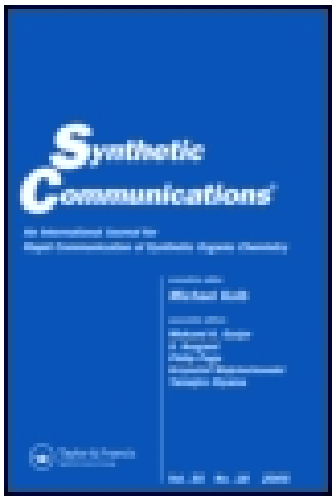

Synthetic Communications: An International Journal for Rapid Communication of Synthetic Organic Chemistry

Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/lsyc20

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Accepted author version posted online: 23 Feb 2015.

Synthesis of novel saccharide hydrazones

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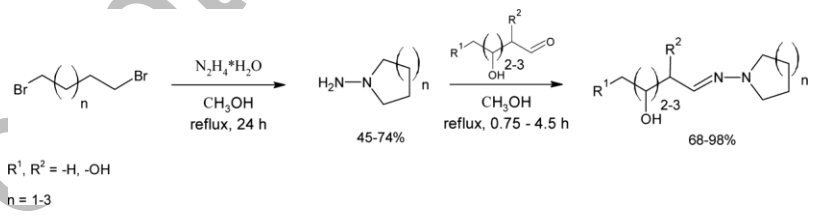
Abstract

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 analogues, were done. L-arabinose N-aminoazepane hydrazone showed minor anti-HIV activity, giving a starting point for further structural modifications.



KEYWORDS: *Heterocycle, hydrazine, saccharide, hydrazone, HIV*

INTRODUCTION

Hydrazones is a class of 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 known, which contain aforementioned fragments[7,8]. However, there are only few methods available for synthesis of these heterocyclic hydrazine derivatives. Most commonly secondary amines are used as a starting material and electrophilic amination[9] or nitrosoylation[10] with subsequent reduction[11] is carried out. These methods have some setbacks – electrophilic amination includes using unstable reagents such as chloramines and hydroxylamines while nitrosoylation route provides highly toxic and carcinogenic nitroso-intermediates.

Synthesis of *N*-aminopyrrolidine, *N*-aminopiperidine and *N*-aminoazepane can be also carried out via polyanion strategy[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 in 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[16,17], but they were found out to be either unoptimized or non-reproducible. 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 in order 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 in order to keep the reaction medium basic. This procedure gave desired products in very low yields (1.6-7.2%), probably due to 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, a polymerization occurred in our initial experiments. We speculate that the polymerization was favored against the cyclization 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 higher 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 to good yields (45-74%) of hydrazine derivatives (see Scheme 3) considering that the synthesis is a simple one-step process and inexpensive and easily accessible reagents were used. Yields can be probably improved somewhat further, when to use 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).

Procedure used for these condensation reactions were analogous to the one used in German scientists H. Stroh and H. Scharnow's work[18].

All the reactions were clean according to the TLC and NMR and were completed within several hours. After the completion, solvents and other volatiles were removed in vacuum and 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.

18 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 non-toxic to mammalian cells at the concentration of 1 mM. Saccharide hydrazones **4-21** were also tested for anti-HIV properties. It was found out, that compound **6** have minor effect ($IC_{50} \approx 400$ μM) against HIV. Also, compound **21** showed some activity ($IC_{50} \wp$ 1 mM) against HIV. Other 16 hydrazones turned out to be ineffective towards inhibiting HIV.

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 70-230 mesh silica gel was used.

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 cm^{-1}.

NMR spectra were recorded with Bruker Avance II 200 and Bruker Avance III HD spectrometers. ^{1}H NMR spectra were measured at frequencies 200 MHz and 700 MHz. ^{13}C NMR spectra were measured at 50 MHz and 176 MHz. TMS was used as an internal standard. Chemical shifts are presented in ppm, decoupling constants in Hz.

HRMS spectra were measured on Thermo Electron LTQ Orbitrap spectrometer with ESI ethod.

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. Mixture was heated to reflux and 1,4-dibromobutane (17.91 ml, 0.15 mol) was added dropwise within 1 h. 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_2O. Combined extracts were dried on anhydrous Na_2SO_4 and filtrated. Solvent was removed under reduced pressure and the product was purified by vacuum distillation (bp 46 ˚C/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. Solution was heated to reflux and 1,5-dibromopentane (13.62 ml, 0.1 mol) was added dropwise within 1 h. Reaction mixture was left stirring at reflux for 24 h. Methanol was removed under reduced pressure and residue was basified with 40 g of 40% NaOH solution. Mixture was extracted 8 times with Et_2O and combined extracts were dried on anhydrous Na_2SO_4 and filtrated. Solvent was removed under reduced pressure (40 ˚C at 40 mbar). 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. 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. Residue was basified with 55 g of 40% NaOH solution and extracted 10 times with Et_2O. Combined extracts were dried on anhydrous Na_2SO_4 and filtrated. Solvent was removed under reduced pressure (40 ˚C at 25 mbar). Process yielded 7.695 g (45%) 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. 5 mmol of monosaccharide was added and mixture was stirred at the methanol reflux. After 0.75 – 4.5 h of stirring, the reaction was complete according to the TLC. Volatiles were removed under reduced pressure and the residue was recrystallized in MTBE-ethanol mixture or purified by column chromarography (eluent: ethanol-benzene-triethylamine 29:10:1). Additional details about the specific procedures can be found in Table 1 and in the supplementary information.

