Bioorganic & Medicinal Chemistry 18 (2010) 4374–4384

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Design, synthesis, and biological evaluation of novel coxsackievirus B3 inhibitors

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ARTICLE INFO

Article history:
Received 18 February 2010
Revised 21 April 2010
Accepted 25 April 2010
Available online 29 April 2010

Keywords:
6-Chloropurine
Coxsackievirus B3
Mitsunobu reaction
Bicyclo[2.2.1]heptane
Fluorination

ABSTRACT

The synthesis and SAR study of a novel class of coxsackievirus B3 (CVB3) inhibitors are reported. These compounds could be considered as the 6-chloropurines substituted at position 9 with variously substituted bicyclic scaffolds (bicyclo[2.2.1]heptane/ene—norbornane or norbornene). The synthesis and biological evaluation of 31 target compounds are described. Several of the analogues inhibited CVB3 in the low micromolar range (0.66–2 μM). Minimal or no cytotoxicity was observed.

1. Introduction

Coxsackieviruses are non-enveloped, single-stranded (+) RNA viruses belonging to the Picornaviridae family.[1] Coxsackieviruses are subdivided into two subgroups—CVA and CVB, based on their pathology to newborn mice. Infections with coxsackieviruses, and in particular with CVB3, are the most common cause of viral myocarditis. Moreover, these viruses are reported to be associated with the development of pancreatitis, meningitis, and encephalitis.[2] Other diseases associated with coxsackievirus infections are hand-foot-and-mouth disease and hemorrhagic conjunctivitis.

There is currently no approved antiviral therapy for the treatment of picornaviral infections in man or animals. Numerous compounds were reported in the past as selective inhibitors of enteroviruses.[3] For example, pleconaril entered clinical trials but despite the promising results, it was not approved by the FDA for the treatment of common cold symptoms in HRV-infected patients.[4] Rupintrivir, another compound tested in the clinical trials,[5] failed in natural infection study in patients.[6] A large series of promising novel compounds based on substituted 5-nitro-5-phenoxybenzonitriles was recently discovered. Most of the compounds examined in the study exerted significant activity against CVB3 comparable with the parental analogue MDL-860 (6.5 ± 4.9 μM),[7] and some of them were also tested against other picornaviruses. The compound 2-(2-chlorophenoxy)-5-nitrobenzonitrile possessed broad-spectrum anti-enterovirus activity including activity against Echo 9 at micromolar level unlike the parental compound MDL-860. Anti-enterovirus activity in micromolar range was also reported for compounds derived from 2,6-dihalophenyl-substituted 1*H*,3*H*-thiazolo[3,4*a*]benzimidazoles.[8]

Recently, we reported a series of carbocyclic nucleoside analogues with antiviral activity against coxsackieviruses. These compounds carry bicycloalkanes,[9] bicycloheteroalkanes[10] or tricycloheteroalkanes[11] instead of the glycone part of the nucleoside molecule (compounds **1**–**6**, Fig. 1). The compounds were primarily designed as the conformationally locked analogues of carbocyclic nucleosides.[9] Some of the analogues also exhibited antiviral activity against HIV-1 and HIV-2 on T-lymphocyte (CEM) cells.[9b,c,11a] Activity against CVB3 was in the micromolar range with minimal or no cytotoxicity in Vero cells (Table 1).

This study involves the synthesis of a second generation of this compound class with bicyclic scaffold (variously substituted bicyclo[2.2.1]heptane) connected with a nucleobase (6-chloropurine) at position 2. Our concept was based on the simplification of the previously reported compounds with activity against CVB3.[9–11] We examined which substitutions on the bicyclic part of the molecule are necessary for antiviral activity.

2. Synthetic chemistry

The first group of target compounds were synthesized by the Mitsunobu reaction (Method A). 6-Chloropurine was coupled with

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doi:10.1016/j.bmc.2010.04.081

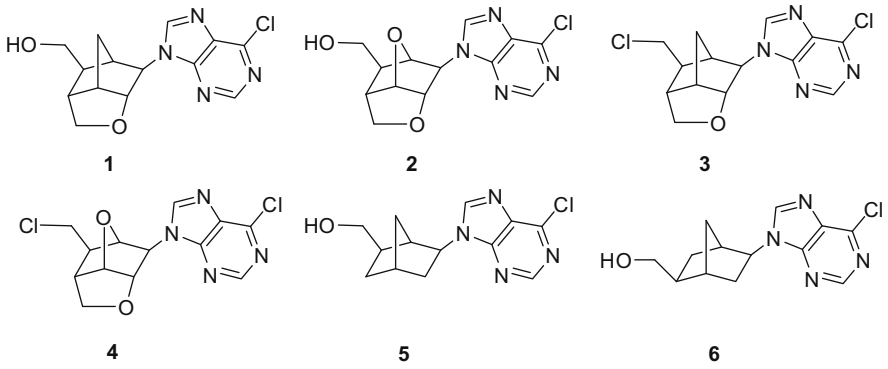


Figure 1. Structure of compounds **1**–**6**.

Table 1

Compound	IC_{50} (μM)	CC_{50} (μM)
1	1.13 ± 0.33	>50
2	1.86 ± 1.13	>50
3	1.00 ± 0.04	>50
4	0.88 ± 0.11	>50
5	0.98 ± 0.17	>50
6	0.91 ± 0.07	>50

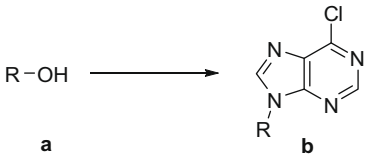
the appropriate alcohol (Table 2). The source of these alcohols is reported in the Supplementary data. Only few derivatives were prepared by construction of the nucleobase at the aminogroup on the corresponding scaffold by a coupling with 4,6-dichloropyrimidin-5-amine and the subsequent ring closure reaction[12] (Method B, Table 3, source of the amines—see Supplementary data). Yields are given in Tables 2 and 3.

Several compounds with a hydroxy group at the scaffold were also prepared and tested for the biological activity. Compound **22** with *cis*-dihydroxy arrangement was prepared from the unsaturated derivative by the simple catalytic *cis*-hydroxylation with osmium tetroxide in high yield (81%). The oxirane ring was introduced by epoxidation of the double bond with *m*-chloroperoxybenzoic acid, compound **23** was obtained in 73% yield (Scheme 1).

Derivatives bearing one hydroxy group were prepared from the commercially available unsaturated acetylderivative **24**, which was hydroborated and the obtained mixture of the acetates were immediately benzoylated (benzoyl chloride/pyridine) (Scheme 2). The acetyl group was then selectively cleaved with potassium carbonate in methanol and the obtained alcohol was oxidized to a ketone function with pyridinium dichromate (PDC) in dichloromethane, yielding a mixture of ketones **25** and **26**. At this stage of the reaction sequence, the isomers were separated by column chromatography (overall yield from the starting acetate **24** was 30% for **25** and 16% for **26**). Ketones **25** and **26** were reduced with sodium borohydride in methanol to obtain the *endo*-hydroxy compounds **27** and **28**.

Benzoylated alcohols afforded protected chloropurine derivatives **29** and **30** under Mitsunobu conditions (Scheme 3). The benzoyl group was removed by a modified version of the Jeong et al. method.[13] Methyl magnesium chloride was used (4.4 equiv) and the reaction time was prolonged. Cleavage of the benzoyl group using classical methods (methanolic ammonia/4 °C, NaCN/methanol, KCN/methanol, diluted aqueous sodium hydroxide/0 °C) was not successful (Isolation of products was not achieved due to the reaction giving a complicated mixture of products). In contrast, only the previously published method[9d] with diisobutylaluminum hydride in dichloromethane gave comparable yield, but isolation of the products from the reaction mixtures appeared rather complicated.

Table 2
B = 6-chloropurin-9-yl



Compound	Alcohol (a)	Product (b)	Yield (%)
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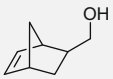
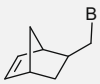
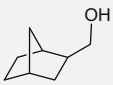
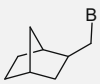
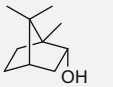
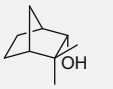
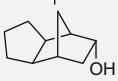
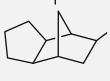
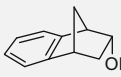
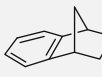
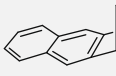
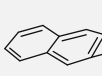
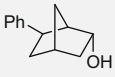
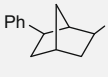
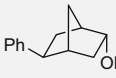
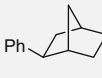
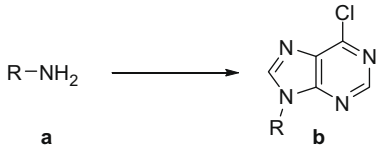
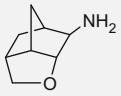
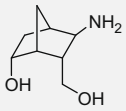
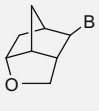
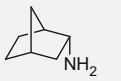
8			62
9			14
10			74
11			68
12			35
13			33
14			46
15			78
16			65
17			65
18			75

Table 3
B = 6-chloropurin-9-yl



Compound	Alcohol (a)	Product (b)	Yield (%)
19			71
20			65
21			40

Configuration of the hydroxy group was inverted to *endo* by simple oxidation to the ketones **33**/**34** and subsequent reduction of the keto group with sodium borohydride (Scheme 4). Inversion of the configuration was not complete. A small portion of the original *exo*-derivative **31** (17%) or **32** (20%) was observed in the reaction mixture after reduction. Ketoderivatives **33** and **34** and *endo*-hydroxy derivatives **37** and **38** were used to synthesize the compounds **39** and **40** bearing the fluorine atom on the bicyclic scaffold. Both fluorinations were carried out with (diethylamino)sulfur trifluoride (DAST) in refluxing dichloromethane in the presence of pyridine as a base. Geminal difluoroderivatives **35**/**36** and monofluoroderivatives **39**/**40** were prepared in moderate to good yields (55% for **35**; 45% for **36**; 73% for **39**; 63% for **40**).

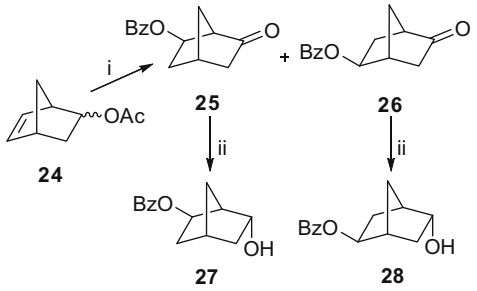
Another group of compounds that were synthesized are derivatives bearing two chloropurine nucleobases. Compounds **41** and **42** with the bicyclic scaffold substituted at positions 2,6 or 2,5 and *exo*-configuration of the substituents were prepared by the Mitsunobu reaction. *Endo*-alcohols **37** and **38** were treated with chloropurine–triphenylphosphine–azodicarboxylate and moderate yields of the final compounds were obtained (50% for **41**, 55% for **42**) (Scheme 5).

Compounds bearing two bases in *trans*-configuration at positions 2 and 3 were prepared by construction of the nucleobase on the appropriate amines (Scheme 6). The unsaturated diamine **43**[14] was coupled with 4 equivalents of the 4,6-dichloropyrimidin-5-amine and the reaction time was prolonged as compared to the classical protocol used for the compounds **19b–21b**. Despite this, the yield of this construction was relatively low (19%). Similar observations were obtained with the saturated diamine **44**.[15] In this case, yield was 20%.

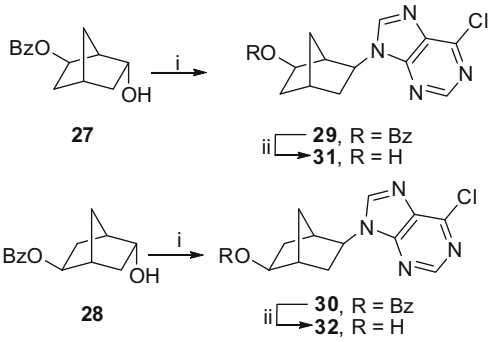
3. Results and discussion

The structures of the prepared compounds were determined by ^{1}H and ^{13}C NMR spectroscopies. Complete assignment of all ^{1}H and ^{13}C signals is based on combination of ^{1}H, ^{13}C APT, H,H-COSY, H,C-HSQC, and H,C-HMBC experiments. The position of the hydroxy or keto group in compounds **25** and **26** was confirmed using HMBC spectra. The hydrogen atom in position 2 (which is easily recognized by its typical chemical shift 4.5–5.5 ppm) exhibits a three-bond correlation cross-peak with carbon 6 and there is usually no cross-peak with carbon 5. In the HMBC spectrum of **25**, we observed a strong cross-peak with a carbonyl carbon (at δ 214.5), which indicates the 2,6-substitution of the bicyclic system. On the other hand, in the HMBC spectrum of **26**, we did not observe the cross-peak to the carbonyl carbon. Similar consecution was used in case of the compounds **17** and **18**. Configuration on carbons 2, 3, 5, and 6 was confirmed using ^{1}H NMR coupling constants—the *endo*-hydrogens do not interact with bridgehead hydrogens (dihedral angle is close to 90°), while the *exo*-hydrogens interact with coupling constant J = 4–5 Hz. Another significant interaction is between the *endo*-hydrogens in positions 2, 3, 5, and 6 and hydrogens in position 7 (W-interaction, J = 1–2 Hz).

