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Carbocyclic analogues of nucleosides containing substituted bicyclic systems

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Ph.D. Thesis Abstract

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Introduction

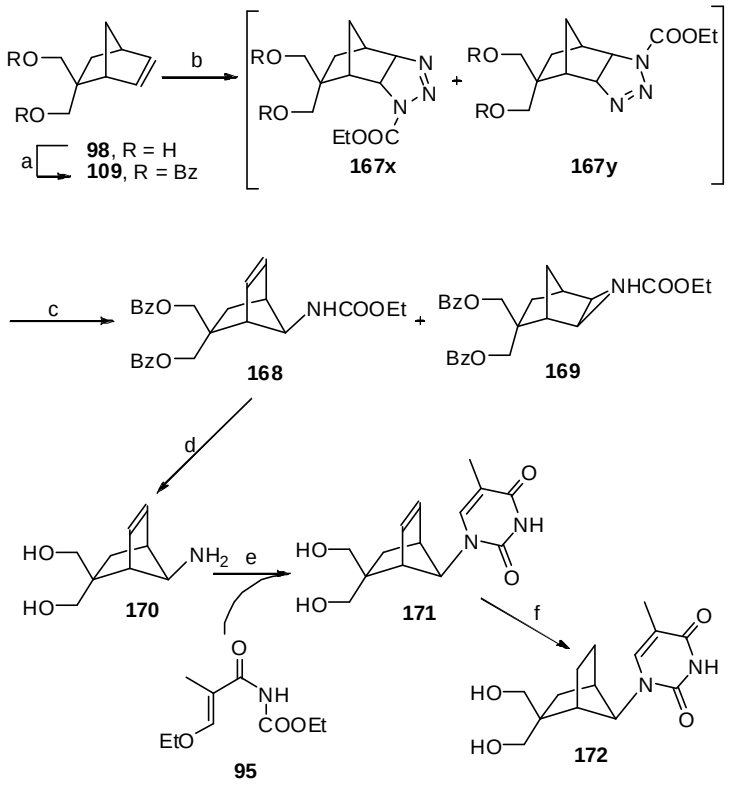
Nucleoside and nucleotide analogues are of fundamental importance for all organisms. Therefore, nucleoside analogues are interesting target for drug discovery and development, mainly as potential antiviral and antitumor agents. A crucial disadvantage of natural nucleosides analogues is cleavage of the N-glycosidic bond by phosphorylases. Modification which increases resistance against enzymatic degradation is substitution of the sugar moiety furanose ring by a hydrocarbon ring. Many of such modified analogues – carbocyclic nucleosides[1] – exhibit interesting antiviral activity. Several analogues containing conformationally locked bicyclic systems were also synthesized. Well known are carbocylic nucleosides with a fused cyclopropane moiety[2] (bicyclo[3.1.0]hexane). Recently, novel conformationally locked carbocyclic nucleosides based on 2-oxabicyclo[2.2.1]heptane ring system were described[3] (as precursors for carbocyclic locked nucleic acids). This thesis concerns the synthesis of biologically active compounds related to the carbocyclic nucleoside analogues. First part of the work is devoted to the synthesis of the conformationally locked carbocyclic nucleosides derived from bicyclo[2.2.1]heptane. These compounds were primarily designed for general biological activity screening. In the course of this work, compounds produced in our laboratory showed interesting antiviral properties against coxsackievirus B3 (Picornaviridae). These very promising findings of the preliminary biological tests led us to start the program targeted on development of novel coxsackievirus B3 inhibitors.

Carbocyclic conformationally locked nucleosides

First part of this chapter attends to a synthesis of novel racemic conformationally locked nucleoside analogues with bicyclo[2.2.1]hept-2-ene or -heptane ring substituted with nucleobase at the position 7 of the bicyclic scaffold (two series, *syn* and *anti* isomers). These bicyclic compounds could be considered as the conformationally locked carbapentofuranose nucleoside analogues.

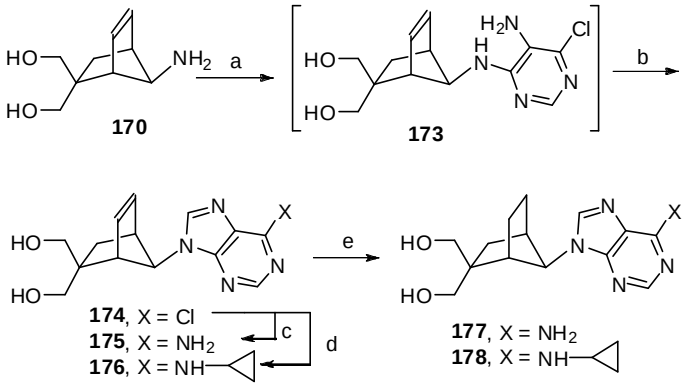
The synthetic strategy was based on construction of the nucleobases at the amino group of an appropriate aminobicycloalkene. The key intermediate in the synthesis of the *syn*-nucleosides was *syn*-amine **170** which was prepared in three simple steps from the commercially available bicyclo[2.2.1]hept-5-ene-2,2-dimethanol (**98**) (Scheme **1**). The dibenzoyl derivative **109** was treated with ethyl azidoformate and intermediate triazoline derivatives **167x** and **167y** were cleaved without isolation with silica gel (Wagner-Meerwein rearrangement). This reaction

afforded a mixture of carbamates **168** and **169** (2:3) which were easily separated by chromatography on a silica gel column. Free amine **170** was obtained in a good yield by deprotection with potassium hydroxide. Thymine nucleoside **171** was prepared in a moderate yield by reaction of amine **170** with carbamate **95** in 1,4-dioxane at 100 °C and following pyrimidine ring closure catalyzed with Dowex 50. Saturated nucleoside **171** was obtained by hydrogenation using palladium(II) hydroxide as a catalyst.



