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Design and synthesis of new *C*-nucleosides as potential adenosine deaminase inhibitors

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ABSTRACT

A number of new pyrazolo[3,4-*c*] and [4,3-*b*]pyridine *C*-nucleosides, which can be viewed as 4- or 6-deazaformycin analogues were synthesized and examined as potential adenosine deaminase (ADA) inhibitors. The compounds were prepared through the condensation of a suitably substituted, lithiated 2- or 4-methylpyridine with tri-*O*-benzyl-D-ribonolactone, followed by borohydride reduction of the resulting hemiacetals, intramolecular Mitsunobu cyclisation of the derived diols, formation of the pyrazolopyridine ring system and subsequent removal of the protecting groups. These derivatives were designed on the structural basis provided by docking simulations performed within the enzyme catalytic site, however they demonstrated weak ADA inhibitory activity. Theoretical calculations assisted in the interpretation of the obtained biological data, thus providing guidance for rational structural modifications within this molecular scaffold.

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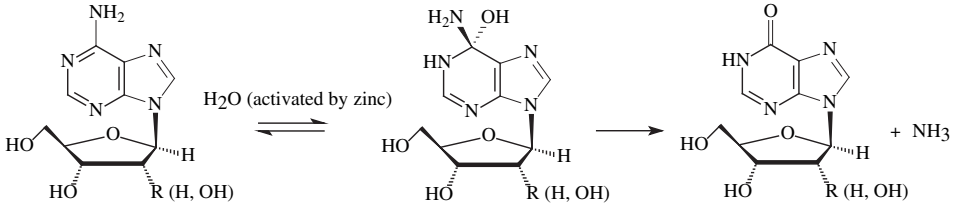
1. Introduction

Adenosine deaminase (ADA), a monomeric, 41KDa zinc metalloenzyme, plays a key role in purine metabolism and catalyzes the irreversible hydrolytic degradation of both adenosine and 2′-deoxyadenosine to the corresponding inosine nucleosides and ammonia, probably via a tetrahedral high energy complex intermediate (Scheme 1).[1] The rate of the deamination reaction is accelerated by approximately 12 orders of magnitude, in the presence of the enzyme.[2]

ADA is ubiquitous to almost all human tissues, regulating both intra- and extra-cellular adenosine concentrations, and consequently, a decrease or increase in its levels triggers several pathological conditions. For instance, genetic deficiency of ADA results in a severe combined immunodeficiency disease (SCID) caused by the accumulation of lymphotoxic adenosine and 2′-deoxyadenosine in B- and T-cells.[3] On the other hand, ADA is an essential enzyme for vital homeostasis, as adenosine plays a crucial role in the differentiation and maturation of the immune system and, additionally it is considered as an important factor in the attenuation of inflammation, signalled through the A2a adenosine receptor.[4] Therefore, immunosuppressive ADA inhibitors have already been proposed for the treatment of lymphoproliferative malignancies, they demonstrate therapeutic potential for the treatment of



Scheme 1. Deamination of heteroaromatic compounds via an unstable hydrated intermediate.

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cerebral and myocardial ischemia,[5] and could also serve as novel anti-inflammatory drugs, presenting limited side effects.[6] Furthermore, it is known that ADA inhibitors can prevent the metabolic breakdown, and thus exhibit synergism with adenosine-based antiviral and antitumour drugs.[7] A number of ADA inhibitors with various degrees of potency have been reported.[8] They are generally grouped into two main classes designated as 'transition-state inhibitors', and 'ground-state inhibitors'. Current 'transition-state inhibitors', including pentostatin, the only ADA inhibitor in clinical use, suffer from poor pharmacokinetics, and moreover they bind to the enzyme so tightly that their activity is nearly irreversible, giving rise to many toxic effects.[9] On the other hand, the usually rapid metabolism of 'ground-state inhibitors' allows a fast recovery of the ADA catalytic activity, which eventually leads to poor therapeutic results.[10] In recent years research efforts are mainly focused on the better understanding of ADA-ligand interactions[11] and the design of new derivatives presenting improved metabolic and pharmacokinetic profiles.[8b,10]

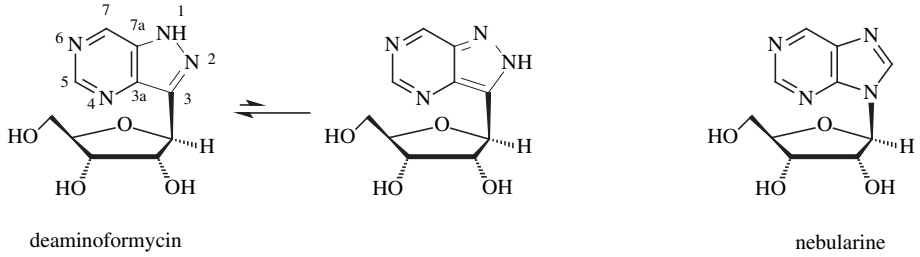
As a continuation of an ongoing medicinal chemistry project towards the design and synthesis of new metabolically stable *C*-nucleoside antibiotics,[12] we have focused our efforts on the exploitation of the deazaformycin nucleoside scaffold for the development of potential ADA inhibitors, using deaminoformycin as lead compound (Scheme 2). This *C*-nucleoside is one of the most promising ADA inhibitors, endowed with a fairly good inhibitory activity (IC_{50} 5 μM), demonstrating an 18-fold affinity improvement for the enzyme, compared to the structurally related analogue nebularine.[13]

In this respect and in conjunction with molecular modelling studies, we have planned the replacement of the pyrimidine heterocyclic core of deaminoformycin by pyridine, in order to evaluate the chemical and biological properties of the resulting new pyrazolo[3,4-*c*] or [4,3-*b*]pyridine *C*-nucleoside derivatives. The introduction of a small 5-amino substitution was also explored, in accordance with previous observations concerning the molecular recognition pattern of the enzyme.[14]

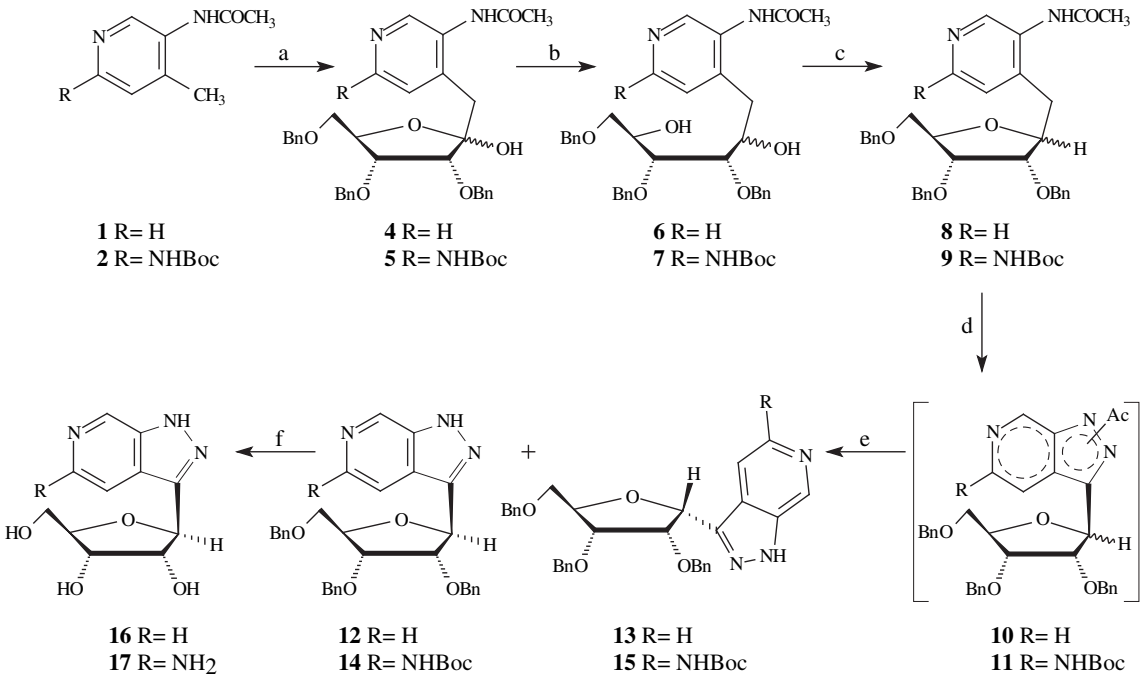
2. Results and discussion

2.1. Molecular modelling

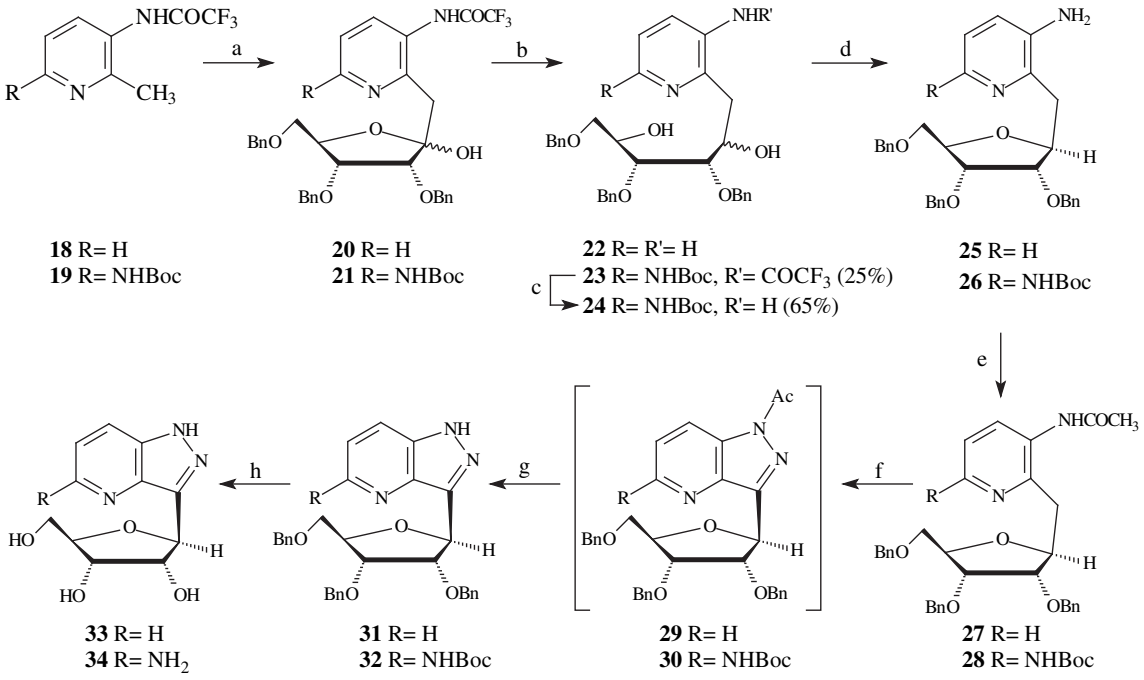
As a first step, docking calculations of the novel nucleosides **16**, **17** (Scheme 3), **33** and **34** (Scheme 4) within the ADA catalytic site were carried out. Results strongly suggested that the designed chemical modifications would possibly enhance stabilizing interactions between the novel analogues and specific residues belonging to the enzyme active site. The theoretical lowest energy structures revealed a favourable binding mode for all four



Scheme 2. Structures of deaminoformycin and nebularine.



