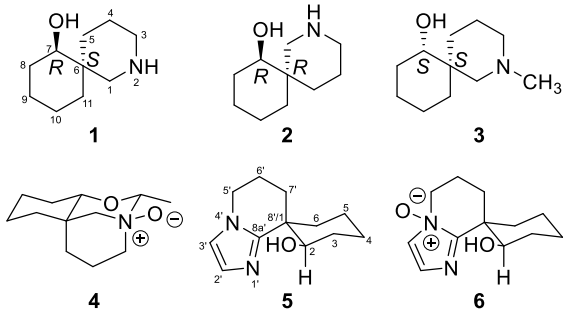
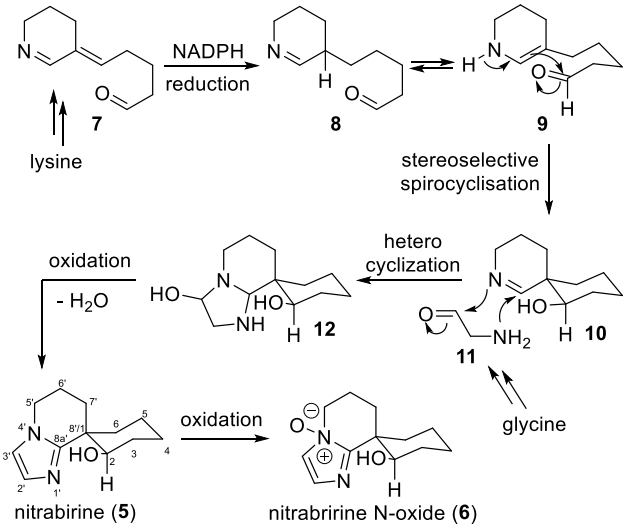


Figure 1. Structures of simple *Nitraria* spiranic alkaloids

Most of the simple *Nitraria* spiranic alkaloids including (+)-nitramine (**1**), (+)-isonitramine (**2**), (–)-sibirine (**3**) and (–)-sibirinine (**4**) have already been successfully targeted by racemic or enantioselective total syntheses,[9–14] which contributed to their structure elucidation enabling the X-ray diffraction of their single crystals finally revealing their stereo configurations. However, to the best of our knowledge, no synthesis of nitrabirine (**5**) or nitrabirine-*N*-oxide (**6**) has been reported so far, completely elucidating their stereochemistry.

The biosynthesis of nitrabirine (**5**) and nitrabirine-*N*-oxide (**6**) was proposed in 2010 by Poupon and Gravel[1] based on the initial reduction of the pivotal, lysine-derived achiral intermediate **7** releasing intermediate **8**, which is in equilibrium with its tautomer **9** as outlined in Scheme 1. A stereoselective spirocyclization of the enamine **9** might lead to the spirocyclic imine **10**, which undergoes a late-stage heterocyclization with the glycine-derived α-aminoaldehyde **11** leading after final elimination of water and oxidation of the 8a' Position to the formation of nitrabirine (**5**).

Scheme 1. The biosynthetic hypothesis for nitrabirine (**5**) and nitrabirine *N*-oxide (**6**).

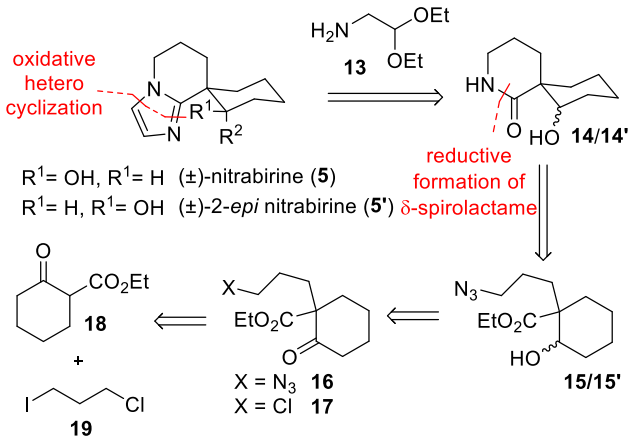


Inspired by this proposed biosynthetic late-stage heterocyclization, we envisaged the first total synthesis of nitrabirine (**5**) and derivatives to elucidate its relative stereochemistry (in particular at the C2 position) and enable first biological evaluation as well as structure-activity relationships studies of this natural product.

RESULTS AND DISCUSSION

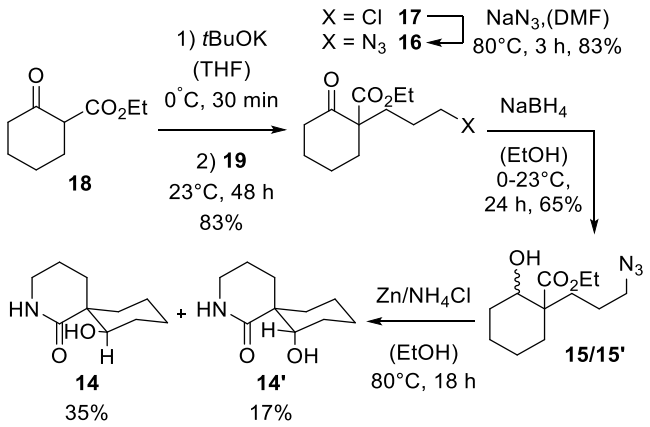
We planned to achieve the racemic total synthesis of nitrabirine (**5**) and 2-*epi* nitrabirine (**5'**) via biomimetic, oxidative late-stage heterocyclization of the spirolactame precursors **14/14'** with 2-aminoacetyaldehyde diethylacetal **13** under acidic open flask conditions as outlined in the retrosynthetic analysis of Scheme 2. As the second key step for the construction of the spiranic *Nitraria* alkaloid skeleton, we envisaged the formation of the δ-spirolactone through azide reduction of the azido-precursors **15/15'**, which should be accessible from commercially available β-ketoester **18** via three-step sequence as pointed out in Scheme 2. The synthesis of the precursors **15/15'** for the construction of the spiranic skeleton started with the alkylation of β-ketoester **18** with 1-chloro-3-iodopropane (**19**) in the presence of KO*t*Bu according to a known procedure of Pearson *et al.*[15] affording compound **17** in 83% yield (Scheme 3).

Scheme 2. Retrosynthetic analysis for nitrabirine (**5**)



Subsequent substitution of the chloride in the presence of sodium azide in dimethylformamide at 80°C yielded the known[16] azide **16** in 83% yield.[17] Reduction of the β-oxo group of **16** in the presence of sodium borohydride in ethanol gave the known[18] secondary alcohols **15** and **15'** in a 1:1 mixture of the diastereomers. Finally, the reduction of the azide moieties of **15** and **15'** to the amino groups in the presence of Zn/NH_4Cl in ethanol:water/3:1[19] followed by spontaneous ring closure to the δ-spiro lactame at 80°C led to a 2:1-mixture of the known[11] diastereomers **14** and **14'**, which could be separated by flash column chromatography through silica gel and were obtained in 35% and 17%, respectively.

Scheme 3. Synthesis of spirolactams **14** and **14'**.



Unfortunately, our initial attempts to facilitate the desired late-stage heterocyclization with aminoacetaldehyde diethylacetal (**13**) were not successful utilizing a variety of Lewis acids with both spirolactams **14** and **14'** as starting materials. Neither tin tetrachloride nor titan tetrachloride[20] nor phosphorus pentachloride[21] could enable the formation of nitrabirine (**5**) or 2-*epi* nitrabirine (**5'**) and led Lewis acids promoted rather the polymerization of **13** instead. Additionally, the Lewis acid free reaction of **14** and **14'** with **13** at high temperatures could not furnish the formation of **5** or **5'**. As we felt, that the free hydroxy moieties might cause the problems, we first oxidized both hydroxy

spirolactames **14** and **14'** to their corresponding oxo-spirolactam **20** in the presence of pyridinium chlorochromate (Scheme 4) as reported earlier by Keppens and De Kimpe.[11] Later on, the lactam moiety of **20** was activated using Meerwein's reagent, which was subsequently displaced by the nucleophilic attack of aminoacetaldehyde diethylacetal (**13**). The final heterocyclization under acidic conditions at 100°C gave previously uncharacterized compound **21** already bearing the tricyclic tetrahydro-5'H-spiro[cyclo-hexane-1,8'-imidazo[1,2-a]pyridin]-2-ol skeleton with a yield of 76%. Finally, the reduction of **21** using sodium borohydride afforded nitrabirine (**5**) and its previously undescribed epimer 2-*epi* nitrabirine (**5'**) with yields of 23% and 39% respectively. In contrast, the reduction in the presence of DIBAL-H led to **5** and **5'** with yields of 40% and 20%, respectively. Moreover, both epimers were easily convertible into the corresponding ketone **21** by Jones oxidation, confirming their diastereomeric nature. The analytical data of compound **5** were in complete accordance with the data published for the isolated natural product nitrabirine (see Supporting Information Table S5).[22] To finally determine the relative configuration of nitrabirine **5** and **5'** we analyzed lanthanide-induced shifts (LIS) in the ^{1}H-NMR of both compounds utilizing (tris(7,7,-dimethyl-1,1,1,2,2,3,3-heptafluoroocta-4,6-dionato)europium(III) or simply (Eu(fod)$_3$) as shift reagent (structure depicted in Figure 2). Since the discovery by Hinckley in 1969 that a europium (III) complex produces a large isotropic chemical shift in the ^{1}H-NMR spectrum of cholesterol, the technique of lanthanide-induced shifts (LIS) has become one of the most powerful stereochemical tools, complementary to other techniques such as the NOE and spin-decoupling.[23] The direction of the shift, this is, upfield or downfield, depends primarily upon the lanthanide complex used. Thus, complexes of europium, erbium, thulium, and ytterbium shift resonances to lower fields while complexes of cerium, praseodymium, neodymium, samarium, terbium, and holmium tend to shift resonances to a higher field. Most of the lanthanide complexes also give considerable line broadening at higher concentrations. This effect is undesirable due to loss of resolution. Complexes of europium and praseodymium are by far the best shift reagents in this respect giving shift broadenings of only 0.003 and 0.005 Hz/Hz of shift, respectively.[24]

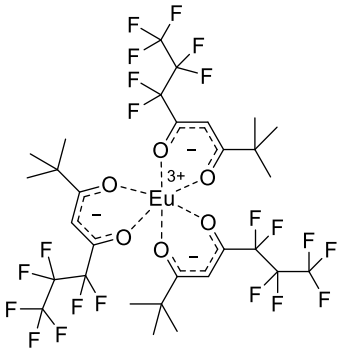
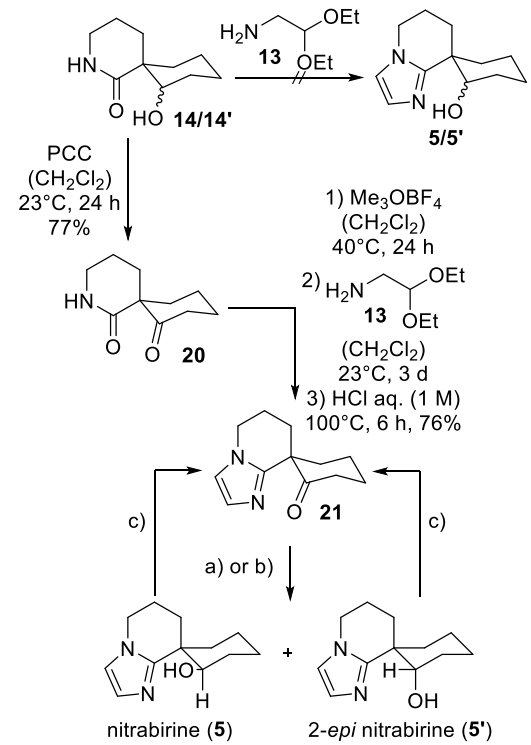


Figure 2. Structure of Eu(fod)$_3$

The principle is based on chelate-like interactions between the europium and the lone-pair electrons of the oxygen of the hydroxyl group and the sp^2 nitrogen of the imidazole ring of **5** on one hand and of its epimer **5'** on the other hand. The chemical shift difference increases with the increasing concentration of shift reagent and with the stability of the complexes. In addition, the shift difference is dependent on the angle and distance of the protons relative to the site of complexation.