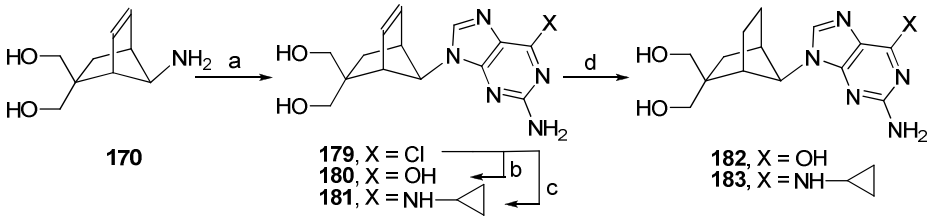
Scheme **1**. a) BzCl, pyridine, r.t., 91%; b) ethyl azidoformate, toluene, 6 h, 80 °C; c) silicagel, CH_2Cl_2, 2 h, 33% for **168**, 47% for **169**; d) KOH, EtOH-H_2O, reflux, 14 h, 83%; e) 1. 1,4-dioxane, 100 °C, 3 h, 2. Dowex 50 (H^+ cycle), 1,4-dioxane, 100 °C, 2.5 h, 50%; f) H_2, $Pd(OH)_2$/C, MeOH-H_2O, 3 d, 71%.

Amine **170** was also treated with 4,6-dichloropyrimidine-5-amine in ethanol in the presence of triethylamine (Scheme **2**) and the purine ring was subsequently closed with triethyl orthoformate in the presence of concentrated hydrochloric acid to give 6-chloropurine derivative **174**. Finally, position 6 of the purine ring was derivatized by ammonolysis (**175**) and nucleophilic substitution of the chlorine atom with cyclopropylamino group (**176**). Both unsaturated nucleosides were hydrogenated to give saturated nucleosides **177** and **178**.



Scheme **2**. a) 4,6-dichlorpyrimidine-5-amine, Et_3N, EtOH, 100 °C, 6 d, 97%; b) 1. $HC(OEt)_3$, HCl, 3 d 2. HCl, THF-H_2O, 4 h, 79%; c) NH_3 (l), 75 °C, 2 d, 95%; d) cyclopropylamine, MeOH, r.t., overnight, 92%; e) H_2, $Pd(OH)_2/C$, MeOH-H_2O, 87% for **177**, 85% for **178**.

Amine **170** was coupled with 4,6-dichloropyrimidine-2,5-diamine in the presence of triethylamine in ethanol, the obtained intermediate was treated with triethyl orthoformate and concentrated hydrochloric acid to give 2-amino-6-chloropurine nucleoside **179** (Scheme **3**). The nucleoside analogue **179** was converted to guanine compound **180** by treatment with trifluoroacetic acid. The reaction of **179** with cyclopropylamine in methanol afforded compound **181**. Saturated derivatives **182** and **183** were prepared by hydrogenation.

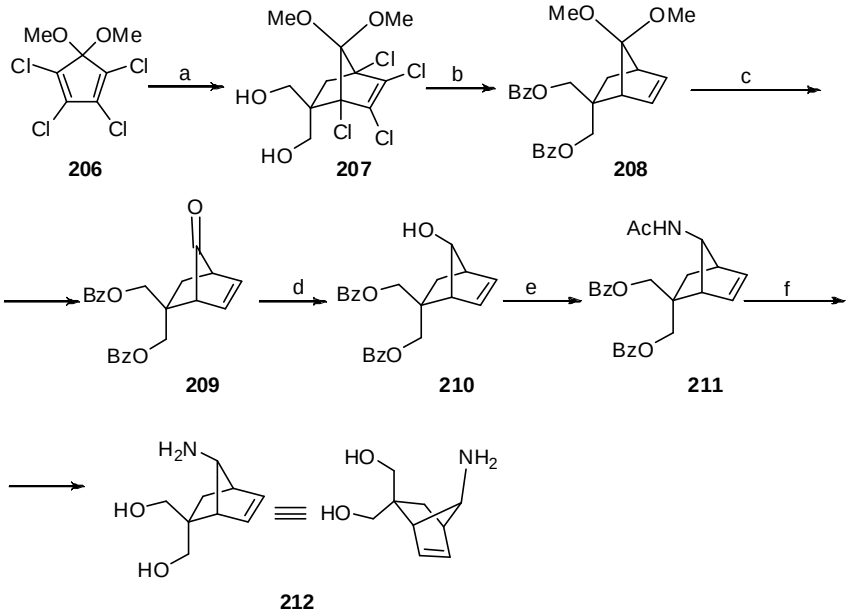


Scheme **3**. a) 1. 4,6-dichlorpyrimidine-2,5-diamine, Et_3N, EtOH, 100 °C, 6 d, 2. $HC(OEt)_3$, HCl, 4 d, 3. HCl, THF-H_2O, 4 h, 79%; b) CF_3COOH, H_2O, 3 d, 94%; c) cyclopropylamine, MeOH, overnight, 94%; d) H_2, $Pd(OH)_2/C$, DMF, 87% for **182**, MeOH-H_2O, 80% for **183**.

