Short thesis for the degree of doctor of philosophy (PhD)

Synthesis of morpholine ring-containing nucleoside analogues

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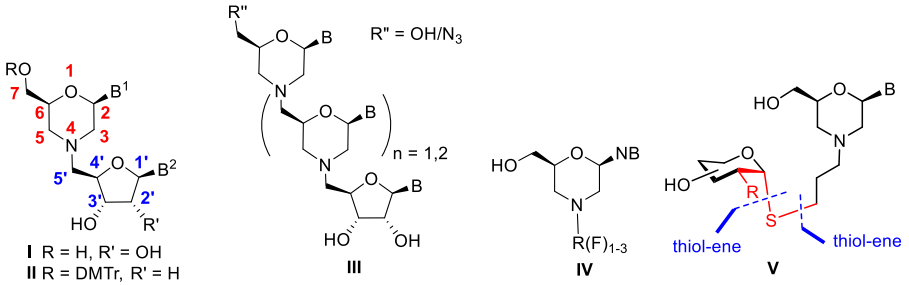
1. Introduction

Nucleosides and nucleotide analogues have a long and rich history in medicinal chemistry. Naturally occurring nucleosides are unique starting points for drug design because they are involved in many key biological processes and because they are essential building blocks for both DNA and RNA synthesis. Due to their polyanionic phosphodiester backbone, nucleic acids are highly sensitive to degradation by nucleases and have a low ability to penetrate cells, therefore the biological applicability of natural oligonucleotides is limited. The replacement of the phosphodiester bond creates an opportunity to produce artificial oligonucleotides and take advantage of their better properties.

Morpholino is a compound derived from a nucleoside, which contains a tetrahydro-1,4-oxazine (morpholine) skeleton instead of the ribose ring, and the nucleobase is connected to it in position 2 with an *N*-glycosidic bond. These compounds are often used in medicinal chemistry due to their beneficial physicochemical, biological and metabolic properties. During my doctoral work, I have dealt with the synthesis of nucleoside analogues in which the morpholine ring was present (Figure 1):

- synthesis of nucleoside-morpholino dimers and oligomers (**I-III**)
- production of fluorine-containing morpholine ring derivatives (**IV**)
- synthesis of potential glycosyltransferase inhibitors containing a morpholine ring (**V**)



Scheme 1: The structure of the planned compounds

2. Applied methods

During the synthetic work, the progress of the reaction was monitored by thin-layer chromatography, the purification of the crude products was performed by flash column chromatography, except for compound **48**, which was purified on a thick layer. The organic phases were dried over Na_2SO_4 and, after filtration, concentrated under reduced pressure using a rotary vacuum evaporator in a 40-50 °C water bath. Optical rotation determination, one- and two-dimensional (^{1}H-^{1}H COSY, ^{1}H-^{13}C HSQC and ^{1}H-^{13}C HMBC) NMR spectroscopy, as well as MALDI-TOF and ESI-QTOF mass spectroscopy were used to identify the produced compounds.

3. New scientific results of the dissertation

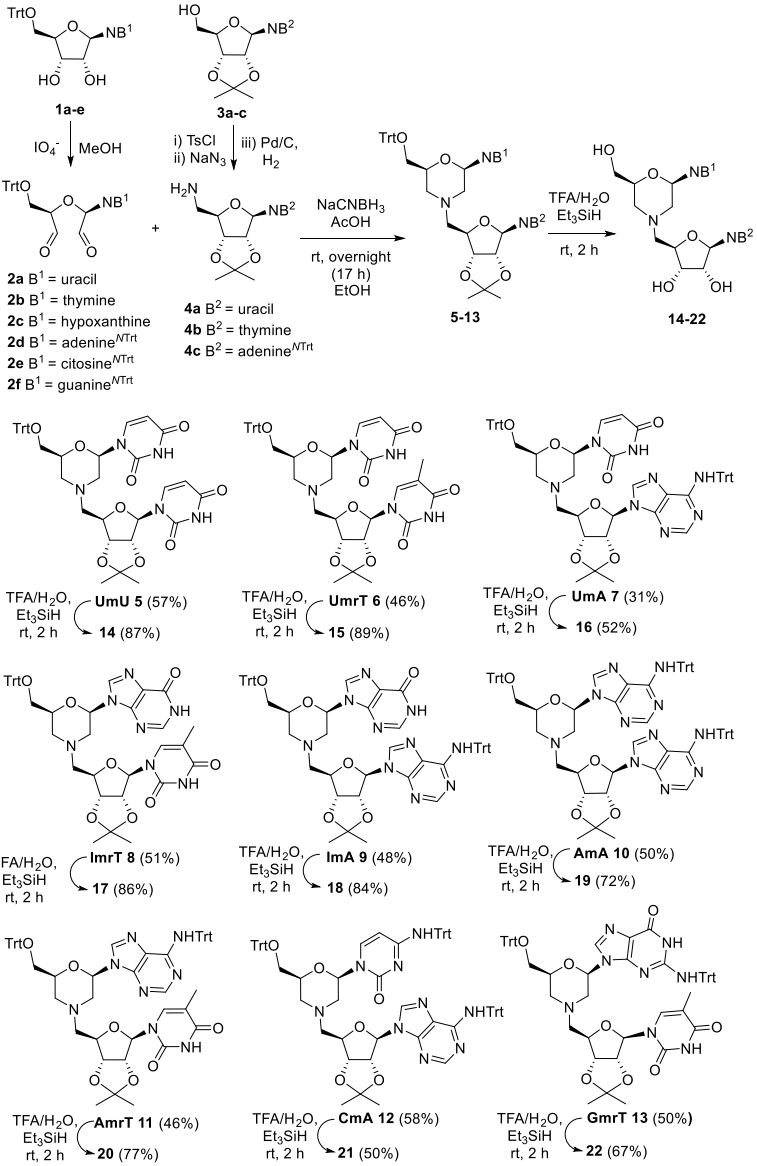
3.1 Morpholino-nucleoside chimeras

We prepared di- and oligonucleotides with different base sequences, in which the carbon atom 5 of ribose and the nitrogen of the morpholine ring are directly connected to each other. The nitrogen in these compounds is part of a tertiary