Scheme 4. Synthesis of **5** and **5'**.



a) $NaBH_4$, (EtOH), 0-23°C, 24 h: 23% of **5** and 39% of **5'.** b) DIBAL-H, (CH_2Cl_2), -78°C, 3 h: 40% of **5** and 20% of **5'.** c) CrO_3/H_2SO_4, (acetone/CH_2Cl_2), 0-23°C, 14 h: 60% (from **5**), 54% (from **5'**)

To evaluate the influence of the paramagnetic Europium on the surroundings protons to the coordinating atoms of both **5** and **5'**, the ^{1}H chemical shift difference was plotted as a function of the molar ratio **n** (Eu(fod)$_3$)/**n** (**5** or **5'**), showing a linear correlation between 0.0 and 0.2-mole ratio (see Tables S1, S2, Figures S1 and S3 in the Supporting Information). Foremost, the signals of all the protons of nitrabirine (**5**) were shifted at low concentrations of the shift reagent. Besides, only the signals of protons 2-H, 2'-H, and H_{OH} were significantly shifted downfield (see Figure S2 in the Supporting Information).

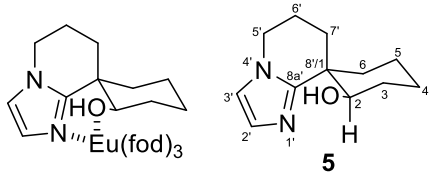


Figure 3. Plausible coordination to Eu(fod)$_3$ and relative configuration for **5**.

One reason for this could be the decreasing distances of these protons from the coordination sites. This is in agreement with the well-known fact that the influence of the paramagnetic Europium centre on all protons in its surrounding increases with decreasing distances of these protons. The other reason could be the existence of complexation as depicted in Figure 3, represented by a chelate-like

interaction. Moreover, the other signals were not considerably shifted downfield because of their increasing distances from the coordination sites. Thus, one can assume the hydroxyl group is oriented towards the imidazole ring in the equatorial position, while proton 2-H is directed in the same plane with the imidazole ring that means in the axial position. Thus, the most probable relative configuration for nitrabirine (**5**) in particular with regards to the 2-position is in accordance with previously reported configuration by Ibragimov *et al*.[5] In addition, we were able to obtain single crystals of the synthesized nitrabirine (**5**) enabling X-ray diffraction analysis, which confirmed this relative configuration as depicted in Figure 4.

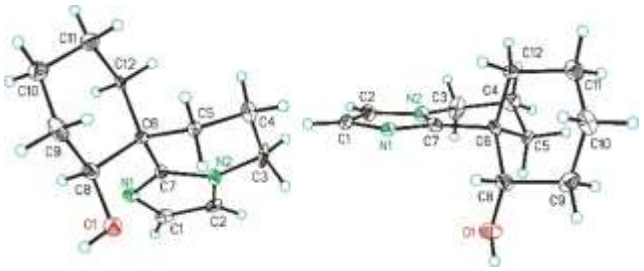


Figure 4: ORTEP diagram (40 % probability ellipsoids) of the molecular structure of **5** in two different perspective views. Of disordered atoms only one atomic position is shown.

Furthermore, the signals of the protons 5'a-H and 5'b-H were slightly more shifted than the signals of the other protons of **5'** (see Figure S4 in the Supporting Information). This can be explained by the presence of a complexation rather between the nitrogen in 4' position of the imidazole ring and europium as represented in Figure 5. Thus, the hydroxyl group is assumedly directed in the same plane with the imidazole ring (axial position), whereas the proton 2-H is directed toward the imidazole ring (equatorial position).

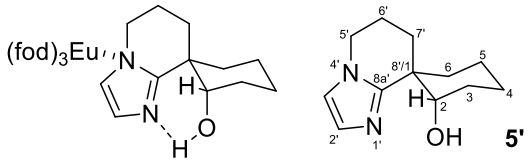
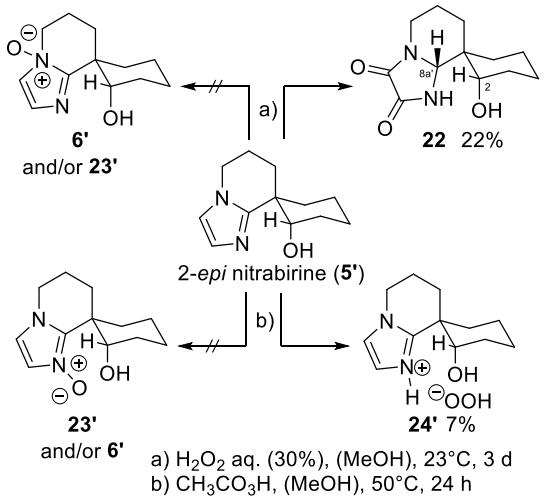


Figure 5. Plausible coordination to Eu(fod)$_3$ and relative configuration for **5'**.

In addition, there is no chelate-like interaction for **5'** probably because of the existence of a hydrogen bridge between the proton of the hydroxyl group and the 1' position of the imidazole ring as shown in Figure 5. The most probable conformation for 2-*epi* nitrabirine (**8'**) is represented in Figure 5.

With the first natural product in hand, next, we aimed to synthesize the corresponding nitrabirine *N*-oxide (**6**). Although it is known that imidazole-*N*-oxides are difficult to generate via direct *N*-oxidation from their corresponding imidazoles,[25] in 2001 Tulyaganov *et al*.[6] have reported the direct *N*-oxidation of **5** by the use of aqueous hydrogen peroxide (30%) obtaining nitrabirine *N*-oxide (**6**). However, in our hands, the same reaction conditions applied to 2-*epi* nitrabirine (**5'**) did not furnish the formation of the expected *N*-oxides (neither **6'** nor **23'**) but surprisingly gave the previously uncharacterized compound **22** with the yield of 22% as shown in Scheme 5. The NOESY spectrum of **22** (see Supporting Information Page 38) showed a clear cross-peak of the 2-H and 8a'-H so that we concluded 8a'-H is in a syn position compared to 2-H, defining the relative configuration in line with the X-ray diffraction analysis as depicted in Figure 6.

Scheme 5. Oxidation of **5'**.



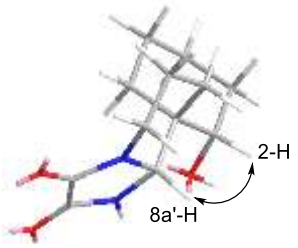
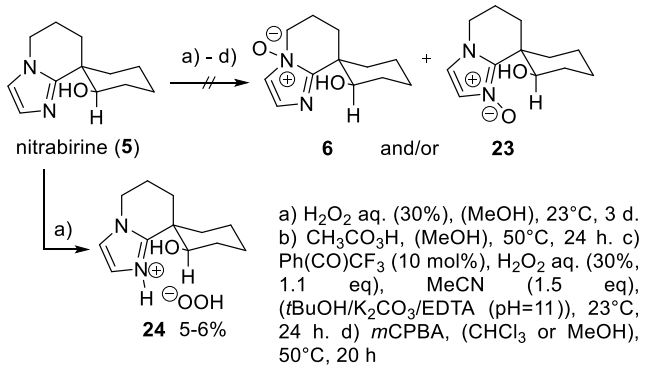


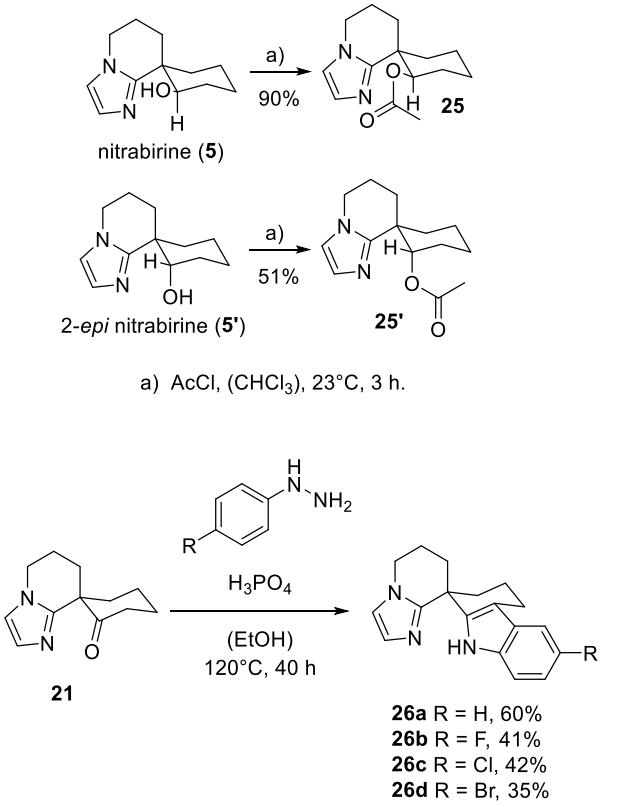
Figure 6. NOESY correlation between 2-H and 8a'-H in **22** (Chem3D illustration).

Further attempts to enable *N*-oxidation **5'** in the presence of peroxoacidic acid in methanol at 50°C failed, however, led to a low yield (7%) of the corresponding imidazolium hydroperoxide salt **24'**. In line with our findings for **5'**, in our hands, the reaction of **5** in the presence of aqueous hydrogen peroxide (30%) did not lead to the formation of the desired *N*-oxides **6** or **23**, as described by Tulyaganov *et. al*..[6] In contrast, the basic character of **5** was highlighted affording the salt **24** in low yield (5%) in presence of hydrogen peroxide (30%) as shown in Scheme 6. It is worth mentioning that, when the reactions leading to **24** and **24'** are performed in NMR tubes with deuterated methanol, they lead to yields above 90%. By comparison of the ^{1}H NMR data, Tulyaganov and co-workers assumably prepared the same salt **24** and mistakenly assigned it to the structure of **6**. Further attempts to achieve *N*-oxidation of **5** in the presence of peroxo carboxylic acids or the use of catalytic amounts of 2,2,2-trifluoroacetophenone[26] in the presence of hydrogen peroxide for the *in situ* formation of the corresponding dioxirane could not provide access to the desired *N*-oxides. Similarly, attempts for the *N*-methylation and *N*-acylation of **5** and **5'** were not successful. The low nucleophilicity of the imidazole nitrogens in **5** and **5'** in the course of the *N*-oxidation, *N*-methylation, and *N*-acylation is in line with the studies of Mayr and co-workers,[27] who attributed the weak reactivity to the relevantly higher reorganization energies for the reaction of imidazoles with electrophiles compared to other amines.

Scheme 6. Oxidation of **5**.



Scheme 7. Synthesis of the acetates **25** and **25'** and the tetracyclic indole derivatives **26a-d**.



Next, we attempted to generate derivatives of **5** and **5'**. Therefore, the *O*-acetyl products **25** and **25'** (Scheme 7) were formed in 90% and 51%, respectively, in the presence of acylchloride. Compound **25** was prior prepared by Ibragimov *et al.*[5] but was not yet characterized till now. Furthermore, we identified the 2-oxo moiety in intermediate **21** as an ideal starting point for the synthesis of novel nitrabirine derivatives bearing indole moieties[28] via the Fischer indole reaction (Scheme 7). Thus, **21** was reacted with different phenyl hydrazines in the presence of phosphoric acid at 120°C in ethanol which led to the novel nitrabirine indoles **26a-d** in moderate yields of 35-60%.

Finally, the compounds **5**, **5'**, **21**, **22**, **25**, **25'** and **26a–d** were evaluated for their antimicrobial activity against various bacteria and fungi (including the bacteria *Staphylococcus aureus, Bacillus subtilis, Mycobacterium smegmatis, Escherichia coli, Pseudomonas aeruginosa, Chromobacterium violaceum, Acinetobacter baumannii* and the fungi *Schizosaccharomyces pombe, Pichia anomala, Mucor hiemalis, Candida albicans, Rhodotorula glutinis*), as described previously.[29] While most of the compounds including the natural product **5** did not show antimicrobial effects against any of the tested microbes, compounds **25'** and **26a** both displayed weak antibacterial activity against *B. subtilis* with MIC values of 66.6 µg/mL. Furthermore, the two novel nitrabirine indole derivatives, **26c** and **26d** with a chlorine or bromine respectively at the 6-position of the indole core exhibited weak to moderate antimicrobial activities against several of the tested bacteria and fungi (see Table S6 in the Supporting Information). The most potent antibacterial activity for both, **26c** and **26d**, were observed against *B. subtilis* with a MIC of 16.6 µg/mL, equally potent compared to Oxytetracyclin. In addition, **26c** and **26d** demonstrated weak activity against *S. aureus* with MICs of 33.3 µg/mL each.