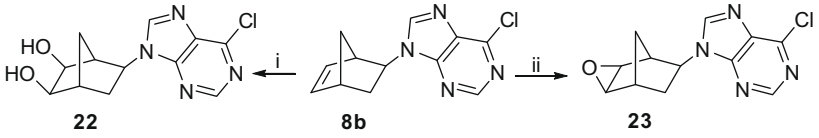
All target compounds were evaluated for antiviral activity against coxsackievirus B3 (Table 4). TTP-8307 was included as a reference compound.[16] Antiviral activity was expressed by means of EC_{50} or EC_{90} values, whereas CC_{50} values were calculated for cytotoxicity. Most compounds efficiently inhibited coxsackievirus B3 replication with minimal cytotoxicity to Vero cells. Only



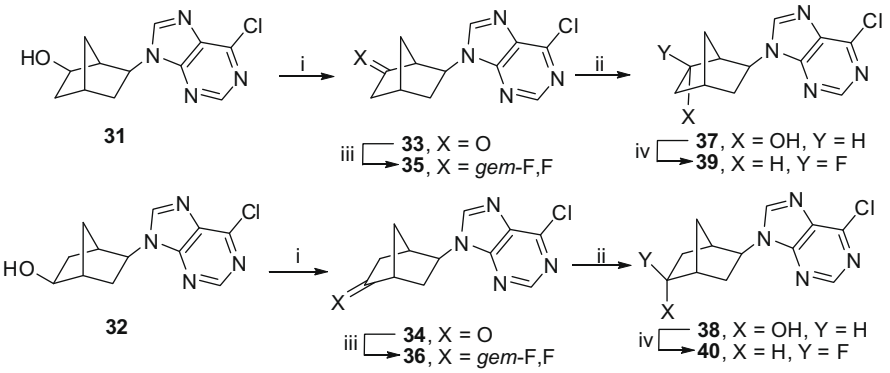
Scheme 2. Reagents and conditions: (i) (1) BH_3–THF, THF, 0 °C, 3 h, (2) $NaBO_3$, H_2O–THF, rt, overnight, (3) BzCl, pyridin, rt, overnight, (4) K_2CO_3, MeOH, rt, 45 min, (5) PDC, CH_2Cl_2, rt, overnight, **25** (30%), **26** (16%); (ii) $NaBH_4$, MeOH, 0 °C, 1 h, 90% for **27**, 89% for **28**.



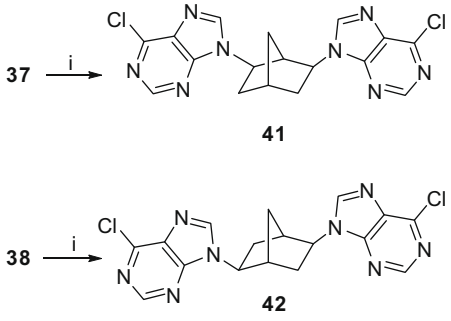
Scheme 3. Reagents and conditions: (i) 6-chloropurine, PPh_3, DIAD, THF, rt to reflux, 63% for **29**, 48% for **30**; (ii) CH_3MgCl, THF, 0 °C, overnight, 58% for **31**, 72% for **32**.



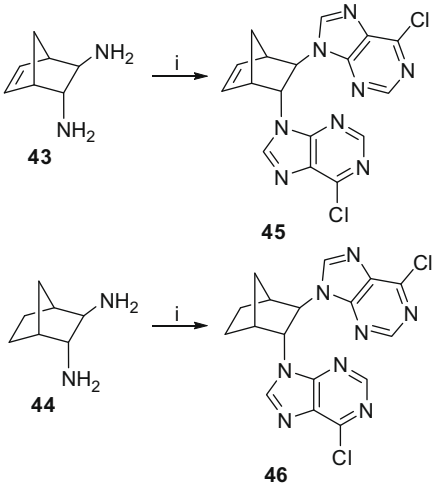
Scheme 1. Reagents and conditions: (i) OsO_4, NMMO, acetone–H_2O, rt, overnight, 81%; (ii) *m*CPBA, $CHCl_3$, rt, overnight, 73%.



Scheme 4. Reagents and conditions: (i) PDC, $CHCl_3$, rt, overnight, 88% for **33**, 92% for **34**; (ii) $NaBH_4$, MeOH, 0 °C, 1 h, 66% for **37**, 63% for **38**; (iii) DAST, CH_2Cl_2, pyridine, reflux, 30 h, 55% for **35**, 45% for **36**; (iv) DAST, CH_2Cl_2, pyridine, reflux, 12 h, 73% for **39**, 63% for **40**.



Scheme 5. Reagents and conditions: (i) 6-chloropurine, PPh_3, DIAD, THF, rt 15 h to reflux 10 h, 50% for **41**, 55% for **42**.



Scheme 6. Reagents and conditions: (i) (1) 4,6-dichloropyrimidin-5-amine, Et_3N, EtOH, 100 °C, 10 days, (2) $HC(OEt)_3$, HCl, 3 days, 19% for **45**, 20% for **46**.

compounds **17b** and **18b** bearing phenyl substituent either in position 5 or 6 on the bicyclic scaffold showed considerable cytotoxicity.

4. Conclusion

In summary, we have synthesized a novel class of coxsackievirus inhibitors derived from a bicyclo[2.2.1]heptane scaffold. Two methodologies were employed for introduction of the chloropurine moiety to the scaffold: (i) the Mitsunobu reaction of the chloropurine nucleobase with appropriate alcohols and (ii) a built-up strategy from appropriate amines. All compounds were evaluated for antiviral activity against coxsackievirus B3. Most analogues showed activity in the low micromolar range with minimal cytotoxicity to the cell line used in this study. Several compounds showed comparable or slightly better activity than previously reported derivatives. Studies on the mode of action of the most promising compounds (**7b** and **15b**) are ongoing.

Table 4
Antiviral evaluation against CVB3 of the 9-substitutedpurines in Vero cells, all data are mean values ± standard deviation for at least three independent experiments

Entry	Compound	EC_{50} (μM)	EC_{90} (μM)	CC_{50} (μM)
1	**7b**	0.81 ± 0.20	1.82 ± 0.91	>50
2	**8b**	2.43 ± 1.43	5.87 ± 4.15	>50
3	**9b**	1.52 ± 0.12	ND	43.85 ± 8.70
4	**10b**	3.58 ± 0.98	8.49 ± 4.32	>50
5	**11b**	3.83 ± 0.76	9.43 ± 4.72	>50
6	**12b**	3.47 ± 1.42	36.26 ± 26.57	>50
7	**13b**	10.88 ± 7.89	>50	>50
8	**14b**	1.90 ± 1.20	ND	43.35 ± 9.40
9	**15b**	0.66 ± 0.35	>50	>50
10	**16b**	>50	>50	>50
11	**17b**	2.98 ± 2.07	ND	13.38 ± 9.34
12	**18b**	5.72 ± 0.69	ND	16.98 ± 4.21
13	**19b**	1.27 ± 0.50	ND	>50
14	**20b**	1.00 ± 0.01	ND	>50
15	**21b**	>50	>50	>50
16	**22**	2.26 ± 2.04	36.52 ± 26.40	>50
17	**23**	1.03 ± 0.20	3.46 ± 2.91	>50
18	**31**	1.10 ± 0.03	ND	47.85 ± 3.05
19	**32**	2.44 ± 1.95	ND	>50
20	**33**	1.57 ± 0.95	ND	>50
21	**34**	0.90 ± 0.04	1.78 ± 0.89	>50
22	**37**	1.30 ± 0.40	ND	>50
23	**38**	1.05 ± 0.01	ND	>50
24	**35**	1.16 ± 0.24	34.40 ± 27.97	>50
25	**36**	1.00 ± 0.02	ND	>50
26	**39**	0.83 ± 0.05	1.70 ± 0.85	>50
27	**40**	0.95 ± 0.02	1.96 ± 0.98	>50
28	**41**	7.83 ± 4.90	ND	>50
29	**42**	22.50 ± 4.37	ND	>50
30	**45**	3.69 ± 0.51	8.81 ± 4.48	49.76 ± 0.35
31	**46**	3.70 ± 2.23	ND	19.07 ± 1.67
32	TTP-8307	1.2 ± 0.1	ND	>50

5. Experimental section

5.1. Chemistry

Melting points were determined on a Büchi B-540 apparatus. NMR spectra (δ, ppm; J, Hz) were measured on a Bruker Avance

II-600 and/or Bruker Avance II-500 instruments (600.1 or 500.0 MHz for ^{1}H and 150.9 or 125.7 MHz for ^{13}C) in hexadeuterated dimethyl sulfoxide and referenced to the solvent signal (δ 2.50 and 39.70, respectively). Mass spectra were measured on a LTQ Orbitrap XL (Thermo Fisher Scientific) using electrospray ionization (ESI) and a GCT Premier (Waters) using EI. Column chromatography was performed on Silica gel 60 (Fluka) and thin-layer chromatography (TLC) on Silica gel 60 F254 foils (Merck). Solvents were evaporated at 2 kPa and bath temperature 30–60 °C; the compounds were dried at 13 Pa and 50 °C. The elemental analyses were obtained on a Perkin–Elmer CHN Analyzer 2400, Series II Sys (Perkin–Elmer). The elemental compositions for all compounds agreed to within ±0.4% of the calculated values. For all the tested compounds satisfactory elemental analysis was obtained supporting >95% purity. Bicyclo[2.2.1]hept-5-en-2-yl acetate **24** (mixture of *endo*/*exo* isomers) was purchased from Sigma–Aldrich.

5.1.1. General method for the Mitsunobu coupling of a alcohol with a nucleobase

To a mixture of the alcohol (0.50 g, 1 equiv), triphenylphosphine (1.3 equiv), and 6-chloropurine (1.3 equiv) in THF (35 mL) a solution of the diisopropyl azadicarboxylate (1.3 equiv) in THF (15 mL) was dropwise added. The resulting mixture was stirred overnight (TLC control) and then eventually heated for reflux until the starting alcohol was consumed (TLC control). The reaction mixture was evaporated and the residue was chromatographed on silica gel column (200 g) in appropriate mobile phase. Finally, the pure product was obtained after crystallization.

5.1.1.1. 9-[(1*R,2*R**,4*S**)-Bicyclo[2.2.1]hept-2-yl]-6-chloro-9*H*-purine (7b).** Yield 41%. Mobile phase: toluene–ethyl acetate (6:1→4:1). Mp 104–105 °C (water–methanol, white crystals). ^{1}H NMR (600.13 MHz): 8.85 (1H, s, H-8), 8.77 (1H, s, H-2), 4.56 (1H, ddd, *J* 8.4, 4.5, 0.9 Hz, H-2′), 2.55 (1H, bd, *J* 4.6 Hz, H-1′), 2.43 (1H, bt, *J* 4.2, 4.2 Hz, H-4′), 2.09–2.05 (1H, m, H-3′*endo*), 2.00 (1H, ddd, *J* 13.4, 8.5, 2.3 Hz, H-3′*exo*), 1.71 (1H, dm, *J* 10.3 Hz, H-7′a), 1.62 (1H, tt, *J* 12.2, 12.2, 4.5, 4.5 Hz, H-6′*endo*), 1.56–1.54 (1H, m, H-5′*endo*), 1.40 (1H, dddd, *J* 12.1, 9.1, 4.1, 2.3 Hz, H-6′*exo*), 1.29–1.27 (1H, m, H-7′b), 1.29–1.25 (1H, m, H-5′*exo*). ^{13}C NMR (150.92 MHz): 152.11 (C-4), 151.45 (C-2), 149.11 (C-6), 145.62 (C-8), 131.43 (C-5), 58.37 (C-2′), 42.16 (C-1′), 37.76 (C-3′), 35.93 (C-4′), 35.82 (C-7′), 27.95 (C-5′), 26.94 (C-6′). ESI MS, *m/z* (rel%): 249/251 (100/35) [M+H]. For $C_{12}H_{13}ClN_4$ (248.7) calcd: C, 57.95; H, 5.27; Cl, 14.25; N, 22.53; found: C, 57.93; H, 5.32; Cl, 14.29; N, 22.34.

5.1.1.2. 9-[(1*R,2*S**,4*R**)-Bicyclo[2.2.1]hept-5-en-2-yl]-6-chloro-9*H*-purine (8b).** Yield 62%. Mobile phase: toluene–ethyl acetate (6:1→4:1). Mp 117.5–118.5 °C (water–methanol, white crystals). ^{1}H NMR (600.13 MHz): 8.93 (1H, s, H-8), 8.78 (1H, s, H-2), 6.37 (1H, dd, *J* 5.7, 2.9 Hz, H-5′), 6.26 (1H, dd, *J* 5.7, 3.2 Hz, H-6′), 4.46 (1H, ddd, *J* 8.3, 3.9, 1.6 Hz, H-2′), 3.23–3.19 (1H, m, H-1′), 3.07–3.04 (1H, m, H-4′), 2.18 (1H, dt, *J* 12.6, 3.7, 3.7 Hz, H-3′*exo*), 1.88 (1H, ddd, *J* 12.6, 8.3, 2.6 Hz, H-3′*endo*), 1.82–1.76 (1H, dm, *J* 9.2 Hz, H-7′b), 1.59–1.52 (1H, dm, *J* 9.2 Hz, H-7′a). ^{13}C NMR (150.92 MHz): 152.60 (C-4), 151.49 (C-2), 149.14 (C-6), 145.96 (C-8), 140.21 (C-5′), 134.48 (C-6′), 131.51 (C-5), 55.82 (C-2′), 47.55 (C-1′), 46.23 (C-7′), 41.28 (C-4′), 32.14 (C-3′). ESI MS, *m/z* (rel%): 343 (100), 269/271(90/22) [M+Na], 247/249 (38/12) [M+H]. For $C_{12}H_{11}ClN_4$ (246.7) calcd: C, 58.42; H, 4.49; Cl, 14.37; N, 22.71; found: C, 58.44; H, 4.38; Cl, 14.34; N, 22.44.