alkyl amine, which has a basic property, so it can facilitate entry into the cell. The synthesis was carried out by the reductive amination ring-closing reaction of properly protected nucleoside-dialdehydes and 5'-amino nucleosides and the subsequent removal of the protecting group.

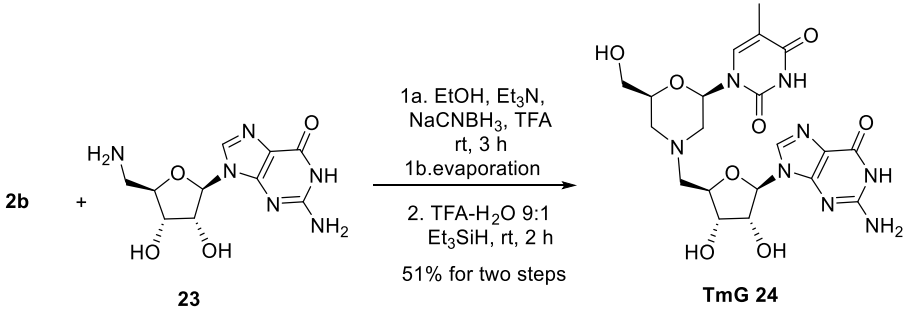
For the synthesis of dinucleotide analogues **14-22**, the corresponding secodialdehydes (**2a-e**) were formed by oxidative cleavage of trityl (Trt)-protected uridine, ribothymidine, inosine, adenosine, cytidine and guanosine derivatives (**1a-e**). In parallel, the nucleoside-5'-amines (**4a-c**) were prepared from 2',3'-*O*-isopropylidenated uridine, ribothymidine and adenosine (**3a-c**) in three steps. The key step of the synthetic protocol is the ring-closing double reductive amination of dialdehydes **2a-e** with amines **4a-c** using the reducing agent sodium cyanoborohydride ($NaCNBH_3$) in the presence of acetic acid, which resulted in the protected dinucleotide analogues **5-13**. The free derivatives (**14-22**) were obtained by simultaneous removal of the isopropylidene and trityl protecting groups using 90% trifluoroacetic acid (TFA) and triethylsilane (Et_3SiH) (Scheme 2).

Due to the lower yield experienced in the case of the protected UmA dimer (**7**), we performed optimization reactions, investigated the effect of the amount of the amine, the amount and quality of the reducing agent, and the quality of the acid and solvent, and from the 12 optimization reactions, the conditions using 1.5 equiv. of amine, 2.5 equiv. of $NaCNBH_3$, and 1.0 equiv. of TFA in EtOH were the most suitable, so the initial 31% was improved to 62%. In the case of non-optimized reactions, side products were formed, for which we proposed a reaction mechanism.



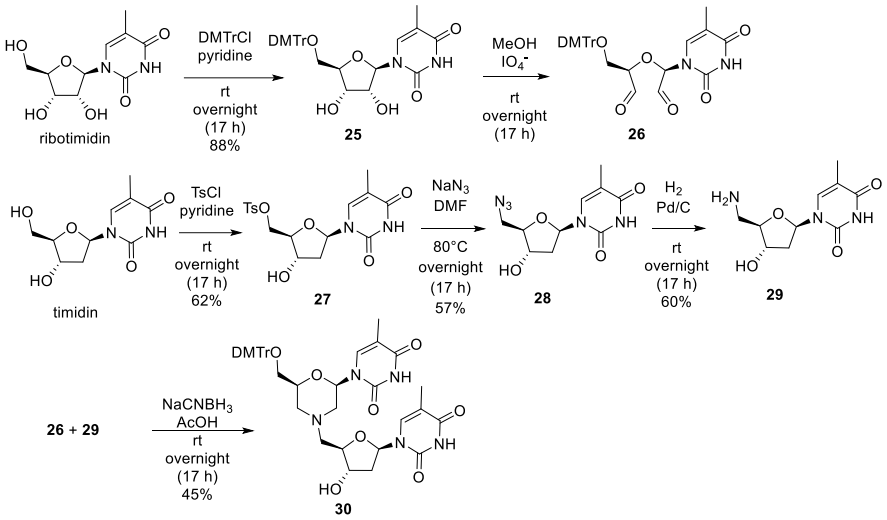
Scheme 2: The structure of morpholino-nucleoside dimers

Starting from reactants **2b** and **23**, we developed an efficient, so-called one-pot method for the preparation of the free derivative without isolating the protected product (Scheme 3). After the reductive amination step, the reaction mixture was evaporated and the protective groups were removed without purification. Thus, the expected dimer **24** was isolated with a total yield of 51%.