Scheme 3. Reagents and conditions:(a) (i) *n*-BuLi, THF, −78 °C. (ii) 2,3,5-tri-*O*-benzyl-D-ribonolactone (**3**), THF, −30 °C, 43% (for **4**), 25% (for **5**); (b) $NaBH_4$, MeOH, rt, 2 h, 93% (for **6**), 80% (for **7**); (c) PPh_3, DEAD, reflux 90 min, 40% (for **8**), 38% (for **9**); (d) AcOK, Ac_2O, isoamyl nitrite, toluene dry, reflux, 15 h; (e) NH_3, dry CH_3OH, rt, 12 h, 40–70% (two steps); (f) BCl_3, CH_2Cl_2, −78 °C, 2 h 60–70%.



Scheme 4. Reagents and conditions: (a) (i) *n*-BuLi, THF, −78 °C, (ii) 2,3,5-tri-*O*-benzyl-D-ribonolactone (**3**), THF, −30 °C 44–55%; (b) $NaBH_4$, MeOH, rt, 2 h 80%; (c) K_2CO_3, MeOH, H_2O, 60 °C, 15 h, 65%; (d) PPh_3, DEAD, reflux 90 min; 77–89%; (e) Ac_2O, CH_2Cl_2, 15 h, rt 90%; (f) AcOK, Ac_2O, isoamyl nitrite, toluene dry, reflux; (g) NH_3, dry CH_3OH, rt; 85% (two steps); (h) BCl_3, CH_2Cl_2, −78 °C 79–96%.

compounds, indicating potential interaction partners within ADA binding pocket and thus supporting the proposed modifications (Fig. 1).

The N(1)H, which appears in the predominant tautomeric form of the molecule, is positioned in close proximity to the side chain carboxylate of Asp296, forming a strong hydrogen bond with it. Conversely, according to calculations, the N(2)H tautomer is not expected to form a similar interaction, since its orientation is not optimal for the formation of a hydrogen bond with the aforementioned aspartate residue. This observation is in full agreement with the experimental binding mode of a transition state purine analogue as it appears in the crystal structure of murine ADA.[15] Furthermore, the introduction of an amino group at position 5 was strongly encouraged by calculations. Position 5 of the nucleoside is oriented towards the side chain of Glu217 and thus, as modelling suggested, the amine moiety of the analogues could form a hydrogen bond with the glutamate carboxylate. This modification was also in accordance with existing SAR data indicating that the introduction of an amine at this particular position favours affinity, however in an inversely proportional fashion to the bulk of the amine substituent.[14] Finally, the conversion of the heterocyclic nitrogen of position 4 into carbon was justified by calculations as potentially favourable, as this part of the molecule is oriented towards a rather hydrophobic sub-site of the binding pocket. This part of the pocket is comprised by the side chains of Leu62, Gly184 and Leu58 and, as a result, a hydrophobic carbon atom could possibly be accommodated in such an environment under more favourable terms than the corresponding polar nitrogen. The permutation of the carbon atom between positions 4 and 6 was finally performed in order to fully elucidate the biological functionality of N(6).

2.2. Synthesis

For the synthesis of the pyrazolo[3,4-*c*]pyridine analogues, we used as starting material the previously prepared 3-acetamido-4-methylpyridine (**1**)[16] and the corresponding 6-*tert*-butyl carbamate **2**[17] (Scheme 3).

The coupling of the lithiated derivative of **1** or **2** with 2,3,5-tri-*O*-benzyl-D-ribonolactone (**3**)[18] provided a 6:1, α/β-D anomeric mixture of the hemiacetals **4** (43%) and **5** (25%), respectively. A small amount of a side product is also produced, resulting from the attack of the anion formed on the acetamide methyl upon the lactone **3**. The unambiguous structural elucidation of compounds **4** and **5** was made through the examination of the corresponding HMBC spectroscopic data, where the protons of the acetamide methyl group correlate with the carbonyl and at the same time, the methylene group possesses a strong correlation peak with both the anomeric carbon and the aromatic carbons 4 (^2J coupling) and 5 (^3J coupling). The hemiacetals were then reduced with sodium borohydride into their corresponding *S*/*R* diols **6** (93%) and **7** (80%). Very good diastereoselectivity was observed in this reduction step, with a stereofacial preference for the *R* epimer (ratio *S*/*R*: 1/7 for **6** and 1/6 for **7**, respectively, as indicated by ^{1}H NMR data). Intramolecular Mitsunobu cyclisation of the diols **6** and **7** provided an inseparable mixture of the α,β-*C*-glycosides **8** (ratio α/β 1/7) and **9** (ratio α/β 1/2.5) in moderate yield (40%). The α,β anomeric mixture of each derivative was treated with isoamyl nitrite in toluene at reflux,[19] in the presence of acetic anhydride and potassium acetate, to give the rearranged intermediate *N*-nitroso compounds, giving a mixture of 1- and 2-acetylpyrazolopyridines (**10** and **11**, respectively). Subsequent methanolic ammonia deacetylation of **10** and **11**, resulted in the β-pyrazolopyridine *C*-nucleosides **12** and **14**, which were separated from the corresponding α isomeric counterparts **13** and **15** by column chromatography (50–70% isolated yield). The stereochemistry of the compounds was unambiguously determined on the basis of NOE data, where a strong correlation peak between the H-1′ and H-4′ was indicative of the β anomers. Concomitant removal of the protective groups with boron trichloride in a dichloromethane solution yielded the desired 4-deazaformycin analogue **16**, as well as the 5-amino derivative **17**.

In order to get access to the pyrazolo[4,3-*b*]pyridine analogues the 3-trifluoroacetamido-2-methyl pyridine (**18**) as well as the 6-carbamate derivative **19** were used as starting materials (Scheme 4). The trifluoracetamide **18** was prepared upon diazotisation of the

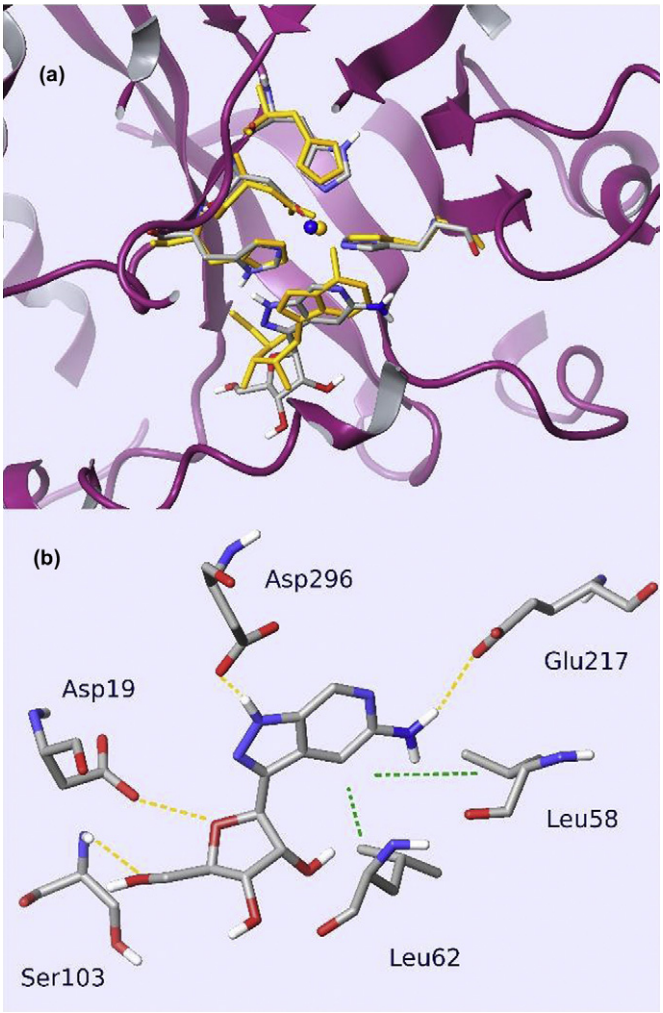


Fig. 1. (a) Superposition of the theoretical binding mode of compound **17** and the experimental binding geometry of 6-hydroxyl-1,6-dihydropurine ribonucleoside (yellow-pdb id: 2ADA) within the catalytic site of murine adenosine deaminase. The coordinated zinc cation is visible as a sphere between His15, His17, His214 and Asp295. The protein is depicted in a ribbon representation. The nearly-identical interaction mode of the two molecules is fairly reasonable since both compounds share a high degree of structural similarity. (b) The proposed binding mode of compound **17**, as determined by docking calculations within the catalytic site of murine ADA. Two hydrogen bonds (yellow dashed lines) stabilize the pyrazolopyridine ring of the nucleoside inside the protein. Those bonds are formed between the 5-amino group and the side chain of Glu217 and between the N1(H), which appears in the dominant tautomeric form of **17** and the side chain of Asp296. Two additional bonds are formed between the sugar moiety and the residues Asp19 and Ser103. The hydrophobic sub-site of the pocket, which is formed by the side chains of Leu58, Leu62 and Gly184 (not visible) is oriented towards position 4 of the heteroaromatic system and hydrophobic interactions are depicted in green dashed lines.

known 6-amino-3-nitro-2-picoline,[20] followed by chlorination,[21] reduction and trifluoracetylation.[22] Analogously, trifluoracetylation of *tert*-butyl-*N*-(3-amino-2-methylpyridin-6-yl)carbamate[17] provided **19**.[23] As already reported, concerning the lithiation of aminosubstituted 2-picoline, it is preferable to use the trifluoracetamide instead of the corresponding acetamide in order to avoid the formation of a substantial amount of side-products.[24] The lithiation of the trifluoroacetamides **18** and **19** was accomplished according to the above mentioned methodology to result in a 3:1 and a 8:1 α/β-D anomeric mixture of the hemiacetals **20** (55%) and **21** (44%), respectively. The borohydride reduction of the hemiacetals **20** afforded the corresponding *R*- and *S*-aminodiols **22** in 80% yield (ratio *R*/*S* 1/3), whereas the reduction of **21** provided a mixture of the *R*-aminodiols **24** (65% yield) together with the trifluoroacetamide derivative **23** (25% yield). This latter can be easily and selectively deprotected to **24** in mild basic media. The aminodiols **22** and **24** were subjected to intramolecular Mitsunobu cyclisation and were converted successively into the corresponding β-*C*-glycoside derivatives **25** (77% yield) and **26**, respectively and then to the acetates **27** and **28** (89% yield two steps). The unambiguous NMR characterization of these analogues (**25**–**28**) proved to be more convenient when performed on the acetates.