5.1.1.3. 6-Chloro-9-[(1*R,3*S**,4*S**)-tricyclo[2.2.1.0^{2,6}]hept-3-yl]-9*H*-purine (9b).** Yield 14%. Mobile phase: toluene–ethyl acetate (4:1). Mp 92–93 °C (hexanes–ether, white crystals). ^{1}H NMR (499.95 MHz): 8.77 (1H, s, H-2), 8.65 (1H, s, H-8), 4.42 (1H, t, *J* 1.7, 1.7(H-3′), 2.51–2.47 (1H, m, H-4′), 1.84 (1H, tdd, *J* 5.2, 5.2, 1.4, 1.0 Hz, H-2′), 1.69–1.62 (1H, dm, *J* 10.6 Hz, H-5′b), 1.52–1.50 (2H, m, H-1′, H-6′), 1.45–1.39 (1H, dm, *J* 10.6 Hz, H-5′a), 1.29–1.22 (1H, dm, *J* 11.4 Hz, H-7′b), 1.10–1.02 (1H, dm, *J* 11.3 Hz, H-7′a). ^{13}C NMR (125.73 MHz): 152.22 (C-4), 151.54 (C-2), 149.16 (C-6), 146.58 (C-8), 131.52 (C-5), 60.54 (C-3′), 33.49 (C-4′), 31.38 (C-5′), 29.17 (C-7′), 12.92 (C-2′), 12.10 (C-6′), 11.05 (C-1′). ESI MS, *m/z* (rel%): 247/249 (100/31) [M+H]. For $C_{12}H_{11}ClN_4$ (246.7) calcd: C, 58.42; H, 4.49; Cl, 14.37; N, 22.71; found: C, 58.27; H, 4.41; Cl, 14.23; N, 22.53.

5.1.1.4. 9-[(1*R,2*S**,4*R**)-Bicyclo[2.2.1]hept-5-en-2-ylmethyl]-6-chloro-9*H*-purine (10b).** Yield 74%. Mobile phase: toluene–ethyl acetate (4:1), viscous yellowish oil. ^{1}H NMR (499.95 MHz): 8.79 (1H, s, H-8), 8.78 (1H, s, H-2), 6.08 (1H, dd, *J* 5.7, 2.9 Hz, H-5′), 6.04 (1H, dd, *J* 5.7, 3.1 Hz, H-6′), 4.38–4.29 (2H, m, CH_2N), 2.87–2.83 (1H, m, H-4′), 2.55–2.51 (1H, m, H-1′), 2.02–1.98 (1H, m, H-2′), 1.56–1.48 (1H, dm, *J* 8.6 Hz, H-7′b), 1.35–1.29 (2H, m, H-3′*exo*, H-7′a), 1.18–1.14 (1H, m, H-3′*endo*). ^{13}C NMR (125.73 MHz): 152.25 (C-4), 151.72 (C-2), 149.22 (C-6), 147.82 (C-8), 137.19 (C-5′), 136.26 (C-6′), 131.00 (C-5), 48.83 (CH_2N), 44.92 (C-7′), 43.99 (C-1′), 41.58 (C-4′), 39.17 (C-2′), 30.33 (C-3′). ESI MS, *m/z* (rel%): 261/263 (100/33) [M+H]. For $C_{13}H_{13}ClN_4$ (260.7) calcd: C, 59.89; H, 5.03; Cl, 13.60; N, 21.49; found: C, 59.49; H, 5.08; Cl, 13.95; N, 21.15.

5.1.1.5. 9-[(1*R,2*R**,4*S**)-Bicyclo[2.2.1]hept-2-ylmethyl]-6-chloro-9*H*-purine (11b).** Yield 68%. Mobile phase: toluene–ethyl acetate (4:1), viscous yellowish oil. ^{1}H NMR (499.95 MHz): 8.77 (1H, s, H-2), 8.76 (1H, s, H-8), 4.15 (1H, dd, *J* 13.9, 8.7 Hz, CH_2N), 4.01 (1H, dd, *J* 13.9, 7.4 Hz, CH_2N), 2.22–2.18 (1H, m, H-4′), 2.11–2.07 (1H, m, H-2′), 1.93–1.89 (1H, m, H-1′), 1.49–1.43 (1H, dm, *J* 9.8 Hz, H-7′b), 1.42–1.38 (2H, m, H-5′*exo*, H-6′*exo*), 1.28 (1H, ddd, *J* 13.2, 8.6, 2.3 Hz, H-3′*endo*), 1.16–1.12 (1H, m, H-3′*exo*), 1.14–1.06 (1H, dm, *J* 9.8, H-7′a), 1.07–1.03 (2H, m, H-5′*endo*, H-6′*endo*). ^{13}C NMR (125.73 MHz): 152.26 (C-4), 151.72 (C-2), 149.22 (C-6), 147.85 (C-8), 130.93 (C-5), 48.37 (CH_2N), 41.97 (C-2′), 38.66 (C-1′), 36.18 (C-4′), 34.99 (C-7′), 34.71 (C-3′), 29.23 (C-6′), 28.46 (C-5′). ESI MS, *m/z* (rel%): 263/265 (100/33) [M+H]. For $C_{13}H_{15}ClN_4$ (262.7) calcd: C, 59.43; H, 5.75; Cl, 13.49; N, 21.32; found: C, 59.25; H, 5.90; Cl, 13.30; N, 21.05.

5.1.1.6. 6-Chloro-9-[(1*R,2*R**,4*R**)-1,7,7-trimethylbicyclo[2.2.1]hept-2-yl]-9*H*-purine (12b).** Yield 35%. Mobile phase: toluene–ethyl acetate (5:1). Mp 132.3–134.5 °C (water–methanol, white crystals). ^{1}H NMR (600.13 MHz): 9.00 (1H, s, H-8), 8.79 (1H, s, H-2), 4.70 (1H, dd, *J* 9.5, 6.9 Hz, H-2′), 2.77 (1H, dddd, *J* 13.3, 6.9, 4.0, 3.2 Hz, H-3′*exo*), 1.99 (1H, dd, *J* 13.2, 9.6 Hz, H-3′*endo*), 1.95 (1H, t, *J* 4.2, 4.2 Hz, H-4′), 1.84–1.80 (1H, m, H-5′*exo*), 1.66 (1H, td, *J* 12.2, 12.2, 4.9 Hz, H-6′*exo*), 1.45 (1H, ddd, *J* 12.4, 9.2, 3.6 Hz, H-6′*endo*), 1.33 (1H, ddd, *J* 12.3, 9.3, 4.9 Hz, H-5′*endo*), 0.99 (3H, s, 7′-CH_3), 0.85 (3H, s, 7′-CH_3), 0.65 (3H, s, 1′-CH_3). ^{13}C NMR (150.92 MHz): 153.48 (C-4), 151.41 (C-2), 149.28 (C-6), 146.77 (C-8), 131.18 (C-5), 63.15 (C-2′), 50.66 (C-1′), 47.24 (C-7′), 44.59 (C-4′), 36.64 (C-6′), 34.31 (C-3′), 26.55 (C-5′), 21.13 and 19.82 (2 × 7′-CH_3), 12.06 (1′-CH_3). For $C_{15}H_{19}ClN_4$ (290.8) calcd: C, 61.96; H, 6.59; Cl, 12.19; N, 19.27; found: C, 61.89; H, 6.70; Cl, 12.31; N, 19.07.

5.1.1.7. 6-Chloro-9-[(1*R,2*S**,4*S**)-3,3-dimethylbicyclo[2.2.1]hept-2-yl]-9*H*-purine (13b).** Yield 35%. Mobile phase: toluene–ethyl acetate (5:1). Mp 112.3–114 °C (water–methanol, white crystals). ^{1}H NMR (600.13 MHz): 8.78 (1H, s, H-8), 8.78 (1H, s, H-2). 4.15 (1H, d, *J* 1.6 Hz, H-2′), 2.70 (1H, dm, *J* 4.8 Hz, H-1′), 2.28 (1H, dm, *J* 10.8 Hz, H-7′b), 1.92 (1H, dm, *J* 3.6 Hz, H-4′), 1.76–1.72 (2H, m, H-5′*endo*, H-6′*exo*), 1.48–1.40 (3H, m, H-5′*exo*, H-6′*endo*, H-

7′a), 1.27 (3H, s, 3′-CH_3), 0.39 (3H, s, 3′-CH_3). ^{13}C NMR (150.92 MHz): 152.86 (C-4), 151.82 (C-2), 149.22 (C-6), 145.68 (C-8), 131.57 (C-5), 68.08 (C-2′), 48.03 (C-4′), 44.46 (C-3′), 42.68 (C-1′), 37.09 (C-7′), 28.22 (C-6′), 26.14 (3′-CH_3), 23.59 (C-5′), 23.37 (3′-CH_3). ESI MS, *m/z* (rel%): 277/279 (100/45) [M+H]. For $C_{14}H_{17}ClN_4$ (276.8) calcd: C, 60.76; H, 6.19; Cl, 12.81; N, 20.24; found: C, 60.48; H, 6.36; Cl, 12.57; N, 19.99.

5.1.1.8. 6-Chloro-9-[(1*R,2*R**,6*R**,7*R**,8*S**)-tricyclo[5.2.1.0^{2,6}]dec-8-yl]-9*H*-purine (14b).** Yield 46%. Mobile phase: toluene–ethyl acetate (7:1). Mp 104.5–106.5 °C (water–acetone, white powder). ^{1}H NMR (600.13 MHz): 8.86 (1H, s, H-8), 8.77 (1H, s, H-2), 4.48 (1H, ddd, *J* 8.4, 4.2, 1.0 Hz, H-8′), 2.33 (1H, s, H-7′), 2.15 (1H, d, *J* 4.4 Hz, H-1′), 2.05 (1H, dt, *J* 13.3, 4.3, 4.3 Hz, H-9′*exo*), 2.04–2.01 (1H, m, H-6′), 1.95–1.91 (1H, m, H-9′*endo*), 1.93–1.89 (1H, m, H-2′), 1.90–1.83 (2H, m, H-3′b, H-5′b), 1.67–1.63 (1H, m, H-4′), 1.51–1.53 (1H, dm, *J* 11.1 Hz, H-10′b), 1.48–1.40 (1H, dm, *J* 11.1 Hz, H-10′a), 1.26–1.17 (2H, m, H-4′), 1.01–0.93 (2H, m, H-3′a, H-5′a). ^{13}C NMR (150.92 MHz): 152.25 (C-4), 151.47 (C-2), 149.12 (C-6), 145.67 (C-8), 131.48 (C-5), 57.90 (C-8′), 46.93 (C-2′), 46.40 (C-7′), 45.42 (C-6′), 40.21 (C-1′), 36.81 (C-9′), 31.87 (C-5′), 31.48 (C-3′), 29.95 (C-10′), 27.46 (C-4′). ESI MS, *m/z* (rel%): 289/291 (100/29) [M+H]. For $C_{15}H_{17}ClN_4$ (288.8) calcd: C, 62.39; H, 5.93; Cl, 12.28; N, 19.40; found: C, 62.25; H, 5.86; Cl, 12.53; N, 19.17.

5.1.1.9. 6-Chloro-9-[(1*R,8*R**,9*S**)-tricyclo[6.2.1.0^{2,7}]undeca-2,4,6-trien-9-yl]-9*H*-purine (15b).** Yield 78%. Mobile phase: toluene–ethyl acetate (8:1→6:1). Mp 202–203.5 °C (water–methanol, white crystals). ^{1}H NMR (499.84 MHz): 9.03 (1H, s, H-8), 8.80 (1H, s, H-2), 7.37–7.33 (1H, m, H-6′), 7.30–7.26 (1H, m, H-3′), 7.17–7.12 (2H, m, H-4′, H-5′), 4.53 (1H, ddd, *J* 8.3, 4.2, 1.0 Hz, H-9′), 3.73 (1H, bs, H-8′), 3.58–3.54 (1H, m, H-1′), 2.52 (1H, dt, *J* 12.9, 4.0, 4.0 Hz, H-10′*exo*), 2.20 (1H, dt, *J* 9.8, 1.4, 1.4 Hz, H-11′b), 1.99 (1H, ddd, *J* 12.9, 8.4, 2.5 Hz, H-10′*endo*), 1.88 (1H, d, *J* 9.7 Hz, H-11′a). ^{13}C NMR (125.70 MHz): 152.52 (C-4), 151.63 (C-2), 149.23 (C-6), 148.51 (C-2′), 146.07 (C-8), 144.91 (C-7′), 131.56 (C-5), 126.95 and 126.22 (C-4′ and C-5′), 122.10 (C-6′), 121.31 (C-3′), 57.16 (C-9′), 49.36 (C-8′), 46.90 (C-11′), 43.12 (C-1′), 34.91 (C-10′). ESI MS, *m/z* (rel%): 297/299 (100/30) [M+H]. For $C_{16}H_{13}ClN_4 \times \frac{1}{4}\ H_2O$ (301.3) calcd: C, 64.76; H, 4.42; Cl, 11.95; N, 18.88; found: C, 64.89; H, 4.21; Cl, 12.19; N, 18.53.