After evaluating the MICs of all compounds listed above, the anti-biofilm activities were investigated. More concretely, the dispersal effects on *C. albicans* and *S. aureus* accompanied by the inhibition of biofilm formation of *S. aureus* and *P. aeruginosa* were tested at sub-MIC concentrations, following previously established protocols.[30] Compounds **26a**, **26c**, and **26d** demonstrated moderate dispersal effects by *ca*. 60-70% against *C. albicans* at the concentration of 62.5 µg/mL relative to the negative control (= 0%). Moreover, compounds **26c** and **26d** caused by *ca*. 50 % dispersal effects at 31.3 µg/mL and compound **26d** showed 39% dispersal effects even at the concentration of 15.6 µg/mL (see Table S7 in the Supporting Information). It is similar active as the positive control farnesol. In comparison of these three compounds, the other candidates **5, 5', 22, 25,** and **26b** exhibited weak dispersal effects around 29-73% when applied at the highest concentration of 250 µg/mL. For the inhibition of formation of biofilms and dispersive effects on preformed biofilms of *S. aureus*, compounds **5**, **5'**, **21, 22, 25, 25', 26a,** and **26b** were tested. Due to their MICs of 33.3 µg/ml against *S. aureus,* which were lower than the highest anti-biofilm testing concentration, compound **26c** and **26d** were not investigated. Whereas just compound **26a** has anti-biofilm activities against the formation of biofilms and dispersal effects on *S. aureus* merely at the concentration of 250 µg/mL with ca. 90% as well as *ca*. 51%, respectively. All compounds were detected for inhibition effects against biofilms of *P. aeruginosa,* however, none of the compounds showed inhibition effects in the case.

Moreover, **5**, **5'**, **21**, **22**, **25'**, **25',** and **26a–d** were evaluated for their antiproliferative effects against two mammalian tumor cell lines namely human cervix carcinoma cells KB3.1 and mouse connective tissue fibroblasts L929 (see Table S9 in the Supporting Information), as described previously.[29,31] Again, the natural product **5** did not show an antiproliferative effect. Compounds **26a** and **26b** showed very weak antiproliferative activity without displaying changed cell shapes or cytotoxic effects against both cancer cell lines. Similarly, **25'** showed weak antiproliferative activity without displaying changed cell shapes or cytotoxic effects against L929 but gave moderate cytotoxic activity with an IC_{50} of 16 µg/mL against KB3.1 cells. Whereas, compounds **26c** and **26d** exhibited potent cytotoxic activities against L929 with IC_{50} values of 6.1 µg/mL and 8.5 µg/mL, respectively. Their cytotoxicity was even more pronounced

against KB3.1 cells with IC_{50} values of 2.4 µg/mL and 2.3 µg/mL, respectively. Based on the potent antiproliferative activity found for **26c** and **26d** we expanded the antiproliferative activity screening towards further human cancer cell lines and could demonstrate a potent antiproliferative activity of these compounds against human lung carcinoma A549, breast adenocarcinoma MCF-7, ovary adenocarcinoma SK-OV-3, prostate carcinoma PC-3 and epidermic carcinoma A431 cells (see Table S9 in the Supporting Information). The remaining compounds showed neither antiproliferative inhibition nor cytotoxic effect against the evaluated cancer cell lines (see Table S9 in the Supporting Information).

CONCLUSION

In conclusion, the racemic total synthesis of nitrabirine (**5**) together with the prior undescribed 2-*epi* nitrabirine (**5'**) was accomplished via a six-step synthesis including the reductive formation of the *δ*-spirolactane core and a biomimetic late-stage heterocyclization enabling the assignment of the relative configuration by the lanthanide-induced shifts (LIS) experiment, later on confirmed by X-ray diffraction analysis. Studies for direct *N*-oxidation of **5** and **5'** in the presence of hydrogen peroxide did not lead to the related natural product nitrabirine *N*-oxide (**6**) as described earlier by Tulyaganov *et al.*.[6] In contrast, the reaction of **5** with hydrogen peroxide yielded the salt **24**, which was assumably misinterpreted by Tulyaganov *et al*.. Surprisingly, under the same conditions, **5'** led to the beforehand undescribed product **22**. Further, *N*-methylation, and *N*-acetylation studies revealed a poor nucleophilicity of the imidazole ring of **5** and **5'**. Finally, the Fischer-indole reaction of **21** provided a simple derivatization opportunity leading to four novel nitrabirine indole derivatives **26a–d**. Compounds **5**, **5'**, **21**, **22**, **25**, **25',** and **26a–d** were biologically evaluated for their antimicrobial, antibiofilm, and antiproliferative activities. While the natural product **5** and **5'** were mostly inactive against all tested bacteria and fungi, the two novel nitrabirine indole derivatives **26c** and **26d** exhibited the most potent antimicrobial activity against *B. subtilis* with MICs of 16.6 µg/mL each and also displayed potent cytotoxic effect against a panel of mammalian cancer cell lines including KB3.1 cells with IC_{50} values of 2.4 µg/mL and 2.3 µg/mL, respectively. In addition, a general biofilm dispersal effect of the series of compounds was found against *C. albicans*, with the tetracyclic indole derivatives **26a**, **26c** and **26d** again being most active. Thereby, derivative **26a** exhibited neither antifungal effects at sub-MIC concentrations against *C. albicans* nor cytotoxic effects against the evaluated cell lines.

EXPERIMENTALS

General remarks. All reactions dealing with air- or moisture-sensitive compounds were carried out in a dry reaction vessel under a positive pressure of nitrogen. Air- and moisture-sensitive liquids and solutions were transferred via a syringe. All reactions were carried out with freshly distilled, in some cases, dry solvents. Anhydrous solvents were distilled immediately before use. Flash column chromatography was performed using silica gel (0.04–0.063 mm) as a stationary phase purchased from Fluka, (Germany) and aluminum oxide 90 active neutral (0.063–0.200 mm) purchased from Merck KGaA (Germany). Thin-layer chromatography (TLC) was carried out on POLYGRAM SIL G/UV254 and POLYGRAM Alox N UV254, ready foils from the MACHEREY-NAGEL (Germany). The solvents used, are mentioned in the experimental procedures. Commercially available starting materials were purchased from Acros Organics (Belgium), Sigma-Aldrich (Germany), TCI (Germany), Merck KGaA (Germany), abcr (Germany), Fluka (Germany). Compounds **16**[16], **17**[15], and **20**[11] were prepared according to the cited literature. NMR spectra were recorded with a ASCEND 250 FT spectrometer (Bruker Corp., Billerica, MA, USA) operating at 250 MHz for ^{1}H NMR and 69.9 MHz for ^{13}C NMR; and ASCEND 600 FT spectrometer (Bruker Corp., Billerica, MA, USA) operating at 600 MHz for ^{1}H NMR and 150.9 MHz for ^{13}C NMR ^{1}H NMR and ^{13}C NMR signals were referenced relative to residual solvent signals. Data are presented as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, quint = quintet, sext = sextet, sept = septet, m = multiplet, br =broad), coupling constants in hertz (Hz), followed by the number of hydrogen atoms. Assignments of NMR signals were further supported by correlation spectroscopy, heteronuclear single quantum correlation, and heteronuclear multiple bond correlation 2D-NMR methods and also by comparison of the data of homologous compounds reported in the literature. IR spectra were recorded with a Bruker Tensor 27 IR spectrometer (with ATR-technique) and with a Nicolet™ iS™ 5 spectrometer (Thermo Fisher Scientific Inc., Waltham, MA, USA) in a KBr cuvette for liquids (0.12 mm thickness) on one hand and the other hand measured as KBr pellet for solids. The measurement of the high-resolution mass spectra was carried out on a micrOTOF QII spectrometer (Bruker Corp., Billerica, MA, USA) utilizing an electrospray-ionization technique (source= Apollo II ESI). Melting points (mp) were recorded by the BOETIUS method on a heating apparatus from VEB Analytik Dresden PHMK 74/0032. Diffraction data for **5** was collected with an Oxford Gemini S diffractometer, with graphite-monochromated Mo Kα radiation (λ = 0.71073 Å). The structure of **5** was solved by direct methods and refined by full-matrix least-squares procedures. The graphics of the molecular structure of **5** have been created by using ORTEP and Diamond (Version 3.2k). Optical rotation values were measured on an MCP150 Modular Circular Polarimeter (Anton Paar). The lanthanide-induced shifts (LIS) experiment was performed in $CDCl_3$ (as NMR solvent and as a standard, δ = 7.26 ppm). 10 mg of **5** and **5'** was dissolved each in 0.6 mL of $CDCl_3$. A 0.2 M solution of $Eu(fod)_3$ in $CDCl_3$ was prepared by dissolving 100 mg of $Eu(fod)_3$ in 0.5 mL of $CDCl_3$ (see the titration volumes in Tables S1 and S2 of the Supporting Information and compare spectra).

*Synthesis of the ethyl 1-(3-azidopropyl)-2-hydroxycyclohexane-1-carboxylates (**15** and **15'**)*

17 (3.50 g, 13.8 mmol) was placed in a two-necked round bottomed flask under N_2 flow and dissolved with dry ethanol (8.8 mL). The mixture was cooled to 0 °C and $NaBH_4$ (0.44 g, 11.6 mmol) was added portionwise within 30 min. The reaction mixture was then allowed to stir overnight at 23°C, treated with brine (200 mL), and extracted with ethyl acetate (6×60 mL). The combined organic phases were dried over anhydrous $MgSO_4$ and the solvent was evaporated by a rotary evaporator. The crude product was purified by column chromatography on silica gel with diethyl

ether/*n*-hexane (3:1) as an isocratic eluent. The yellow oily liquid product could be obtained as a mixture of two diastereomers with a yield of 2.30 g (9.0 mmol, 65%). According to the **^{1}H-NMR** spectrum, we could assert that the mixture was made up of 43% of one diastereomer and 57% of the other. This can be explained by the presence in the spectrum of two doublets of doublet at 3.96 ppm and 3.41 ppm integrating, respectively for 0.43 and 0.57, both attributable to protons at the alpha position of the hydroxy group. ^{1}H NMR ($CDCl_3$, 600 MHz): δ 4.13– 4.23 (m, 2H, -O-C$\underline{H}_2$-CH_3), 3.96 (dd, $^3J_{H,H}$ = 8.6, 3.7 Hz, ½(1H), HO-C$\underline{H}$-) and 3.41 (dd, $^3J_{H,H}$ = 10.1, 3.7 Hz, ½(1H), HO-C$\underline{H}$-), 3.18–3.30 (m, 2H, -CH_2-C$\underline{H}_2$-N_3), 2.10–2.17 (m, 1H), 1.79–1.92 (m, 2H), 1.36–1.76 (m, 8H), 1.26 (t, $^3J_{H,H}$ =7.1 Hz, 3H, -OCH_2-C$\underline{H}_3$), 1.28 (t, $^3J_{H,H}$ = 7.1 Hz, 3H, -OCH_2-C$\underline{H}_3$) The signal of the proton of the hydroxy group (-O$\underline{H}$) was not found. ^{13}C{^{1}H} NMR ($CDCl_3$, 150.9 MHz): δ 176.7 (s, O=$\underline{C}$-O-), 74.9 and 71.4 (d, HO-$\underline{C}$H-), 60.7 and 60.6 (t, -O-$\underline{C}H_2$-CH_3), 51.7 and 51.6 (t, -CH_2-$\underline{C}H_2$-N_3), 51.1 and 50.2 (s, CH_2-$\underline{C}$-C), 34.2 (t, O=C-$\underline{C}H_2$-CH_2-), 32.3 (t, -$\underline{C}H_2$-), 29.6 and 29.4 (t, -$\underline{C}H_2$-), 23.8 and 23.7 (t, -$\underline{C}H_2$-), 22.5 (t, -$\underline{C}H_2$-), 21.3 (t, -$\underline{C}H_2$-), 14.2 (q, -OCH_2-$\underline{C}H_3$).