The acetates **27** and **28** were refluxed in toluene with isoamyl nitrite in the presence of acetic anhydride and potassium acetate, as already described, to give the β-1-acetylpyrazolopyridines **29** and **30**, respectively. The acetyl groups were easily cleaved upon treatment of **29** or **30** with methanolic ammonia, and the protective groups were then removed with boron trichloride in a dichloromethane solution to yield the target β-*C*-nucleosides **33** and **34**.

2.3. Assessment of the bioactivity

The compounds were tested regarding the inhibition of calf spleen ADA and the results are presented in Table 1. Several concentrations were tested ranging from 0.01 to 1 mM. The compounds proved to be very weak inhibitors of ADA, even in high concentrations and therefore K_i determinations were not performed. Nevertheless, for each pair of isomeric pyrazolopyridines, it is the 5-aminosubstituted analogue that appears to be the more potent inhibitor of the enzyme.

Table 1
Compounds inhibitory activities against calf spleen ADA

Compound	C (μM)	% Inhibition
16	319	8.2
17	237	18
33	295	19
34	237	25

According to results obtained by initial docking calculations (Section 2.1), it was anticipated that the new *C*-nucleoside derivatives could ultimately present enhanced binding affinity towards ADA. However, the biological data (Table 1) did not confirm this hypothesis. These rather unexpected results prompted us to investigate alternative factors that could possibly influence the biological activity of the new compounds. Some structurally related triazolotriazine nucleoside derivatives possessing strong ADA inhibitory activity were previously studied by the use of theoretical calculations.[14] Reported results suggested that the biological activity of nebularine-related derivatives could be partially attributed to the relative stability of their corresponding covalent hydrated transition state analogues based on calculated values for stabilities. However, it is still unclear whether binding of the nucleoside framework or the tendency of these molecules to undergo the hydration reaction is predominant for the activity. Consequently, the newly synthesized nucleosides were evaluated with respect to their potential to form stable hydrates. A theoretical study (Section 4.2) was undertaken using high-level quantum mechanical calculations. A full vibrational and thermochemical analysis was performed for the nucleosides and their corresponding hydrates, as well as for the molecule of water at the restricted HF level of theory using the triple zeta LACV3P basis set, augmented with full polarisation and diffusion functions. The enthalpy of hydration was calculated for compounds **16**, **17**, **33** and **34** as well as for nebularine and deaminoformycin at the HF/LACV3P**++ level, in accordance with the formalism followed in previous studies,[14] as the difference of heat of formation between reactants and products for the reaction: nucleoside+water→hydrated nucleoside.

In all cases the calculated enthalpy of hydration values were positive, ranging from 19.9 to 26.8 kcal/mol. The corresponding values for nebularine and deaminoformycin were found to be negative, suggesting that hydration of pyrazolopyridines is less favoured.

These results could provide a strong indication that hydration is a critical factor, concerning the ADA inhibitory profile of nucleoside derivatives with an aglycone resembling to 6-unsubstituted purines, and cannot be effectively counterbalanced by moderate enhancement of intermolecular interactions between the enzyme and the potential inhibitor.

3. Conclusion

In conclusion, we have performed the synthesis of four new *C*-nucleosides through the lithiation of suitable aminopicolines, reduction, application of the Mitsunobu cyclodehydration and elaboration of the pyrazolopyridine ring system. The compounds were evaluated as ADA inhibitors and they proved to be very weak inhibitors of ADA, in spite of the favourable ADA binding affinity predicted by docking studies. This result strongly supports the hypothesis of poor covalent hydrate stability observed in theoretical studies, and argues in favour of the importance of the hydration of the heterocyclic ring system, which seems to be the critical factor for effective ADA inhibition, concerning these 7-unsubstituted nucleoside derivatives.

4. Experimental

4.1. Chemistry

Melting points were determined on a Büchi apparatus and are uncorrected. Optical rotations were obtained on a Perkin–Elmer 341 Polarimeter. FT-IR spectra were recorded on a Perkin–Elmer RX 1 FT-IR spectrometer. ^{1}H NMR spectra and 2-D spectra were recorded on a Bruker Avanche 400 instrument, whereas ^{13}C NMR spectra were recorded on a Bruker AC 200 spectrometer in deuterated solvents and were referenced to TMS (δ scale). The signals of ^{1}H and ^{13}C spectra were unambiguously assigned by using 2D NMR techniques: ^{1}H^{1}H COSY, NOESY HMQC and HMBC. Flash chromatography was performed on Merck silica gel 60 (0.040–0.063 mm). Analytical thin layer chromatography (TLC) was carried out on precoated (0.25 mm) Merck silica gel F-254 plates. Elemental analyses were within ±0.4% of the theoretical values.

*4.1.1. N-[4-(2,3,5-Tri-O-benzyl-1-hydroxy-α,β-D-ribofuranosylmethyl)pyridin-3-yl]acetamide (**4**).* To a solution of 4-methylpyridine **1**[16] (910 mg, 6.06 mmol) in dry THF (70 mL) at −78 °C was added under argon *n*-BuLi (9.30 mL, 15.16 mmol, 1.6 M solution in hexanes), the resulting solution was stirred at −78 °C for 15 min and the temperature then raised to −25 °C for 90 min. The solution was cooled to −78 °C and a solution of D-ribonolactone **3**[18] (3.03 g, 7.28 mmol) in dry THF (20 mL) was added dropwise. The resulting mixture was stirred at −78 °C for 10 min and then at −20 °C for 3 h. A saturated ammonium chloride solution was then added to the yellow solution to quench the excess *n*-BuLi. The solvent was vacuum-evaporated, water was added to the residue and then extracted with CH_2Cl_2 (4×50 mL). The organic extracts were dried (Na_2SO_4) and the solvent was evaporated under reduced pressure to give an orange oil. Flash chromatography purification (silica gel) using a mixture of CH_2Cl_2/MeOH 50/1 as the eluent afforded a light yellow oil consisting of a mixture of the hemiacetals **4** (1.5 g, 43.5%), with a clear predominance of the α-anomer (α/β ratio 6:1, as determined by ^{1}H NMR). Data for the α-anomer: IR (film) ν_{max} 3400–3270 (br), 1694, 1568, 1518, 1416, 1116 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.06 (s, 3H, CH_3), 2.90 (d, 1H, 4-CH_2, J=13.65 Hz), 3.07 (d, 1H, 4-CH_2, J=13.65 Hz), 3.34 (dd, 1H, H-5′, $J_{5'-4'}$=3.75 Hz, $J_{5'-5'}$=10.59 Hz), 3.39 (dd, 1H, H-5′, $J_{5'-4'}$=3.75 Hz, $J_{5'-5'}$=10.59 Hz), 3.76 (d, 1H, H-2′, $J_{2'-3'}$=4.78 Hz), 3.90–3.95 (m, 1H, H-3′), 4.33–4.64 (m, 7H, 3×CH_2–Ph, H-4′), 6.77 (d, 1H, H-5, J_{5-6}=5.12 Hz), 7.17–7.40 (m, 15H, 3×C_6H_5), 8.15 (d, 1H, H-6, $J_{6,5}$=5.12 Hz), 8.92 (s, 1H, NH, D_2O exch.), 9.03 (s, 1H, H-2); ^{13}C NMR (50 MHz, $CDCl_3$) δ 23.91 (CH_3), 39.45 (CH_2), 69.81 (C-5′), 72.34, 72.43, 73.37 (CH_2–Ph), 76.52 (C-3′), 78.24 (C-2′), 80.72 (C-4′), 104.54 (C-1′), 126.44 (C-5), 127.60, 127.70, 127.86, 127.94, 128.13, 128.22, 128.46, 128.52 [CH(Ph)], 134.14 (C-3), 136.25, 136.56, 136.68, 137.25 [C(Ph), C-4], 144.69 (C-6), 145.46 (C-2), 168.59 (CO). Anal. Calcd for $C_{34}H_{36}N_2O_6$: C, 71.81; H, 6.38; N, 4.93. Found: C, 71.57; H, 6.73; N, 5.14.

*4.1.2. tert-Butyl-N-[5-acetamido-4-(2,3,5-tri-O-benzyl-1-hydroxy-α,β-D-ribofuranosylmethyl)-pyridin-2-yl]carbamate (**5**).* This compound was prepared by a procedure analogous to that of **4**, starting from **2**[17] to result in the hemiacetals **5** (yellow oil, 25%, α/β ratio 6:1, as determined by ^{1}H NMR). Data for the α-anomer: IR (film) ν_{max} 3365–3290 (br), 1719, 1686, 1543, 1403, 1159 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.55 (s, 9H, $(CH_3)_3$), 2.08 (s, 3H, CH_3), 3.04 (d, 1H, 4-CH_2, J=13.74 Hz), 3.11 (d, 1H, 4-CH_2, J=13.74 Hz), 3.32–3.42 (m, 2H, H-5′), 3.83–3.89 (m, 2H, H-2′, H-3′), 4.33–4.38 (m, 1H, H-4′), 4.38–4.59 (m, 6H, 3×CH_2–Ph), 7.20–7.41 (m, 15H, 3×C_6H_5), 7.93 (s, 1H, H-3), 8.70 (s, 1H, H-6), 8.78 (s, 1H, NHAc, D_2O exch.), 9.08 (s, 1H, NHBoc, D_2O exch.); ^{13}C NMR (50 MHz, $CDCl_3$) δ 24.01 (CH_3), 28.39 [$(CH_3)_3$], 40.22 (CH_2), 69.84 (C-5′), 72.61, 72.95, 73.56 (CH_2–Ph), 76.88 (C-3′), 78.75 (C-2′), 80.67 (C-4′), 80.81 [$(CH_3)_3C$], 104.84 (C-1′), 114.89 (C-3), 127.70, 128.00, 128.31, 128.53, 128.60, 128.66 [CH(Ph)], 129.39 (C-5), 136.84, 137.07, 137.51 [C(Ph)], 139.37 (C-4), 143.83 (C-6), 148.77 (C-2), 152.72 (OCONH), 168.72 ($COCH_3$). Anal. Calcd for $C_{39}H_{45}N_3O_8$: C, 68.50; H, 6.63; N, 6.15. Found: C, 68.79; H, 6.44; N, 5.78.