5.1.1.10. 6-Chloro-9-[(1*R,2*S**,4*R**)-1,2,3,4-tetrahydroanthracen-2-yl]-9*H*-purine (16b).** Yield 65%. Mobile phase: toluene–ethyl acetate (10:1). Mp 267–268.5 °C (methanol, white powder, decomposition). ^{1}H NMR (499.84 MHz): 9.08 (1H, s, H-8), 8.83 (1H, s, H-2), 7.84 (1H, s, H-9′), 7.82–7.92 (2H, m, H-5′, H-8′), 7.74 (1H, s, H-10′), 7.48–7.44 (2H, m, H-6′, H-7′), 4.71–4.65 (1H, m, H-2′), 3.90–3.85 (1H, m, H-1′), 3.73–3.69 (1H, m, H-4′), 2.66 (1H, dt, *J* 13.0, 4.2, 4.2 Hz, H-3′*exo*), 2.36–2.30 (1H, dm, *J* 9.9 Hz, H-11′b), 2.12 (1H, ddd, *J* 13.0, 8.6, 2.3 Hz, H-3′*endo*), 1.99–1.92 (1H, dm, *J* 9.9 Hz, H-11′a). ^{13}C NMR (125.72 MHz): 152.51 (C-4), 151.67 (C-2), 149.27 (C-6), 146.58 (C-4′a), 146.10 (C-8), 143.43 (C-9′a), 132.96 (C-10′a), 132.54 (C-8′a), 131.62 (C-5), 128.07 and 127.89 (C-5′ and C-8′), 125.75 and 125.47 (C-6′ and C-7′), 120.17 (C-9′), 118.98 (C-10′), 57.38 (C-2′), 49.21 (C-1′), 45.82 (C-11′), 42.90 (C-4′), 35.45 (C-3′). ESI MS, *m/z* (rel%): 369/371 (100/33) [M+Na]. For $C_{20}H_{15}ClN_4$ (346.8) calcd: C, 69.26; H, 4.36; Cl, 10.22; N, 16.15; found: C, 69.22; H, 4.51; Cl, 10.37; N, 15.75.

5.1.1.11. 6-Chloro-9-[(1*R,2*S**,4*R**,6*R**)-6-phenylbicyclo[2.2.1]hept-2-yl]-9*H*-purine (17b).** Yield 65%. Mobile phase: toluene–ethyl acetate (5:1). Mp 102–103.5 °C (ether, white powder). ^{1}H NMR (499.95 MHz): 8.91 (1H, s, H-8), 8.78 (1H, s, H-2), 7.31–7.26 (4H, m, H-2″, H-3″), 7.19–7.15 (1H, m, H-4″), 4.77 (1H, dd, *J* 8.0, 4.2 Hz, H-2′), 3.04 (1H, dd, *J* 9.0, 5.5 Hz, H-6′), 2.76 (1H, bs, H-1′), 2.57–2.53 (1H, m, H-4′), 2.19–2.08 (2H, m, H-3′), 1.89 (1H, ddd, *J* 12.3, 8.9, 1.9 Hz, H-5′*endo*), 1.71–1.64 (2H, m, H-5′*exo*, H-7′), 1.60–1.54 (1H, dm, *J* 10.7 Hz, H-7′a). ^{13}C NMR (125.73 MHz): 152.25 (C-4), 151.51 (C-2), 149.13 (C-6), 145.74 (C-8), 145.59 (C-1″), 131.54 (C-5), 128.62 (C-3″), 127.28 (C-2″), 126.06 (C-4″), 58.96 (C-2′), 48.21 (C-1′), 44.20 (C-6′), 37.47 (C-5′), 36.90 (C-3′), 36.42 (C-4′), 33.82 (C-7′). ESI MS, *m/z* (rel%): 325/327 (100/33) [M+H]. For $C_{18}H_{17}ClN_4$ (324.8) calcd: C, 66.56; H, 5.28; Cl, 10.92; N, 17.25; found: C, 66.29; H, 5.30; Cl, 10.88; N, 16.99.

5.1.1.12. 6-Chloro-9-[(1*R,2*S**,4*R**,5*S**)-5-phenylbicyclo[2.2.1]hept-2-yl]-9*H*-purine (18b).** Yield 75%. Mobile phase: toluene–ethyl acetate (5:1). Mp 129–131 °C (ether, white powder). ^{1}H NMR (499.95 MHz): 8.90 (1H, s, H-8), 8.79 (1H, s, H-2), 7.32–7.25 (4H, m, H-2″, H-3″), 7.20–7.15 (1H, m, H-4″), 4.70 (1H, bdd, *J* 8.2, 4.9 Hz, H-2′), 2.91 (1H, dd, *J* 8.9, 5.6 Hz, H-5′), 2.68 (1H, bd, *J* 4.6 Hz, H-1′), 2.52–2.48 (1H, m, H-4′), 2.25–2.15 (2H, m, H-3′), 2.00 (1H, ddd, *J* 12.9, 9.0, 2.4 Hz, H-6′*endo*), 1.77 (1H, dt, *J* 12.9, 5.1, 5.1 Hz, H-6′*exo*), 1.75–1.72 (1H, m, H-7′b), 1.60–1.54 (1H, dm, *J* 10.7 Hz, H-7′a). ^{13}C NMR (125.73 MHz): 152.23 (C-4), 151.52 (C-2), 149.19 (C-6), 146.19 (C-1″), 145.69 (C-8), 131.51 (C-5), 128.60 (C-3″), 127.17 (C-2″), 125.99 (C-4″), 58.02 (C-2′), 45.42 (C-5′), 42.87 and 42.85 (C-1′ and C-4′), 38.21 (C-3′), 35.71 (C-6′), 33.59 (C-7′). ESI MS, *m/z* (rel%): 325/327 (100/33) [M+H], 329 (65). For $C_{18}H_{17}ClN_4$ (324.8) calcd: C, 66.56; H, 5.28; Cl, 10.92; N, 17.25; found: C, 66.38; H, 5.20; Cl, 11.15; N, 16.94.

5.1.2. General method for the nucleobase built-up from amines

A mixture of amine (3 mmol), 4,6-dichloropyrimidin-5-amine (4.5 mmol), and triethylamine (1.8 mL) in ethanol (9 mL) was heated in a pressure vessel at 105 °C for 6 days and, after cooling, was evaporated. The residue was chromatographed on a column of silica gel (200 g). The pyrimidine intermediate was eluted with appropriate mobile phase and this intermediate was immediately used in the next step. Concentrated hydrochloric acid (1 mL) was added to a suspension of pyrimidine intermediate in triethyl orthoformate (80 mL) and the reaction mixture was vigorously stirred for 3 days at room temperature. Solution was evaporated and the residue was crystallized from water–methanol (95:5) to afford product.

5.1.2.1. 6-Chloro-9-[(1*R,2*S**,3*S**,6*S**,7*R**)-4-oxatricyclo[4.2.1.0^{3,7}]non-2-yl]-9*H*-purine (19b).** Yield 71%. Mobile phase: toluene–ethyl acetate (5:1→1:1). Mp 166–167 °C (water–methanol). ^{1}H NMR (600.13 MHz): 8.78 (1H, s, H-8), 8.77 (1H, s, H-2), 4.76 (1H, dd, *J* 5.0, 1.4 Hz, H-3′), 4.14 (1H, d, *J* 2.1 Hz, H-2′), 3.80 (1H, dd, *J* 8.0, 4.2 Hz, H-5′b), 3.75 (1H, d, *J* 8.0 Hz, H-5′a), 2.76–2.72 (1H, m, H-7′), 2.62–2.58 (1H, m, H-1′), 2.50–2.46 (1H, m, H-6′), 2.06 (1H, ddd, *J* 12.8, 10.8, 4.5 Hz, H-9′*exo*), 1.88–1.83 (1H, dm, *J* 11.3 Hz, H-8′b), 1.72–1.65 (1H, dm, *J* 11.4 Hz, H-8′a), 1.29 (1H, dt, *J* 12.8, 2.3 Hz, H-9′*endo*). ^{13}C NMR (150.92 MHz): 152.30 (C-4), 151.57 (C-2), 149.23 (C-6), 145.79 (C-8), 131.30 (C-5), 83.92 (C-3′), 74.51 (C-5′), 66.85 (C-2′), 45.78 (C-7′), 39.03 (C-1′), 37.16 (C-6′), 35.45 (C-8′), 35.13 (C-9′). ESI MS, *m/z* (rel%): 299/301 (100/35) [M+Na], 277/279 (73/23) [M+H]. For $C_{13}H_{13}ClN_4O$ (276.7) calcd: C, 56.42; H, 4.74; Cl, 12.81; N, 20.25; found: C, 56.21; H, 4.56; Cl, 13.08; N, 19.96.

5.1.2.2. 6-Chloro-9-[(1*R,3*R**,6*R**,7*R**,9*R**)-4-oxatricyclo[4.2.1.0^{3,7}]non-9-yl]-9*H*-purine (20b).** Yield 65%. Mobile phase: toluene–ethyl acetate (5:1→1:1). Mp 196–198 °C (water–methanol, white crystals). ^{1}H NMR (600.13 MHz): 8.88 (1H, s, H-8), 8.79 (1H, s, H-2), 4.37 (1H, dd, *J* 7.7, 4.9 Hz, H-3′), 4.33 (1H, m, H-9′), 3.94 (1H, d, *J* 8.5 Hz, H-5′a), 3.80 (1H, dd, *J* 8.5, 4.6 Hz, H-5′b), 3.04 (1H, bt, *J* 5.2, 5.2 Hz, H-6′), 2.74 (1H, tq, *J* 4.8, 4.8, 1.4, 1.4, 1.4 Hz,

H-7′), 2.58 (1H, bd, *J* 3.9 Hz, H-1′), 1.99–1.93 (1H, dm, *J* 11.2 Hz, H-8′b), 1.73 (1H, ddd, *J* 13.3, 7.7, 4.0 Hz, H-2′*exo*), 1.54–1.47 (1H, dm, *J* 11.2 Hz, H-8′a), 1.22 (1H, dd, *J* 13.3, 3.3 Hz, H-2′*endo*). ^{13}C NMR (150.92 MHz): 152.22 (C-4), 151.57 (C-2), 149.17 (C-6), 145.72 (C-8), 131.49 (C-5), 77.84 (C-3′), 71.58 (C-5′), 65.78 (C-9′), 46.70 (C-6′), 45.76 (C-7′), 39.50 (C-1′), 38.16 (C-2′), 35.18 (C-8′). ESI MS, *m/z* (rel%): 277/279 (100/20) [M+H]. For $C_{13}H_{13}ClN_4O$ (276.7) calcd: C, 56.42; H, 4.74; Cl, 12.81; N, 20.25; found: C, 56.31; H, 4.75; Cl, 12.72; N, 20.04.

5.1.2.3. 9-[(1*R,2*S**,4*S**)-Bicyclo[2.2.1]hept-2-yl]-6-chloro-9*H*-purine (21b).** Yield 40%. Mobile phase: toluene–ethyl acetate (5:1→2:1). Mp 99–100.5 °C (water–methanol, white crystals). ^{1}H NMR (499.84 MHz): 8.87 (1H, s, H-8), 8.76 (1H, s, H-2), 4.96–4.92 (1H, m, H-2′), 2.74–2.72 (1H, m, H-1′), 2.40–2.36 (1H, m, H-4′), 2.20–2.18 (1H, m, H-3′*exo*), 1.98 (1H, ddd, *J* 13.3, 5.0, 3.0 Hz, H-3′*endo*), 1.69–1.62 (1H, dm, *J* 9.9 Hz, H-7′b), 1.59–1.53 (2H, m, H-5′), 1.46–1.40 (1H, dm, *J* 9.9 Hz, H-7′a), 1.34–1.31 (1H, m, H-6′*exo*), 0.94–0.91 (1H, m, H-6′*endo*). ^{13}C NMR (125.70 MHz): 152.81 (C-4), 151.52 (C-2), 149.22 (C-6), 146.50 (C-8), 131.53 (C-5), 57.11 (C-2′), 41.11 (C-1′), 37.88 (C-7′), 36.41 (C-4′), 32.38 (C-3′), 28.61 (C-5′), 21.79 (C-6′). ESI MS, *m/z* (rel%): 249/251 (100/33) [M+H]. For $C_{12}H_{13}ClN_4$ (248.7) calcd: C, 57.95; H, 5.27; Cl, 14.25; N, 22.53; found: C, 57.88; H, 5.32; Cl, 14.45; N, 22.21.

5.1.3. (1*R,2*R**,3*S**,4*S**,5*S**)-5-(6-Chloro-9*H*-purin-9-yl)bicyclo-[2.2.1]heptane-2,3-diol (22)**

Unsaturated derivative **8b** (250 mg, 1.01 mmol) was dissolved in mixture of water–acetone (15 mL, 1:1). Solution of NMMO (50% in water, 2 mL) and water solution of osmium tetroxide (50 μL, 100 mg/5 mL) was added and the reaction mixture was stirred at rt overnight. The reaction mixture was evaporated and the residue was chromatographed on silica gel column (100 g) in ethyl acetate–acetone–ethanol–water (225:15:6:4). It was obtained 230 mg (81%) of the product **22**. Mp 198–200 °C (water, white powder). ^{1}H NMR (600.13 MHz): 8.84 (1H, s, H-8′), 8.76 (1H, s, H-2′), 4.82 (1H, d, *J* 6.0 Hz, 3-OH), 4.80 (1H, d, *J* 6.1 Hz, 2-OH), 4.49 (1H, dd, *J* 8.5, 4.3 Hz, H-6), 3.80–3.76 (1H, m, H-2), 3.68–3.64 (1H, m, H-3), 2.40 (1H, bs, H-1), 2.22–2.16 (1H, dm, *J* 4.7 Hz, H-4), 2.01–1.97 (1H, m, H-5*exo*), 1.89 (1H, ddd, *J* 13.8, 8.5, 2.2 Hz, H-5*endo*), 1.83–1.77 (1H, dm, *J* 10.8 Hz, H-7b), 1.57–1.51 (1H, dm, *J* 10.8 Hz, H-7a). ^{13}C NMR (150.92 MHz): 152.15 (C-4′), 151.45 (C-2′), 149.13 (C-6′), 145.67 (C-8′), 131.48 (C-5′), 72.51 (C-3), 71.28 (C-2), 54.61 (C-6), 49.10 (C-1), 42.77 (C-4), 32.96 (C-5), 29.70 (C-7). ESI MS, *m/z* (rel%): 281/283 (100/30) [M+H]. For $C_{12}H_{13}ClN_4O_2 \times ½\ H_2O$ (289.7) calcd: C, 49.57; H, 4.87; Cl, 12.24; N, 19.34; found: C, 49.82; H, 4.55; Cl, 12.51; N, 19.19.