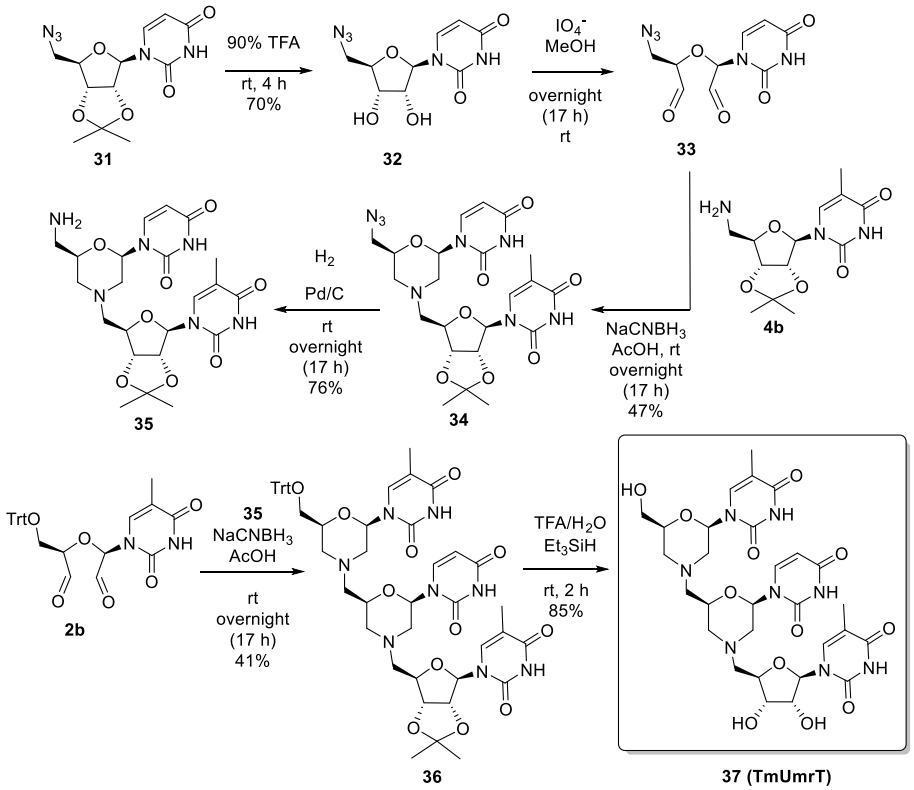
Scheme 3: One-pot reaction

We have prepared a nucleosyl-morpholino dinucleotide derivative (**30**) suitable for incorporation into DNA oligonucleotides using a solid phase automated method. To achieve this goal, the trityl protecting group was replaced by dimethoxytrityl (DMTr) and the ribonucleoside by 2'-deoxynucleoside (Scheme 4). The DMTr-protected nucleoside **25** was oxidized to dialdehyde **26** and subjected to reductive amination-cyclization with the 5'-amino derivative **29** obtained from thymidine, resulting in the dinucleotide target compound **30**.



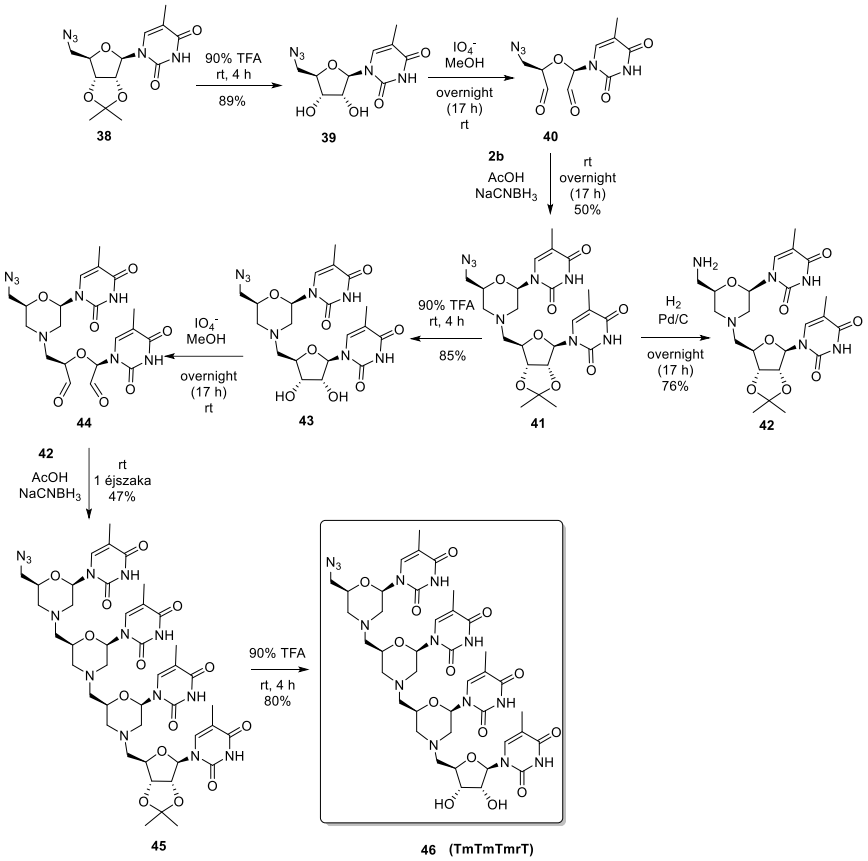
Scheme 4: Synthesis of 2'-Deoxy dimer for solid phase oligonucleotide synthesis

We extended our method to the synthesis of a trinucleotide analogue. First, the dinucleotide derivative **34** was prepared by double reductive amination of **33** with 5′-azidodialdehyde **4b** (Scheme 5). The azido function of compound **34** was converted to an amine by catalytic hydrogenation. The reductive amination cyclization reaction of the dimer amino building block **35** and dialdehyde **2b** resulted in trinucleotide **36** in good yield. By removing the protecting groups of **36** in one step, we obtained the free derivative **37** (TmUmrT).