*4.1.3. N-[4-[(2RS,5S)-3,4,6-Tri-O-benzyl-2,5-dihydroxyhexane]pyridin-3-yl]acetamide (**6**).* To a solution of the hemiacetals **4** (510 mg, 0.89 mmol) in dry MeOH (15 mL) was added cautiously sodium borohydride (84 mg, 2.24 mmol) and the mixture was stirred at room temperature for 90 min. The reaction was quenched with ice-water, neutralized with a 0.1 M HCl solution and extracted with CH_2Cl_2 (3×50 mL) and AcOEt (2×50 mL). The combined organic extracts were dried (Na_2SO_4) and concentrated to dryness. The residue was purified by flash chromatography (silica gel) using a mixture of CH_2Cl_2/MeOH 100/3 as the eluent to result in a mixture of the diols **6** (480 mg, 93%) as a white foam. IR (film) ν_{max} 3465–3170 (br), 1678, 1568, 1525, 1453, 1417, 1216 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.05 (s, 3H, CH_3), 2.48 (m, 1H, H-1′), 3.03 (dd, 1H, H-1′, $J_{1'-1'}$=13.54 Hz, $J_{1'-2'}$=9.88 Hz), 3.65 (dd, 1H, H-6′, $J_{6'-5'}$=4.76 Hz, $J_{6'-6'}$=9.51 Hz), 3.70 (dd, 1H, H-6′, $J_{6'-5'}$=2.93 Hz, $J_{6'-6'}$=9.51 Hz), 3.77–3.81 (m, 1H, H-3′), 3.92 (dd, 1H, H-4′, $J_{4'-3'}$=1.46 Hz, $J_{4'-5'}$=8.40 Hz), 3.96–4.01 (m, 1H, H-5′), 4.10–4.14 (m, 1H, H-2′), 4.46–4.85 (m, 6H, 3×CH_2–Ph), 6.95 (br s, 1H, H-5), 7.20–7.43 (m, 15H, 3×C_6H_5), 8.15 (br s,1H, H-6), 8.99 (s, 1H, H-2), 9.29 (s, 1H, NH, D_2O exch.); ^{13}C NMR (50 MHz, $CDCl_3$) δ 24.04 (CH_3), 36.97 (C-1′), 69.65 (C-5′), 70.95 (C-6′), 73.21 (C-2′), 73.07, 73.58, 74.54 (CH_2–Ph), 79.99 (C-3′), 80.58 (C-4′), 125.51 (C-5), 128.08, 128.17, 128.23, 128.57, 128.60 [CH(Ph)], 134.38 (C-3), 137.34, 137.67, 137.74 [C(Ph)], 140.38 (C-4), 145.03 (C-2, C-6), 168.90 (CO). Anal. Calcd for $C_{34}H_{38}N_2O_6$: C, 71.56; H, 6.71; N, 4.91. Found: C, 71.35; H, 6.57; N, 4.79.

*4.1.4. tert-Butyl-N-[5-acetamido-4-[(2RS,5S)-3,4,6-tri-O-benzyl-2,5-dihydroxyhexane]pyridin-2-yl]carbamate (**7**).* Following an analogous procedure to that described for **6**, the mixture of the diols **7** was obtained as transparent oil in 80% yield (chromatographic purification was effected using a mixture of CH_2Cl_2/MeOH 40/1 as the eluent). IR (film) ν_{max} 3410–3147 (br), 1723, 1678, 1552, 1404, 1160 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.53 (s, 9H, $(CH_3)_3$), 2.07 (s,

3H, CH_3), 2.52–2.59 (m, 1H, H-1′), 3.10 (dd, 1H, H-1′, J=13.70, 9.39 Hz), 3.66 (dd, 1H, H-6′, $J_{6'-5'}$=5.48 Hz, $J_{6'-6'}$=9.78 Hz), 3.72 (dd, 1H, H-6′, $J_{6'-5'}$=2.74 Hz, $J_{6'-6'}$=9.78 Hz), 3.77–3.84 (m, 1H, H-3′), 3.87 (dd, 1H, H-4′, $J_{4'-3'}$=1.56 Hz, $J_{4'-5'}$=8.60 Hz), 4.02–4.10 (m, 1H, H-5′), 4.16–4.19 (m, 1H, H-2′), 4.50–4.86 (m, 6H, 3×CH_2–Ph), 7.25–7.39 (m, 15H, 3×C_6H_5), 7.87 (s, 1H, H-3), 8.71 (s, 1H, H-6), 9.18 (s, 1H, NHAc, D_2O exch.), 9.23 (s, 1H, NHBoc, D_2O exch.); ^{13}C NMR (50 MHz, $CDCl_3$) δ 23.86 (CH_3), 28.31 [(CH_3)$_3$], 37.46 (C-1′), 69.31 (C-5′), 71.00 (C-6′), 73.18 (CH_2–Ph), 73.34 (C-2′), 73.41, 74.31 (CH_2–Ph), 79.98 (C-3′), 80.76 [(CH_3)$_3$–*C*], 81.09 (C-4′), 113.64 (C-3), 127.97, 128.07, 128.12, 128.49, 129.37 [CH(Ph), C-5], 137.34, 137.58, 137.66 [C(Ph)], 143.06 (C-4), 143.18 (C-6), 148.77 (C-2), 152.90 (*O*CONH), 168.78 (*C*OCH_3). Anal. Calcd for $C_{39}H_{47}N_3O_8$: C, 68.30; H, 6.91; N, 6.13. Found: C, 68.04; H, 7.17; N, 6.20.

*4.1.5. N-[4-(2,3,5-Tri-O-benzyl-α,β-D-ribofuranosylmethyl)pyridin-3-yl]acetamide (**8**).* Triphenylphosphine (470 mg, 1.78 mmol) was added under argon to a solution of the diols **6** (510 mg, 0.894 mmol) in dry THF (15 mL) and the resulting mixture was heated at reflux. Diethylazodicarboxylate (0.27 mL, 1.78 mmol) was then added dropwise at this temperature and heating was continued for 90 min. The solvent was evaporated and the resulting oil was purified by flash chromatography (silica gel) using a mixture of CH_2Cl_2/AcOEt 5/2 as the eluent to give **8** (200 mg, 40%) as an oil. An adequate quantity of pure β-anomer could be isolated from the mixture for identification. Data for the β-anomer: oil; IR (film) ν_{max} 3318, 1693, 1519, 1415, 1275 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.01 (s, 3H, CH_3), 2.70 (dd, 1H, CH_2, J=6.14, 14.34 Hz), 3.03 (dd, 1H, CH_2, J=2.73, 14.34 Hz), 3.29 (dd, 1H, H-5′, $J_{5'-4'}$=4.78 Hz, $J_{5'-5'}$=10.24 Hz), 3.41 (dd, 1H, H-5′, $J_{5'-4'}$=3.75 Hz, $J_{5'-5'}$=10.24 Hz), 3.51 (dd, 1H, H-2′, $J_{2'-1'}$=8.19 Hz, $J_{2'-3'}$=5.46 Hz), 3.72 (dd, 1H, H-3′, $J_{3'-2'}$=5.46 Hz, $J_{3'-4'}$=3.07 Hz), 4.19–4.24 (m, 1H, H-4′), 4.26–4.33 (m, 1H, H-1′), 4.34–4.75 (m, 6H, 3×CH_2–Ph), 6.79 (br s, 1H, H-5), 7.17–7.45 (m, 15H, 3×C_6H_5), 8.21 (br s, 1H, H-6), 8.80 (s, 1H, NH, D_2O exch.), 8.98 (s, 1H, H-2); ^{13}C NMR (50 MHz, $CDCl_3$) δ 23.89 (CH_3), 34.23 (CH_2), 70.11 (C-5′), 72.06, 72.42, 73.49 (CH_2–Ph), 76.39 (C-3′), 78.91 (C-2′), 81.21 (C-1′), 82.22 (C-4′), 125.71 (C-5), 128.11, 128.17, 128.30, 128.36, 128.44, 128.59, 128.61, 128.70 [CH(Ph)], 134.24 (C-3), 137.32 (C-4), 137.40, 137.91 [C(Ph)], 145.53 (C-6), 146.24 (C-2), 168.65 (CO). Anal. Calcd for $C_{34}H_{36}N_2O_5$: C, 73.89; H, 6.57; N, 5.07. Found: C, 73.62; H, 6.44; N, 5.26.