The analytical data were in complete accordance with prior published ones.[18]

*Synthesis of the 7-hydroxy-2-azaspiro[5.5]undecan-1-ones (**14** and **14'**)*

To the mixture **15/15'** (3.00 g, 11.7 mmol) and ammonium chloride (1.30 g, 23.5 mmol, 2eq.) in ethanol (36 mL) and water (6 mL), zinc powder (1.20 g, 18.4 mmol, 1.5 eq.) was added portionwise at room temperature within 1 h. The evolution of N_2 was monitored with an oil bubbler during the addition of zinc powder. The reaction mixture was then stirred vigorously at 80 °C for 18 h. Then, ethyl acetate (80 mL) and aqueous ammonia (NH_4OH) (6 mL) were added. The mixture was filtered through celite pad and extracted with ethyl acetate (200 mL). The filtrate was washed with brine (6×30 mL) and dried over anhydrous Na_2SO_4. After removal of the solvent under reduced pressure, the residue was purified by column chromatography using ethyl acetate/ methanol/ NH_4OH as eluent, in the ratio 100:10:1 to give two diastereomers; **14´** with the yield 0.75 g (4.1 mmol, 35%) and **14** with the yield 0.36 g (2.0 mmol, 17%). The analytical data were in complete accordance with prior published ones.[11]

*Synthesis of 6',7'-dihydro-5'H-spiro[cyclohexane-1,8'-imidazo[1,2-a]pyridin]-2-one (**21**)*

To a dried Schlenk flask was added trimethyloxoniumtetrafluoroborate (1.47 g, 10.0 mmol) and dry dichloromethane (10 mL). To the mixture was added under nitrogen a solution of spirolactam **20** (1.50 g, 8.0 mmol) and dry dichloromethane (10 mL). The yellow reaction mixture was then refluxed for 19 h to afford a brown solution which was concentrated at the rotary evaporator. The obtained brown gummy slurry was dissolved in dichloromethane (20 mL) and aminoacetaldehyde diethyl acetal (**13**) (3 mL, 2.75 g, 20.7 mmol, 2.5 eq.) was added dropwise under nitrogen. The reaction mixture was then stirred at room temperature for 3 days and concentrated at the rotary evaporator to produce a brown oily residue to which was added hydrochloric acid (140 mL, 1 mol/L). The reaction solution was heated at 100 °C for 6 h. The reaction mixture was cooled down and a saturated aqueous solution of $NaHCO_3$ (50 mL) was added to neutralize the solution, and then it was extracted with ethyl acetate (6×30 mL). The organic layer was washed with brine (6×30 mL) and dried over anhydrous $MgSO_4$. The solvent was removed under reduced pressure and the resulting crude product was purified by column chromatography using ethyl acetate/ methanol/ NH_4OH as an isocratic eluent, in the ratio 100:5:0.5 with *Rf* = 0.35. The pure compound **22** was obtained as a yellow oil with a yield of 1.30 g (6.4 mmol, 76%). ^{1}H NMR ($CDCl_3$, 600 MHz): δ 7.00 (d, $^3J_{H,H}$ = 1.1 Hz, 1H, -CH=C$\underline{H}$-N=), 6.79 (d, $^3J_{H,H}$ = 1.1 Hz, 1H, -N-C$\underline{H}$=CH-), 3.96 (dt, $^2J_{H,H}$ = 12.2, $^3J_{H,H}$ = 5.0 Hz, 1H, -CH_2-C$\underline{H}_2$-N-), 3.83 (ddd, $^2J_{H,H}$ = 12.2, $^3J_{H,H}$ = 9.6, $^3J_{H,H}$ = 5.2 Hz, 1H, -CH_2-C$\underline{H}_2$-N-), 2.93 (brddd, $J_{H,H}$ = 14.2, 9.4, 5.8 Hz, 1H, -C$\underline{H}_2$-), 2.45–2.53 (m, 2H, -C$\underline{H}_2$-), 2.43 (brddd, $J_{H,H}$ = 14.1, 8.5, 5.4 Hz, 1H, -C$\underline{H}_2$-), 2.20–2.27 (m, 1H, -C$\underline{H}_2$-), 1.84–2.05 (m, 4H, -C$\underline{H}_2$-), 1.69–1.80 (m, 2H, -C$\underline{H}_2$-), 1.62 (brddd, $J_{H,H}$ = 14.1, 11.2, 3.1 Hz, 1H, -C$\underline{H}_2$-). ^{13}C{^{1}H} NMR ($CDCl_3$, 150.9 MHz): δ 210.7 (s, -$\underline{C}$=O), 145.6 (s, -N=$\underline{C}$-), 127.6 (d, C-2', -CH=$\underline{C}$H-N=), 118.6 (d, C-3', -N-$\underline{C}$H=CH-), 51.1 (s, -$\underline{C}$-C=N-), 44.5 (t, -CH_2-$\underline{C}H_2$-N-), 39.1 (t, -$\underline{C}H_2$-CO-), 38.9 (t, -$\underline{C}H_2$-), 31.0 (t, -$\underline{C}H_2$-), 26.9 (t, -$\underline{C}H_2$-), 20.9 (t, -$\underline{C}H_2$-), 19.9 (t, -$\underline{C}H_2$-). IR (ATR); υ_{max} (cm^{-1}) 2941, 2866, 1704, 1519, 1447, 1315, 1260, 1203, 1122, 1083, 923, 890, 747, 724, 677. HRMS (ESI) *m/z*: calcd for $C_{12}H_{17}N_2O$, $[M+H]^+$ 205.1341; found, 205.1338.

*Synthesis of (±)- 6',7'-dihydro-5'H-spiro[cyclohexane-1,8'-imidazo[1,2-a]pyridin]-2-ol (**5**)*

Method using $NaBH_4$. **21** (1.20 g, 5.9 mmol) was placed in a two-necked flask and dissolved in dry ethanol (50 mL) under N_2 inert gas flow. The mixture was cooled to 0 °C and $NaBH_4$ (3.51 g, 88.2 mmol) was added portionwise within 15 minutes. The reaction mixture was then allowed to stir overnight at room temperature. Then, it was treated with brine (200 mL) and extracted with ethyl acetate (6×30 mL). The organic phase was dried over anhydrous $MgSO_4$ and the solvent was removed by a rotary evaporator. The crude product was purified by silica gel column chromatography using ethyl acetate/ methanol/ NH_4OH as an isocratic eluent, in the ratio 100:10:1, to afford separately nitrabirine (**5**) with *Rf* = 0.32 and its new epimer, named epinitrabirine (**5'**) with *Rf* = 0.66, each as a white amorphous solid and with the yields 285 mg (1.4 mmol, 23%) and 473 mg (2.3 mmol, 39%) respectively. The single crystals of nitrabirine (**5**) appropriate for X-ray diffraction were obtained by slow evaporation in chloroform.

Method using DIBAL-H. To a solution of ketone **21** (458 mg, 2.2 mmol) in CH_2Cl_2 (10 mL) was added DIBAL-H (6.70 mL, 25% solution in hexanes, 6.7 mmol, 3.0 eq.) dropwise at –78 °C. After stirring at –78 °C for 2 hours, the reaction mixture was quenched with a saturated aqueous solution of Rochelle's salt (20 mL) and stirred at room temperature overnight. The phases were separated, and the aqueous phase was extracted with CH_2Cl_2 (4×25 mL). The combined organic phases were dried over $MgSO_4$, filtered, and concentrated under reduced pressure by a rotary evaporator. The crude product was purified as described in the previous method affording **5** and **5'** as a white amorphous solid and with the yields 171 mg (0.8 mmol, 40%) and 92 mg (0.4 mmol, 20%) respectively. mp. 193–195 °C; $[\alpha]_D^{25°C}$ = ±0.241° (c =8.3 mg/mL in MeOH); ^{1}H NMR ($CDCl_3$, 250 MHz): δ 7.01 (d, $^3J_{H,H}$ = 1.1 Hz, 1H, -CH=C$\underline{H}$-N=), 6.75 (d, $^3J_{H,H}$ = 1.2 Hz, 1H, -N-C$\underline{H}$=CH-), 4.43 (dd, $^3J_{H,H}$ = 11.2, 4.3 Hz, 1H, -CH_2-C$\underline{H}$-OH), 3.95 (m, 2H, -CH_2-C$\underline{H}_2$-N-), 2.15 (s, 1H, -O$\underline{H}$), 1.48–2.08 (m, 12H, -C$\underline{H}_2$-). ^{13}C{^{1}H} NMR ($CDCl_3$, 69.9 MHz): δ 150.8 (s, -N=$\underline{C}$-), 127.4 (d, C-2', -CH=$\underline{C}$H-N=), 118.1 (d, C-3', -N-$\underline{C}$H=CH-), 74.8 (d, -H$\underline{C}$-OH), 44.8 (t, -CH_2-$\underline{C}H_2$-N-

), 42.7 (s, -C-C=N-), 35.2 (t, -CH$_2$-), 28.9 (t, -CH$_2$-CH-OH), 24.5 (t, -CH$_2$-), 21.3 (t, -CH$_2$-), 20.7 (t, -CH$_2$-), 19.6 (t, -CH$_2$-). ^{1}H NMR (CDCl$_3$, 600 MHz): δ 6.96 (d, $^3J_{H,H}$ = 1.2 Hz, 1H, =CH-N=), 6.72 (d, $^3J_{H,H}$ = 1.2 Hz, 1H, -N-CH=CH-), 4.37 (brdd, $^3J_{H,H}$ = 11.4, $^3J_{H,H}$ = 4.2 Hz, 1H, -CH$_2$-CH-OH), 3.95 (brdt, $^2J_{H,H}$ = 12.3, $^3J_{H,H}$ = 4.6 Hz, 1H, -CH$_2$-CH$_2$-N-), 3.85 (brddd, $^3J_{H,H}$ = 15.8, $^2J_{H,H}$ = 12.0, $^3J_{H,H}$ = 4.8 Hz, 1H, -CH$_2$-CH$_2$-N-), 2.58 (s, 1H, -OH), 1.97–2.06 (m, 3H, -CH$_2$-), 1.90–1.95 (m, 1H, -CH$_2$-), 1.75–1.81 (m, 3H, -CH$_2$-), 1.72 (brtdd, $J_{H,H}$ = 13.9, 3.9, 1.0 Hz, 1H, -CH$_2$-), 1.48–1.53 (m, 2H, -CH$_2$-), 1.43–1.47 (m, 1H, -CH$_2$-), 1.37 (brdt, $J_{H,H}$ = 12.9, 3.8 Hz, 1H, -CH$_2$-). ^{13}C{^{1}H} NMR (CDCl$_3$, 150.9 MHz): δ 150.9 (s, -N=C-), 127.4 (d, C-2', -CH=CH-N=), 118.0 (d, C-3', -N-CH=CH-), 74.6 (d, -HC-OH), 44.7 (t, -CH$_2$-CH$_2$-N-), 42.6 (s, -C-C=N-), 35.3 (t, -CH$_2$-), 29.0 (t, -CH$_2$-CH-OH-), 24.5 (t, -CH$_2$-), 21.3 (t, -CH$_2$-), 20.7 (t, -CH$_2$-), 19.6 (t, -CH$_2$-). IR (ATR); υ_{max} (cm^{-1}) 3231, 2964, 2932, 2863, 1524, 1483 1441, 1353, 1311, 1266, 1208. 1128, 1070, 1021, 984, 928, 903, 755, 729, 651, 597. HRMS (ESI) *m/z*: calcd for $C_{12}H_{19}N_2O$, $[M+H]^+$, 207.1492; found, 207.1501.

*Synthesis of (±)-6',7'-dihydro-5'H-spiro[cyclohexane-1,8'-imidazo[1,2-a]pyridin]-2-ol (**5'**)*

mp. 98–100 °C; $[\alpha]_D^{25°C}$ = ±0.361° (c =8.3 mg/mL in MeOH); ^{1}H NMR (CDCl$_3$, 600 MHz): δ 6.90 (brd, $^3J_{H,H}$ = 1.3 Hz, 1H, =CH-N=), 6.73 (brd, $^3J_{H,H}$ = 1.2 Hz, 1H, N-CH=CH-), 3.95 (brdt, $^2J_{H,H}$ = 11.8, $^3J_{H,H}$ = 4.4 Hz, 1H, -CH$_2$-CH$_2$-N-), 3.82 (ddd, $^2J_{H,H}$ = 12.1, $^3J_{H,H}$ = 10.5, $^3J_{H,H}$ = 5.1 Hz, 1H, -CH$_2$-CH$_2$-N-), 3.58 (vbrt, $^3J_{H,H}$ = 3.3 Hz, 1H, -CH$_2$-CH-OH), 2.10–2.17 (m, 2H, -CH$_2$-), 1.89–2.03 (m, 2H, -CH$_2$-), 1.87 (vbrdt, $J_{H,H}$ = 13.0, 4.3 Hz, 1H, -CH$_2$-), 1.78 (vbrdd, $J_{H,H}$ = 14.1, 3.4 Hz, 1H, -CH$_2$-), 1.61 (brtdd, $J_{H,H}$ = 13.6, 4.1, 2.5 Hz, 1H, -CH$_2$-), 1.49–1.55 (m, 2H, -CH$_2$-), 1.40–1.49 (m, 2H, -CH$_2$-), 1.36–1.41 (m, 1H, -CH$_2$-). The proton signal of the hydroxyl group (-OH) was not found.

