SYNTHESIS OF NOVEL SACCHARIDE HYDRAZONES

Mihkel Ilisson,1 Kristjan Tomson,1 Anastasia Selyutina,2 Silver Türk,3 and Uno Mäeorg1

1Institute of Chemistry, University of Tartu, Tartu, Estonia
2Institute of Technology, University of Tartu, Tartu, Estonia
3Faculty of Medicine, University of Tartu, Tartu, Estonia

GRAPHICAL ABSTRACT

Abstract Synthesis of important heterocyclic hydrazine derivatives N-aminopyrrolidine, N-aminopiperidine, and N-aminoazepane from hydrazine hydrate and dihalogenides were examined and optimized. These heterocyclic hydrazine derivatives were used in condensation reactions with six different monosaccharides to form corresponding hydrazones. Biological evaluations of these novel compounds, which are simple acyclic nucleoside mimetics, were done. L-Arabinose N-aminoazepane hydrazone showed minor anti-HIV activity, giving a starting point for further structural modifications.

Keywords Heterocycle; HIV; hydrazine; hydrazone; saccharide

INTRODUCTION

Hydrazones are chemical compounds analogous with imines. They can be synthesized with a condensation reaction from a hydrazine derivative and a carbonyl compound. Saccharide hydrazones, which contain heterocycles, can be observed as acyclic nucleoside mimetics. These compounds have shown biological activity against bacteria,[1–3] viruses,[4,5] fungi,[1,2] and cancer cells.[6]

Structurally simple heterocyclic hydrazine derivatives, such as N-aminopyrrolidine, N-aminopiperidine, and N-aminoazepane, are important building blocks in organic synthesis. There are several bioactive molecules that contain aforementioned fragments.[7,8] However, there are only a few methods available
for the synthesis of these heterocyclic hydrazine derivatives. Most commonly secondary amines are used as a starting material, and electrophilic amination\cite{9} or nitrosoylation\cite{10} with subsequent reduction\cite{11} is carried out. These methods have some setbacks; for example, electrophilic amination includes using unstable reagents such as chloramines and hydroxylamines while the nitrosoylation route provides highly toxic and carcinogenic nitroso-intermediates.

Synthesis of \( N \)-aminopyrrolidine, \( N \)-aminopiperidine, and \( N \)-aminoazepane can be also carried out via a polyanion strategy\cite{12–15} developed out in our workgroup. However, this route requires using protection–deprotection steps and column chromatography as a purification method, which makes this method inconvenient to use on a larger scale.

In current work, efforts were concentrated on synthesis of heterocyclic hydrazine derivatives using hydrazine hydrate and suitable dihalogenides as starting materials. Previously, some procedures for the synthesis of \( N \)-aminopyrrolidine and \( N \)-aminopiperidine have been described,\cite{16,17} but they were found out to be either unoptimized or nonreproducible. To the best of our knowledge, \( N \)-aminoazepane have not been synthesized from hydrazine hydrate and 1,6-dihalohexane before.

Synthesized hydrazine derivatives were used in condensation reactions with monosaccharides to form corresponding hydrazones. These novel compounds were used in experiments against human immunodeficiency virus (HIV)–based virus-like particles, bacteria, and fungi to characterize their antiretroviral, antibacterial, and antifungal activities.

**RESULTS AND DISCUSSION**

**Synthesis of Heterocyclic Hydrazine Derivatives**

Initial experiments were started using just a slight excess (1.2 eq) of hydrazine hydrate compared to the dihalogenides. Also, sodium hydroxide solution was used to keep the reaction medium basic. This procedure gave desired products in very poor yields (1.6–7.2\%), probably because of formation of \( N,N' \)-bisubstituted hydrazine derivatives and elimination and substitution reactions caused by the use of sodium hydroxide (Scheme 1). Therefore we decided to significantly increase the excess of hydrazine hydrate, which decreases the amount of disubstitution and also binds the hydrogen halide formed during the reaction, making the use of inorganic base unnecessary.