5.1.4. 6-Chloro-9-[(1*R,2*R**,4*S**,5*S**,6*S**)-3-oxatricyclo[3.2.1.0^{2,4}] oct-6-yl]-9*H*-purine (23)**

To a solution of unsaturated derivative **8b** (250 mg, 1.01 mmol) in dichloromethane (45 mL) was added *m*-chloroperbenzoic acid (400 mg, 1.5 mmol, 65%) and the solution was stirred at rt overnight. Reaction mixture was washed with saturated aqueous $Na_2S_2O_3$ (20 mL), saturated $NaHCO_3$ (3 × 20 mL), dried over anhydrous sodium sulfate and evaporated. Residue was crystallized from water–methanol to afford 193 mg (73%) of the product **23** as white crystals. Mp 159–163 °C (water–methanol). ^{1}H NMR (600.13 MHz): 8.88 (1H, s, H-8), 8.79 (1H, s, H-2), 4.60 (1H, ddd, *J* 8.0, 4.5, 1.5 Hz, H-6′), 3.38 (1H, dd, *J* 3.8, 1.7 Hz, H-2′), 3.34 (1H, dd, *J* 3.7, 1.4 Hz, H-3′), 2.92–2.86 (1H, m, H-1′), 2.64–2.60 (1H, m, H-4′), 2.14–2.06 (2H, m, H-5′*endo*, H-5′*exo*), 1.38–1.31 (1H, dm, *J* 10.6 Hz, H-7′b), 1.32–1.24 (1H, dm, *J* 10.7 Hz, H-7′a). ^{13}C NMR (150.92 MHz): 152.20 (C-4), 151.53 (C-2), 149.18 (C-6), 145.96 (C-8), 131.38 (C-5), 54.21 (C-6′), 50.83 (C-3′), 48.91 (C-2′), 42.68 (C-1′), 36.88 (C-4′), 33.84 (C-5′), 23.95 (C-7′). ESI MS, *m/z* (rel%): 263/265 (100/31) [M+H]. For $C_{12}H_{11}ClN_4O$ (262.7) calcd: C, 54.87; H, 4.22; Cl, 13.50; N, 21.33; found: C, 54.63; H, 4.07; Cl, 13.79; N, 20.59.

5.1.5. (1*R,2*R**,4*S**)-6-Oxobicyclo[2.2.1]hept-2-yl benzoate (25) and (1*R**,2*S**,4*R**)-5-oxobicyclo[2.2.1]hept-2-yl benzoate (26)**

To a mixture of bicyclo[2.2.1]hept-5-en-2-yl acetate **24** (mixture of *endo*/*exo* isomers, 11.27 g, 74 mmol) in dry THF (15 mL) BH_3–THF complex (1 M solution in THF, 35 mL) at 0 °C was dropwise added. The reaction mixture was stirred under argon for 2 hours at 0 °C. Next, a few drops of water were carefully added followed by a suspension of $NaBO_3 \times 4\ H_2O$ (33 g, 215 mmol) in water (70 mL). The reaction mixture was vigorously stirred overnight at room temperature. Inorganic salts were removed by filtration and the filtrate was extracted with ether (4 × 150 mL). The organic phase was dried over anhydrous sodium sulfate and evaporated (9.55 g). Benzoyl chloride (8 mL, 69 mmol) was added to a stirred and cooled solution hydroborated intermediate in pyridine (100 mL) and the mixture was left at room temperature overnight. Water (3 mL) was then added and, after 15 min, the solvent was evaporated. The residue was partitioned between ethyl acetate (250 mL) and water (200 mL). The organic phase was washed with water (150 mL), 5% hydrochloric acid (120 mL), saturated aqueous $NaHCO_3$ (3 × 150 mL), dried over anhydrous sodium sulfate, and the solvent was evaporated (15.12 g of benzoylated intermediate). This crude intermediate was dissolved in methanol (300 mL) and to this solution K_2CO_3 (2 g, 14.5 mmol). The reaction mixture was stirred for 1.5 hour, neutralized with 5% solution of hydrochloric acid and evaporated. The residue was partitioned between ethyl acetate (300 mL) and water (100 mL). The organic phase was washed with water (100 mL), dried over anhydrous sodium sulfate and evaporated (12.47 g of deacetylated intermediate). A mixture of pyridinium dichromate (30 g, 79.7 mmol), molecular sieves (3A, powdered, 30 g) and dichloromethane (250 mL) was stirred at room temperature for 15 min. A solution of the deacetylated intermediate (12.47 g) in dichloromethane (100 mL) was added to the mixture. After 15 h stirring at room temperature, the mixture was filtered and the filtrates were evaporated. The residue was stirred with ether, the mixture was filtered with a Celite pad and the filtrates were taken down. Chromatography of the residue on silica gel (400 g) in toluene–ethyl acetate (30:1) afforded 5.09 g (30%) of ketobenzoate **25** and 2.76 g (16%) of ketobenzoate **26**, both as colorless oils.

5.1.5.1. (1*R,2*R**,4*S**)-6-Oxobicyclo[2.2.1]hept-2-yl benzoate (25).** ^{1}H NMR (499.95 MHz): 7.99–7.93 (2H, m, H-2′), 7.68–7.64 (1H, m, H-4′), 7.49–7.55 m, 2H (H-3′), 5.03–4.96 (1H, dm, *J* 7.1 Hz, H-2), 2.80–2.76 (2H, m, H-1, H-4), 2.14–2.06 (1H, dm, *J* 17.8 Hz, H-5*exo*), 2.13 (1H, ddd, *J* 13.8, 7.2, 2.4 Hz, H-3*endo*), 2.02–1.96 (1H, dm, *J* 10.5 Hz, H-7b), 1.88–1.81 (3H, m, H-3*exo*, H-5*endo*, H-7a). ^{13}C NMR (125.73 MHz): 214.52 (C-6), 165.33 (COO), 133.70 (C-4′), 129.81 (C-1′), 129.43 (C-2′), 128.97 (C-3′), 72.56 (C-2), 56.69 (C-1), 44.17 (C-5), 37.65 (C-3), 35.01 (C-7), 34.75 (C-4). EI MS, *m/z* (rel%): 230 (11) [M], 202 (52), 105 (100) [Bz], 77 (55). For $C_{14}H_{13}O_3$ (230.3) calcd: C, 73.03; H, 6.13; found: C, 72.69; H, 6.02.

5.1.5.2. (1*R,2*S**,4*R**)-5-Oxobicyclo[2.2.1]hept-2-yl benzoate (26).** ^{1}H NMR (499.95 MHz): 8.02–7.96 (2H, m, H-2′), 7.68–7.64 (1H, m, H-4′), 7.56–7.50 (2H, m, H-3′), 5.09–5.03 (1H, dm, *J* 6.9 Hz, H-2), 2.79–2.74 (1H, dm, *J* 5.2 Hz, H-1), 2.56–2.50 (1H, dm, *J* 4.9 Hz, H-4), 2.13 (1H, ddd, *J* 17.7, 5.2, 1.2 Hz, H-6*exo*), 2.03 (1H, ddd, *J* 14.3, 6.9, 2.4 Hz, H-3*endo*), 1.96–1.89 (2H, m, H-6*endo*, H-7b), 1.85 (1H, dddd, *J* 14.3, 5.0, 2.5, 1.4 Hz, H-3*exo*), 1.81–1.75 (1H, dm, *J* 10.4 Hz, H-7a). ^{13}C NMR (125.73 MHz): 215.00 (C-6), 165.47 (COO), 133.62 (C-4′), 129.98 (C-1′), 129.41 (C-2′), 128.97

(C-3′), 75.50 (C-2), 48.46 (C-4), 40.63 (C-1), 39.40 (C-6), 34.12 (C-7), 33.22 (C-3). EI MS, *m/z* (rel%): 230 (31) [M], 212 (15), 108 (90), 105 (92) [Bz], 77 (100), 66 (93). For $C_{14}H_{13}O_3$ (230.3) calcd: C, 73.03; H, 6.13; found: C, 72.91; H, 6.09.

5.1.6. (1*R**,2*S**,4*S**,6*S**)-6-Hydroxybicyclo[2.2.1]hept-2-yl benzoate (27) and (1*R**,2*S**,4*R**,5*R**)-5-hydroxybicyclo[2.2.1]hept-2-yl benzoate (28)

Ketobenzoate **25** or **26** (5.17 g, 22.45 mmol) was dissolved in methanol (210 mL) and the solution was immersed in an ice-bath. Sodium borohydride (517 mg, 13.7 mmol) was added in small portions during 20 min. The reaction mixture was stirred at 0 °C for 1 h and evaporated, co-distilled with methanol (50 mL) and the residue was partitionated between ethyl acetate (200 mL) and brine (100 mL). The organic phase was washed with brine (100 mL), dried over anhydrous sodium sulfate and evaporated. Residue was chromatographed on a silica gel (350 g) in toluene–ethyl acetate (7:1) to afford alcohol.

5.1.6.1. (1*R,2*S**,4*S**,6*S**)-6-Hydroxybicyclo[2.2.1]hept-2-yl benzoate (27).** Yield 5.05 g (95%), colorless oil. ^{1}H NMR (499.95 MHz): 7.97–7.92 (1H, m, H-2′), 7.67–7.63 (1H, m, H-4′), 7.53–7.49 (2H, m, H-3′), 5.43–5.39 (1H, m, H-2), 4.88 (1H, d, *J* 3.8 Hz, OH), 4.14–4.10 (1H, m, H-6), 2.41 (1H, d, *J* 4.8 Hz, H-1), 2.22 (1H, bt, *J* 4.6, 4.6 Hz, H-4), 1.95 (1H, ddd, *J* 13.3, 7.1, 2.4 Hz, H-3*endo*), 1.82 (1H, dddd, *J* 12.6, 10.2, 4.6, 3.0 Hz, H-5*exo*), 1.62–1.54 (1H, dm, *J* 10.2 Hz, H-7b), 1.55–1.51 (1H, m, H-3*exo*), 1.33–1.27 (1H, dm, *J* 10.1 Hz, H-7a), 0.79 (1H, dt, *J* 12.6, 3.4, 3.4 Hz, H-5*endo*). ^{13}C NMR (125.73 MHz): 165.54 (COO), 133.38 (C-4′), 130.49 (C-1′), 129.27 (C-2′), 128.93 (C-3′), 72.92 (C-2), 68.86 (C-6), 47.87 (C-1), 40.38 (C-3), 38.06 (C-5), 36.31 (C-4), 34.83 (C-7). EI MS, *m/z* (rel%): 232 (37) [M], 188 (26), 166 (11), 123 (60), 110 (35), 105 (100) [Bz], 77 (60). For $C_{14}H_{16}O_3$ (232.3) calcd: C, 72.39; H, 6.94; found: C, 72.12; H, 7.13.

5.1.6.2. (1*R,2*S**,4*R**,5*R**)-5-Hydroxybicyclo[2.2.1]hept-2-yl benzoate (28).** Yield 4.75 g (91%), colorless oil. ^{1}H NMR (499.95 MHz): 7.98–7.92 (1H, m, H-2′), 7.67–7.63 (1H, m, H-4′), 7.55–7.49 (2H, m, H-3′), 4.81–4.75 (1H, dm, *J* 7.1 Hz, H-2), 4.69 (1H, d, *J* 4.1 Hz, OH), 4.03–5.97 (1H, m, H-5), 2.56 (1H, bd, *J* 5.7 Hz, H-1), 2.44 (1H, ddd, *J* 13.6, 7.1, 2.4 Hz, H-3*endo*), 2.22–2.17 (1H, m, H-4), 1.89 (1H, ddd, *J* 13.2, 10.1, 5.5 Hz, H-6*exo*), 1.60–1.55 (1H, dm, *J* 10.2 Hz, H-7b), 1.38–1.30 (1H, dm, *J* 13.6 Hz, H-3*exo*), 1.33–1.27 (1H, dm, *J* 10.1 Hz, H-7a), 0.71 (1H, dt, *J* 13.2, 3.5, 3.5 Hz, H-6*endo*). ^{13}C NMR (125.73 MHz): 165.48 (COO), 133.44 (C-4′), 130.36 (C-1′), 129.28 (C-2′), 128.95 (C-3′), 77.54 (C-2), 69.31 (C-5), 42.05 (C-1), 41.30 (C-4), 34.49 (C-6), 33.87 (C-7), 30.49 (C-3). EI MS, *m/z* (rel%): 232 (10) [M], 230 (8), 166 (15), 110 (90), 105 (100) [Bz], 77 (71). For $C_{14}H_{16}O_3$ (232.3) calcd: C, 72.39; H, 6.94; found: C, 72.08; H, 7.03.