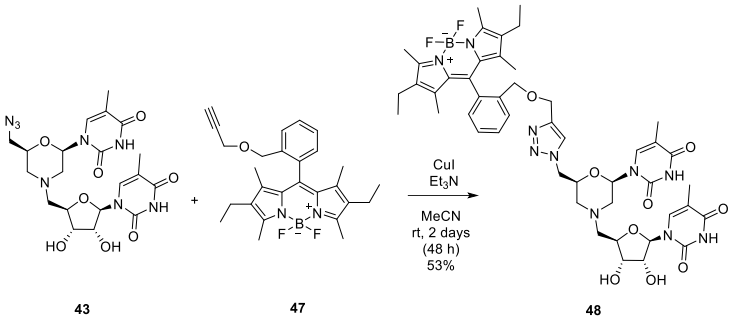
Scheme 5: Synthesis of nucleoside morpholino trimer

To further study the efficiency of the process for the production of higher oligomers, we successfully prepared a tetramer by reductive aminocyclization of a dimer amine (**42**) and a dimer dialdehyde (**44**) (Scheme 6).



Scheme 6: Synthesis of 7'-azido functionalized tetramer

To study the cell penetration ability of the morpholino-nucleoside chimeras, a fluorescent conjugate (**48**) was prepared by the Cu(I)-iodide-catalyzed cycloaddition reaction of the 7'-azido dimer (**43**) and BODIPY (**47**) functionalized with a propargyl group (Scheme 7).

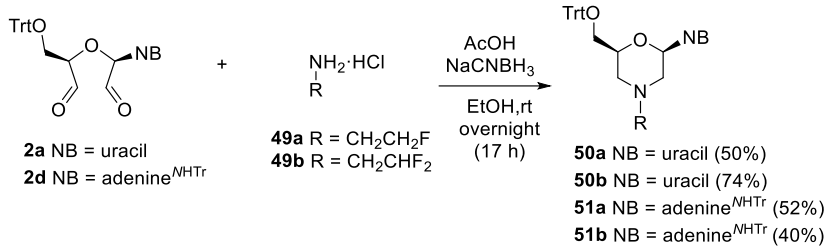


Scheme 7: Reaction of 5'-azido dimer with functionalized BODIPY

Fluorescence microscopy of the immortalized intestinal epithelial cell line Caco-2 (colorectal adenocarcinoma) revealed that the BODIPY-nucleotide dimer conjugate (**48**) entered the cytoplasm, but not the nucleus. The nuclei are stained blue in the microscopic images, and the BODIPY conjugate stains the cytoplasm green.

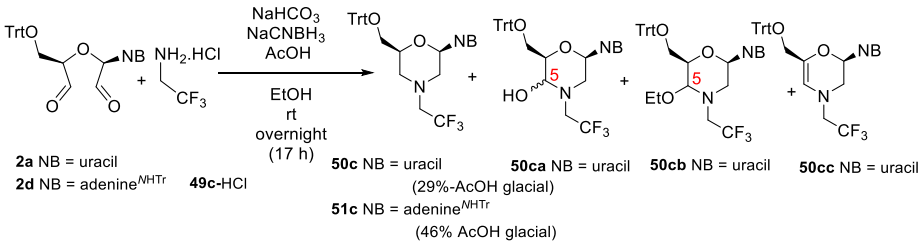
3.2 N-fluoroalkylated morpholinos - a new type of nucleoside analogues

We were the first to produce fluorine-containing morpholino derivatives in the reductive cyclization reaction of fluorinated ethyl and propylamines and nucleoside dialdehydes. During the syntheses, 5'-*O*-Trt-uridine- (**2a**) and 5'-*O*-Trt-*N*-Trt-adenosine dialdehydes (**2d**) were reacted with commercially available 2-fluoroethyl- (**49a**), 2,2-difluoroethyl- (**49b**), 2,2,2-trifluoroethyl (**49c**) and 3,3,3-trifluoropropyl (**49d**) amines. Using mono- and difluoroethylamines, the reactions took place with moderate to good yields (Scheme 8).



Scheme 8: Preparation of *N*-fluoroalkylated morpholinos by reductive amination cyclization reaction

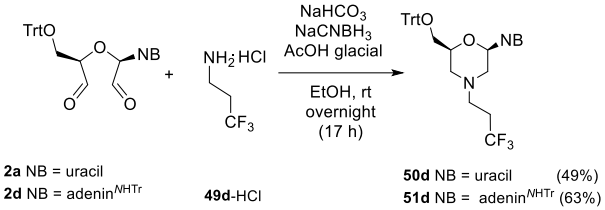
During the reactions with 2,2,2-trifluoroethylamine (**49c**-HCl) using the literature protocol, the expected product was not formed (Scheme 9), instead ethoxy- (**50cb**) and hydroxyl group-containing (**50ca**) derivatives, and elimination products (a.g. **50cc**) were isolated.