In the synthesis of \( N \)-aminoazepane, polymerization occurred in our initial experiments. We speculate that the polymerization was favored against the cyclization

![Scheme 1. Possible reaction paths in the synthesis of heterocyclic hydrazine derivatives.](image-url)
because of the conformational hindrances and high concentration of reagents (see Scheme 2). Formation of seven-membred rings has high energy barrier and the polymerization is preferred at greater concentrations. Therefore, the next experiments for the synthesis of N-aminoazepane were carried out in significantly diluted solutions and without the presence of sodium hydroxide to avoid the polymerization.

N-Aminopyrrolidine was purified by vacuum distillation. For N-aminopiperidine and N-aminoazepane, the distillation was not applied because of the decomposition, which occurred during the distillation process. On the other hand, the corresponding high-purity products were already obtained by extraction of the reaction mixture and evaporation of the solvent.

During the optimization of the reaction conditions, we reached good yields (45–74%) of hydrazine derivatives (see Scheme 3), considering that the synthesis is a simple one-step process and that inexpensive and easily accessible reagents were used. Yields can be probably improved somewhat further, when using even larger excess of hydrazine hydrate in the reactions.

**Synthesis and Biological Evaluation of Saccharide Hydrazones**

For the synthesis of saccharide hydrazones, previously synthesized heterocyclic hydrazine derivatives 1–3 and six different aldoses (L-arabinose, D-galactose, D-mannose, D-ribose, L-rhamnose, and 2-deoxy-D-ribose) were used (see Table 1). Procedures used for these condensation reactions were analogous to the one used in Stroh and Scharnow’s work.\[^{18}\]

All the reactions were clean according to the thin-layer chromatography (TLC) and NMR and were completed within several hours. After the completion, solvents and other volatiles were removed in vacuum and the residue was recrystallized in a suitable solvent. In some cases, the product was a liquid and therefore column chromatography was used for purification. Even though triethylamine-doped eluent was used, we could see some decomposition by TLC. However, this decomposition turned out to be insignificant, as we got excellent yields and did not detect any impurities with NMR.

Eighteen synthesized novel hydrazones 4–21 were subjected to biological evaluations. Their activity was examined against *E. coli*, *S. aureus*, and *C. albicans*. In these experiments it was confirmed that selected compounds did not have any effect on these bacteria and fungi.

Toxicology tests revealed that all synthesized hydrazones are nontoxic to mammalian cells at the concentration of 1 mM. Saccharide hydrazones 4–21 were also

![Scheme 2. Cyclization and polymerization route of (6-bromohexyl)hydrazine.](image)
tested for anti-HIV properties. It was found that compound 6 had a minor effect (IC\textsubscript{50} ≈ 400 µM) against HIV. Also, compound 21 showed some activity (IC\textsubscript{50} > 1 mM) against HIV. The other 16 hydrazones turned out to be ineffective for inhibiting HIV.

\textbf{Table 1.} Procedure details for the synthesis of saccharide hydrazones

| Hydrazone | Hydrazine derivative | Monosaccharide | Reaction time (h) | Purification method\textsuperscript{a} | Isolated yield (\%) |
|-----------|----------------------|----------------|-------------------|------------------------------------------|---------------------|
| 4         | 1                    | L-Arabinose    | 3                 | 50:50                                    | 93                  |
| 5         | 2                    |                | 3.5               | 50:50                                    | 85                  |
| 6         | 3                    |                | 3                 | 50:50                                    | 85                  |
| 7         | 1                    | D-Galactose    | 4                 | 25:75                                    | 96                  |
| 8         | 2                    |                | 4.5               | 50:50                                    | 77                  |
| 9         | 3                    |                | 2                 | 67:33                                    | 71                  |
| 10        | 1                    | D-Mannose      | 3.5               | 50:50                                    | 93                  |
| 11        | 2                    |                | 4                 | 67:33                                    | 77                  |
| 12        | 3                    |                | 2                 | 67:33                                    | 68                  |
| 13        | 1                    | D-Ribose       | 3.25              | CC                                       | 85                  |
| 14        | 2                    |                | 4                 | 80:20                                    | 83                  |
| 15        | 3                    |                | 3                 | 100:0                                    | 71                  |
| 16        | 1                    | L-Rhamnose     | 2.5               | 80:20                                    | 80                  |
| 17        | 2                    |                | 3.75              | 75:25                                    | 83                  |
| 18        | 3                    |                | 3.25              | 100:0                                    | 74                  |
| 19        | 1                    | 2-Deoxy-D-ribose| 1.5               | CC                                       | 88                  |
| 20        | 2                    |                | 1                 | CC                                       | 98                  |
| 21        | 3                    |                | 0.75              | 100:0                                    | 73                  |