Synthesis of other *syn*-nucleosides (without any substitution or bearing only one hydroxymethyl or hydroxy group) was realized in similar way as described above.

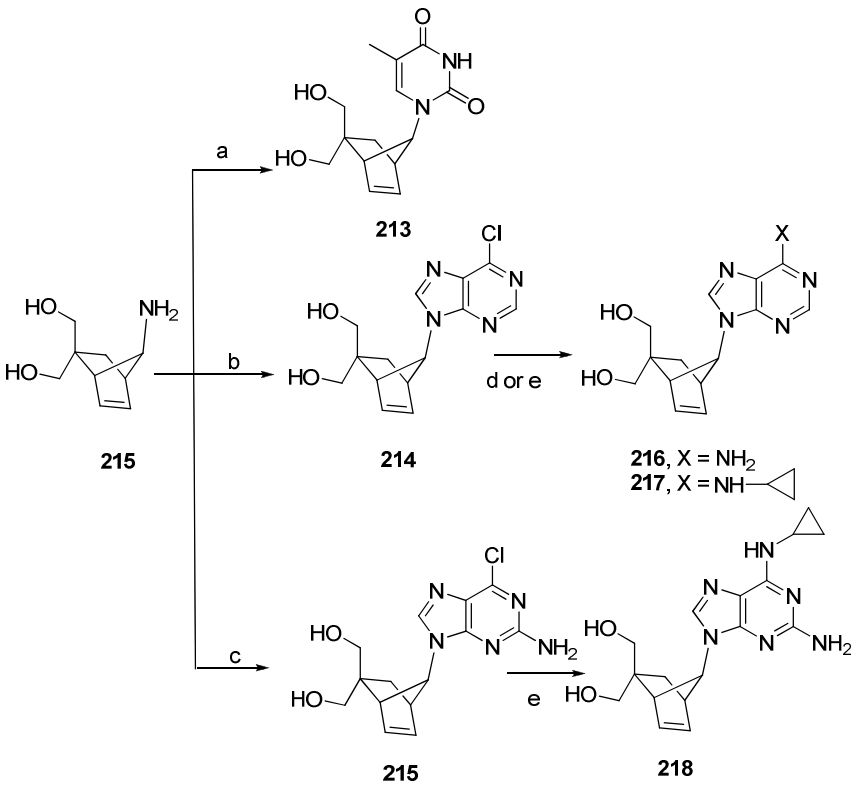
Also, the synthesis of the *anti*-nucleosides was based on the construction of nucleobases at the amino group (Scheme **4**). *Anti*-amine **212** was prepared in six steps, starting from commercially available 1,2,3,4-tetrachloro-5,5-dimethoxycyclopentadiene **206**. The chlorocyclopentadiene **206** was treated with acrolein, and the Diels-Alder intermediate - formyl derivative - was reacted with formaldehyde under basic conditions (NaOH) to yield the dihydroxymethyl derivative **207**. Chlorine atoms were removed with sodium in liquid

ammonia, and the free hydroxy groups were immediately protected by benzoylation. Ketone **209** was then prepared by deketalization (Dowex 50, H^+ cycle) in refluxing dioxane-water mixture. Reduction of the keto group was achieved by reaction with sodium borohydride in mixture of tetrahydrofuran and water. Due to the steric hindrance (hydroxymethyl group against double bond) only one isomer (*anti*) was obtained in very good yield. Finally, protected amino group was introduced by Ritter reaction with retention of the configuration. The free amine **212** was finally released by basic deprotection (KOH).

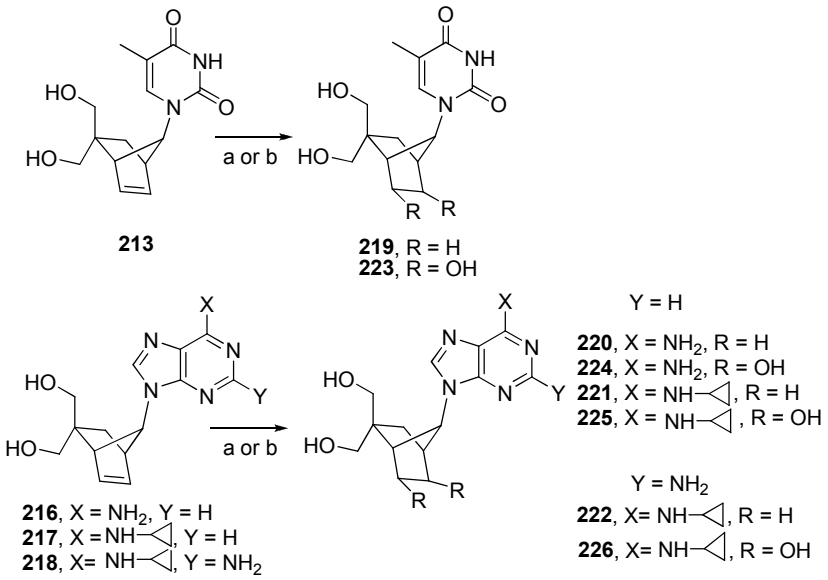


Scheme **4**. a) 1. acrolein, 55 °C, 8 h, 2. aq. HCHO, NaOH, 55 °C, 4 h, 3. $NaBH_4$, MeOH, overnight, 82%; b) 1. Na, NH_3 (l), THF-EtOH, -45 °C, 2. BzCl, pyridine, overnight, 78 %; c) Dowex 50 (H^+ cycle), 1,4-dioxane-water, reflux, 10 h, 75%; d) $NaBH_4$, THF- H_2O, 0 °C, 30 min, 88%; e) CH_3CN, H_2SO_4-AcOH, r.t., 1 h, 84%; f) KOH, EtOH-H_2O, reflux, 9 h, 90%.