*4.1.6. tert-Butyl-N-[5-acetamido-4-(2,3,5,tri-O-benzyl-α,β-D-ribofuranosylmethyl)pyridin-2-yl]carbamate (**9**).* The anomeric mixture of **9** was prepared by a procedure analogous to that described for **8** in 38% yield. Pure β-anomer could be isolated from the mixture. Data for the β-anomer: oil; IR (film) ν_{max} 3325, 1717, 1671, 1540, 1408, 1160 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.56 (s, 9H, (CH_3)$_3$), 1.99 (s, 3H, CH_3), 2.74 (dd, 1H, CH_2, J=6.65, 14.08 Hz), 3.06 (dd, 1H, CH_2, J=3.13, 14.08 Hz), 3.30 (dd, 1H, H-5′, $J_{5'-4'}$=3.08 Hz, $J_{5'-5'}$=10.57 Hz), 3.45 (dd, 1H, H-5′, $J_{5'-4'}$=3.53 Hz, $J_{5'-5'}$=10.57 Hz), 3.61 (dd, 1H, H-2′, $J_{2'-1'}$=5.09 Hz, $J_{2'-3'}$=7.44 Hz), 3.67 (dd, 1H, H-3′, $J_{3'-2'}$=5.09 Hz, $J_{3'-4'}$=3.52 Hz), 4.16–4.23 (m, 1H, H-4′), 4.33 (dd, 1H, H-1′, $J_{1'-2'}$=5.09, 7.40 Hz), 4.37–4.67 (m, 6H, 3×CH_2–Ph), 7.18–7.45 (m, 15H, 3×C_6H_5), 7.88 (s, 1H, H-3), 8.37 (s, 1H, H-6), 8.61 (s, 1H, NHBoc, D_2O exch.), 8.66 (s, 1H, NHAc, D_2O exch.); ^{13}C NMR (50 MHz, $CDCl_3$) δ 23.78 (CH_3), 28.41 [(CH_3)$_3$], 34.97 (CH_2), 70.12 (C-5′), 72.06, 72.72, 73.50 (CH_2–Ph), 76.55 (C-3′), 79.42 (C-2′), 80.97 [(CH_3)$_3$–*C*], 81.59 (C-1′), 81.86 (C-4′), 114.02 (C-3), 127.73, 127.99, 128.19, 128.29, 128.61 [CH(Ph)], 137.40, 137.57, 137.65, 137.73 [C(Ph), C-5], 141.36 (C-4), 144.24 (C-6), 148.84 (C-2), 152.60 (*O*CONH), 168.78 (*C*OCH_3). Anal. Calcd for $C_{39}H_{45}N_3O_7$: C, 70.14; H, 6.79; N, 6.29. Found: C, 70.31; H, 6.57; N, 6.46.

*4.1.7. 3-(2,3,5-Tri-O-benzyl-β-D-ribofuranosyl)pyrazolo[3,4-c]pyridine (**12**).* To a suspension of **8** (250 mg, 0.452 mmol) in dry toluene (30 mL) were added under argon AcOK (46 mg, 0.292 mmol) and Ac_2O (133 μL, 1.35 mmol). The reaction mixture was heated at 80 °C, isoamyl nitrite (155 μL, 1.13 mmol) was then added dropwise and the resulting mixture was heated at 100 °C for 16 h. The insoluble material was filtered off, washed with hot toluene and the combined filtrates were evaporated to dryness to afford an orange oil corresponding to a mixture of the acetamides **10**. A saturated methanolic ammonia solution (30 mL) was added to the above mixture and the resulting solution was stirred at room temperature overnight. The solvent was removed in vacuo and the residue was purified by flash chromatography (silica gel) using a mixture of cyclohexane/AcOEt 2/1 as the eluent to give the β-anomer **12** (170 mg, 72%) and the α-anomer **13** (24 mg, 10%) as light yellow oils.

*4.1.8. Data for 3-(2,3,5-Tri-O-benzyl-β-D-ribofuranosyl)pyrazolo[3,4-c]pyridine (**12**).* $[\alpha]_D^{22}$ −55 (*c* 0.41, MeOH); IR (film) ν_{max} 3286, 1454, 1360, 1268, cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.66 (dd, 1H, H-5′, $J_{5'-4'}$=3.75 Hz, $J_{5'-5'}$=10.58 Hz), 3.79 (dd, 1H, H-5′, $J_{5'4'}$=3.41 Hz, $J_{5'-5'}$=10.58 Hz), 4.18–4.22 (m, 1H, H-3′), 4.39 (dd, 1H, H-2′, $J_{2'-1'}$=6.82 Hz, $J_{2'-3'}$=5.47 Hz), 4.42–4.48 (m, 1H, H-4′), 4.50–4.79 (m, 6H, 3×CH_2–Ph), 5.57 (d, 1H, H-1′, $J_{1'-2'}$=6.82 Hz), 7.08–7.41 (m, 15H, 3×C_6H_5), 7.74 (d, 1H, H-4, J_{4-5}=5.80 Hz), 8.06 (d, 1H, H-5, J_{5-4}=5.80), 9.00 (s, 1H, H-7); ^{13}C NMR (50 MHz, $CDCl_3$) δ 70.33 (C-5′), 72.31, 72.41, 73.68 (CH_2–Ph), 77.51 (C-3′), 78.43 (C-1′), 81.10 (C-2′), 82.43 (C-4′), 115.47 (C-4), 125.04 (C-3α), 128.02, 128.28, 128.56 [CH(Ph)], 134.85 (C-7), 137.41, 137.89 [C(Ph)], 138.00 (C-7α), 138.16 (C-5), 138.35 [C(Ph)], 144.38 (C-3). Anal. Calcd for $C_{32}H_{31}N_3O_4$: C, 73.68; H, 5.99; N, 8.06. Found: C, 73.81; H, 5.82; N, 7.89.

*4.1.9. Data for 3-(2,3,5- tri-O-benzyl-α-D-ribofuranosyl)pyrazolo[3,4-c]pyridine (**13**).* $[\alpha]_D^{22}$ −3 (*c* 0.25, MeOH); IR (film) ν_{max} 3271, 1458, 1361, 1264, 1113 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.98 (dd, 1H, H-5′, $J_{5'-4'}$=4.09 Hz, $J_{5'-5'}$=10.24 Hz), 4.05 (dd, 1H, H-5′, $J_{5'-4'}$=6.83 Hz, $J_{5'-5'}$=10.24 Hz), 4.09 (d, 1H, CH_2–Ph, J=11.95 Hz), 4.26 (d, 1H, CH_2–Ph, J=11.95 Hz), 4.33–4.43 (m, 3H, H-2′, H-3′, H-4′), 4.51–4.73 (m, 4H, 2×CH_2–Ph), 5.49 (d, 1H, H-1′, $J_{1'-2'}$=4.77 Hz), 6.76–7.42 (m, 15H, 3×C_6H_5), 7.98 (d, 1H, H-4, J_{4-5}=5.37 Hz), 8.05 (d, 1H, H-5, J_{5-4}=5.37 Hz), 8.96 (s, 1H, H-7); ^{13}C NMR (50 MHz, $CDCl_3$) δ 69.92 (C-5′), 73.19, 73.60, 73.66 (CH_2–Ph), 77.67 (C-1′), 78.61, 79.41, 80.47 (C-3′, C-2′, C-4′), 117.45 (C-4), 127.50, 127.62, 127.81, 127.96, 128.02, 128.05, 128.30 [CH(Ph)], 128.53 (C-3α), 134.78 (C-7), 137.57 [C(Ph)], 137.66 (C-5), 137.86, 138.26 [C(Ph)], 138.71 (C-7α), 142.91 (C-3). Anal. Calcd for $C_{32}H_{31}N_3O_4$: C, 73.68; H, 5.99; N, 8.06. Found: C, 73.92; H, 5.78; N, 7.96.

*4.1.10. tert-Butyl-N-[3-(2,3,5-tri-O-benzyl-β-D-ribofuranosyl)pyrazolo[3,4-c]pyridin-5-yl]carbamate (**14**).* Both anomers **14** and **15** were prepared by a procedure analogous to that of the corresponding compounds **12** and **13**, starting from the acetamides **9**.

*4.1.11. Data for tert-butyl-N-[3-(2,3,5-tri-O-benzyl-β-D-ribofuranosyl) pyrazolo[3,4-c]pyridin-5-yl]carbamate (**14**).* Oil; Yield: 63%; $[\alpha]_D^{22}$ −13 (*c* 0.14, MeOH); IR (film) ν_{max} 3248, 1718, 1450, 1272, 1117 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.53 (s, 9H, (CH_3)$_3$), 3.62–3.68 (m, 1H, H-5′), 3.73–3.78 (m, 1H, H-5′), 4.14–4.17 (m, 1H, H-3′), 4.37–4.42 (m, 1H, H-4′), 4.50–4.67 (m, 7H, 3×CH_2–Ph, H-2′), 5.51 (d, 1H, H-1′, $J_{1'-2'}$=6.26 Hz), 7.10–7.34 (m, 15H, 3×C_6H_5), 8.28 (s, 1H, H-4), 8.52 (br s, 1H, NHBoc, D_2O exch.), 8.58 (s, 1H, H-7); ^{13}C NMR (50 MHz, $CDCl_3$) δ 28.43 [(CH_3)$_3$], 69.79 (C-5′), 72.15, 72.33, 73.36 (CH_2–Ph), 77.71 (C-1′), 80.25 (C-3′), 80.34 (C-4′), 80.44 [(CH_3)$_3$–*C*], 81.81 (C-2′), 101.09 (C-4), 127.65, 127.69, 127.80, 127.94, 127.98, 128.16, 128.18, 128.38 [CH(Ph)], 132.64 (C-7), 135.58 (C-7α), 137.69 (C-3α), 137.91 (C-3), 143.08, 143.62, 143.85 [C(Ph)], 152.73 (C-5), 153.65 (CO). Anal. Calcd for $C_{37}H_{40}N_4O_6$: C, 69.79; H, 6.33; N, 8.80. Found: C, 69.93; H, 6.18; N, 8.61.

*4.1.12. Data for tert-butyl-N-[3-(2,3,5-tri-O-benzyl-α-D-ribofuranosyl) pyrazolo[3,4-c]pyridin-5-yl]carbamate (**15**).* Oil; Yield: 12%; $[\alpha]_D^{22}$ −24 (*c* 0.44, MeOH); IR (film) ν_{max} 3263, 1722, 1458, 1264, 1152, 1117 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.55 (s, 9H, (CH_3)$_3$), 4.00–4.08

(m, 2H, H-5′), 4.19–4.25 (m, 1H, H-3′), 4.35–4.38 (m, 1H, H-4′), 4.40–4.44 (m, 1H, H-2′), 4.57–4.78 (m, 6H, 3×CH_2–Ph), 5.53 (d, 1H, H-1′, $J_{1'-2'}$=5.47 Hz), 6.90–7.40 (m, 15H, 3×C_6H_5), 8.41 (s, 1H, H-4), 8.85 (br s, 1H, NHBoc, D_2O exch.), 8.89 (s, 1H, H-7); ^{13}C NMR (50 MHz, $CDCl_3$) δ 28.49 [$(CH_3)_3$], 69.76 (C-5′), 73.16, 73.65, 73.75 (CH_2–Ph), 75.37 (C-1′), 78.88 (C-3′), 78.96 (C-4′), 79.04 (C-2′), 80.07 [$(CH_3)_3$–*C*], 102.22 (C-4), 127.72, 127.94, 128.11, 128.28, 128.45, 128.61 [CH(Ph)], 131.31 (C-7), 132.20 (C-7α), 133.32 (C-3α), 137.44 (C-3), 137.75, 137.86, 138.14 [C(Ph)], 152.36 (C-5), 152.71 (CO). Anal. Calcd for $C_{37}H_{40}N_4O_6$: C, 69.79; H, 6.33; N, 8.80. Found: C, 69.65; H, 6.06; N, 8.99.