Scheme 9: Reactions of protected dialdehydes and trifluoroethylamine

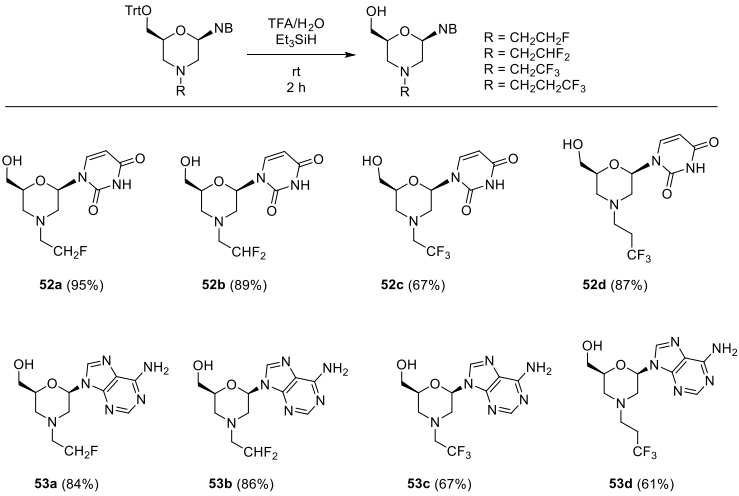
In order to prepare **50c**, we performed optimization reactions. Using the $NaCNBH_3$/ glacial acetic acid combination, we isolated **50c** with a 29% yield, but the $NaCNBH_3/ZnCl_2$ combination gave the highest yield (71%). In the case of adenosine, in the presence of glacial acetic acid, the expected product (**51c**) was isolated with a yield of 46% (Scheme 9).

In the case of 3,3,3-trifluoropropylamine (**49d**-HCl), the expected products (**50d**, **51d**) were isolated from both dialdehydes in good yield using glacial acetic acid (Scheme 10).



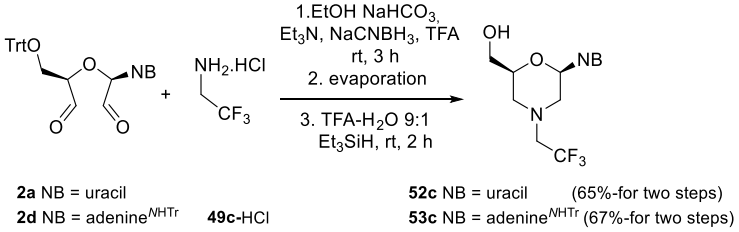
Scheme 10: Synthesis of morpholinos containing 3,3,3-trifluoropropylamine

With the use of 90% TFA and Et_3SiH reducing agent, the 8 fluorine-containing morpholino was produced in free form (**52a-d, 53a-d**) with excellent yield (Scheme 11).



Scheme 11: Structure of the free *N*-alkylated morpholinos

The one-pot synthesis developed during the synthesis of nucleosyl-morpholino hybrids was also used in the case of 2,2,2-trifluoroethylamine hydrochloride (**49c**-HCl), which produced excellent yields with both uridine and adenosine dialdehydes (Scheme 12).

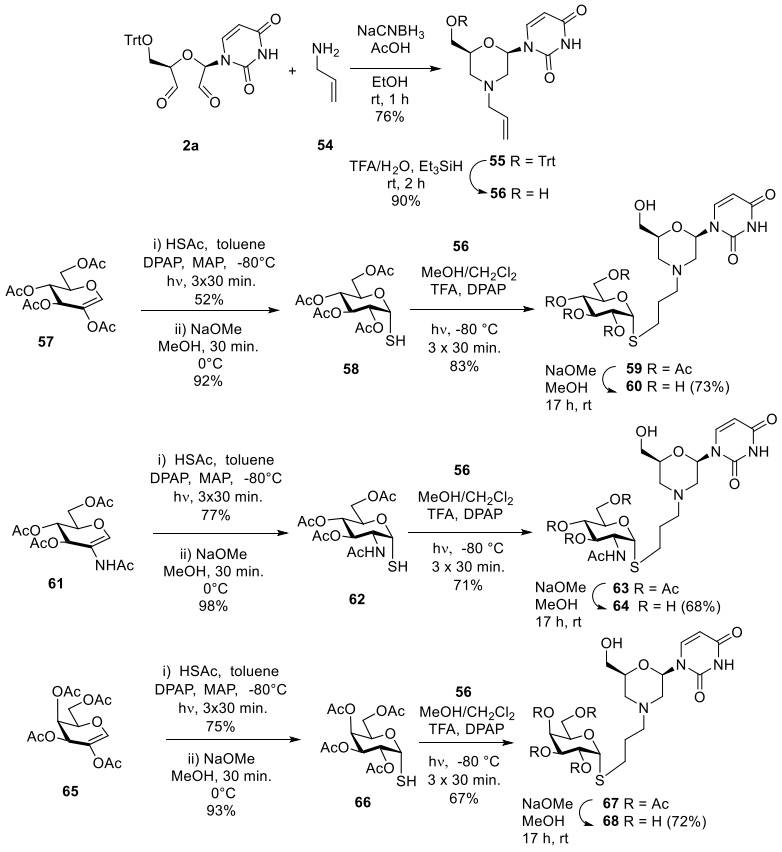


Scheme 12: One-pot reactions

3.3 Synthesis of potential glycosyltransferase inhibitors by thio-click reactions

Potential donor-substrate analogue glycosyltransferase inhibitors were produced, in which the nucleoside unit was replaced by a morpholino and the phosphate ester bond was replaced by a thioether linker, in which the anomeric carbon atom of the sulfur and the carbohydrate part are connected to each other by an α-glycosidic bond, as in natural donor substrates. In order to prepare the corresponding α-glycosidic UDP-sugar analogues, α-glycosylthiols **58**, **62** and **66** were prepared by photoinitiated addition of HSAc to the corresponding 2-substituted glycals followed by selective S-deacetylation (Scheme 13). Using a synergistic combination of 4-methoxyacetophenone (MAP) and 2,2-dimethoxy-2-phenylacetophenone (DPAP) as an initiator, thioacetic acid additions took place in good yield. After selective *S*-deacetylation, the obtained glycosyl-α-1-