Amine **212** was then used for the construction of target compounds (Scheme **5**) by the same procedures as in the case of the *syn*-nucleosides. The double bond in each of the nucleosides **213**, **216**, **217**, **218** was utilized in two ways (Scheme **6**). Saturated compounds **219**, **220**, **221**, **222** were obtained after hydrogenation over palladium hydroxide on charcoal. The compounds **223**, **224**, **225**, and **226** with two *cis*-hydroxy groups were prepared by osmium tetroxide catalyzed *cis*-hydroxylation in an acetone-water mixture with *N*-methylmorpholine-*N*-oxide (NMMO) as a recovering agent for osmium catalytic cycle.



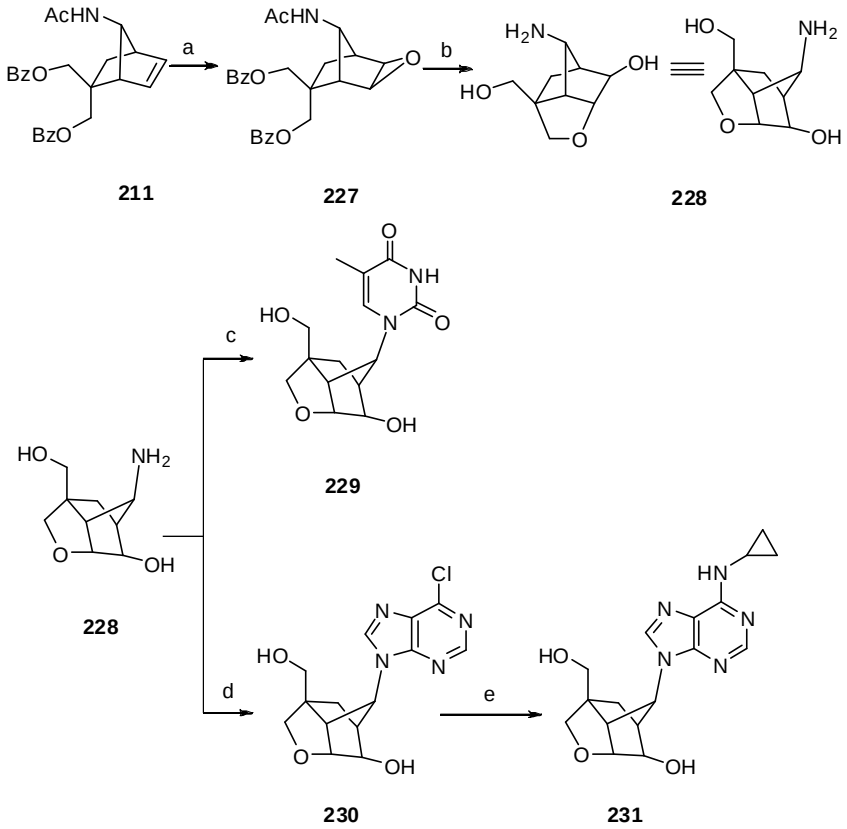
Scheme **5**. a) 1. **95**, 1,4-dioxane, 100 °C, 3 h, 2. Dowex 50 (H^+ cycle), 1,4-dioxane, 100 °C, 2.5 h, 55%; b) 1. 4,6-dichlorpyrimidine-5-amine, Et_3N, EtOH, 100 °C, 6 d, 2. $HC(OEt)_3$, HCl, 2 d, 3. HCl, THF-H_2O, 3 h, 63%; c) 1. 4,6-dichlorpyrimidine-2,5-diamine, Et_3N,EtOH, 100 °C, 6 d, 2. $HC(OEt)_3$, HCl, 5 d 3. HCl, THF-H_2O, 4 h, 67%; d) NH_3 (l), 75 °C, 2 d, 91%; e) cyclopropylamine, MeOH, overnight, 92% for **217**, 80% for **218**.



Scheme **6**. a) H_2, $Pd(OH)_2$/C, MeOH-H_2O; b) OsO_4, NMMO, acetone-H_2O.

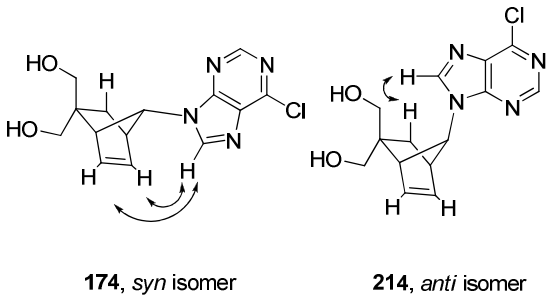
Compounds with a tricyclic skeleton were synthesized starting from the protected amide **211** (Scheme **7**). This compound was treated with *m*-chloroperbenzoic acid in dichloromethane

and the epoxide **227** afforded the amine **228** by means of intramolecular oxirane ring opening under mild basic conditions (deprotection of the benzoyl groups by potassium carbonate). After that, acetyl protecting group was removed by potassium hydroxide. Nucleosides **229**, **230**, 2**31** were prepared starting from amine **228**, using the same procedures as described above.