*4.1.13. 3-(β-D-Ribofuranosyl)pyrazolo[3,4-c]pyridine (**16**).* A solution of BCl_3 (1.61 mL, 1 M in hexane) was added dropwise under argon at −78 °C to a solution of **12** (70 mg, 0.134 mmol) in dry CH_2Cl_2 (10 mL). The reaction mixture was stirred at −78 °C for 90 min and then quenched with a cold solution of CH_2Cl_2/MeOH (1/2). The solvents were evaporated and the residue was purified by flash chromatography (silica gel), using a mixture of CH_2Cl_2/MeOH 8.5/1.5 as the eluent to give pure **16** (25 mg, 74%) as a clear oil. $[\alpha]_D^{22}$ −11 (*c* 0.09, MeOH); IR (film) ν_{max} 3457–3170 (br), 1458, 1377, 1264 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD) δ 3.76 (dd, 1H, H-5′, $J_{5'-4'}$=4.70 Hz, $J_{5'-5'}$=11.74 Hz), 3.84 (dd, 1H, H-5′, $J_{5'-4'}$=3.52 Hz, $J_{5'-5'}$=11.74 Hz), 4.05–4.09 (m, 1H, H-4′), 4.20 (dd, 1H, H-3′, *J*=4.30, 5.48 Hz), 4.38 (t, 1H, H-2′, *J*=6.26 Hz), 5.21 (d, 1H, H-1′, $J_{1'-2'}$=7.04 Hz), 8.02 (d, 1H, H-4, J_{4-5}=5.87 Hz), 8.19 (d, 1H, H-5, J_{5-4}=5.87 Hz), 8.97 (s, 1H, H-7); ^{13}C NMR (50 MHz, CD_3OD) δ 63.53 (C-5′), 72.90 (C-3′), 76.89 (C-2′), 80.48 (C-1′), 87.04 (C-4′), 116.82 (C-4), 126.13 (C-3α), 135.70 (C-7), 135.90 (C-7α), 138.49 (C-5), 146.90 (C-3). Anal. Calcd for $C_{11}H_{13}N_3O_4$: C, 52.59; H, 5.22; N, 16.72. Found: C, 52.36; H, 5.08; N, 16.49.

*4.1.14. 5-Amino-3-(β-D-ribofuranosyl)pyrazolo[3,4-c]pyridine (**17**).* This compound was prepared by a procedure analogous to that of **16**, starting from **14**. Yield: 60%; oil; $[\alpha]_D^{22}$ −21 (*c* 0.15, MeOH); IR (film) ν_{max} 3534–3093 (br), 1442, 1261, 1106 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD) δ 3.77 (dd, 1H, H-5′, $J_{5'-4'}$=4.69 Hz, $J_{5'-5'}$=12.13 Hz), 3.83 (dd, 1H, H-5′, $J_{5'-4'}$=3.52 Hz, $J_{5'-5'}$=12.13 Hz), 4.04–4.09 (m, 1H, H-4′), 4.18 (dd, 1H, H-3′, $J_{3'-2'}$=5.48 Hz, $J_{3'-4'}$=3.92 Hz), 4.37 (dd, 1H, H-2′, $J_{2'-3'}$=5.48 Hz, $J_{2'-1'}$=7.04 Hz), 5.13 (d, 1H, H-1′, $J_{1'-2'}$=7.04 Hz), 7.56 (s, 1H, H-4), 8.66 (s, 1H, H-7); ^{13}C NMR (50 MHz, CD_3OD) δ 63.51 (C-5′), 72.95 (C-3′), 76.41 (C-2′), 80.15 (C-1′), 87.26 (C-4′), 102.27 (C-4), 124.06 (C-7), 133.66 (C-7α), 134.40 (C-3α), 144.99 (C-3), 148.36 (C-5). Anal. Calcd for $C_{11}H_{14}N_4O_4$: C, 49.62; H, 5.30; N, 21.04. Found: C, 49.78; H, 5.53; N, 20.96.

*4.1.15. N-[2-(2,3,5-Tri-O-benzyl-1-hydroxy-α,β-D-ribofuranosyl) methylpyridin-3-yl]trifluoroacetamide (**20**).* This compound was prepared by a procedure analogous to that of **4**, starting from **18**. Yield: 55%; Yellow syrup. Data for the predominant α-anomer: IR (film) ν_{max} 3403–3240 (br), 1749, 1455, 1202, 1130 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.35–3.53 (m, 3H, 2×CH_2, H-5′), 3.54–3.71 (m, 1H, H-5′), 3.80–3.86 (m, 1H, H-3′), 4.09 (d, 1H, H-2′, $J_{2'-3'}$=4.69 Hz), 4.35–4.40 (m, 1H, H-4′), 4.41–4.82 (m, 6H, CH_2–Ph), 6.11 (s, 1H, OH, D_2O exch.), 7.15–7.46 (m, 16H, 3×C_6H_5, H-5), 8.06 (d, 1H, H-4, J_{4-5}=8.21 Hz), 8.36 (d, 1H, H-6, J_{6-5}=4.69 Hz), 10.50 (s, 1H, NH, D_2O exch.). Anal. Calcd for $C_{34}H_{33}F_3N_2O_6$: C, 65.59; H, 5.34; N, 4.50. Found: C, 65.78; H, 5.51; N, 4.36.

*4.1.16. tert-Butyl-N-[3-trifluoroacetamido-2-(2,3,5-tri-O-benzyl-1-hydroxy-α,β-D-ribofuranosylmethyl)pyridin-6-yl] carbamate (**21**).* This compound was prepared by a procedure analogous to that of **4**, starting from **19**. Yield: 44%; oil. Data for the predominant α-anomer: IR (film) ν_{max} 3380–3217 (br), 1735, 1595, 1514, 1396, 1270, 1153 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.57 (s, 9H, $(CH_3)_3$), 3.15–3.25 (m, 2H, CH_2), 3.37 (dd, 1H, H-5′, $J_{5'-4'}$=3.42 Hz, $J_{5'-5'}$=10.58 Hz), 3.43 (dd, 1H, H-5′, $J_{5'-4'}$=3.74 Hz, $J_{5'-5'}$=10.58 Hz), 3.75–3.78 (m, 1H, H-3′), 3.92 (d, 1H, H-2′, $J_{3'-2'}$=4.78 Hz), 4.29–4.34 (m, 1H, H-4′), 4.34–4.62 (m, 6H, 3×CH_2–Ph), 4.94 (s, 1H, OH, D_2O exch.), 7.04 (br s, 1H, NHBoc, D_2O exch.), 7.07–7.40 (m, 15H, 3×C_6H_5), 7.77 (d, 1H, H-5, J_{5-4}=8.88 Hz), 7.89 (d, 1H, H-4, J_{4-5}=8.88 Hz), 10.04 (s, 1H, $NHCOCF_3$, D_2O exch.); ^{13}C NMR (50 MHZ, $CDCl_3$) δ 28.38 [$(CH_3)_3$], 42.85 (CH_2), 69.43 (C-5′), 72.74, 72.88, 73.54 (CH_2–Ph), 76.89 (C-3′), 77.90 (C-2′), 80.62 (C-4′), 81.29 [$(CH_3)_3$–*C*], 104.80 (C-1′), 107.38, 113.20, 118.93, 124.66 (CF_3), 110.66 (C-5), 126.86 (C-3), 127.73, 127.81, 127.99, 128.26, 128.54, 128.64 [CH(Ph)], 133.72 (C-4), 136.99, 137.21, 137.54 [C(Ph)], 146.85 (C-2), 148.78 (C-6), 152.03 (OCONH), 154.32, 155.06, 155.80, 156.55 (*C*OCF_3). Anal. Calcd for $C_{39}H_{42}F_3N_3O_8$: C, 63.49; H, 5.74; N, 5.70. Found: C, 63.18; H, 5.90; N, 5.51.

*4.1.17. 2-[(2RS,5S)-2,5-Dihydroxy-3,4,6-tri-O-benzylhexane]pyridin-3-yl-amine (**22**).* This compound was prepared by a procedure analogous to that of **6**, starting from **20**. Yield 80%; oil; IR (film) ν_{max} 3490–3186 (br), 1454, 1308, 1093 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.89 (dd, 1H, H-1′, $J_{1'-2'}$=4.30 Hz, $J_{1'-1'}$=14.87 Hz), 3.02 (dd, 1H, H-1′, $J_{1'-2'}$=7.04 Hz, $J_{1'-1'}$=14.87 Hz), 3.68–3.80 (m, 2H, H-6′), 3.94–4.18 (br s, 2H, NH_2, D_2O exch.), 3.96–4.00 (m, 1H, H-3′), 4.01–4.08 (m, 1H, H-4′), 4.11–4.18 (m, 1H, H-5′), 4.51–4.88 (m, 7H, 3×CH_2–Ph, H-2′), 6.85–7.01 (m, 2H, H-5, H-4), 7.25–7.45 (m, 15H, 3×C_6H_5), 7.86–7.91 (m, 1H, H-6); ^{13}C NMR (50 MHz, $CDCl_3$) δ 36.64 (C-1′), 70.11 (C-5′), 70.60 (C-2′), 71.52 (C-6′), 73.01, 73.44, 74.13 (CH_2–Ph), 79.99 (C-3′), 80.29 (C-4′), 122.28 (C-5), 122.49 (C-4), 125.85, 127.32, 127.65, 127.74, 127.97, 128.16, 128.37 [CH(Ph)], 138.17 (C-6), 138.36, 138.58 [C(Ph)], 142.16 (C-3), 144.90 (C-2). Anal. Calcd for $C_{32}H_{36}N_2O_5$: C, 72.70; H, 6.86; N, 5.30. Found: C, 72.94; H, 7.07; N, 5.13.

*4.1.18. tert-Butyl-N-[3-amino-2-[(2RS,5S)-2,5-dihydroxy-3,4,6-tri-O-benzylhexane]pyridin-6-yl]carbamate (**24**).* This compound was prepared by a procedure analogous to that of **6**, starting from **21**. Chromatographic purification was made using a mixture of AcOEt/CH_2Cl_2 1/4 as the eluent and provided first the trifluoroacetamide diols **23** (25%) then the aminodiols **24** (65%) as light yellow oils.