5.1.7. (1*R**,2*S**,4*S**,6*R**)-6-(6-Chloro-9*H*-purin-9-yl)bicyclo[2.2.1]hept-2-yl benzoate (29) and (1*R**,2*S**,4*R**,5*S**)-5-(6-chloro-9*H*-purin-9-yl)bicyclo[2.2.1]hept-2-yl benzoate (30)

To a mixture of the alcohol **27** or **28** (2.52 g, 10.8 mmol), triphenylphosphine (3.7 g, 14.1 mmol), and 6-chloropurine (2.18 g, 14.1 mmol) in THF (50 mL) a solution of the diisopropyl azadicarboxylate (2.8 mL, 14.2 mmol) in THF (30 mL) was dropwise added. The resulting mixture was stirred overnight (TLC control) and then heated to reflux for 3 hours. The reaction mixture was evaporated and the residue was chromatographed on silica gel (200 g) in toluene–ethyl acetate (3:1).

5.1.7.1. (1*R,2*S**,4*S**,6*R**)-6-(6-Chloro-9*H*-purin-9-yl)bicyclo[2.2.1]hept-2-yl benzoate (29).** Yield 2.50 g (62.8%). Mp 162–164 °C (water–ethanol (1:1), white crystals). ^{1}H NMR (499.95 MHz): 8.88 (1H, s, H-8′), 8.80 (1H, s, H-2′), 7.97–7.93 (2H, m, H-2″), 7.67–7.63 (1H, m, H-4″), 7.55–7.49 (2H, m, H-3″), 5.14–5.10 (1H, dm, *J* 7.0 Hz, H-2), 4.73 (1H, bt, *J* 6.5, 6.5 Hz, H-6), 2.86 (1H, bs, H-1), 2.58–2.55 (1H, m, H-4), 2.05–2.07 (2H, m, H-5), 2.00 (1H, ddd, *J* 13.7, 7.1, 2.3 Hz, H-3*endo*), 1.90–1.86 (1H, dm, *J* 10.8 Hz, H-7b), 1.80–1.72 (1H, dm, *J* 10.8 Hz, H-7a), 1.65–1.60 (1H, dm, *J* 13.7 Hz, H-3*exo*). ^{13}C NMR (125.73 MHz): 165.38 (COO), 152.15 (C-4′), 151.53 (C-2′), 149.20 (C-6′), 145.77 (C-8′), 133.60 (C-4″), 131.49 (C-5′), 129.99 (C-1″), 129.34 (C-2″), 128.96 (C-3″), 75.42 (C-2), 54.33 (C-6), 47.52 (C-1), 38.26 (C-3), 36.82 (C-5), 35.19 (C-4), 33.57 (C-7). ESI MS, *m/z* (rel%): 369/371 (100/33) [M+H]. For $C_{19}H_{17}ClN_4O_2$ (368.8) calcd: C, 61.87; H, 4.65; Cl, 9.61; N, 15.19; found: C, 61.54; H, 4.61; Cl, 9.49; N, 14.82.

5.1.7.2. (1*R,2*S**,4*R**,5*S**)-5-(6-Chloro-9*H*-purin-9-yl)bicyclo[2.2.1]hept-2-yl benzoate (30).** Yield 3.44 g (contaminated with hydrazine byproduct), product crystallized water–ethanol (1:1) to afford pure compound as white crystals, 1.92 g (48%). Mp 166–168 °C (water–ethanol (1:1)). ^{1}H NMR (499.95 MHz): 8.90 (1H, s, H-8′), 8.79 (1H, s, H-2′), 8.00–7.94 (2H, m, H-2″), 7.68–7.64 (1H, m, H-4″), 7.56–7.50 (2H, m, H-3″), 4.99–4.90 (1H, dm, *J* 7.1 Hz, H-2), 4.61 (1H, bdd, *J* 8.4, 3.9 Hz, H-5), 2.72 (1H, bd, *J* 5.0 Hz, H-4), 2.61 (1H, bd, *J* 5.0 Hz, H-1), 2.24 (1H, dt, *J* 14.2, 4.5, 4.5 Hz, H-6*exo*), 2.13–2.06 (2H, m, H-3*endo*, H-6*endo*), 1.79–1.68 (3H, m, H-3*exo*, H-7). ^{13}C NMR (125.73 MHz): 165.55 (COO), 152.26 (C-4′), 151.50 (C-2′), 149.19 (C-6′), 145.70 (C-8′), 133.53 (C-4″), 131.53 (C-5′), 130.13 (C-1″), 129.35 (C-2″), 128.95 (C-3″), 76.42 (C-2), 57.27 (C-5), 41.30 and 41.27 (C-1, C-4), 36.56 (C-3), 32.90 (C-7), 31.81 (C-6). ESI MS, *m/z* (rel%): 369/371 (100/33) [M+H]. For $C_{19}H_{17}ClN_4O_2$ (368.8) calcd: C, 61.87; H, 4.65; Cl, 9.61; N, 15.19; found: C, 61.84; H, 4.63; Cl, 9.79; N, 15.14.

5.1.8. (1*R**,2*S**,4*S**,6*R**)-6-(6-Chloro-9*H*-purin-9-yl)bicyclo[2.2.1]heptan-2-ol (31) and (1*R**,2*S**,4*R**,5*S**)-5-(6-chloro-9*H*-purin-9-yl)bicyclo[2.2.1]heptan-2-ol (32)

To a stirred solution of a benzoylated compound **29** or **30** (1.8 g, 4.88 mmol) in THF (55 mL) was added methylmagnesium chloride (7.2 mL, 3 M solution in THF, 21.6 mmol) at 0 °C and the mixture was stirred at 0 °C overnight. Reaction was quenched with diluted AcOH (added until the inorganic precipitated salt dissolved again) and reaction mixture was evaporated and adsorbed on silica gel from minimum volume of water–methanol mixture. This silica gel was placed on the top of the silica gel column (200 g). Chromatography with ethyl acetate→ethyl acetate–toluene–acetone–ethanol (17:4:3:1) afforded product. Compounds were crystallized from appropriate mixture of solvents.

5.1.8.1. (1*R,2*S**,4*S**,6*R**)-6-(6-Chloro-9*H*-purin-9-yl)bicyclo[2.2.1]heptan-2-ol (31).** Yield 750 mg (58%). Mp 155–156 °C (ethyl acetate–hexanes, white crystals). ^{1}H NMR (499.95 MHz): 8.85 (1H, s, H-8′), 8.77 (1H, s, H-2′), 4.84 (1H, d, *J* 3.8 Hz, OH), 4.45 (1H, dd, *J* 8.4, 4.8 Hz, H-6), 3.89–3.86 (1H, m, H-2), 2.43–2.37 (2H, m, H-1, H-4), 1.97–1.93 (1H, m, H-5*exo*), 1.87 (1H, ddd, *J* 13.1, 8.5, 1.4 Hz, H-5*endo*), 1.68 (1H, ddd, *J* 12.9, 7.0, 1.6 Hz, H-3*endo*), 1.67–1.63 (2H, m, H-7), 1.30–1.26 (1H, m, H-3*exo*). ^{13}C NMR (125.73 MHz): 152.13 (C-4′), 151.45 (C-2′), 149.15 (C-6′), 145.69 (C-8′), 131.45 (C-5′), 71.00 (C-2), 54.67 (C-6), 50.25 (C-1), 40.51 (C-3), 37.00 (C-5), 34.96 (C-4), 32.42 (C-7). ESI MS, *m/z* (rel%): 287/289 (40/32) [M+Na], 265/267 (100/30) [M+H]. For $C_{12}H_{13}ClN_4O$ (264.7) calcd: C, 54.45; H, 4.95; Cl, 13.39; N, 21.17; found: C, 54.28; H, 4.89; Cl, 13.73; N, 20.92.

5.1.8.2. (1*R,2*S**,4*R**,5*S**)-5-(6-Chloro-9*H*-purin-9-yl)bicyclo[2.2.1]heptan-2-ol (32).** Yield 930 mg (72%). Mp 202–203 °C (ethyl acetate, white crystals). ^{1}H NMR (600.13 MHz): 8.85 (1H, s, H-8′), 8.75 (1H, s, H-2′), 4.73 (1H, d, *J* 3.7 Hz, OH), 4.42 (1H, ddd,

J 8.5, 3.9, 1.1 Hz, H-5), 3.73–3.69 (1H, m, H-2), 2.54 (1H, bd, *J* 5.0 Hz, H-4), 2.22 (1H, bd, *J* 5.4 Hz, H-1), 2.02 (1H, dddd, *J* 13.9, 3.9, 5.0, 1.0 Hz, H-3*exo*), 1.83 (1H, ddd, *J* 13.9, 8.4, 2.2 Hz, H-6*endo*), 1.78 (1H, ddd, *J* 13.3, 6.8, 2.4 Hz, H-3*endo*), 1.68–1.62 (1H, dm, *J* 10.5 Hz, H-7b), 1.58–1.52 (1H, dm, *J* 10.4 Hz, H-7a), 1.31 (1H, dddd, *J* 13.3, 5.0, 2.4, 1.0 Hz, H-3*exo*). ^{13}C NMR (150.92 MHz): 152.22 (C-4′), 151.43 (C-2′), 149.11 (C-6′), 145.60 (C-8′), 131.46 (C-5′), 71.84 (C-2), 57.65 (C-5), 43.73 (C-1), 41.22 (C-4), 38.98 (C-3), 32.34 (C-6), 31.84 (C-7). ESI MS, *m/z* (rel%): 288 (100), 265/267 (44/14) [M+H]. For $C_{12}H_{13}ClN_4O$ (264.7) calcd: C, 54.45; H, 4.95; Cl, 13.39; N, 21.17; found: C, 54.75; H, 4.91; Cl, 13.19; N, 21.00.

5.1.9. (1*R**,4*S**,6*R**)-6-(6-Chloro-9*H*-purin-9-yl)bicyclo[2.2.1]heptan-2-one (33) and (1*R**,4*R**,5*S**)-5-(6-chloro-9*H*-purin-9-yl)bicyclo[2.2.1]heptan-2-one (34)

A mixture of PDC (3.28 g, 8.6 mmol), molecular sieves (3A, powder, 5 g) and dichloromethane (50 mL) was stirred at room temperature for 15 min. A solution of alcohol **31** or **32** (1.15 g, 4.34 mmol) in a mixture of dichloromethane (50 mL) and chloroform (20 mL) was added to the reaction mixture. After 5 days of stirring at room temperature, the mixture was filtered and the filtrates were evaporated. The residue was chromatographed on silica gel (200 g).

5.1.9.1. (1*R,4*S**,6*R**)-6-(6-Chloro-9*H*-purin-9-yl)bicyclo[2.2.1]heptan-2-one (33).** Yield 1.01 g (88%). Mobile phase: toluene–ethyl acetate (3:2). Mp 181.5–182.5 °C (ethyl acetate, white powder). ^{1}H NMR (499.95 MHz): 8.94 (1H, s, H-8′), 8.79 (1H, s, H-2′), 4.88 (1H, bdd, *J* 8.5, 4.4 Hz, H-6), 2.92 (1H, bs, H-1), 2.90–2.86 (1H, m, H-4), 2.46–2.42 (1H, m, H-5*exo*), 2.33 (1H, ddd, *J* 13.6, 8.5, 2.2 Hz, H-5*endo*), 2.21–2.16 (2H, m, H-3*exo*, H-7b), 2.00 (1H, dd, *J* 17.9, 4.4 Hz, H-3*endo*), 1.91–1.83 (1H, dm, *J* 11.1 Hz, H-7a). ^{13}C NMR (125.73 MHz): 214.26 (C-2), 152.16 (C-4′), 151.64 (C-2′), 149.24 (C-6′), 145.86 (C-8′), 131.54 (C-5′), 56.36 (C-1), 53.27 (C-6), 43.84 (C-3), 35.67 (C-5), 35.41 (C-4), 35.40 (C-7). ESI MS, *m/z* (rel%): 263/265 (100/34) [M+H]. For $C_{12}H_{11}ClN_4O$ (262.7) calcd: C, 54.87; H, 4.22; Cl, 13.50; N, 21.33; found: C, 54.55; H, 4.03; Cl, 13.90; N, 20.94.

5.1.9.2. (1*R,4*R**,5*S**)-5-(6-Chloro-9*H*-purin-9-yl)bicyclo[2.2.1]heptan-2-one (34).** Yield 1.05 g (92%). Mobile phase: toluene–ethyl acetate (4:1). Mp 153–154 °C (ethyl acetate, white powder). ^{1}H NMR (499.95 MHz): 8.95 (1H, s, H-8′), 8.80 (1H, s, H-2′), 4.89 (1H, ddd, *J* 8.3, 4.3, 1.2, H-5), 3.02 (1H, bd, *J* 5.4 Hz, H-4), 2.66 (1H, bd, *J* 4.9 Hz, H-1), 2.48–2.44 (1H, m, H-6*exo*), 2.29–2.25 (1H, m, H-3*exo*), 2.22 (1H, ddd, *J* 14.1, 8.4, 2.3 Hz, H-6*endo*), 2.13–2.07 (2H, m, H-3*endo*, H-7b), 1.89–1.81 (1H, dm, *J* 12.1 Hz, H-7a). ^{13}C NMR (125.73 MHz): 214.85 (C-2), 152.28 (C-4′), 151.57 (C-2′), 149.23 (C-6′), 145.86 (C-8′), 131.55 (C-5′), 56.57 (C-5), 49.29 (C-1), 42.19 (C-3), 41.31 (C-4), 34.70 (C-7), 31.20 (C-6). ESI MS, *m/z* (rel%): 263/265 (100/32) [M+H], 437 (82). For $C_{12}H_{11}ClN_4O$ (262.7) calcd: C, 54.87; H, 4.22; Cl, 13.50; N, 21.33; found: C, 54.95; H, 4.23; Cl, 13.50; N, 21.00.