\textsuperscript{a}For purification method MTBE/EtOH solvent ratio is written if the product was purified by recrystallization. CC indicates purification by column chromatography with standard eluent composition (ethanol–benzene–triethylamine 29:10:1).
EXPERIMENTAL

Synthesis

All the used reagents and solvents were obtained commercially from Sigma-Aldrich company and used without further purification.

Thin-layer chromatography was performed on Macherey-Nagel Alugram SIL G/UV 254 silica-gel plates. For visualization, 1% phosphomolybdic acid solution in ethanol or 10% sulfuric acid solution in ethanol were used. For column chromatography, Merck Kieselgel 70- to 230-mesh silica gel was used.

The FTIR spectra were measured with Perkin-Elmer Spectrum BXII FTIR spectrometer equipped with Interspectrum (Estonia) zinc selenide ATR crystal. Wavelenghts in spectra are presented in centimeters\(^{-1}\).

The NMR spectra were recorded with Bruker Avance II 200 and Bruker Avance III HD spectrometers. \(^1\)H NMR spectra were measured at frequencies of 200 and 700 MHz. \(^13\)C NMR spectra were measured at 50 and 176 MHz. Tetramethylsilane (TMS) was used as an internal standard. Chemical shifts are presented in parts per million (ppm), with decoupling constants in hertz (Hz).

The HRMS spectra were measured on Thermo Electron LTQ Orbitrap spectrometer with the electrospray ionization (ESI) method.

Procedure for Preparation of \(N\)-Aminopyrrolidine (1)

In a 100-ml, two-necked flask, hydrazine hydrate (24.25 ml, 0.5 mol) was dissolved in 40 ml of methanol. The mixture was heated to reflux and 1,4-dibromobutane (17.91 ml, 0.15 mol) was added dropwise within 1 h. The reaction mixture was left stirring at reflux for 24 h. Methanol was removed by fractional distillation. Residue was basified with 75 g of 40% NaOH and extracted 10 times with Et\(_2\)O. Combined extracts were dried on anhydrous Na\(_2\)SO\(_4\) and filtered. The solvent was removed under reduced pressure and the product was purified by vacuum distillation (bp 46 °C at 30 mbar). This process yielded 6.374 g (49%) of \(N\)-aminopyrrolidine,\(^{17}\) a clear colorless liquid.

Procedure for Preparation of \(N\)-Aminopiperidine (2)

In a 100-ml, two-necked flask, hydrazine hydrate (14.55 ml, 0.3 mol) was dissolved in 25 ml of methanol. The solution was heated to reflux and 1,5-dibromopentane (13.62 ml, 0.1 mol) was added dropwise within 1 h. The reaction mixture was left stirring at reflux for 24 h. Methanol was removed under reduced pressure, and the residue was basified with 40 g of 40% NaOH solution. The mixture was extracted eight times with Et\(_2\)O and combined extracts were dried on anhydrous Na\(_2\)SO\(_4\) and filtrated. Solvent was removed under reduced pressure (40 °C at 40 mbar). The process yielded 7.380 g (74%) of \(N\)-aminopiperidine,\(^{17}\) a light yellow liquid, which was used in subsequent experiments without further purification.

Procedure for Preparation of \(N\)-Aminoazepane (3)

In a 500-ml, two-necked flask, hydrazine hydrate (24.25 ml, 0.5 mol) was dissolved in 360 ml of methanol. The solution was heated to reflux and 1,6-dibromohexane (23.07 ml, 0.15 mol) was added dropwise within 1.5 h. After 24 h of stirring at
reflux, the methanol was evaporated under reduced pressure. The residue was basified with 55 g of 40% NaOH solution and extracted 10 times with Et2O. Combined extracts were dried on anhydrous Na2SO4 and filtered. The solvent was removed under reduced pressure (40 °C at 25 mbar). The process yielded 7.695 g (45%o) of N-aminoazepane,\[16\] a light yellow liquid, which was used in subsequent experiments without further purification.