*4.1.19. Data for tert-butyl-N-[3-trifluoroacetamido-2-[(2RS,5S)-2,5-dihydroxy-3,4,6-tri-O-benzylhexane]pyridin-6-yl]carbamate (**23**).* IR (film) ν_{max} 3457–3178 (br), 1731, 1525, 1396, 1271, 1154 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.51 (s, 9H, $(CH_3)_3$), 2.75–2.86 (m, 1H, H-1′), 3.16 (dd, 1H, H-1′, $J_{1'-1'}$=14.00 Hz, $J_{1'-2'}$=8.20 Hz), 3.64 (dd, 1H, H-6′, $J_{6'-5'}$=4.10 Hz, $J_{6'-6'}$=9.56 Hz), 3.71 (dd, 1H, H-6′, $J_{6'-5'}$=2.05 Hz, $J_{6'-6'}$=9.56 Hz), 3.76–3.85 (m, 1H, H-3′), 3.90–3.99 (m, 2H, H-4′, H-5′), 4.34–4.41 (m, 1H, H-2′), 4.42–4.84 (m, 6H, 3×CH_2–Ph), 7.18–7.40 (m, 16H, 3×C_6H_5, NHBoc), 7.81 (d, 1H, H-5, J_{5-4}=8.87 Hz), 8.13 (d, 1H, H-4, J_{4-5}=8.87 Hz), 10.65 (br s, 1H, $NHCOCF_3$, D_2O exch.); ^{13}C NMR (50 MHz, $CDCl_3$) δ 28.37 [$(CH_3)_3$], 39.41 (C-1′), 69.73 (C-5′), 70.94 (C-6′), 72.23 (C-2′), 73.05, 73.67, 74.81(CH_2–Ph), 79.48 (C-3′), 80.31 (C-4′), 81.32 [$(CH_3)_3$–*C*], 110.62 (C-5), 106.55, 112.60, 118.70, 124.79 (CF_3), 127.03 (C-3), 127.83, 128.09, 128.18, 128.24, 128.30, 128.64, 128.69 [CH(Ph)], 133.40 (C-4), 137.40, 137.70 [C(Ph)], 148.73 (C-6), 149.53 (C-2), 152.16 (OCONH), 154.40, 155.14, 155.88, 156.62 (*C*OCF_3). Anal. Calcd for $C_{39}H_{44}F_3N_3O_8$: C, 63.32; H, 5.99; N, 5.68. Found: C, 63.05; H, 6.27; N, 5.79.

*4.1.20. Data for tert-Butyl-N-[3-amino-2-[(2RS,5S)-2,5-dihydroxy-3,4,6-tri-O-benzylhexane]pyridin-6-yl]carbamate (**24**).* IR (film) ν_{max} 3527–3178 (br), 1722, 1514, 1470, 1282, 1160 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.50 (s, 9H, $(CH_3)_3$), 2.75 (dd, 1H, H-1′, $J_{1'-1'}$=14.68 Hz, $J_{1'-2'}$=3.75 Hz), 2.91 (dd, 1H, H-1′, $J_{1'-1'}$=14.68 Hz, $J_{1'-2'}$=7.51 Hz), 3.67 (dd, 1H, H-6′, $J_{6'-5'}$=5.12 Hz, $J_{6'-6'}$=9.90 Hz), 3.73 (dd, 1H, H-6′, $J_{6'-5'}$=2.73 Hz, $J_{6'-6'}$=9.90 Hz), 3.85–3.91 (m, 1H, H-3′), 3.95 (dd, 1H, H-4′, *J*=2.05, 8.19 Hz), 4.05–4.14 (m, 1H, H-5′), 4.12–4.35 (br s, 2H, NH_2, D_2O exch.), 4.39–4.45 (m, 1H, H-2′), 4.48–4.84 (m, 6H, CH_2–Ph), 6.96 (d, 1H, H-5, J_{5-4}=8.53 Hz), 7.18 (br

s, 1H, NH, D_2O exch.), 7.22–7.39 (m, 15H, 3×C_6H_5), 7.59 (d, 1H, H-4, J_{4-5}=8.53 Hz); ^{13}C NMR (50 MHz, $CDCl_3$) δ 28.41 [$(CH_3)_3$], 36.69 (C-1′), 70.19 (C-5′), 70.88 (C-2′), 71.37 (C-6′), 73.36, 73.56, 74.12 (CH_2–Ph), 80.17 (C-3′), 80.50 (C-4′), 80.56 [$(CH_3)_3$–*C*], 111.47 (C-5), 126.07 (C-4), 127.87, 128.05, 128.31, 128.50 [CH(Ph)], 137.59, 138.10 [C(Ph)], 138.10 (C-3), 142.52 (C-2), 143.05 (C-6), 152.59(O*C*ONH). Anal. Calcd for $C_{37}H_{45}N_3O_7$: C, 69.03; H, 7.05; N, 6.53. Found: C, 68.76; H, 6.91; N, 6.36.

*4.1.21. 2-[(2,3,5-Tri-O-benzyl-β-D-ribofuranosyl)methyl]pyridin-3-ylamine (**25**).* This compound was prepared by a procedure analogous to that of **8**, starting from **22**. Yield. 77%; oil; $[\alpha]_D^{22}$ +24 (*c* 0.15, MeOH); IR (film) ν_{max} 3426, 3348, 1582, 1452, 1268, 1094 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.93 (dd, 1H, CH_2, *J*=7.50, 14.00 Hz), 3.10 (dd, 1H, CH_2, *J*=3.75, 14.00 Hz), 3.42 (dd, 1H, H-5′, $J_{5'-4'}$=4.10 Hz, $J_{5'-5'}$=10.59 Hz), 3.57 (dd, 1H, H-5′, $J_{5'-4'}$=3.07 Hz, $J_{5'-5'}$=10.59 Hz), 3.71 (dd, 1H, H-3′, *J*=5.46, 6.49 Hz), 3.88–4.03 (br s, 2H, NH_2, D_2O exch.), 3.93 (t, 1H, H-2′, *J*=4.43 Hz), 4.15–4.21 (m, 1H, H-4′), 4.35–4.70 (m, 7H, 3×CH_2–Ph, H-1′), 6.79 (dd, 1H, H-4, J_{4-5}=7.85 Hz, J_{4-6}=1.36 Hz), 6.94 (dd, 1H, H-5, J_{5-4}=7.85 Hz, J_{5-6}=4.78 Hz), 7.22–7.41 (m, 15H, 3×C_6H_5), 7.95 (dd, 1H, H-6, J_{6-5}=4.78 Hz, J_{6-4}=1.36 Hz); ^{13}C NMR (50 MHz, $CDCl_3$) δ 38.67 (CH_2), 69.45 (C-5′), 71.97, 72.05, 73.45 (CH_2–Ph), 76.95 (C-3′), 79.53 (C-2′), 80.38 (C-4′), 82.95 (C-1′),122.66 (C-5), 123.16 (C-4), 127.81, 127.95, 128.14, 128.20, 128.46, 128.57 [CH(Ph)], 137.92, 138.00, 138.15 [C(Ph)], 138.71 (C-6), 142.74 (C-3), 144.55 (C-2). Anal. Calcd for $C_{32}H_{34}N_2O_4$: C, 75.27; H, 6.71; N, 5.49. Found: C, 75.01; H, 6.59; N, 5.63.

*4.1.22. N-[2-(2,3,5-Tri-O-benzyl-β-D-ribofuranosyl)methylpyridin-3yl]acetamide (**27**).* To a solution of **25** (40 mg, 0.078 mmol) in dry CH_2Cl_2 (15 mL) was added acetic anhydride (0.009 mL, 0.093 mmol) and the mixture was stirred overnight. The reaction was quenched with a saturated $NaHCO_3$ solution, extracted with CH_2Cl_2 and the organic extracts were dried (Na_2SO_4) and concentrated to dryness. Column chromatography (silica gel) of the residue using a mixture of CH_2Cl_2/MeOH 37.5/1 as the eluent afforded **27** (40 mg, 92%) as an oil. $[\alpha]_D^{22}$ +9 (*c* 0.22, MeOH); IR (film) ν_{max} 3325, 1682, 1589, 1436, 1192, 1119 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.02 (s, 3H, CH_3), 3.03 (dd, 1H, CH_2, *J*=6.48, 14.00 Hz), 3.27–3.36 (m, 2H, CH_2, H-5′), 3.46–3.53 (m, 2H, H-5′, H-3′), 3.80 (t, 1H, H-2′, *J*=5.80 Hz), 4.14–4.20 (m, 1H, H-4′), 4.33–4.70 (m, 7H, 3×CH_2–Ph, H-1′), 7.10–7.42 (m, 16H, 3×C_6H_5, H-5), 8.06 (dd, 1H, H-4, J_{4-5}=7.85 Hz, J_{4-6}=1.36 Hz), 8.28 (dd, 1H, H-6, J_{6-5}=4.78 Hz, J_{6-4}=1.36 Hz), 8.97 (s, 1H, NH, D_2O exch.); ^{13}C NMR (50 MHz, $CDCl_3$) δ 24.18 (CH_3), 38.43 (CH_2), 69.86 (C-5′), 72.20, 72.61, 73.52 (CH_2–Ph), 76.96 (C-3′), 79.33 (C-2′), 81.43 (C-4′), 82.67 (C-1′), 122.34 (C-5), 127.76, 128.01, 128.20, 128.24, 128.52, 128.59 [CH(Ph)], 131.36 (C-4), 134.07 (C-3), 137.52, 137.71, 137.97 [C(Ph)], 144.97 (C-6), 149.66 (C-2), 168.98 (CO). Anal. Calcd for $C_{34}H_{36}N_2O_5$: C, 73.89; H, 6.57; N, 5.07. Found: C, 73.69; H, 6.39; N, 5.22.