5.1.10. 6-Chloro-9-[(1*R**,2*R**,4*S**)-6,6-difluorobicyclo[2.2.1]hept-2-yl]-9*H*-purine (35) and 6-chloro-9-[(1*R**,2*S**,4*R**)-5,5-difluorobicyclo[2.2.1]hept-2-yl]-9*H*-purine (36)

To a stirred mixture of the ketone **33** or **34** (255 mg, 0.97 mmol) in dichloromethane (10 mL) and pyridine (1.2 mL), DAST (1.9 mL, 14.4 mmol) was added at room temperature under argon. The reaction mixture was refluxed under argon for 30 hours. After cooling to room temperature, the reaction mixture was poured onto a saturated solution of $NaHCO_3$ + ice (50 mL). The water phase was extracted with ethyl acetate (100 mL). The organic phase was washed with saturated $NaHCO_3$ (50 mL) and water (50 mL), dried over anhydrous sodium sulfate and evaporated. Residue was chromatographed on silica gel (150 g).

5.1.10.1. 6-Chloro-9-[(1*R,2*R**,4*S**)-6,6-difluorobicyclo[2.2.1]hept-2-yl]-9*H*-purine (35).** Yield 152 mg (55%). Mobile phase: toluene–ethyl acetate (7:1→4:1). Mp 156–158 °C (water–methanol, white solid). ^{1}H NMR (499.95 MHz): 8.93 (1H, s, H-8), 8.80 (1H, s, H-2), 5.06–5.00 (1H, m, H-2′), 2.96 (1H, d, *J*(1′,F) 8.0 Hz, H-1′), 2.66–2.60 (1H, m, H-4′), 2.32–2.22 (2H, m, H-3′), 2.08 m, 1H (H-5′*exo*), 2.03–1.98 (1H, m, H-7′b), 1.89–1.83 (1H, m, H-5′*endo*), 1.78–1.70 (1H, dm, *J* 11.5 Hz, H-7′a). ^{13}C NMR (125.73 MHz): 152.25 (C-4), 151.66 (C-2), 149.21 (C-6), 145.87 (C-8), 131.46 (C-5), 130.38 (dd, *J*(6′,F) 256.4 and 252.9 Hz, C-6′), 51.42 (t, *J*(2′,F) 8.0 Hz, C-2′), 50.69 (dd, *J*(1′,F) 19.4 and 25.6 Hz, C-1′), 41.35 (dd, *J*(5′,F) 21.9 and 23.5 Hz, C-5′), 35.83 (m, C-3′, C-4′), 34.80 (d, *J*(7′,F) 4.0 Hz, C-7′). ESI MS, *m/z* (rel%): 285/287 (100/33) [M+H]. For $C_{12}H_{11}ClF_2N_4$ (284.7) calcd: C, 50.63; H, 3.89; Cl, 12.45; F, 13.35; N, 19.68; found: C, 50.87; H, 3.85; N, 19.51.

5.1.10.2. 6-Chloro-9-[(1*R,2*S**,4*R**)-5,5-difluorobicyclo[2.2.1]hept- 2-yl]-9*H*-purine (36).** Yield 124 mg (45%). Mobile phase: toluene–ethyl acetate (7:1→4:1). Mp 163–164 °C (water–methanol, white crystals). ^{1}H NMR (499.95 MHz): 8.90 (1H, s, H-8), 8.79 (1H, s, H-2), 4.78 (1H, ddd, *J* 8.2, 4.5, 1.2 Hz, H-2′), 2.81 (1H, bd, *J* 4.6 Hz, H-1′), 2.71–2.65 (1H, m, H-4′), 2.37–2.13 (3H, m, H-3′*exo*, 3′*endo*, H-6′*exo*), 2.02–1.92 (2H, m, H-6′*endo*, H-7′b), 1.77–1.69 (1H, dm, *J* 11.1 Hz, H-7′a). ^{13}C NMR (125.73 MHz): 152.22 (C-4),151.54 (C-2), 149.23 (C-6), 145.83 (C-8), 131.55 (C-5), 130.76 (dd, *J*(5′,F) 254.4 and 251.6 Hz, C-5′), 56.51 (d, *J*(2′,F) 1.4 Hz (C-2′), 44.40 (dd, *J*(4′,F) 24.8 and 21.0 (C-4′), 41.72 (t, *J*(1′,F) 3.2 Hz, C-1′), 40.45 (t, *J*(6′,F) 24.1 Hz, C-6′), 34.34 (d, *J*(7′,F) 4.9 Hz, C-7′), 28.89 (dd, *J*(3′,F) 6.8 and 5.3 Hz, C-3′). ESI MS, *m/z* (rel%): 285/287 (100/35) [M+H]. For $C_{12}H_{11}ClF_2N_4$ (284.7) calcd: C, 50.63; H, 3.89; Cl, 12.45; F, 13.35; N, 19.68; found: C, 50.67; H, 3.99; N, 19.31.

5.1.11. (1*R**,2*R**,4*S**,6*R**)-6-(6-Chloro-9*H*-purin-9-yl)bicyclo[2.2.1]heptan-2-ol (37) and (1*R**,2*R**,4*R**,5*S**)-5-(6-chloro-9*H*-purin-9-yl)bicyclo[2.2.1]heptan-2-ol (38)

Ketoderivative **33** or **34** (440 mg, 1.67 mmol) was dissolved in methanol (40 mL) and the solution was cooled to 0 °C, when sodium borohydride (43 mg, 1.14 mmol) was added. The reaction mixture was stirred at 0 °C for 1 hour and evaporated, co-distilled with methanol (50 mL) and evaporated. The residue was chromatographed on silica gel (120 g) in ethyl acetate→ethyl acetate–toluene–acetone–ethanol (17:4:3:1) to afford *endo*-alcohol. A small amount of the *exo*-alcohols **31** (17%) or **32** (20%) was obtained from the reaction mixture as well.

5.1.11.1. (1*R,2*R**,4*S**,6*R**)-6-(6-Chloro-9*H*-purin-9-yl)bicyclo-[2.2.1]heptan-2-ol (37).** Yield 293 mg (66%). Mp 156–157 °C (ether–hexanes, white crystals). ^{1}H NMR (499.84 MHz): 8.86 (1H, s, H-8′), 8.78 (1H, s, H-2′), 5.30 (1H, bdd, *J* 8.8, 4.5 Hz, H-6), 5.10 (1H, d, *J* 3.8 Hz, OH), 4.21–4.16 (1H, m, H-2), 2.57–2.51 (1H, m, H-1), 2.36–2.32 (1H, m, H-4), 2.12 (1H, ddd, *J* 12.8, 8.7, 2.3 Hz, H-5*endo*), 1.99–1.92 (1H, m, H-5*exo*), 1.94–1.88 (1H, m, H-3*endo*), 1.89–1.81 (1H, dm, *J* 10.7 Hz, H-7b), 1.46–1.37 (1H, dm, *J* 10.7 Hz, H-7a), 0.88 (1H, dt, *J* 12.6, 3.5, 3.5 Hz, H-3*exo*). ^{13}C NMR (125.70 MHz): 152.17 (C-4′), 151.50 (C-2′), 149.13 (C-6′), 146.04 (C-8′), 131.29 (C-5′), 69.49 (C-2), 50.94 (C-6), 48.50 (C-1), 39.57 (C-5), 37.59 (C-3), 36.99 (C-4), 35.28 (C-7). ESI MS, *m/z* (rel%): 265/267 (100/33) [M+H]. For $C_{12}H_{13}ClN_4O$ (264.7) calcd: C, 54.45; H, 4.95; Cl, 13.39; N, 21.17; found: C, 54.65; H, 5.15; Cl, 13.67; N, 20.87.

5.1.11.2. (1*R,2*R**,4*R**,5*S**)-5-(6-Chloro-9*H*-purin-9-yl)bicyclo-[2.2.1]heptan-2-ol (38).** Yield 280 mg (63%). Mp 172.5–174 °C (ethyl acetate, white crystals). ^{1}H NMR (600.13 MHz): 8.87 (1H, s, H-8′), 8.77 (1H, s, H-2′), 4.83 (1H, d, *J* 4.0 Hz, OH), 4.58 (1H, ddd,

J 8.6, 4.2, 1.2 Hz, H-5), 4.12–4.06 (1H, m, H-2), 2.63 (1H, ddd, *J* 13.4, 8.6, 2.4 Hz, H-6*endo*), 2.44 (1H, bd, *J* 5.1 Hz, H-4), 2.35–2.30 (1H, m, H-1), 1.98 (1H, ddd, *J* 13.1, 10.1, 5.2 Hz, H-3*exo*), 1.91 (1H, dtt, *J* 13.5, 4.2, 4.2, 1.1, 1.1 Hz, H-6*exo*), 1.75–1.67 (1H, dm, *J* 10.7 Hz, H-7b), 1.41–1.33 (1H, dm, *J* 10.7 Hz, H-7a), 0.95 (1H, dt, *J* 13.1, 3.5, 3.5 Hz, H-3*endo*). ^{13}C NMR (150.92 MHz): 152.15 (C-4′), 151.49 (C-2′), 149.14 (C-6′), 145.70 (C-8′), 131.45 (C-5′), 69.39 (C-2), 58.28 (C-5), 42.92 (C-4), 42.14 (C-1), 37.44 (C-3), 34.58 (C-7), 28.93 (C-6). ESI MS, *m/z* (rel%): 265/267 (100/33) [M+H]. For $C_{12}H_{13}ClN_4O$ (264.7) calcd: C, 54.45; H, 4.95; Cl, 13.39; N, 21.17; found: C, 54.41; H, 4.78; Cl, 13.20; N, 21.05.

5.1.12. 6-Chloro-9-[(1*S**,2*S**,4*R**,6*R**)-6-fluorobicyclo[2.2.1]hept-2-yl]-9*H*-purine (39) and 6-chloro-9-[(1*R**,2*S**,4*R**,5*S**)-5-fluorobicyclo[2.2.1]hept-2-yl]-9*H*-purine (40)

To a stirred mixture of *endo*-alcohol **37** or **38** (300 mg, 1.13 mmol) in dichloromethane (10 mL) and pyridine (1.2 mL), DAST (0.7 mL, 5.3 mmol) was added at room temperature under argon. The reaction mixture was refluxed under argon for 12 hours. After cooling to room temperature., the reaction mixture was poured onto saturated solution of $NaHCO_3$ + ice (50 mL).The water phase was extracted with ethyl acetate (100 mL). The organic phase was washed with saturated $NaHCO_3$ (50 mL) and water (50 mL), dried over anhydrous sodium sulfate and evaporated. Residue was chromatographed on silica gel (150 g) in toluene–ethyl acetate (2:1) to afford product.

5.1.12.1. 6-Chloro-9-[(1*S**,2*S**,4*R**,6*R**)-6-fluorobicyclo[2.2.1]hept-2-yl]-9*H*-purine (39).

Yield 219 mg (73%). Mp 127.5–129 °C (water–methanol, white crystals). ^{1}H NMR (499.95 MHz): 8.87 (1H, s, H-8), 8.79 (1H, s, H-2), 4.98 (1H, ddm, *J*(6′,F) 54.5, 6.4 Hz, H-6′), 4.52 (1H, dd, *J* 8.3, 5.0 Hz, H-2′), 2.85 (1H, bd, *J*(1′,F) 8.8 Hz, H-1′), 2.54–2.49 (1H, m, H-4′), 2.02–1.98 (1H, m, H-3′*exo*), 1.93 (1H, ddd, *J* 13.0, 8.3, 2.2 Hz, H-3′*endo*), 1.88–1.84 (1H, m, H-5′*endo*), 1.87–1.81 (1H, m, H-7′b), 1.64–1.59 (1H, m, H-7′a), 1.63–1.57 (1H, m, H-5′*exo*). ^{13}C NMR (125.73 MHz): 152.15 (C-4), 151.51 (C-2), 149.19 (C-6), 145.79 (C-8), 131.49 (C-5), 93.71 (d, *J*(6′,F) 181.6 Hz, C-6′), 52.85 (d, *J*(2′,F) 14.5 Hz, C-2′), 48.11 (d, *J*(1′,F) 21.9 Hz, C-1′), 38.45 (d, *J*(5′,F) 19.7 Hz, C-5′), 36.54 (C-3′), 34.74 (C-4′), 32.90 (C-7′). ESI MS, *m/z* (rel%): 267/269 (76/23) [M+H], 288 (100). For $C_{12}H_{12}ClFN_4$ (266.7) calcd: C, 54.04; H, 4.54; Cl, 13.29; F, 7.12; N, 21.01; found: C, 53.92; H, 4.31; N, 20.85.

5.1.12.2. 6-Chloro-9-[(1*R**,2*S**,4*R**,5*S**)-5-fluorobicyclo[2.2.1]hept-2-yl]-9*H*-purine (40).