**General Procedure for Preparation of Saccharide Hydrazones (4–21)**

In a 100-ml flask, 8 mmol of hydrazine derivative (N-aminopyrrolidine, N-aminopiperidine, or N-aminoazepane) was dissolved in 15 ml of methanol, and 5 mmol of monosaccharide was added. The mixture was stirred at the methanol reflux. After 0.75–4.5 h of stirring, the reaction was complete according to TLC. Volatiles were removed under reduced pressure and the residue was recrystallized in MTBE–ethanol mixture or purified by column chromatography (eluent: ethanol–benzene–triethylamine 29:10:1). Additional details about the specific procedures can be found in 1 and in the supplementary information.

**Biological Evaluation**

**Antiretroviral evaluation.** Dimethyl sulfoxide (DMSO) and polybrene were purchased from Sigma Aldrich (USA). All reagents and media used for cell cultivation were purchased from Naxo OÜ (Estonia). U2OS human osteosarcoma cells were obtained from ATCC and grown in Iscove’s modified Dulbecco’s medium (IMDM) (supplemented with 10% fetal bovine serum (FBS), 100 U penicillin, and 100 µg/ml streptomycin (pen/strep)) at 37 °C in the presence of 5% CO2.

**Cytotoxicity assay.** Cytotoxicity was measured using the xCELLigence RTCA DP Instrument (ACEA Biosciences, Inc). The U2OS cells were seeded on E-plate 16 (3.5 × 10^3 cells per well) and incubated for 24 h. Then compounds (4–21) were dissolved in dimethylsulfoxide (DMSO; or equivalent amount of DMSO as a vehicle control) and incubation was continued for another 24 h. Impedance signals were recorded throughout the incubation.

**Analysis of antiviral activity.** Antiretroviral activity was analyzed using HIV-1-based virus-like particles (VLPs), obtained using ViraPower Lentiviral Expression System according to the manufacture (Invitrogen) instructions. The RNA packed into VLPs was engineered to encode a reporter gene (Gaussia luciferase (Gluc)), which is, however, expressed only when RNA is reverse transcribed into DNA by HIV reverse transcriptase present in VLPs. The day before the experiment U2OS cells were seeded on 24-well plate (5 × 10^4 cells per well). The next day cells were infected with HIV-1 VLPs at a multiplicity of infection (MOI) = 0.01 infectious units/cell in the presence of polybrene 6 µg/ml and analyzed compounds (or DMSO as a vehicle control). After 1 h of incubation (37 °C, 5% CO2), cells were washed, and full media with analyzed compounds (or DMSO) was added. Cells were incubated for 24 h with compounds and then in fresh media without compounds for another 24 h. After this, cells were lysed and Gluc activity in cells was measured using Renilla Luciferase Assay System and Glomax 20/20 Luminometer (Promega). Gluc signal was normalized to the total protein concentration (measured with Bio-Rad protein assay).
Antimicrobial evaluation. Synthesized hydrazones (4–21) were tested for antimicrobial activity against *Escherichia coli* ATCC 700336, *Staphylococcus aureus* ATCC 43300, and *Candida albicans* ATCC MYA-2876 by disk-diffusion assay. Müller–Hinton plates (Oxoid, Basingstoke, UK) were inoculated with bacteria suspended in distilled water (turbidity McFarland 0.5) by using sterile cotton swabs. Sterilized filter paper disks were placed on the inoculated plates, and 50 µg hydrazones in 50 microliters of distilled water were pipetted onto the disks. Then the plates were incubated for 24 h at 37 °C. None of the tested hydrazones formed zones of inhibition against any of the tested microorganisms.

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**SUPPLEMENTARY INFORMATION**

Supplemental data for this article can be accessed on the publisher’s website.

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