^{13}C{^{1}H} NMR (CDCl$_3$, 150.9 MHz): δ 151.2 (s, -N=C-), 126.7 (d, C-2', -CH=CH-N=), 118.0 (d, C-3', -N-CH=CH-), 72.4 (d, -HC-OH), 44.7 (t, -CH$_2$-CH$_2$-N-), 39.2 (s, -C-C=N-), 29.9 (t, -CH$_2$-), 27.6 (t, -CH$_2$-CH-OH-), 27.0 (t, -CH$_2$-), 20.5 (t, -CH$_2$-), 19.1 (t, -CH$_2$-), 18.7 (t, -CH$_2$-). IR (ATR); υ_{max} (cm^{-1}) 3291, 2935, 2860, 1441, 1319, 1289, 1202, 1147, 1127, 1080, 1041, 1003, 979, 927, 878, 724, 649, 595. HRMS (ESI) *m/z*: calcd for $C_{12}H_{19}N_2O$, $[M+H]^+$, 207.1492; found, 207.1489.

*Synthesis of 2-hydroxytetrahydro-5'H-spiro[cyclohexane-1,8'-imidazo[1,2-a]pyridine]-2',3'-dione (**22**)*

To a stirred solution of **5'** (40 mg, 0.2 mmol) in MeOH (1 mL), H_2O_2 (0.07 mL, 0.6 mmol, 30%) was added *via* a syringe over 10 min at room temperature. The reaction mixture was stirred at room temperature for 3 days and cooled to 0 °C. Then, a saturated solution of $Na_2S_2O_5$ (5 mL) was added *via* a syringe over 5 min and stirred for 30 min. The mixture was later on extracted with dichloromethane (6×10 mL) and the organic layers were combined, dried over anhydrous $MgSO_4$, and filtrated. After this, the filtrate was concentrated by a rotary evaporator. The crude yellow oily product was purified by column chromatography on silica gel with methylen dichloride/methanol as an isocratic eluent, in the ratio 10:1, to afford **22** with *Rf* = 0.33 as a white solid and with the yield 10 mg (0.04 mmol, 22%). mp. 246–248 °C; ^{1}H NMR (CD$_3$OD, 600 MHz): δ 4.76 (s, 1H, -NCH-NH-), 4.20 (dtd, $^2J_{H,H}$ = 13.3, $^3J_{H,H}$ = 4.3, $^3J_{H,H}$ = 2.1 Hz, 1H, -CH$_2$-CH$_2$-N-), 3.65 (brt, $^3J_{H,H}$ = 3.2 Hz, 1H, -CH$_2$-CH-OH), 2.99 (ddd, $^2J_{H,H}$ = 13.2, $^3J_{H,H}$ = 9.3, $^3J_{H,H}$ = 6.7 Hz, 1H, -CH$_2$-CH$_2$-N-), 2.18 (tdd, $J_{H,H}$ = 14.4, 3.5, 1.7 Hz, 1H, -CH$_2$-), 1.78 (ddd, $J_{H,H}$ = 13.7, 4.4, 2.4 Hz, 1H, -CH$_2$-), 1.60–1.69 (m, 4H, -CH$_2$-), 1.44–1.48 (m, 1H, -CH$_2$-), 1.49–1.55 (m, 2H, -CH$_2$-), 1.39 (dt, $J_{H,H}$ = 13.0, 3.5 Hz, 1H, -CH$_2$-), 1.26–1.33 (m, 1H, -CH$_2$-), 1.16–1.21 (m, 1H, -CH$_2$-). ^{13}C{^{1}H} NMR (CD$_3$OD, 150.9 MHz): δ 161.4 (s, C-3', -N-C=O), 158.6 (s, C-2', O=C-NH-), 75.2 (d, -HC-OH), 73.4 (d, C-5', -CH-NH), 43.2 (s, -C-C-NH-), 40.8 (t, -CH$_2$-CH$_2$-N-), 30.0 (t, -CH$_2$-CH-OH), 28.8 (t, -CH$_2$-), 21.0 (t, -CH$_2$-), 20.8 (t, -CH$_2$-), 20.1 (t, -CH$_2$-), 19.3 (t, -CH$_2$-). IR (KBr pellet); υ_{max} (cm^{-1}) 3458, 3251, 2940, 2862, 1731, 1710, 1471, 1447, 1297. HRMS (ESI) *m/z*: calcd for $C_{12}H_{18}N_2NaO_3$, $[M+Na]^+$ 261.1215; found, 261.1207.

*Synthesis of 2-hydroxy-6',7'-dihydro-5'H-spiro[cyclohexane-1,8'-imidazo[1,2-a]pyridin]-1'-ium hydroperoxide (**24**)*

Method using H_2O_2 30%. To a stirred solution of nitrabirine (**5**) (75 mg, 0.4 mmol) in MeOH (1 mL), H_2O_2 (0.1 mL, 0.9 mmol, 30%) was added *via* a syringe over 10 min at room temperature. The reaction mixture was stirred for 3 days and cooled to 0 °C. Then, a saturated solution of $Na_2S_2O_5$ (5 mL) was added *via* a syringe over 5 min and after stirring for 30 min, the mixture was extracted with dichloromethane (6×10 mL). The organic layers were combined, dried over anhydrous $MgSO_4$, and concentrated by a rotary evaporator affording the crude product as a yellow oil which was, in fact, a mixture of **25** and spirolactam **15** in the ratio **15/25** = 6/1, determined by integration of the proton NMR spectrum. The crude product was washed with THF, and the THF insoluble residue was concentrated at a rotary evaporator and yielded **24** with 4 mg (0.02 mmol, 5%) as a yellow bulky oil.

Method using peracetic acid. To a stirred solution of **5** (275 mg, 1.3 mmol) in MeOH (5 mL), 1 mL of the prepared peracetic acid solution[22] was added *via* a syringe over 10 min at room temperature. The reaction mixture was stirred at 50 °C for 24 hours. Then a saturated solution of $Na_2S_2O_5$ (10 mL) was added and the mixture was extracted with chloroform (6×10 mL). The organic layers were combined, dried over anhydrous $MgSO_4$ and concentrated by a rotary evaporator affording the crude product as a yellow oil. The crude product was washed with THF, and the THF insoluble residue was concentrated by a rotary evaporator and yielded **24** with 19 mg (0.08 mmol, 6%) as a yellow bulky. ^{1}H NMR (CDCl$_3$, 600 MHz): δ 7.23 (d, $^2J_{H,H}$ = 1.8 Hz, 1H, -CH=CH-N=), 6.92 (d, $^2J_{H,H}$ = 1.8 Hz, 1H, -N-CH=CH-), 4.51 (dd, $^3J_{H,H}$ = 11.1, $^3J_{H,H}$ = 4.2 Hz, 1H, -CH$_2$-CH-OH), 4.03 (dd, $J_{H,H}$ = 9.1, 4.1 Hz, 2H, -CH$_2$-CH$_2$-N-), 2.14–2.23 (m, 2H, -CH$_2$-), 1.90–2.02 (m, 3H, -CH$_2$-), 1.73–1.87 (m, 3H, -CH$_2$-), 1.48–1.60 (m, 3H, -CH$_2$-), 1.35–1.42 (m, 1H, -CH$_2$-). The signals of the hydroxylic proton, hydroperoxide proton and of the ammonium proton were not found. ^{13}C{^{1}H} NMR (CDCl$_3$, 150.9 MHz): δ 150.7 (s, -N=C), 121.9 (d, C-2', -CH=CH-N=), 119.1 (d, C-3', -N-CH=CH-), 73.7 (d, -HC-OH), 45.8 (t, -CH$_2$-CH$_2$-N-), 42.8 (s, -C-C=N-), 35.1 (t, -CH$_2$-), 29.6 (t, -CH$_2$-CH-OH-), 24.0 (t, -CH$_2$-), 21.0 (t, -CH$_2$-), 20.4 (t, -CH$_2$-), 19.4 (t, -CH$_2$-). ^{1}H NMR (CD$_3$OD, 600 MHz): δ 7.39 (d, $^3J_{H,H}$ = 2.0 Hz, 1H, -CH=CH-N=).7.31 (d, $^3J_{H,H}$ = 2.0 Hz, 1H, -N-CH=CH-), 4.8 (td, $^2J_{H,H}$ = 12.9, $^3J_{H,H}$ = 4.7 Hz, 1H, -CH$_2$-CH$_2$-N-), 4.08 (dd, $^3J_{H,H}$ = 11.2, $^3J_{H,H}$ = 3.7 Hz, 1H, -CH$_2$-CH-OH), 4.03 (ddd, $^2J_{H,H}$ = 12.9, $^3J_{H,H}$ = 10.0 Hz, $^3J_{H,H}$ = 4.3 Hz, 1H, -CH$_2$-CH$_2$-N-), 2.15–2.23 (m, 2H, -CH$_2$-), 1.99–2.06 (m, 2H, -CH$_2$-), 1.91–1.96 (m, 1H, -CH$_2$-), 1.79–1.86 (m, 2H, -CH$_2$-), 1.66–1.74 (m, 1H, -CH$_2$-), 1.56–1.62 (m, 2H, -CH$_2$-), 1.49–1.54 (m, 2H, -CH$_2$-). ^{13}C{^{1}H} NMR (CD$_3$OD, 150.9 MHz): δ 152.0 (s, -N=C). 121.7 (d, C-3', -NCH=CH-), 121.2 (d, C-2', -CH=CH-N=), 75.8 (d, -HC-OH), 47.2

(t, -CH_2-$\underline{C}H_2$-N-), 43.9 (s, -$\underline{C}$-C=N-), 36.2 (t, -$\underline{C}H_2$-CH-OH-), 30.9 (t, -CH_2-), 25.3 (t, -CH_2-), 22.3 (t, -CH_2-), 21.2 (t, -CH_2-), 20.5 (t, -CH_2-). IR (KBr, $CDCl_3$); υ_{max} (cm^{-1}) 3345, 2939, 2873, 1666, 1448, 1125, 907, 732, 644. HRMS (ESI) *m/z*: calcd for $C_{12}H_{21}N_2O_3$, $[M+H]^+$ 241.1547; found, 241.1522.

*Synthesis of -2-hydroxy-6',7'-dihydro-5'H-spiro[cyclohexane-1,8'-imidazo[1,2-a]pyridin]-1'-ium hydroperoxide (**24'**)*