Yield 190 mg (63%). Mp 111–112 °C (water–methanol, white crystals). ^{1}H NMR (499.95 MHz): 8.87 (1H, s, H-8), 8.77 (1H, s, H-2), 4.81 (1H, ddm, *J*(5′,F) 56.0, 6.3 Hz, H-5′), 4.47 (1H, ddm, *J* 8.5, 3.8 Hz, H-2′), 2.69 (1H, bd, *J* 5.0 Hz, H-1′), 2.61–2.56 (1H, m, H-4′), 2.18–2.13 (1H m, H-3′*exo*), 1.99 (1H, ddd, *J*(6′en,F) 18.2, 14.4, 6.3, 2.7 Hz, H-6′*endo*), 1.88 (1H, ddm, *J* 14.4, 8.3 Hz, H-3′*endo*), 1.76–1.68 (1H, dm, *J* 11.0 Hz, H-7′b), 1.64 (1H, ddt, *J*(6′ex,F) 39.1, 14.4, 5.1, 1.5, 1.5 (H-6′*exo*), 1.63–1.57 (1H, m, H-7′a). ^{13}C NMR (125.73 MHz): 152.26 (C-4), 151.46 (C-2), 149.17 (C-6), 145.68 (C-8), 131.50 (C-5), 94.46 (d, *J*(5′,F) 180.8 Hz, C-5′), 57.03 (C-2′), 41.74 (d, *J*(4′,F) 20.7 Hz, C-4′), 40.75 (C-1′), 36.94 (d, *J*(6′,F) 20.7 Hz, C-6′), 32.22 (C-7′), 29.96 (d, *J*(3′,F) 11.5 Hz, C-3′). ESI MS, *m/z* (rel%): 267/269 (100/33) [M+H]. For $C_{12}H_{12}ClFN_4$ (266.7) calcd: C, 54.04; H, 4.54; Cl, 13.29; F, 7.12; N, 21.01; found: C, 53.91; H, 4.31; N, 20.71.

5.1.13. 9,9′-(2*R**,6*S**)-Bicyclo[2.2.1]heptane-2,6-diylbis(6-chloro-9*H*-purine) (41)

A solution of the diisopropyl azadicarboxylate (0.4 mL, 1.96 mmol) in THF (10 mL) was added dropwise to a mixture of the hydroxyderivative **37** (400 mg, 1.51 mmol), triphenylphosphine (515 mg, 1.96 mmol) and 6-chloropurine (267 mg, 1.66 mmol) in THF (30 mL). The resulting mixture was stirred overnight and then heated to reflux for 10 hours. The reaction mixture was evaporated and the residue was chromatographed on silica gel column (200 g) in ethyl acetate→ethyl acetate–toluene–acetone–ethanol (17:4:3:1). 303 mg (50%) of the product **41** was obtained. Compound was crystallized from water–methanol. Mp 272–274 °C (decomposition, white powder). ^{1}H NMR (499.84 MHz): 8.89 (2H, s, H-8), 8.80 (2H, s, H-2), 4.93 (2H, dd, *J* 8.0, 4.5 Hz, H-2′, H-6′), 3.14 (1H, bs, H-1′), 2.66 (1H, m, H-4′), 2.24–2.14 (4H, m, H-3′, H-5′), 1.92 (2H, bs, H-7′). ^{13}C NMR (125.70 MHz): 152.17 (C-4), 151.62 (C-2), 149.26 (C-6), 145.67 (C-8), 131.48 (C-5), 56.29 (C-2′ and C-6′), 47.72 (C-1′), 36.85 (C-3′ and C-5′), 36.01 (C-4′), 34.12 (C-7′). ESI MS, *m/z* (rel%): 401/403 (100/60) [M+H]. For $C_{17}H_{14}Cl_2N_8 \times \frac{3}{4}\ H_2O$ (414.8) calcd: C, 48.01; H, 3.95; Cl, 16.67; N, 26.35; found: C, 48.01; H, 3.95; Cl, 16.95; N, 26.15.

5.1.14. 9,9′-(1*R**,2*S**,4*R**,5*S**)-Bicyclo[2.2.1]heptane-2,5-diylbis-(6-chloro-9*H*-purine) (42)

A solution of the diisopropyl azadicarboxylate (0.4 mL, 1.96 mmol) in THF (10 mL)was dropwise added to a mixture of the hydroxyderivative **38** (400 mg, 1.51 mmol), triphenylphosphine (515 mg, 1.96 mmol) and 6-chloropurine (267 mg, 1.66 mmol) in THF (30 mL). The resulting mixture was stirred overnight and then heated to reflux for 10 hours. The reaction mixture was evaporated to the one half and the solid product was filtered off, washed with THF, methanol and water. 150 mg (25%) of the product **42** was obtained. The filtrates were evaporated and the residue was chromatographed on silica gel column (200 g) in ethyl acetate→ethyl acetate–toluene–acetone–ethanol (17:4:3:1). 180 mg (30%) of second crop of the product **42** was obtained. Mp >300 °C (THF, white powder). ^{1}H NMR (499.95 MHz): 8.92 (2H, s, H-8), 8.81 (2H, s, H-2), 4.78 (2H, dd, *J* 7.3, 5.6 Hz, H-2′, H-5′), 2.87–2.83 (2H, m, H-1′, H-4′), 2.32–2.30 (4H, m, H-3′, H-6′), 1.89 (2H, bs, H-7′). ^{13}C NMR (125.73 MHz): 152.27 (C-4), 151.52 (C-2), 149.20 (C-6), 145.77 (C-8), 131.54 (C-5), 57.33 (C-2′ and C-5′), 42.14 (C-1′ and C-4′), 34.76 (C-3′ and C-6′), 33.66 (C-7′). ESI MS, *m/z* (rel%): 401/403 (94/56) [M+H]. For $C_{17}H_{14}Cl_2N_8$ (401.3) calcd: C, 50.89; H, 3.52; Cl, 17.67; N, 27.93; found: C, 50.86; H, 3.38; Cl, 17.99; N, 27.62.

5.1.15. 9,9′-(1*R**,2*S**,3*S**,4*S**)-Bicyclo[2.2.1]hept-5-ene-2,3-diylbis-(6-chloro-9*H*-purine) (45)

A mixture of (1*R**,2*S**,3*S**,4*S**)-bicyclo[2.2.1]hept-5-ene-2,3-diamine[13] **43** (750 mg, 6 mmol), 4,6-dichloropyrimidin-5-amine (3.94 g, 24 mmol), and triethylamine (3.6 mL) in ethanol (9 mL) was heated in a pressure vessel at 105 °C for 10 days and, after cooling, was evaporated. The residue was chromatographed on a column of silica gel (200 g). The pyrimidine intermediate was eluted with toluene–ethyl acetate (4:1) and this intermediate was immediately used in the next step. Concentrated hydrochloric acid (2 mL) was added to a suspension of pyrimidine intermediate in triethyl orthoformate (160 mL) and THF (30 mL) and the reaction mixture was vigorously stirred for 3 days at room temperature. Reaction mixture was evaporated and residue was chromatographed on silica gel column in ethyl acetate to afford 450 mg (19%) of the product **45**. Mp 219–220.5 °C (water–methanol, white powder). ^{1}H NMR (499.84 MHz): 9.01 (1H, s, H-8), 8.80 (1H, s, H-8″), 8.63 (1H, s, H-2″), 8.55 (1H, s, H-2), 6.70 (1H, dd, *J* 5.6, 3.3 Hz, H-6′), 6.30 (1H, dd, *J* 5.7, 2.8 Hz, H-5′), 5.75 (1H, t, *J* 3.7, 3.7 Hz, H-3′), 5.15 (1H, dd, *J* 3.8, 2.2 Hz, H-2′), 3.65–3.62 (1H, m, H-1′), 3.57–3.55 (1H, m, H-4′), 2.51–2.47 (1H, m, H-7′b), 1.92–1.86 (1H, dm, *J* 9.9 Hz, H-7′a). ^{13}C NMR (125.70 MHz): 152.68 and 152.63 (C-4 and C-4″), 151.58 and 151.48 (C-2 and C-2″), 149.22 (C-6 and C-6″), 146.19 (C-8), 145.68 (C-8″), 138.28 (C-6′), 136.05 (C-5′), 131.55 (C-5), 131.09 (C-5″), 61.15 (C-2′), 60.91 (C-3′), 47.86 (C-1′), 46.72 (C-4′), 46.25 (C-7′). ESI MS, *m/z* (rel%):

399/401 (100/63) [M+H]. For $C_{17}H_{12}Cl_2N_8$ (399.2) calcd: C, 51.14; H, 3.03; Cl, 17.76; N, 28.07; found: C, 51.02; H, 2.86; Cl, 17.81; N, 27.85.

5.1.16. 9,9′-(1*R**,2*S**,3*S**,4*S**)-Bicyclo[2.2.1]heptane-2,3-diylbis(6-chloro-9*H*-purine) (46)

A mixture of (1*R**,2*S**,3*S**,4*S**)-bicyclo[2.2.1]heptane-2,3-diamine[14] **44** (750 mg, 6 mmol), 4,6-dichloropyrimidin-5-amine (3.94 g, 24 mmol), and triethylamine (3.6 mL) in ethanol (9 mL) was heated in a pressure vessel at 105 °C for 10 days and, after cooling, was evaporated. The residue was chromatographed on a column of silica gel (200 g). Pyrimidine intermediate was eluted with toluene–ethyl acetate (4:1) and this intermediate was immediately used in the next step. Concentrated hydrochloric acid (2 mL) was added to a suspension of pyrimidine intermediate in triethyl orthoformate (160 mL) and THF (30 mL) and the reaction mixture was vigorously stirred for 3 days at room temperature. The reaction mixture was evaporated and residue was chromatographed on silica gel column in ethyl acetate to afford 490 mg (20%) of the product **46**. Mp 191–192.5 °C (water–methanol, white powder). ^{1}H NMR (499.95 MHz): 9.03 (1H, s, H-8), 8.95 (1H, s, H-8″), 8.69 (1H, s, H-2), 8.56 (1H, s, H-2″), 5.53 (1H, dd, *J* 5.0, 1.9 Hz, H-3′), 5.45–5.41 (1H, m, H-2′), 3.00–2.96 (2H, m, H-1′, H-4′), 2.45–2.39 (1H, dm, *J* 10.9 Hz, H-7′b), 1.97–1.93 (1H, m, H-5′*endo*), 1.83–1.79 (1H, m, H-5′*exo*), 1.69–1.63 (1H, dm, *J* 10.9 Hz, H-7′a), 1.56–1.52 (1H, m, H-6′*exo*), 1.28–1.24 (1H, m, H-6′*endo*). ^{13}C NMR (125.73 MHz): 152.75 (C-4), 152.25 (C-4″), 151.51 and 151.42 (C-2 and C-2″), 149.31 (C-6), 149.14 (C-6″), 146.92 (C-8), 145.81 (C-8″), 131.42 and 131.36 (C-5 and C-5″), 64.94 (C-2′), 60.19 (C-3′), 42.25 and 42.02 (C-1′ and C-4′), 35.97 (C-7′), 27.33 (C-5′), 21.28 (C-6′). ESI MS, *m/z* (rel%): 401/403 (100/65) [M+H]. For $C_{17}H_{14}Cl_2N_8$ (401.3) calcd: C, 50.89; H, 3.52; Cl, 17.67; N, 27.93; found: C, 50.68; H, 3.50; Cl, 17.87; N, 27.59.

5.2. Antiviral assay

5.2.1. Anti-coxsackievirus assay

The antiviral activity of the selected compounds was determined using an MTS-based cytopathic effect (CPE) reduction assay and was expressed as the 50% effective concentration (EC_{50}), being the compound concentration that inhibits virus-induced cytopathic effect formation by 50%. Cells, grown to confluency in 96-well plates, were infected with 100 $CCID_{50}$ of virus, one $CCID_{50}$ being the 50% cell culture infective dose. After an adsorption period of two hours at 37 °C, virus was removed and serial dilutions of the compounds were added. The cultures were further incubated at 37 °C for 3 days, until complete CPE was observed in the infected and untreated virus control (VC). After removal of the medium, 90 μL medium and 10 μL MTS/PMS (Promega, Leiden, The Netherlands) were added to each well. After an incubation period of 2 hours at 37 °C the optical density of each well was read at 498 nm in a microplate reader. CPE values were calculated as follows: $\% \text{ CPE} = 100^{*}[OD_{CC} - OD_{CVB3+Compound}]/[OD_{CC} - OD_{VC}]$. In these formulae, OD_{CC} corresponds to the optical density of the uninfected and untreated cell cultures, OD_{VC} represents the infected and untreated cell cultures and $OD_{CVB3+Compound}$ are CVB3-infected cell cultures, treated with a given concentration of compound.

5.2.2. Cytostatic activity assays

The cytotoxicity of the compounds was evaluated by the MTS-method and the 50% cytotoxic concentration (CC_{50}) was calculated. Briefly, the same experimental set-up was used as for the antiviral assay, but for cytotoxicity determination, uninfected cultures were incubated with serial dilution of compound for three days at 37 °C. The cytotoxic activity was calculated using the following formula: $\% \text{ CPE} = 100^{*}[OD_{CC} - OD_{Compound}]/OD_{CC}$, where OD_{CC} corresponds to the optical density of the uninfected and untreated cell cultures and $OD_{Compound}$ are uninfected cultures, treated with compound.

Acknowledgments

This work is a part of the research project Z4 055 0506. It was supported by the 'Centre for New Antivirals and Antineoplastics' (Ministry of Education, Youth and Sports of the Czech Republic, 1M0508) and by Gilead Sciences, Inc. (Foster City, CA, U.S.A.). Armando M. De Palma is a postdoctoral fellow of the FWO-Vlaanderen. The author (M.S.) would like to thank to Ms. Eliška Procházková for her crucial role in preparing of the manuscript.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2010.04.081.

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