Scheme **7**. a) *m*CPBA, CH_2Cl_2, overnight, quant.; b) 1. K_2CO_3, MeOH, 2. KOH, EtOH-H_2O, reflux, 9 h, 85%; c) 1. **95**, 1,4-dioxane, 100 °C, 3h, 2. Dowex 50 (H^+ cycle), 1,4-dioxane, 100 °C, 2.5 h, 56%; d) 1. 4,6-dichlorpyrimidine-5-amine, Et_3N, EtOH, 100 °C, 6 d, 2. $HC(OEt)_3$, HCl, 2 d, 2. HCl, THF-H_2O, 3 h, 30%; e) cyclopropylamine, MeOH, overnight, 92%.

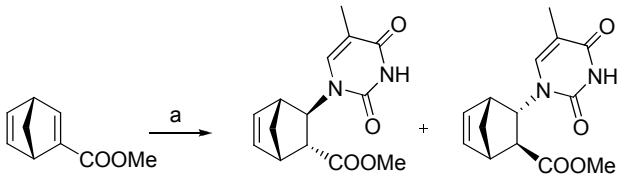
The relative configuration (*syn*/*anti*) at the position 7 was confirmed by NOE experiment (compounds **174** and **214**). For **174**, a correlation was found between proton H-8´ (8.47 ppm) and protons H-5 (6.02 ppm) and H-6 (6.07 ppm) indicating *syn*-position of the nucleobase, while for **214**, interaction between proton H-8´ (8.76 ppm) and proton H-3*exo* (1.85 ppm) indicating *anti*-position of the nucleobase was observed (Fig. **1**).



174, *syn* isomer **214**, *anti* isomer

Fig. **1**. Important NOE interactions in compounds **174** and **214**.

Second part of the carbocyclic nucleoside project concerns the synthesis of the compounds with vicinal position of the nucleobase and hydroxymethyl group. Synthetic strategy was originally based on Michael addition of the nucleobase to the activated double bond. Addition of thymine to ester **232** in the presence of the base (DBU, K_2CO_3) gave moderate yields of isomers **233a** and **233b** (Scheme **8**). Only isomers with *trans* configuration were observed in the reaction mixture and these were easily separated by column chromatography. The ratio of the *exo*-**233a** and *endo*-**233b** depended on the used base. In the case of DBU the ratio **233a**/**233b** was 3.5:1, whereas when potassium carbonate was used, the ratio was almost completely inversed - 1:2.5. However, this conjugate addition is not suitable for preparing purine nucleoside derivatives, yields of these additions were low and complicated reaction mixtures were obtained.

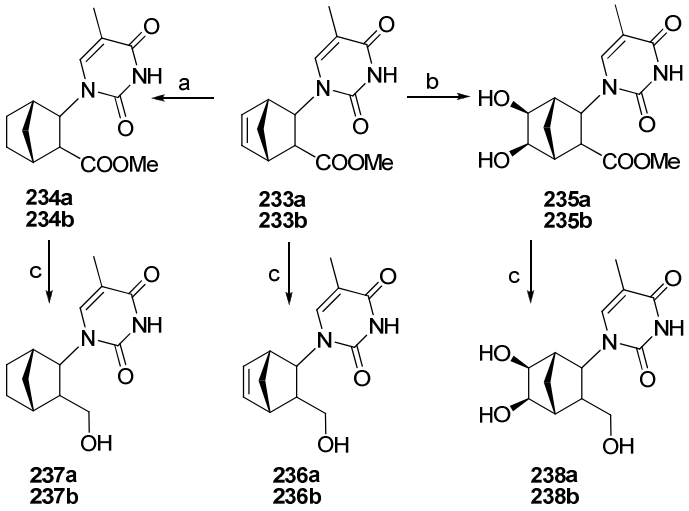


232 **233a** **233b**

Scheme **8**. a) Method I: thymine, DBU, DMF, 75 °C, 72 h, 35% for **233a**, 10% for **233b**; a) Method II: thymine, K_2CO_3, DMF, 75 °C, 72 h, 16% for **233a**, 40% for **233b.**

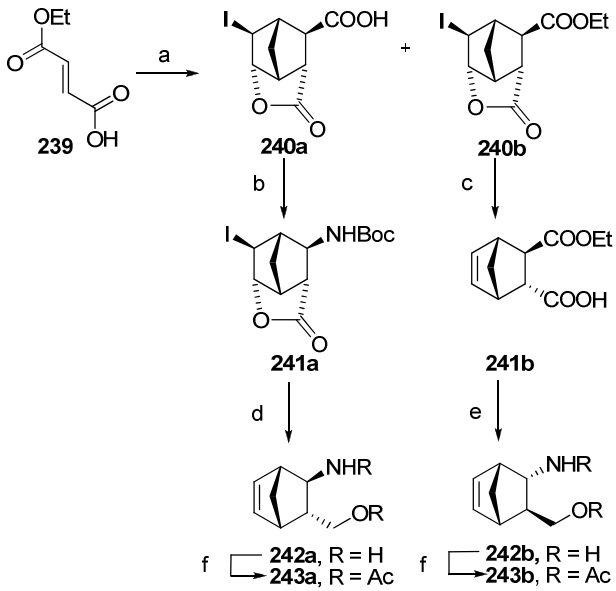
Target thymine nucleosides were prepared by standard reaction procedures (Scheme **9**). Ester group on unsaturated esters **233a** and **233b** was reduced with lithium aluminium hydride in tetrahydrofurane to afford hydroxymethyl group (**236a** and **236b**). Saturated nucleosides **237a** and **237b** were prepared in two simple steps: the double bond was hydrogenated on palladium hydroxide and then the ester group was reduced to hydroxymethyl group with lithium aluminium hydride under the same conditions as for unsaturated compounds giving **237a** and **237b**. Compounds **238a** and **238b** with two *cis*-hydroxy groups at the skeleton were prepared