To a stirred solution of **5'** (295 mg, 1.4 mmol) in MeOH (5 mL), 1.2 mL of the prepared peracetic acid solution[32] was added *via* a syringe over 20 min at room temperature. The reaction mixture was stirred at 50 °C for 24 hours. Then a saturated solution of $Na_2S_2O_5$ (10 mL) was added, and the mixture was extracted with chloroform (6×15 mL). The organic layers were combined, dried over anhydrous $MgSO_4$. After filtration and concentration by a rotary evaporator, the salt **24** was yielded 17 mg (0.07 mmol, 5%) as a yellow oil. ^{1}H NMR ($CDCl_3$, 600 MHz): δ 13.07 (s, 1H, =CH-N$\underline{H}$=), 7.39 (t, $^3J_{H,H}$ = 2.0 Hz, 1H, -CH=C$\underline{H}$-N=), 7.11 (t, $^3J_{H,H}$ = 2.0 Hz, 1H, -N-C$\underline{H}$=CH-), 4.20 (ddd, $J_{H,H}$ = 12.6, 7.0, 5.3 Hz, 1H, -CH_2-C$\underline{H}_2$-N-), 4.06–4.12 (m, 1H, -CH_2-C$\underline{H}_2$-N-), 3.85 (brt, $^3J_{H,H}$ = 2.8 Hz, 1H, -CH_2-C$\underline{H}$-OH), 2.32 (td, $J_{H,H}$ = 13.3, 4.0 Hz, 1H, -C$\underline{H}_2$-), 2.12–2.19 (m, 1H, -C$\underline{H}_2$-), 2.04–2.09 (m, 1H, -C$\underline{H}_2$-), 1.98–2.02 (m, 2H, -C$\underline{H}_2$-), 1.83–1.90 (m, 2H, -C$\underline{H}_2$-), 1.72–1.80 (m, 1H, -C$\underline{H}_2$-), 1.64–1.68 (m, 1H, -C$\underline{H}_2$-), 1.40–1.57 (m, 3H, -C$\underline{H}_2$-). The signal of the hydroperoxide proton was not found. ^{13}C{^{1}H} NMR ($CDCl_3$, 150.9 MHz): δ 148.8 (s, -N=$\underline{C}$), 120.0 (d, C-3', -N-$\underline{C}$H=CH-), 119.6 (d, C-2', -CH=$\underline{C}$H-N=), 69.6 (d, -H$\underline{C}$-OH), 46.2 (t, -CH_2-$\underline{C}H_2$-N-), 40.9 (s, -$\underline{C}$-C=N-), 30.6 (t, -CH_2-), 27.8 (t, -$\underline{C}H_2$-CH-OH-), 27.3 (t, -CH_2-), 20.0 (t, -CH_2-), 18.5 (t, -CH_2-), 18.1 (t, -CH_2-). ^{1}H NMR (CD_3OD, 600 MHz): δ 7.41 (d, $^3J_{H,H}$ = 2.0 Hz, 1H, -CH=C$\underline{H}$-N=), 7.39 (d, $^3J_{H,H}$ = 2.0 Hz, 1H, -N-C$\underline{H}$=CH-), 3.79 (brd, $^3J_{H,H}$ = 3.4 Hz, 1H, -CH_2-C$\underline{H}$-OH), 2.20 (td, $J_{H,H}$ = 14.4, 8.9 Hz, 1H, -C$\underline{H}_2$-), 2.09–2.13 (m, 2H, -C$\underline{H}_2$-), 2.06–2.08 (m, 2H, -C$\underline{H}_2$-), 1.84–1.90 (m, 2H, -C$\underline{H}_2$-), 1.75 (dt, $J_{H,H}$ = 10.2, 3.4 Hz, 1H, -C$\underline{H}_2$-), 1.58–1.65 (m, 3H, -C$\underline{H}_2$-), 1.48–1.55 (m, 1H, -C$\underline{H}_2$-). ^{13}C{^{1}H} NMR (CD_3OD, 150.9 MHz): δ 150.2 (s, -N=$\underline{C}$), 122.1 (d, C-2', -CH=$\underline{C}$H-N=), 119.4 (d, C-3', -N-$\underline{C}$H=CH-), 70.4 (d, -H$\underline{C}$-OH), 47.4 (t, -CH_2-$\underline{C}H_2$-N-), 41.9 (s, -$\underline{C}$-C=N-), 31.6 (t, -$\underline{C}H_2$-CH-OH-), 29.1 (t, -CH_2-), 28.3 (t, -CH_2-), 21.1 (t, -CH_2-), 19.6 (t, 2C, -CH_2-). IR (KBr, $CDCl_3$); υ_{max} (cm^{-1}) 3414, 2942, 2860, 1603, 1128, 1008, 907, 732, 650. HRMS (ESI) *m/z*: calcd for $C_{12}H_{21}N_2O_3$, $[M+H]^+$, 241.1547; found, 241.1550.

*Synthesis of 6',7'-dihydro-5'H-spiro[cyclohexane-1,8'-imidazo[1,2-a]pyridin]-2-yl acetate (**25'**);* **A general method for the synthesis of 25 and 25' was followed**. The preparation of **25** is representative. **5** (206 mg, 1 mmol) dissolved in chloroform (5 mL) was introduced to a three-necked 25 mL round-bottomed flask. Acetyl chloride (0.1 mL, 94 mg, 1.2 mmol) was taken up in a syringe, and the needle is inserted into the flask *via* the septum. After the addition was complete, the mixture was stirred for 3 h, during which time the solution cools to room temperature. The reaction mixture was transferred to a 25 mL round-bottomed flask and the solvent was concentrated by rotary evaporation to give a yellow oil. The crude product was purified by column chromatography on neutral alumina with hexanes/ethyl acetate, as an isocratic eluent, in the ratio 3:1 to afford the product **25** (*Rf* = 0.1) as a yellow oil with the yield 223 mg (0.9 mmol, 90%). ^{1}H NMR ($CDCl_3$, 600 MHz): δ 6.96 (d, $^3J_{H,H}$ = 1.1 Hz, 1H, =C$\underline{H}$-N=), 6.69 (d, $^3J_{H,H}$ = 1.2 Hz, 1H, -N-C$\underline{H}$=CH-), 5.36 (brdd, $^3J_{H,H}$ = 10.9, $^3J_{H,H}$ = 4.1 Hz, 1H, -CH_2-C$\underline{H}$-C=O), 3.92 (brdt, $^2J_{H,H}$ = 12.2, $^3J_{H,H}$ = 44.7 Hz, 1H, -CH_2-C$\underline{H}_2$-N-), 3.86 (brddd, $^2J_{H,H}$ = 12.0, $^3J_{H,H}$ = 9.2, $^3J_{H,H}$ = 4.5 Hz, 1H, -CH_2-C$\underline{H}_2$-N-), 2.00–2.10 (m, 3H, -C$\underline{H}_2$-), 1.88–1.97 (m, 3H, -C$\underline{H}_2$-), 1.82 (s, 3H, O=C-C$\underline{H}_3$), 1.70–1.80 (m, 2H, -C$\underline{H}_2$-), 1.60–1.66 (m, 1H, -C$\underline{H}_2$-), 1.50–1.57 (m, 1H, -C$\underline{H}_2$-), 1.37–1.50 (m, 2H, -C$\underline{H}_2$-). ^{13}C{^{1}H} NMR ($CDCl_3$, 150.9 MHz): δ 169.5 (s, O=$\underline{C}$-CH_3), 149.5 (s, -N=$\underline{C}$-), 127.7 (d, C-2', -CH=$\underline{C}$H-N=), 117.6 (d, C-3', -N-$\underline{C}$H=CH-), 77.5 (d, -H$\underline{C}$-OH), 44.6 (t, -CH_2-$\underline{C}H_2$-N-), 40.2 (s, -$\underline{C}$-C=N-), 35.8 (t, -CH_2-), 26.7 (t, -$\underline{C}H_2$-CH-OH-), 24.4 (t, -CH_2-), 23.6 (t, -CH_2-), 21.1 (q, O=C-$\underline{C}H_3$), 20.7 (t, -CH_2-), 19.9 (t, -CH_2-). IR (ATR); υ_{max} (cm^{-1}) 2936, 2864, 1734, 1482, 1371, 1318, 1244, 1036, 929, 721. HRMS (ESI) *m/z*: calcd for $C_{14}H_{19}O_2N_2$, $[M+H]^+$, 249.1598; found, 249.1596.

*6',7'-dihydro-5'H-spiro[cyclohexane-1,8'-imidazo[1,2-a]pyridin]-2-yl acetate (**25'**)*

25' was isolated at *Rf* = 0.3 (hexanes/ethyl acetate in the ratio 3:1) as a yellow oil with the yield of 126 mg (0.5 mmol, 51%) from 206 mg (1 mmol) of **5'**. ^{1}H NMR ($CDCl_3$, 600 MHz): δ 7.00 (brd, $^3J_{H,H}$ = 1.2 Hz, 1H, =C$\underline{H}$-N=), 6.79 (brd, $^3J_{H,H}$ = 1.2 Hz, 1H, N-C$\underline{H}$=CH-), 4.90 (brdd, $^3J_{H,H}$ = 8.3, $^3J_{H,H}$ = 3.7 Hz, 1H, -CH_2-C$\underline{H}$-C=O), 3.95 (ddd, $^2J_{H,H}$ = 12.3, $^3J_{H,H}$ = 6.7, $^3J_{H,H}$ = 5.7 Hz, 1H, -CH_2-C$\underline{H}_2$-N-), 3.90 (ddd, $^2J_{H,H}$ = 12.3, $^3J_{H,H}$ = 6.8, $^3J_{H,H}$ = 3.2 Hz, 1H, -CH_2-C$\underline{H}_2$-N-), 2.18–2.37 (m, 2H, -C$\underline{H}_2$-), 2.12 (brddd, $J_{H,H}$ = 13.5, 8.8, 3.2 Hz, 1H, -C$\underline{H}_2$-), 1.98–2.06 (m, 2H, -C$\underline{H}_2$-), 1.96 (s, 3H, O=C-C$\underline{H}_3$), 1.88–1.94 (m, 1H, -C$\underline{H}_2$-), 1.78–1.88 (m, 2H, -C$\underline{H}_2$-), 1.62 (brtdd, $J_{H,H}$ = 13.6, 8.9, 3.3 Hz, 1H, -C$\underline{H}_2$-), 1.42–1.51 (m, 2H, -C$\underline{H}_2$-), 1.35 (brddd, $J_{H,H}$ = 13.2, 9.2, 3.7 Hz, 1H, -C$\underline{H}_2$-). ^{13}C{^{1}H} NMR ($CDCl_3$, 150.9 MHz): δ 170.8 (s, O=$\underline{C}$-CH_3), 147.5 (s, -N=$\underline{C}$-), 127.3 (d, C-2', -CH=$\underline{C}$H-N=), 118.3 (d, C-3', -N-$\underline{C}$H=CH-), 76.2 (d, -H$\underline{C}$-OH), 44.7 (t, -CH_2-$\underline{C}H_2$-N-), 39.4 (s, -$\underline{C}$-C=N-), 35.6 (t, -$\underline{C}H_2$-), 32.9 (t, -$\underline{C}H_2$-CH-OH-), 27.2 (t, -$\underline{C}H_2$-), 22.7 (t, -$\underline{C}H_2$-), 21.4 (q, O=C-$\underline{C}H_3$), 20.8 (t, -$\underline{C}H_2$-), 19.3 (t, -$\underline{C}H_2$-). IR (ATR); υ_{max} (cm^{-1}) 2934, 2862, 1731, 1481, 1439, 1370, 1314, 1242, 1136, 1030, 927, 724. HRMS (ESI) *m/z*: calcd for $C_{14}H_{19}O_2N_2$, $[M+H]^+$, 249.1598; found, 249.1599.

*Synthesis of 2,3,4,6',7',9-hexahydro-5'H-spiro-[carbazole-1,8'-imidazo[1,2-a]pyridine] (**26a**)*

A dried 50 mL two-necked, round-bottomed flask was charged with the ketone **21** (300 mg, 1.5 mmol) dissolved in 2 mL EtOH, under nitrogen flow. Phenylhydrazine (0.22 mL, 243 mg, 2.2 mmol, 1.5 eq.) was then added *via* a syringe. Phosphoric acid (0.75 mL) was later added slowly over 1 min, and the mixture was stirred for 1 h at room temperature and then brought to 120 °C and stirred for 40 h. The reaction mixture was cooled down at room temperature and 20 mL water was carefully added. The resulting mixture was then extracted with dichloromethane (30 mL). The water phase was afterwards neutralized with a saturated aqueous solution of $NaHCO_3$ (30 mL) and then extracted with dichloromethane (6×20 mL). The organic layers were combined, washed with brine (4×10 mL) and dried over anhydrous $MgSO_4$. The resulting mixture was filtered, and the filtrate was concentrated at reduced pressure by a rotary evaporator to afford a brown oily crude product. The latter was purified by column chromatography on neutral alumina with hexanes/ethyl acetate, 0 to 50% gradient to afford the product **26a** (*Rf* = 0.12, at 50% hexanes/ethyl acetate) as a brown foam with the yield 250 mg (0.9 mmol, 60%). ^{1}H NMR ($CDCl_3$, 600 MHz): δ 8.72(s, 1H, -N$\underline{H}$-), 7.44 (d, $^3J_{H,H}$ = 7.7 Hz, 1H, Ar$\underline{H}$), 7.23 (d, $^3J_{H,H}$ = 7.9 Hz, 1H, Ar$\underline{H}$), 7.05 (dd, $^3J_{H,H}$ = 7.0 Hz, 1H, ArH), 6.63 (s, 1H, =N-C$\underline{H}$=CH-), 6.59 (s, 1H, -N-C$\underline{H}$=CH-), 4.12 (ddd, $^3J_{H,H}$ = 13.8 Hz, $^2J_{H,H}$ = 8.4 Hz, $^3J_{H,H}$ =5.8 Hz, 1H, -N-C$\underline{H}_2$-CH_2-), 3.96 (dt, $^2J_{H,H}$ = 9.1 Hz, $^3J_{H,}$

$_H$ = 4.3 Hz, 1H -N-C$\underline{H}_2$-CH$_2$-), 2.74–2.81 (m, 2H, -C$\underline{H}_2$-), 2.23–2.36 (m, 2H, -C$\underline{H}_2$-), 2.07–2.13 (m, 3H, -C$\underline{H}_2$-), 1.97–2.05 (m, 2H, -C$\underline{H}_2$-), 1.85–1.94 (m, 1H, -C$\underline{H}_2$-). ^{13}C{^{1}H} NMR (CDCl$_3$, 150.9 MHz): δ 149.1 (s, -N-$\underline{C}$=N-), 136.5 (s, -NH-$\underline{C}$=C-), 136.3 (s, -NH-$\underline{C}$=CH-), 127.2 (s, -NH-C=$\underline{C}$-CH=), 125.4 (d, C-2',-CH=$\underline{C}$H-N=), 121.6 (d, -CH=$\underline{C}$H-CH=), 118.9 (d, C-3', -N-$\underline{C}$H=CH-), 118.8 (d, =CH-$\underline{C}$H=CH-), 118.3 (d, =C-$\underline{C}$H=CH-), 111.3 (d, -C=$\underline{C}$-CH$_2$-), 111.2 (d, =C-$\underline{C}$H=CH-), 45.3 (t, -CH$_2$-$\underline{C}$H$_2$-N-), 37.7 (s, -$\underline{C}$-C=N-), 36.7 (t, -$\underline{C}$H$_2$-), 33.7 (t, -$\underline{C}$H$_2$-), 20.8 (t, -$\underline{C}$H$_2$-), 19.7 (t, -$\underline{C}$H$_2$-), 19.6 (t, -$\underline{C}$H$_2$-). IR (ATR); υ_{max} (cm^{-1}) 3148, 2934, 2851, 1597, 1458, 1319, 1295, 1208, 1141, 1030, 740, 625. HRMS (ESI) *m/z*: calcd for $C_{18}H_{20}N_3$, $[M+H]^+$, 278.1652; found, 278.1654.