Biological Evaluation

ANTI-RETROVIRAL EVALUATION

Materials Used

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% CO_2.

CYTOTOXICITY ASSAY

Cytotoxicity was measured using the xCELLigence RTCA DP Instrument (ACEA Biosciences, Inc). U2OS cells were seeded on E-plate 16 ($3{,}5*10^3$ cells per well) and incubated for 24 hours. Then compounds (4-21) dissolved in DMSO (or equivalent amount of DMSO as a vehicle control) were added and incubation was continued for another 24 hours. 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). 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 hour of incubation (37°C, 5%CO_2) cells were washed and full media with analyzed compounds (or DMSO) was added. Cells were

incubated for 24 hours with compounds and then in fresh media without compounds for another 24 hours. 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).

ANTI-MICROBIAL EVALUATION

Synthesized hydrazones (4-21) was tested for antimicrobial activity against *Escherischia 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. 50 µg hydrazones in 50 microliters of distilled water were pipetted onto the disks. Then the plates were incubated for 24 hours at 37°. None of the tested hydrazones formed zones of inhibition against any of the tested microorganisms.

SUPPLEMENTARY INFORMATION

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

ACKNOWLEDGEMENTS

This work was supported by Estonian Science Foundation (grant No. 8794) and Research Targeted Financing projects SF0180032s12, institutional research funding projects

IUT20-17 of the Estonian Ministry of Education and Research and Competence center Mesosystems - Theory and Applications project TK-114.

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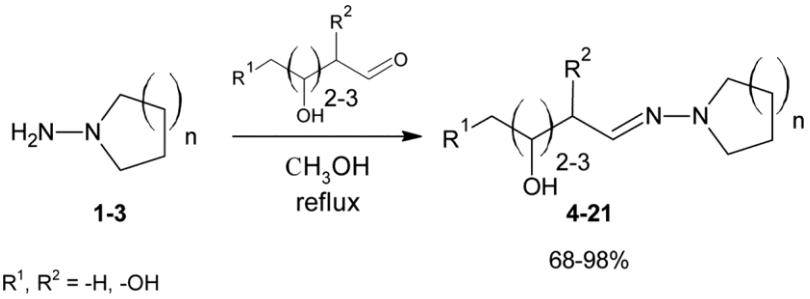
Table 1. Procedure details for the synthesis of saccharide



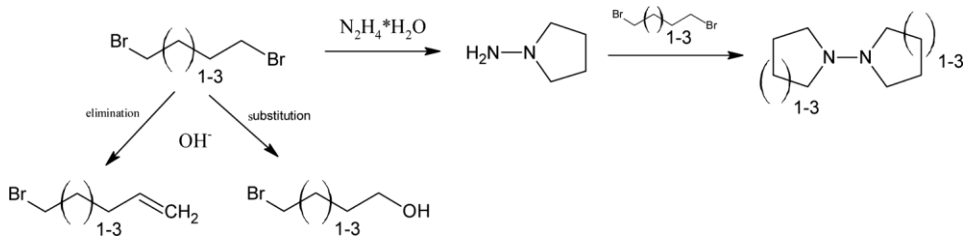
hydrazones.n = 1-3

Hydrazone	Hydrazine derivative	Monosaccharide	Reaction time (h)	Purification method*	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**	Dmannose	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

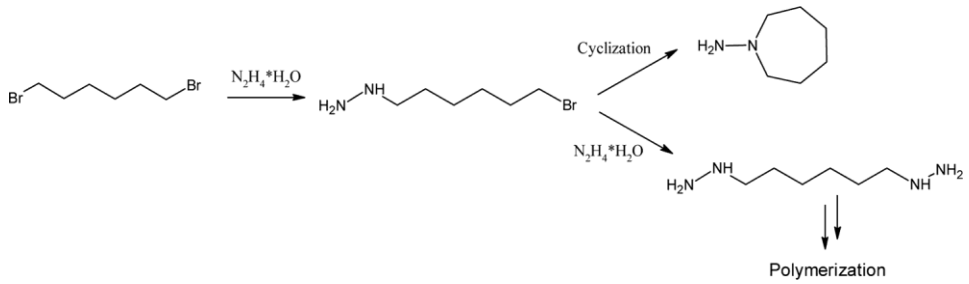
19	**1**	2-deoxy-D-ribose	1.5	CC	88
20	**2**		1	CC	98
21	**3**		0.75	100:0	73

* 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).

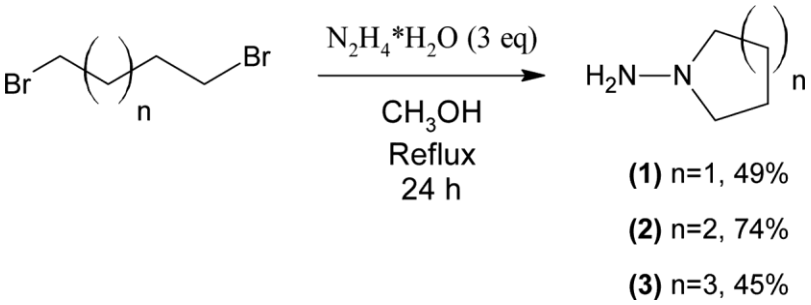
Scheme 1. Possible reaction paths in the synthesis of heterocyclic hydrazine derivatives.



Scheme 2. Cyclization and polymerization route of (6-bromohexyl)hydrazine.



Scheme 3. Yields and optimized reaction conditions for the synthesis of heterocyclic hydrazine derivatives.



(1) n=1, 49%

(2) n=2, 74%

(3) n=3, 45%