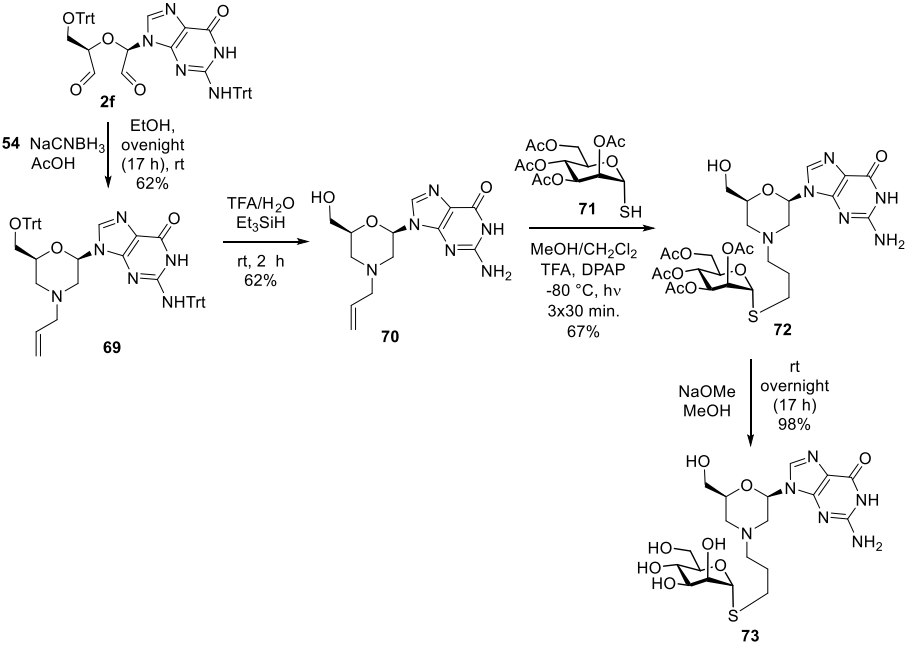
thiols were subjected to a thiol-ene coupling reaction with uracil-*N*-allyl-morpholino (**56**), which was obtained by ring-closing reductive amination reaction of uridine dialdehyde (**2a**) and allylamine (**54**) followed by detritylation. The photoinitiated thiol addition reactions under optimized conditions (in the presence of TFA and DPAP, at -80 °C for 3x30 min irradiation with UV light, λmax = 365 nm) resulted in the expected morpholino-thiosugar conjugates (**59**, **63** and **67**). After Zemplén deacetylation, the new UDP-sugar analogues **60**, **64** and **68** were obtained in good overall yield.



Scheme 13: Synthesis of UDP-sugar analogues

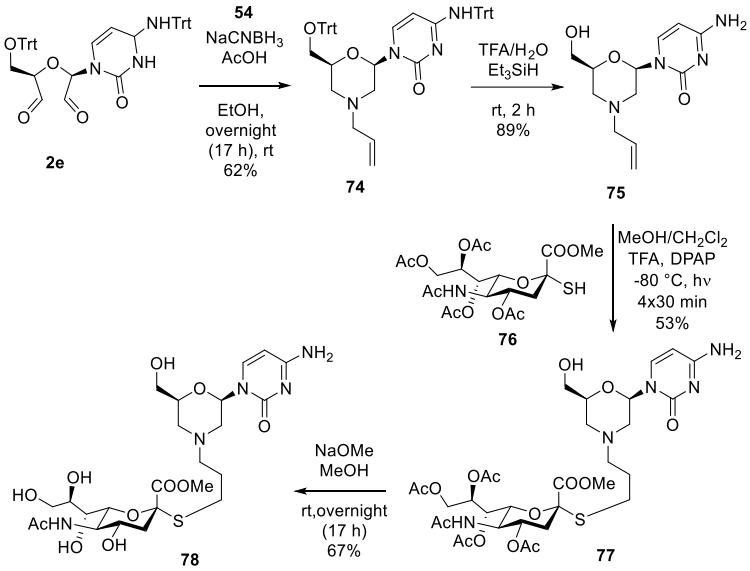
The thiol-ene method was extended to the synthesis of morpholine ring mimetics of guanosine-diphosphate-mannose and cytosine-monophosphate sialic acid. Following the previously established synthetic route, *N*-allyl guanine-morpholino (**70**) was prepared from guanosine secodialdehyde **2f** in two steps, including double reductive amination with allylamine (**54**) and detritylation of the resulting **69**. The thiol addition to **70** took place effectively

with the α-1-thiomannose derivative (**71**) resulting in the guanine-morpholino-thiomannoside conjugate **72** with a yield of 67%, from which the free GDP-mannose analogue was prepared by deacetylation under Zemplén conditions (**73**) (Scheme 14).