by osmium tetroxide catalyzed *cis*-hydroxylation followed by lithium aluminium hydride reduction.



Scheme **9**. a) H_2, $Pd(OH)_2/C$, MeOH-H_2O, 86% for **234a**, 84% for **234b;** b) OsO_4, NMMO, acetone-H_2O, 98% for **235a**, 91% for **235b**; c) $LiAlH_4$, THF, r.t., 54% for **236a**, 69% for **236b** 55% for **237a**, 70% for **237b**, 39% for **238a**, 58% for **238b**.

When the Michael addition strategy failed, it was necessary to find a new route leading to the purine nucleosides. Curtius rearrangement was chosen for the introduction of the amino function (Scheme **10**). The synthesis started with reaction of cyclopentadiene and fumaric acid monoethyl ester **239**. Diels-Alder adducts were treated with iodine and sodium bicarbonate in the presence of potassium iodide in order to obtain to products - iodoacid **240a** and iodoester **240b**. This step allowed the separation of these crucial intermediates by simple acid/base extraction. Both individuals were utilized for preparation of the isomeric aminoalcohols **242a** and **242b** in four steps. Iodoacid **240a** was firstly converted to Boc-protected amine **241a** by Curtius rearrangement. Double bond was restored with zinc in refluxing ethanol-water solution. Protected amino acid was immediately deprotected under acidic conditions and carboxylic acid was subsequently reduced with lithium aluminium hydride in THF to the aminoalcohol **242a**. Synthesis of the aminoalcohol **242b** was realized in a similar way, only the reaction sequence was different. Both amines were used crude for the next step and were fully characterized as acetyl derivatives **243a** and **243b**. The aminoalcohols **242a** and **242b** were used to build the target nucleoside analogues using standard and described synthetic procedures. Both saturated and *cis*-hydroxylated nucleoside analogues were prepared.



Scheme **10.** a) 1. cyclopentadiene, 1,4-dioxane, 60 °C, 1 h, 2. I_2, KI, Na_2CO_3, H_2O-$CHCl_3$, r.t., overnight, 31% for **240a**, 41% for **240b**; b) 1. ClCOOEt, Et_3N, acetone, 0 °C, 1 h, 2. NaN_3, H_2O-acetone, 0°C, 1 h, 3. toluene, *t*-BuOH, reflux, 4 h, 51%; c) Zn, EtOH, reflux, 1 h, quant.; d) 1. Zn, EtOH, reflux, 2 h, 2. HCl, MeOH, 4 d 3. $LiAlH_4$, THF, reflux, 12 h, 61%; e) 1. ClCOOEt, Et_3N, acetone, 0 °C, 1 h, 2. NaN_3, H_2O-acetone, 0 °C, 1 h, 3. toluene, aq. HCl, reflux, 13 h; 4. $LiAlH_4$, THF, reflux, 12 h, 67%; f) Ac_2O, pyridine, r.t., 80% for **243a**, 92% for **243b**.

Unfortunately, none of the prepared carbocyclic nucleosides showed any significant biological activity.

Inhibitors of coxsackie B3 virus replication

Two main groups of the novel coxsackie B3 virus inhibitors were synthesized. First group contained the derivatives with 6-chloropurines substituted in the position 9 with various bicylic systems. Second group of derivatives resulted from the systematic modification of the purine moiety of the selected lead structure **251b**.

The introduction of the nucleobase during the synthesis of 6-chloropurines (Fig. **2**) was usually accomplished by Mitsunobu reaction. This reaction lead to 9-substituted-6-chloropurines with *exo* orientation of the base to the bicyclic scaffold. Only 9-isomers were obtained in yields ranging from 14% to 78% after usual purification. Three derivatives were prepared by the construction of the nucleobase on appropriate amino precursor (Fig. **3**).

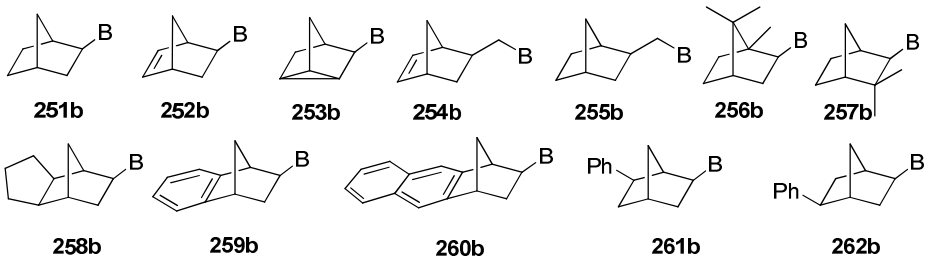


Fig. **2**. Derivatives prepared by Mitsunobu reaction. B = 6-chloropurine-9-yl.