*Synthesis of 6-fluoro-2,3,4,6',7',9-hexahydro-5'H-spiro[carbazole-1,8'-imidazo[1,2-a]pyridine] (**26b**)*

A general procedure was followed for the synthesis of **26b–d**. The preparation of **26b** is representative. A dried 50 mL two-necked, round-bottomed flask was charged with the ketone **21** (300 mg, 1.5 mmol), dissolved in 2 mL EtOH. 4-fluorophenylhydrazine hydrochloride (358 mg, 2.2 mmol, 1.5 eq.) was then added, and stirring was started. Phosphoric acid (0.75 mL) was later added slowly over 1 min and the mixture was stirred for 1 h at room temperature, and then brought to 120 °C, and stirred for 40 h. The reaction mixture was cooled down at room temperature and 20 mL water was carefully added. The resulting mixture was then extracted with dichloromethane (30 mL). The water phase was afterwards neutralized with a saturated aqueous solution of $NaHCO_3$ (40 mL) and then extracted with ethyl acetate (6×20 mL). The organic layers were combined, washed with brine (4×10 mL), and dried over anhydrous $MgSO_4$. The resulting mixture was filtered, and the filtrate was concentrated at reduced pressure by rotary evaporator to afford a brown oily crude product. This latter was purified by column chromatography on neutral alumina with hexane/ethyl acetate, 0 to 50% gradient to afford the desired product as a white solid with the yield 180 mg (0.6 mmol, 40%). TLC (hexanes/ethyl acetate = 1:1 v/v, $KMnO_4$) *Rf* = 0.21; mp. 207–209 °C; ^{1}H NMR (CDCl$_3$, 600 MHz): δ 8.01 (s, 1H, -N$\underline{H}$-), 7.05 (dd, $^3J_{F,\,H}$ = 9.5 Hz, $^4J_{H,\,H}$ = 2.5 Hz, 1H, Ar$\underline{H}$), 6.96 (dd, $^3J_{H,\,H}$ = 8.7 Hz, $^4J_{F,\,H}$ = 4.3 Hz, 1H, Ar$\underline{H}$), 6.85 (s, 1H, -CH=C$\underline{H}$-N=), 6.78 (d, $^3J_{H,\,H}$ = 1.1 Hz, 1H, -N-C$\underline{H}$=CH-), 6.75 (td, $^3J_{F,\,H}$ = 9.1 Hz, $^3J_{H,\,H}$ = 8.7 Hz $^4J_{H,\,H}$ = 2.5 Hz, 1H, Ar$\underline{H}$), 4.02 (dt, $^2J_{H,\,H}$ = 8.4 Hz, $^3J_{H,\,H}$ =4.9 Hz, 1H -N-C$\underline{H}_2$-CH$_2$-), 3.93 (ddd, $^3J_{H,\,H}$ = 11.4 Hz, $^2J_{H,\,H}$ = 8.4 Hz, $^3J_{H,\,H}$ =4.3 Hz, 1H, -N-C$\underline{H}_2$-CH$_2$-), 2.66–2.75 (m, 2H, -C$\underline{H}_2$-), 2.26–2.36 (m, 1H, -C$\underline{H}_2$-), 2.10–2.19 (m, 3H, -C$\underline{H}_2$-), 1.96–2.08 (m, 3H, -C$\underline{H}_2$-), 1.86–1.94 (m, 1H, -C$\underline{H}_2$-). ^{13}C{^{1}H} NMR (CDCl$_3$, 150.9 MHz): δ 157.6 (d, $^1J_{C,\,F}$ = 233.2 Hz, F-$\underline{C}$=), 149.4 (s, -N-$\underline{C}$=N-), 139.7 (s, -NH-$\underline{C}$=C-), 132.7 (s; -NH-$\underline{C}$=CH-), 128.1 (d, C-2',-CH=$\underline{C}$H-N=), 127.9 (d, $^3J_{C,\,F}$ = 9.7 Hz, -NH-C=$\underline{C}$-CH=), 118.2 (d, C-3', -N-$\underline{C}$H=CH-), 111.4 (d, $^4J_{C,\,F}$ = 4.5 Hz, -NH-C=$\underline{C}$-CH$_2$-), 111.2 (d, $^3J_{C,\,F}$ = 9.7 Hz, -NH-C-$\underline{C}$H=CH-), 102.2 (d, $^2J_{C,\,F}$ = 23.0 Hz, -CH=$\underline{C}$H-F), 44.9 (t, -CH$_2$-$\underline{C}$H$_2$-N-), 36.1 (s, -$\underline{C}$-C=N-), 34.1 (t, -$\underline{C}$H$_2$-), 20.8 (t, -$\underline{C}$H$_2$-), 19.8 (t, -$\underline{C}$H$_2$-), 19.4 (t, -$\underline{C}$H$_2$-). IR (ATR); υ_{max} (cm^{-1}) 3151, 2935, 2846, 1584, 1484, 1455, 1311, 1164, 1136, 1107, 1029, 939, 903, 841, 795, 724, 608. HRMS (ESI) *m/z*: calcd for $C_{18}H_{19}FN_3$ $[M+H]^+$, 296.1558; found, 296.1557.

*6-chloro-2,3,4,6',7',9-hexahydro-5'H-spiro[carbazole-1,8'-imidazo[1,2-a]pyridine] (**26c**)*

Colourless needles with the yield 195 mg (0.6 mmol, 42%). TLC (hexanes/ethyl acetate = 1:1 v/v, $KMnO_4$) *Rf* = 0.22; mp. 249–251 °C; ^{1}H NMR (CDCl$_3$, 600 MHz): δ 8.38 (s, 1H, -N$\underline{H}$-), 7.39 (d, $^4J_{H,\,H}$ = 1.9 Hz, 1H, Ar$\underline{H}$), 7.11 (d, $^3J_{H,\,H}$ = 8.5 Hz, 1H, Ar$\underline{H}$), 7.00 (dd, $^3J_{H,\,H}$ = 8.5 Hz, $^4J_{H,\,H}$ = 2.0 Hz, 1H, ArH), 6.74 (s, 1H, =N-C$\underline{H}$=CH-), 6.72 (s, 1H, -N-C$\underline{H}$=CH-), 4.12 (ddd, $^2J_{H,\,H}$ = 12.1 Hz, $^3J_{H,\,H}$ = 9.6 Hz, $^3J_{H,\,H}$ =5.0 Hz, 1H, -N-C$\underline{H}_2$-CH$_2$-), 4.02 (dt, $^2J_{H,\,H}$ = 12.1 Hz, $^3J_{H,\,H}$ = 4.8 Hz, 1H -N-C$\underline{H}_2$-CH$_2$-), 2.71–2.75 (m, 2H, -C$\underline{H}_2$-), 2.23–2.29 (m, 2H, -C$\underline{H}_2$-), 2.08–2.20 (m, 3H, -C$\underline{H}_2$-), 1.99–2.06 (m, 2H, -C$\underline{H}_2$-), 1.85–1.94 (m, 1H, -C$\underline{H}_2$-).^{13}C{^{1}H} NMR (CDCl$_3$, 150.9 MHz): δ 149.0 (s, -N-$\underline{C}$=N-), 138.5 (s, -NH-$\underline{C}$=C-), 134.6 (s; -NH-$\underline{C}$=CH-), 128.5 (s, -NH-C=$\underline{C}$-CH=), 126.6 (d, C-2',-CH=$\underline{C}$H-N=), 124.6 (s, -CH=$\underline{C}$-Cl), 121.7 (d, -CH=$\underline{C}$H-C-Cl), 118.7 (d, C-3', -N-$\underline{C}$H=CH-), 117.8 (d, =C-$\underline{C}$H=C-Cl), 112.1 (d, =C-$\underline{C}$H=CH-C-Cl), 111.2 (s, -NH-C=$\underline{C}$-CH$_2$-), 45.2 (t, -CH$_2$-$\underline{C}$H$_2$-N-), 37.8 (s, -$\underline{C}$-C=N-), 33.7 (t, -$\underline{C}$H$_2$-), 20.6 (t, -$\underline{C}$H$_2$-), 19.7 (t, -$\underline{C}$H$_2$-), 19.4 (t, -$\underline{C}$H$_2$-). IR (ATR); υ_{max} (cm^{-1}) 3133, 3035, 2936, 2847, 1521, 1483, 1444, 1307, 1270, 1208, 1060, 929, 899, 855, 797, 725, 679, 590. HRMS (ESI) *m/z*: calcd for $C_{18}H_{19}ClN_3$, $[M+H]^+$, 312.1262; found, 312.1260.

*6-bromo-2,3,4,6',7',9-hexahydro-5'H-spiro[carbazole-1,8'-imidazo[1,2-a]pyridine] (**26d**)*

Brown foam with the yield 183 mg (0.5 mmol, 35%). TLC (hexanes/ethyl acetate = 1:1 v/v, $KMnO_4$) *Rf* = 0.24; ^{1}H NMR (CDCl$_3$, 600 MHz): δ 8.08 (s, 1H, -N$\underline{H}$-), 7.54 (d, $^4J_{H,\,H}$ = 1.2 Hz, 1H, Ar$\underline{H}$), 7.12 (dd, $^3J_{H,\,H}$ = 8.5 Hz, $^4J_{H,\,H}$ = 1.6 Hz, 1H, ArH), 7.04 (d, $^3J_{H,\,H}$ = 8.5 Hz, 1H, Ar$\underline{H}$), 6.81 (s, 1H, =N-C$\underline{H}$=CH-), 6.77 (s, 1H, -N-C$\underline{H}$=CH-), 4.06–4.09 (m, 2H -N-C$\underline{H}_2$-CH$_2$-), 2.72–2.75 (m, 2H, -C$\underline{H}_2$-), 2.24–2.32 (m, 1H, -C$\underline{H}_2$-), 2.14–2.23 (m, 3H, -C$\underline{H}_2$-), 2.03–2.11 (m, 3H, -C$\underline{H}_2$-), 1.86–1.94 (m, 1H, -C$\underline{H}_2$-). ^{13}C{^{1}H} NMR (CDCl$_3$, 150.9 MHz): δ 149.3 (s, -N-$\underline{C}$=N-), 138.8 (s, -NH-$\underline{C}$=C-), 134.8 (s, -NH-$\underline{C}$=CH-), 129.1 (s, -NH-C=$\underline{C}$-CH=), 127.4 (d, C-2',-CH=$\underline{C}$H-N=), 123.9 (d, -CH=$\underline{C}$H-C-Br), 120.7 (d, =C-$\underline{C}$H=C-Br), 118.4 (d, C-3', -N-$\underline{C}$H=CH-), 112.3 (d, -NH-C-$\underline{C}$H=CH-), 112.0 (s, -CH=$\underline{C}$-Br), 110.8 (s, -NH-C=$\underline{C}$-CH$_2$-), 45.0 (t, -CH$_2$-$\underline{C}$H$_2$-N-), 37.9 (s, -$\underline{C}$-C=N-), 33.9 (t, -$\underline{C}$H$_2$-), 20.7 (t, -$\underline{C}$H$_2$-), 19.8 (t, -$\underline{C}$H$_2$-), 19.4 (t, -$\underline{C}$H$_2$-). IR (ATR); υ_{max} (cm^{-1}) 3134, 2932, 2854, 1484, 1444, 1307, 1208, 1049, 798, 736. HRMS (ESI) *m/z*: calcd for $C_{18}H_{19}BrN_3$, $[M+H]^+$, 356.0757; found, 356.0759.