Scheme 14: Synthesis of GDP-sugar analogue

For the synthesis of the CMP-sialic acid mimetic, the corresponding cytosine *N*-allyl morpholino (**75**) was prepared from cytidine-derived dialdehyde (**2e**) and allylamine **54**. In the thiol addition step, **75** was reacted with α-2-thiosialic acid 76 at -80 °C under UV irradiation to produce the expected morpholino-type thiosialoside (**77**), from which, after Zemplén deacetylation, the free CMP - sialic acid analogue (**78**) was obtained (Scheme 15).



Scheme 14: Synthesis of CMP-sugar analogue

4. Possible application of the results

- In the future, we intend to incorporate the nucleoside chimeras we have produced into RNA and DNA oligomers, and investigate their hybridization capabilities with complementary strands.
- Nucleoside analogues reported in the literature can be proven to have antiviral, antiparasitic and antitumor effects, so in the future we intend to investigate the antiviral, antimalarial and cytotoxic effects of the *N*-fluoralkylated morpholinos we have produced.
- We would like to expand the range of potential GT inhibitors produced so far, and we would also like to investigate their glycosidase inhibitory effect against Leloir glycosyltransferases within the framework of cooperation.

5. List of publications

5.1 Publications in the subject of the Ph.D. thesis

1. **N. Debreczeni**, M. Bege, M. Herczeg, I. Bereczki, Gy. Batta, P. Herczegh, A. Borbás; *Tightly linked morpholino-nucleoside chimeras: new, compact cationic oligonucleotide analogues,* Org. Biomol Chem. **2021** (19) 8711-8721. *IF: 3.89*
2. **N. Debreczeni**, M. Bege, A. Borbás; *Synthesis of Potential Glycosyl Transferase Inhibitors by Thio-Click Reactions,* Eur. J. Org. Chem. **2021** (48) 6743-6747. *IF: 3.261*

5.2 Publications in other subjects

1. V. Kelemen, M. Bege, D. Eszenyi, **N. Debreczeni**, A. Bényei, T. Stürzer, P. Herczegh, A. Borbás; *Stereoselective Thioconjugation by Photoinduced Thiol-ene Coupling Reactions of Hexo- and Pentopyranosyl D-and L-Glycals at Low Temperature-Reactivity and Stereoselectivity Study,* Chem. Eur. J. **2019** (25) 14555-14571. *IF: 4.857*
2. M. Csávás, D. Eszenyi, E. Mező, L. Lázár, **N. Debreczeni**, M. Tóth, L. Somsák, A. Borbás; *Stereoselective Synthesis of Carbon-Sulfur-Bridged Glycomimetics by Photoinitiated Thiol-Ene Coupling Reactions;* Int. J. Mol. Sci. **2020** (21), 573-600. *IF: 5.923*
3. J. József, **N. Debreczeni**, D. Eszenyi, A. Borbás, L. Juhász, L. Somsák; *Synthesis and photoinitiated thiol-ene reactions of exo-mannals - a new*

route to C-β-D-mannosyl derivatives; RSC Adv. **2020** (57) 34825-34836. *IF: 3.361*

4. S. T. Le, D. Páll, E.Rőth, T. Tran, **N. Debreczeni**, M. Bege, I. Bereczki, E. Ostorházi, M. Milánkovits, P. Herczegh, A. Borbás, M. Csávás; *The Very First Modification of Pleuromutilin and Lefamulin by Photoinitiated Radical Addition Reactions-Synthesis and Antibacterial Studies,* Pharmaceutics **2021** (13) 2028-2049. *IF: 6.525*

5.3 Posters

1. **N. Debreczeni**, A. Borbás; *Synthesis of nucleoside conjugates by photoinduced thio-click reactions,* 1st International Conference on Integrative Chemistry, Biology and Translational Medicine, 25-26 February **2019**, New Delhi, India
2. József J., **Debreczeni N.,** Eszenyi D., Juhász L., Borbás A., Somsák L.; *Exo-mannálok fotoiniciált tiol-én reakciója;* I. Fiatal Kémikusok Fóruma Szimpózium, **2019** 04. 3-5. Debrecen, Magyarország
3. **Debreczeni N.**, Bege M., Buzás L., Herczegh P., Borbás A.; *Ikernukleozidok és nukleozid-dipeptidek szintézise,* MKE Vegyészkonferencia, **2019**. 06. 24-26. Eger, Magyarország
4. József J., **Debreczeni N.**, Eszenyi D., Juhász L., Borbás A., Somsák L.; *Exo-mannál származékok szintézise és fényiniciált tioladdícióinak vizsgálata,* MKE Vegyészkonferencia, **2019**. 06. 24-26. Eger, Magyarország

5. L. Juhász, J. József, **N. Debreczeni**, D. Eszenyi, A. Borbás, L. Somsák; *Synthesis and photoinitiated thiol-ene reaction of exo-mannal derivatives.* 20th EUROCARB, 30 June-04 July, **2019**, Leiden, Netherlands
6. M. Bege, **N. Debreczeni**, P. Herczegh, A. Borbás; *Synthesis of twin-nucleosides,* Congressus Pharmaceuticus Hungaricus XVI, 23-25 April, **2020**, Debrecen, Hungary