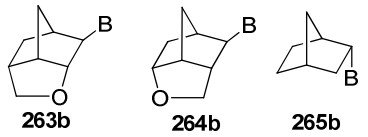


Fig. **3**. Derivatives prepared by nucleobase-construction strategy. B = 6-chloropurine-9-yl.

Compounds bearing various substituents on the norbornane scaffold (e.g. hydroxy, fluorine, ketogroup etc.) were also prepared in order to expand the library of the coxsackievirus inhibitors. Compounds were prepared by linear strategy involving Mitsunobu reaction as a key reaction. For the debenzoylations of protected 6-chloropurines intermediates we improved previously published method.[4] Excess of methylmagnesium chloride in tetrahydrofurane at 0 °C overnight and yields from 35-75% were obtained.

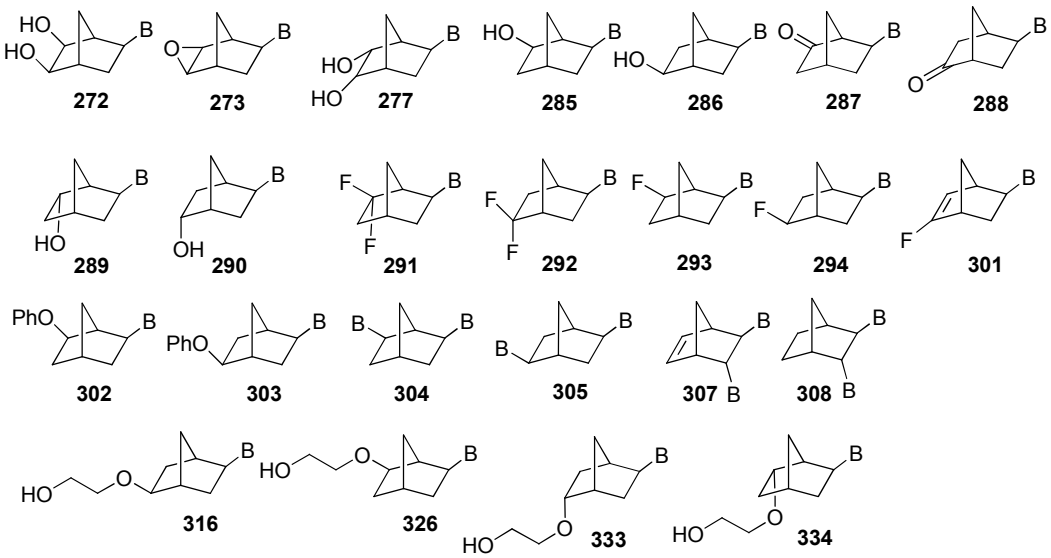


Fig. **4**. Derivatives with variously substituted bicyclic scaffold. B = 6-chloropurine-9-yl.

Second part of the coxsackie B3 virus inhibitors project was focused on the studying of the necessary nucleobase substitution. A small library of compounds was built up using well known synthetic procedures (nucleophilic aromatic substitution, cross-coupling reactions or conversion of the function groups) to modify purine scaffold at the positions 2, 6, 8. Most of

these compounds were prepared by 6-chloropurine derivatization of the lead structure **251b**. This scaffold was chosen for this modifications due to its availability and chemical inactivity of the bicyclic scaffold. Structures of the prepared compounds are shown in the figures **5**, **6** and **7**.

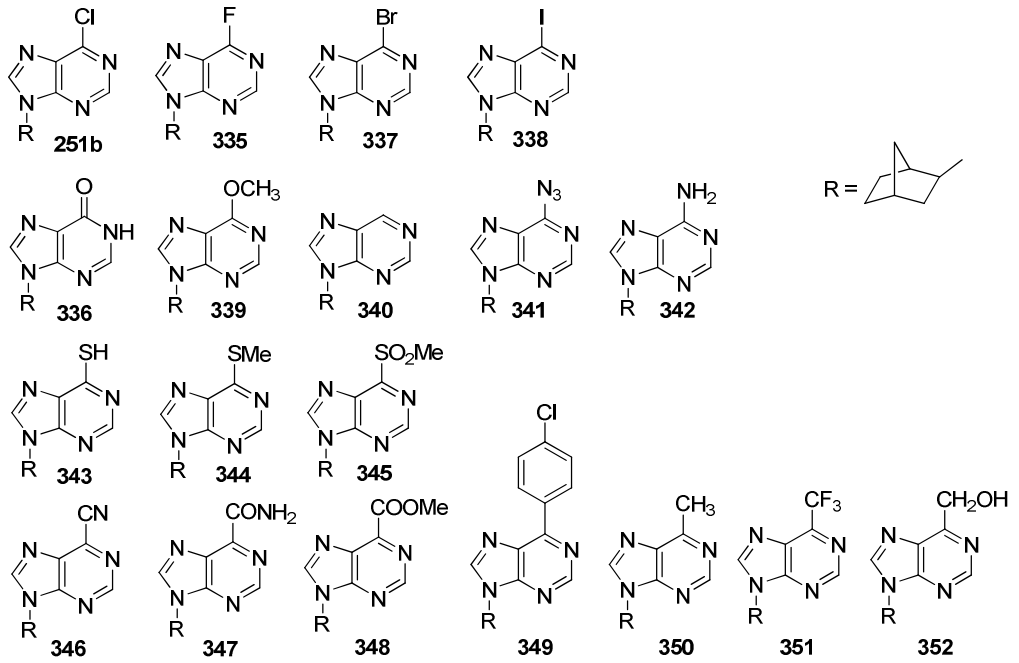


Fig. **5**. Purine modifications, position 6.