ASSOCIATED CONTENT

Supporting Information. LIS experiment for **5** and **5'**, HRMS, NMR spectra, IR spectra, anti-microbial, anti-biofilm and cytotoxicity assay (data and protocols). Description and illustration of the crystal structure of **5**. CCDC 2093114 (**5**) contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre.

AUTHOR INFORMATION

Corresponding Authors

*Mervic D. Kagho, *Dr. Philipp Klahn – Institute of Organic Chemistry, Technische Universität Braunschweig, Hagenring 30, D-38106 Braunschweig, Germany; Organic Chemistry; orcid.org/0000-0003-3714-7738; E-mail: p.klahn@tu-braunschweig.de

*Prof. Dr. Klaus Banert – Organic Chemistry, Technische Universität Chemnitz, Straße der Nationen 62, 09111 Chemnitz, Germany. Prof. Dr. Klaus Banert sadly passed away on September 19, 2020.

Author Contributions

M.D.K. and P.K. wrote the manuscript. M.D.K. synthesized and characterized all target compounds. H.H. supported the preparation of the starting materials **15/15'** under the supervision of A.I.. C.B.,T.R. and H.L. determined the structure of **5** by single crystal X-ray analysis. Biological evaluation of the compounds was planed by M.S.. Antimicrobial and cytotoxicity screens were performed by W.C.. Antibiofilm assays were performed by H.S. and H.Z.. The whole project was supervised by K.B. who also provided experimental guidance. All authors have given approval to the final version of the manuscript.

Funding Sources

We gratefully acknowledge the German Academic Exchange Service (DAAD) for their generous Doctoral research grant to M.D.K. (Personal ref. no.: 91693784).

Notes

The authors declare no conflict of interest.

ACKNOWLEDGMENT

We thank Dr. Andreas Seifert for assistance in NMR spectroscopy. We are also grateful to Dr. Manfred Hagedorn for his assistance with the LIS experiment. Besides, Dr. Manuel Heck is thanked for proofreading the manuscript.

REFERENCES

(1) Gravel, E.; Poupon, E. Biosynthesis and Biomimetic Synthesis of Alkaloids Isolated from Plants of the Nitraria and Myrioneuron Genera: An Unusual Lysine-Based Metabolism. *Nat. Prod. Rep.* **2010**, *27* (1), 32–56. https://doi.org/10.1039/b911866g.

(2) Novgorodova, N. Y.; Maekh, S. K.; Yunusov, S. Y. Nitramine — A New Alkaloid from Nitraria Schoberi. *Chem. Nat. Compd.* **1973**, *9* (2). https://doi.org/10.1007/BF00563342.

(3) Ibragimov, A. A.; Moiseeva, G. P.; Osmanov, Z.; Yunusov, S. Y. Absolute Configuration and Conformational Features of Alkaloids of the Nitramine Group and Their Derivatives. *Chem. Nat. Compd.* **1986**, *22* (6). https://doi.org/10.1007/BF00598349.

(4) Osmanov, Z.; Ibragimov, A. A.; Yunusov, S. Y. Structure of Sibirine. *Chem. Nat. Compd.* **1982**, *18* (2), 206–208. https://doi.org/10.1007/BF00577195.

(5) Ibragimov, A. A.; Osmanov, Z.; Yagudaev, M. R.; Yunusov, S. Y. Alkaloids OfNitraria Sibirica. Structure of Nitrabirine. *Chem. Nat. Compd.* **1983**, *19* (2). https://doi.org/10.1007/BF00580559.

(6) Tulyaganov, T. S.; Allaberdiev, F. K. Alkaloids of Nitraria Sibirica. Dihydroschoberine and Nitrabirine N-Oxide. *Chem. Nat. Compd.* **2001**, *37* (6), 556–558.

(7) Kwon, S. J.; Hwang, S. J.; Jung, Y.; Park, H. geun; Kim, M. hyun; Park, Y.; Lee, H. J. A Synthetic Nitraria Alkaloid, Isonitramine Protects Pancreatic β-Cell and Attenuates Postprandial Hyperglycemia. *Metabolism.* **2017**, *70*, 107–115. https://doi.org/10.1016/j.metabol.2017.02.002.

(8) Turghun, C.; Bakri, M.; Abudulla, R.; Sun, G. Y.; Aisa, H. A. UHPLC-MSn-Assisted Characterization of Bioactive Alkaloids Extracted from Nitraria Sibirica Leaves and Enriched Using Response Surface Method and Adsorption on Macroporous Resin. *Ind. Crops Prod.* **2018**, *125*, 529–536. https://doi.org/10.1016/j.indcrop.2018.09.038.

(9) Hellberg, L. H.; Beeson, C.; Somanthan, R. Synthesis of The Spirocyclic Alkaloid Nitramine. *Tetrahedron Lett.* **1986**, *27* (34), 3955–3956.

(10) Wanner, M. J.; Koomen, G. J. Synthesis of (+/-)-Nitramine, (+/-)-Isonitramine and (+/-)-Sibirine via Diels-Alder Reactions. *Tetrahedron Lett.* **1989**, *30* (17), 2301–2304.

(11) Keppens, M.; De Kimpe, N. Enantioselective Total Syntheses of the Nitraria Alkaloids (-)-Nitramine and (+)-Isonitramine. *J. Org. Chem.* **1995**, *60* (12), 3916–3918. https://doi.org/10.1021/jo00117a053.

(12) Pandey, G.; Prasanna Kumara, C.; Kumar Burugu, S.; Puranik, V. G. Enantioselective Total Syntheses of (-)-Isonitramine, (-)-Sibirine, and (+)-Nitramine by Ring-Closing Metathesis. *European J. Org. Chem.* **2011**, No. 36, 7372–7377. https://doi.org/10.1002/ejoc.201101256.

(13) Numajiri, Y.; Pritchett, B. P.; Chiyoda, K.; Stoltz, B. M. Enantioselective Synthesis of α-Quaternary Mannich Adducts by Palladium-Catalyzed Allylic Alkylation: Total Synthesis of(+)-Sibirinine. *J. Am. Chem. Soc.* **2015**, *137* (3), 1040–1043. https://doi.org/10.1021/ja512124c.

(14) Yang, J.; Park, Y.; Yang, S.; Lee, G.; Ha, M. W.; Kim, M. H.; Hong, S.; Park, H. G. Enantioselective Total Synthesis of Nitraria Alkaloids: (+)-Nitramine, (+)-Isonitramine, (-)-Isonitramine, and (-)-Sibirine via Asymmetric Phase-Transfer Catalytic α-Allylations of α-Carboxylactams. *J. Org. Chem.* **2021**, DOI: 10.1021/acs.joc.0c02573. https://doi.org/10.1021/acs.joc.0c02573.

(15) Pearson, W. H.; Walavalkar, R. A Schmidt Route to 1-Azabicyclo[x.y.0]Alkanes: A Comparison of Carbocation Stabilizing Groups. *Tetrahedron* **2001**, *57* (24), 5081–5089. https://doi.org/10.1016/S0040-4020(01)00353-2.

(16) Milligan, G. L.; Mossman, C. J.; Aubé, J. Intramolecular Schmidt Reactions of Alkyl Azides with Ketones: Scope and Stereochemical Studies. *J. Am. Chem. Soc.* **1995**, *117*, 10449–10459. https://doi.org/10.1016/0040-4020(96)00037-3.

(17) Glindemann, C. P.; Backenköhler, A.; Strieker, M.; Wittstock, U.; Klahn, P. Synthesis and Biochemical Evaluation of an Artificial, Fluorescent Glucosinolate (GSL). *ChemBioChem* **2019**, *19*, 1668–1694. https://doi.org/10.1002/cbic.201900148.

(18) Trost, B. M.; Radinov, R.; Grenzer, E. M.; V, S. U.; May, R. V. Asymmetric Alkylation of -Ketoesters Creating Quaternary Carbon Centers in Which the Absolute Stereochemistry Can Be Controlled by Alkylation Represents a Major Challenge . -Ketoesters Become Interesting Substrates and Selective Manipulation. *J. Am. Chem. Soc.* **1997**, *119*, 7879–7880.

(19) Lin, W.; Zhang, X.; He, Z.; Jin, Y.; Gong, L.; Mi, A. Reduction of Azides to Amines or Amides with Zinc and Ammonium Chloride as Reducing Agent. *Synth. Commun.* **2002**, *32* (21), 3279–3284. https://doi.org/10.1081/SCC-120014032.

(20) Hua, D. H.; Zhang, F.; Chen, J. Condensation of Lactams with 2-Aminoacetylaldehyde Diethylacetal. A One-Pot Synthesis of Bicyclic Imidazoles. *J. Org. Chem.* **1994**, *59*, 5084–5087.

(21) Romine, J. L.; Martin, S. W.; Gribkoff, V. K.; Boissard, C. G.; Dworetzky, S. I.; Natale, J.; Li, Y.; Gao, Q.; Meanwell, N. A.; Starrett Jr., J. E. 4 , 5-Diphenyltriazol-3-Ones : Openers of Large-Conductance Ca 2 + -Activated Potassium (Maxi-K) Channels. *J. Med. Chem.* **2002**, *45*, 2942–2952.

(22) Ibragimov, A. A.; Osmanov, Z.; Yagudaev, M. R.; Yunusov, S. Y. Alkaloids of Nitraria Sibirica: Structure of Nitrabirine. *Khim. Prir. Soedin.* **1983**, 213–216.

(23) Kuhnz, W.; Rembold, H. Application of Lanthanide Induced Shifts in Proton Magnetic Resonance Spectroscopy of Juvenile Hormones. *Zeitschrift für Naturforsch. C* **1977**, *32* (7–8), 563–566. https://doi.org/10.1515/znc-1977-7-814.

(24) Peterson, M. R.; Wahl, G. H. Lanthanide Nmr Shift Reagents. A Powerful New Stereochemical Tool. *J. Chem. Educ.* **1972**, *49* (12), 790. https://doi.org/10.1021/ed049p790.

(25) Murray, J. I.; Spivey, A. C. Preparation of 1-Methylimidazole-N-Oxide (NMI-O). *Org. Synth.* **2016**, *93*, 331–340. https://doi.org/10.15227/orgsyn.093.0331.

(26) Limnios, D.; Kokotos, C. G. 2,2,2-Trifluoroacetophenone as an Organocatalyst for the Oxidation of Tertiary Amines and Azines to N-Oxides. *Chem. - A Eur. J.* **2014**, *20* (2), 559–563. https://doi.org/10.1002/chem.201303360.

(27) Baidya, M.; Brotzel, F.; Mayr, H. Nucleophilicities and Lewis Basicities of Imidazoles, Benzimidazoles, and Benzotriazoles. *Org. Biomol. Chem.* **2010**, *8* (8), 1929–1935. https://doi.org/10.1039/c000965b.

(28) Kaushik, N. K.; Kaushik, N.; Attri, P.; Kumar, N.; Kim, C. H.; Verma, A. K.; Choi, E. H. Biomedical Importance of Indoles. *Molecules* **2013**, *18* (6), 6620–6662. https://doi.org/10.3390/molecules18066620.

(29) Becker, K.; Wessel, A.; Luangsa-ard, J. J.; Stadler, M. Viridistratins A–C, Antimicrobial and Cytotoxic Benzo[j]Fluoranthenes from Stromata of Annulohypoxylon Viridistratum (Hypoxylaceae, Ascomycota). *Biomolecules* **2020**, *10* (5), 805.

https://doi.org/10.3390/biom10050805.
(30) Schriefer, M. G.; Schrey, H.; Zeng, H.; Stadler, M.; Schobert, R. Synthesis of the Fungal Macrolide Berkeleylactone A and Its Inhibition of Microbial Biofilm Formation. *Org. Biomol. Chem.* **2021**, *19* (21), 4743–4751. https://doi.org/10.1039/d1ob00717c.
(31) Klahn, P.; Fetz, V.; Ritter, A.; Collisi, W.; Hinkelmann, B.; Arnold, T.; Tegge, W.; Rox, K.; Hüttel, S.; Mohr, K. I.; Wink, J.; Stadler, M.; Wissing, J.; Jänsch, L.; Brönstrup, M. The Nuclear Export Inhibitor Aminoratjadone Is a Potent Effector in Extracellular-Targeted Drug Conjugates. *Chem. Sci.* **2019**, *10* (20), 5187–5425. https://doi.org/10.1039/c8sc05542d.
(32) Swern, D. *Organic Peroxides*, Vol.II.; Wiley: New York, 1971.