5.4 Lectures

1. **Debreczeni N.**, Borbás A.; *Glükozil- és N-acetil-glükózaminil- transzferáz inhibitorok szintézise tioladdíciós reakcióval*, XLI. Kémiai Előadói Napok, **2018**. 10. 15-17. Szeged, Magyarország
2. **Debreczeni N.**, Bege M., Buzás L., Herczegh P., Borbás A.; *Új típusú nukleozid-dimer vegyületek előállítása biológiai hatásvizsgálatokhoz,* I. Fiatal Kémikusok Fóruma Szimpózium, **2019**. 04. 03-05. Debrecen, Magyarország
3. **N. Debreczeni**, M. Bege, L. Buzás, P. Herczegh, A. Borbás; *Synthesis of New Types of Nucleoside Dimer Compounds,* International Workshop on Chemistry and Chemical Biology of Carbohydrates, Nucleic Acids and Antibiotics, 22-24 May, **2019** Mátrafüred, Hungary
4. J. József, **N. Debreczeni**, D. Eszenyi, L. Juhász, A. Borbás, L. Somsák; *Exo-mannal Derivatives as Substrates of Thiol-ene Reactions,* International Workshop on Chemistry and Chemical Biology of Carbohydrates, Nucleic Acids and Antibiotics, 22-24 May, **2019** Mátrafüred, Hungary

5. V. Kelemen, M. Bege, D. Eszenyi, **N. Debreczeni**, A. Bényei, P. Herczegh, A. Borbás; *Photoinduced Thiol-ene Coupling reactions of Hexo- and Pentopyranosyl D- and L-Glycals at Low Temperature*, International Workshop on Chemistry and Chemical Biology of Carbohydrates, Nucleic Acids and Antibiotics, 22-24 May, **2019** Mátrafüred, Hungary
6. L. Juhász, J. József, **N. Debreczeni**, D. Eszenyi, A. Borbás, L. Somsák; *Synthesis and photoinitiated thiol-ene reaction of exo-mannal derivatives.* 20th EUROCARB, 30 June-04 July, **2019**, Leiden, Netherlands
7. Kelemen V., Bege M., Eszenyi D., **Debreczeni N.,** Herczegh P., Borbás A.; *Fotoiniciált tiol-én addíciós reakciók telítetlen mono- és diszacharidokon*, XLII. Kémiai Előadói Napok, **2019**.10.28-30. Szeged, Magyarország
8. M. Bege, **N. Debreczeni**, P. Herczegh, A. Borbás; *Synthesis of twin-nucleosides;* Congressus Pharmaceuticus Hungaricus XVI; 23-25 April, **2020** Debrecen, Hungary
9. **N. Debreczeni**, A. Borbás*; Synthesis of arylalkylamine-nucleoside conjugates by double reductive amination cyclisation reactions*; UD in house selection for the 3 Minute Thesis competition at the Neuroinnovation Summit. 12 May, **2021**, Debrecen, Hungary
10. **Debreczeni N.**, Bege M., Bereczki I., Herczeg M., Batta Gy., Herczegh P., Borbás A.; Kationos kiméra oligonukleotidok szintézise, MTA Szénhidrát, Nukleinsav és Antibiotikukémiai Munkabizottság és szakmai előadónap, **2021**.06.14. Debrecen, Magyarország

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Subject: PhD Publication List

Candidate: Nóra Hudákné Debreczeni
Doctoral School: Doctoral School of Chemistry
MTMT ID: 10067947

List of publications related to the dissertation

Foreign language scientific articles in international journals (2)

1. **Debreczeni, N.**, Bege, M., Borbás, A.: Synthesis of Potential Glycosyl Transferase Inhibitors by Thio-Click Reactions.
   Eur. J. Org. Chem. 2021 (48), 6743-6747, 2021. ISSN: 1434-193X.
   DOI: http://dx.doi.org/10.1002/ejoc.202101220
   IF: 3.261
2. **Debreczeni, N.**, Bege, M., Herczeg, M., Bereczki, I., Batta, G., Herczegh, P., Borbás, A.: Tightly linked morpholino-nucleoside chimeras: new, compact cationic oligonucleotide analogues.
   Org. Biomol. Chem. 19, 8711-8721, 2021. ISSN: 1477-0520.
   DOI: http://dx.doi.org/10.1039/D1OB01174J
   IF: 3.89

List of other publications

Foreign language scientific articles in international journals (4)

3. Le Thai, S., Páll, D., Róth, E., Tran, T., **Debreczeni, N.**, Bege, M., Bereczki, I., Ostorházi, E., Milánkovits, M., Herczegh, P., Borbás, A., Csávás, M.: The Very First Modification of Pleuromutilin and Lefamulin by Photoinitiated Radical Addition Reactions: synthesis and Antibacterial Studies.
   Pharmaceutics. 13 (12), 1-21, 2021. EISSN: 1999-4923.
   DOI: http://dx.doi.org/10.3390/pharmaceutics13122028
   IF: 6.525
4. Csávás, M., Eszenyi, D., Mező, E., Lázár, L., **Debreczeni, N.**, Tóth, M., Somsák, L., Borbás, A.: Stereoselective Synthesis of Carbon-Sulfur-Bridged Glycomimetics by Photoinitiated Thiol-Ene Coupling Reactions.
   Int. J. Mol. Sci. 21 (2), 1-27, 2020. ISSN: 1661-6596.
   DOI: http://dx.doi.org/10.3390/ijms21020573
   IF: 5.923

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Total IF of journals (all publications): 27,817
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