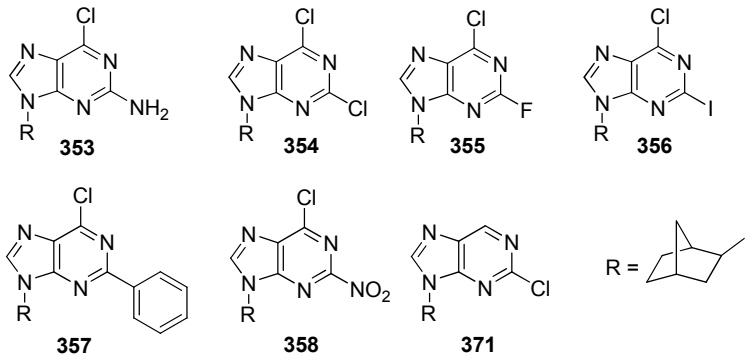


Fig. **6**. Purine modifications, position 2.

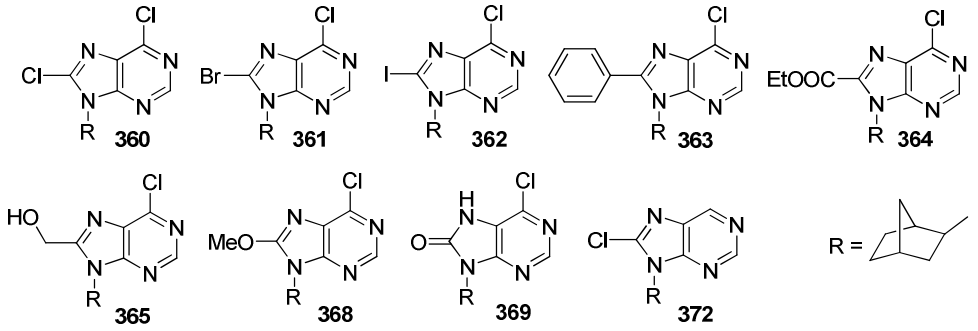


Fig. **7**. Purine modifications, position 8.

The results of the antiviral screening showed some interesting data. Most of the compounds exhibit a significant activity against coxsackie B3 virus with very low level of cytotoxicity to the Vero cells. The presence of the 6-chlorpurine as the nucleobase seems to be essential for the excellent antiviral activity. Most of the modifications in the purine part lead to lower antiviral activity, only compound bearing p-chlorophenyl substituent in position 6 embodied similar value of the EC50 (0.87±0.41 μM) to the parental compound. Several analogues inhibited CVB3 in the low micromolar range (0.66 μM - 2 μM). Some of the compounds showed comparable results or they are slightly better than previously prepared derivatives and are also comparable to the known inhibitor TTP-8307[5]. Compounds **251b** (EC_{50} = 0.81±0.20 μM) and **259b** (EC_{50} = 0.66±0.35 μM) are the best derivatives from this series. Studies to identify the molecular target of these compounds are in progress. Only three compounds from the group of bicyclic modified derivatives showed significant cytotoxicity to the Vero cell line (**261b**, **262b**, **309**), more cytotoxic compounds were found in the group of the purine modified derivatives (**335**, **337**, **354**, **355**, **356**, **358**, **362**).

Conclusion

In the first part of the thesis, three different types of the conformationally locked nucleoside analogues were prepared. In most cases, nucleobase was introduced to the bicyclic scaffold by built up strategy on an appropriate amino function. Three different synthetic pathways were used for preparation of suitable amines. For the 7-*syn* analogues, acid catalyzed decomposition of the intermediate triazolines connected with Wagner-Meervein rearrangement was used. The preparation of the isomeric *anti*-amine required totally different synthetic strategy where the key step was Ritter reaction in order to convert the hydroxy group to the acetamido group in excellent yield and which proceeded with retention of the

configuration to achieve *anti*-configuration. Third used approach for preparation of the amino precursors was Curtius rearrangement of the suitable substituted bicyclic carboxylic acids. This step accompanied with function group transformations (ester reduction) furnished amine-intermediates in good yield. Biological screening of the prepared compounds did not show any considerable activity.

In the second part of the thesis, two large series of the coxsackie B3 virus inhibitors were designed and synthesized. This series uncovered a novel type of the coxsackie B3 virus inhibitors combining norbornane scaffold with 6-chlorpurine base. Two different types of the compounds were synthesized. Firstly, the bicyclic scaffold (OH, F, keto group, saturated, unsaturated scaffold etc.) was modified and as the nucleobase 6-chlorpurine was used. Nucleobases were generally introduced to the scaffold by Mitsunobu reaction in good yields. Second part was devoted to modifications at the purine part of the selected inhibitor (**251b**, easily available, very potent inhibitor), but these modifications did not improved the biological properties. Two most promising compounds **251b** (EC_{50} = 0.81±0.20 μM) and **259b** (EC_{50} = 0.66±0.35 μM) were selected for further investigation.