*4.1.23. tert-Butyl-N-[3-acetamido[2-(2,3,5-tri-O-benzyl-β-D-ribofuranosyl)]methylpyridin-6-yl]carbamate (**28**).* This compound was prepared from **24** by an analogous procedure as described for compounds **25** and **27**. The intermediate carbamate **26** was acetylated without further purification to provide **28**. Yield (two steps) 89%; oil; $[\alpha]_D^{22}$ +14 (*c* 0.48, MeOH); IR (film) ν_{max} 3372–3263 (br), 1731, 1686, 1503, 1368, 1274, 1154 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.55 (s, 9H, $(CH_3)_3$), 1.97 (s, 3H, CH_3), 2.84 (dd, 1H, CH_2, *J*=14.00, 5.80 Hz), 3.16 (dd, 1H, CH_2, *J*=14.00, 3.07 Hz), 3.26 (dd, 1H, H-5′, $J_{5'-4'}$=4.78 Hz, $J_{5'-5'}$=10.58 Hz), 3.44 (dd, 1H, H-5′, $J_{5'-4'}$=3.42 Hz, $J_{5'-5'}$=10.58 Hz), 3.50 (t, 1H, H-3′, *J*=5.12 Hz), 3.73 (t, 1H, H-2′, *J*=5.80 Hz), 4.13–4.18 (m, 1H, H-4′), 4.31–4.69 (m, 7H, 3×CH_2–Ph, H-1′), 6.99 (br s, 1H, NH, D_2O exch.), 7.12–7.42 (m, 15H, 3×C_6H_5), 7.74 (d, 1H, H-5, J_{5-4}=8.88 Hz), 7.90 (d, 1H, H-4, J_{4-5}=8.88 Hz), 8.69 (br s, 1H, NH, D_2O exch.); ^{13}C NMR (50 MHz, $CDCl_3$) δ 23.96 (CH_3), 28.42 [$(CH_3)_3$], 37.49 (CH_2), 68.84 (C-5′), 72.14, 72.34, 73.54 (CH_2–Ph), 76.67 (C-3′), 78.44 (C-2′), 81.01 [$(CH_3)_3$–*C*], 81.30 (C-4′), 82.36 (C-1′), 110.55 (C-5), 127.73, 128.05, 128.25, 128.33, 128.53 [CH(Ph)], 129.17 (C-3), 134.51 (C-4), 137.48, 137.64, 137.97 [C(Ph)], 147.68 (C-2), 148.09 (C-6), 152.24 (OCONH), 168.75 (CO). Anal. Calcd for $C_{39}H_{45}N_3O_7$: C, 70.14; H, 6.79; N, 6.29. Found: C, 70.37; H, 6.53; N, 6.41.

*4.1.24. 3-(2,3,5-Tri-O-benzyl-β-D-ribofuranosyl)pyrazolo-[4.3-b]pyridine (**31**).* This compound was prepared by a procedure analogous to that of **12** starting from **27**. Yield 85%; oil; $[\alpha]_D^{22}$ −27 (*c* 0.39, MeOH); IR (film) ν_{max} 3286, 1481, 1458, 1233, 1079 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.73 (dd, 1H, H-5′, $J_{5'-4'}$=4.78 Hz, $J_{5'-5'}$=10.58 Hz), 3.80 (dd, 1H, H-5′, $J_{5'-4'}$=4.19 Hz, $J_{5'-5'}$=10.58 Hz), 4.36 (t, 1H, H-3′, *J*=5.46 Hz), 4.45–4.72 (m, 7H, 3×CH_2–Ph, H-4′), 4.82 (t, 1H, H-2′, *J*=4.78 Hz), 5.74 (d,1H, H-1′, $J_{1'-2'}$=4.78 Hz), 7.15 (dd, 1H, H-6, J_{6-5}=4.10 Hz, J_{6-7}=8.53 Hz), 7.17–7.40 (m, 15H, 3×C_6H_5), 7.77 (d, 1H, H-7, J_{7-6}=8.53 Hz), 8.47 (d, 1H, H-5, J_{5-6}=4.10 Hz), 11.00–11.60 (v br s, 1H, NH, D_2O exch.); ^{13}C NMR (50 MHz, $CDCl_3$) δ 70.38 (C-5′), 72.20, 72.25, 73.48 (CH_2–Ph), 77.29 (C-1′), 78.20 (C-3′), 79.78 (C-2′), 81.44 (C-4′), 119.02 (C-7), 121.12 (C-6), 127.66, 127.80, 127.86, 128.10, 128.16, 128.26, 128.42 [CH(Ph)], 134.55 (C-7α), 138.07, 138.22, 138.25 [C(Ph)], 138.60 (C-3α), 143.49 (C-3), 145.53 (C-5). Anal. Calcd for $C_{32}H_{31}N_3O_4$: C, 73.68; H, 5.99; N, 8.06. Found: C, 73.56; H, 5.72; N, 8.15.

*4.1.25. tert-Butyl-N-[2-(2,3,5-tri-O-benzyl-β-D-ribofuranosyl)pyrazolo-[4,3-b]pyridin-5-yl]carbamate (**32**).* This compound was prepared by a procedure analogous to that of **12**, starting from **28**. Yield 84%; oil; $[\alpha]_D^{22}$ −17 (*c* 0.29, MeOH); IR (film) ν_{max} 3294, 1725, 1497, 1253, 1156 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.58 (s, 9H, $(CH_3)_3$), 3.67 (dd, 1H, H-5′, $J_{5'-4'}$=4.76 Hz, $J_{5'-5'}$=10.61 Hz), 3.73 (dd, 1H, H-5′, $J_{5'-4'}$=4.02 Hz, $J_{5'-5'}$=10.61 Hz), 4.32 (t, 1H, H-3′, *J*=5.49 Hz), 4.43–4.48 (m, 1H, H-4′), 4.48–4.75 (m, 6H, 3×CH_2–Ph), 4.79 (t, 1H, H-2′, *J*=5.12 Hz), 5.64 (d, 1H, H-1′, $J_{1'-2'}$=5.12 Hz), 7.15–7.39 (m, 15H, 3×C_6H_5), 7.49 (br s, 1H, NH, D_2O exch.), 7.77 (d, 1H, H-7, J_{7-6}=9.15 Hz), 8.02 (d, 1H, H-6, J_{6-7}=9.15 Hz), 10.70–12.10 (v br s, 1H, NH, D_2O exch.); ^{13}C NMR (50 MHz, $CDCl_3$) δ 28.45 [$(CH_3)_3$], 70.22 (C-5′), 72.10, 72.14, 73.44 (CH_2–Ph), 77.36 (C-1′), 77.87 (C-3′), 79.34 (C-2′), 81.09 [$(CH_3)_3$–*C*], 81.42 (C-4′), 112.95 (C-6), 121.35 (C-7), 127.65, 127.71, 127.87, 128.08, 128.19, 128.26, 128.40, 128.45 [CH(Ph)], 132.62 (C-7α), 135.99 (C-3α), 137.98, 138.14, 138.20 [C(Ph)], 141.62 (C-3), 148.25 (C-5), 152.82 (CO). Anal. Calcd for $C_{37}H_{40}N_4O_6$: C, 69.79; H, 6.33; N, 8.80. Found: C, 69.98; H, 6.56; N, 8.57.

*4.1.26. 3-(β-D-Ribofuranosyl)pyrazolo[4,3-b]pyridine (**33**).* This compound was prepared by a procedure analogous to that of **16** starting from **31**. Yield 96%; oil; $[\alpha]_D^{22}$ −50 (*c* 0.26, CH_3OH); IR (film) ν_{max} 3519–3124, 1380, 1078 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD) δ 3.96 (dd, 1H, H-5′, $J_{5'-4'}$=1.95 Hz, $J_{5'-5'}$=11.74 Hz), 4.05 (dd, 1H, H-5′, $J_{5'-4'}$=2.74 Hz, $J_{5'-5'}$=11.74 Hz), 4.19–4.24 (m, 1H, H-4′), 4.28 (t, 1H, H-3′, *J*=4.70 Hz), 4.40 (t, 1H, H-2′, *J*=5.48 Hz), 5.36 (d, 1H, H-1′, $J_{1'-2'}$=5.87 Hz), 8.00 (dd, 1H, H-6, J_{6-5}=5.48 Hz, J_{6-7}=8.60 Hz), 8.83 (dd, 1H, H-5, J_{5-6}=5.48 Hz, J_{5-7}=0.78 Hz), 8.86 (dd, 1H, H-7, J_{7-6}=8.60 Hz, J_{7-5}=0.78 Hz); ^{13}C NMR (50 MHz, CD_3OD) δ 62.55 (C-5′), 73.12 (C-3′), 77.91 (C-2′), 81.64 (C-1′), 86.06 (C-4′), 123.04 (C-6), 129.03 (C-3α), 130.96 (C-7), 138.74 (C-7α), 139.86 (C-5), 142.60 (C-3). Anal. Calcd for $C_{11}H_{13}N_3O_4$: C, 52.59; H, 5.22; N, 16.72. Found: C, 52.74; H, 5.36; N, 16.46.

*4.1.27. 5-Amino-3-(β-D-ribofuranosyl)pyrazolo[4,3-b]pyridine (**34**).* This compound was prepared by a procedure analogous to that of **16** starting from **32**. Yield 79%; oil; $[\alpha]_D^{22}$ −115 (*c* 0.43, CH_3OH); IR (film) ν_{max} 3520–3070, 1373, 1110 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD) δ 3.92 (dd, 1H, H-5′, $J_{5'-4'}$=2.05 Hz, $J_{5'-5'}$=11.95 Hz), 4.01 (dd, 1H, H-5′, J_{5-4}=2.73 Hz, $J_{5'-5'}$=11.95 Hz), 4.12–4.16 (m, 1H, H-4′), 4.20 (t, 1H, H-3′, *J*=4.43 Hz), 4.26 (t, 1H, H-2′, *J*=5.46 Hz), 5.16 (d, 1H, H-1′, $J_{1'-2'}$=5.81 Hz), 7.00 (d, 1H, H-6, J_{6-7}=9.56 Hz), 8.19 (d, 1H, H-7, J_{7-6}=9.56 Hz); ^{13}C NMR (50 MHz,

CD_3OD) δ 62.60 (C-5′), 73.12 (C-3′), 77.86 (C-2′), 81.21 (C-1′), 85.94 (C-4′), 114.35 (C-6), 123.68 (C-3α), 131.41 (C-7), 132.44 (C-7α), 138.74 (C-3), 154.34 (C-5). Anal. Calcd for $C_{11}H_{14}N_4O_4$: C, 49.62; H, 5.30; N, 21.04. Found: C, 49.79; H, 5.49; N, 20.86.

4.2. Molecular modelling

Vibrational analysis and thermochemical calculations were performed on pre-optimized geometry structures of the nucleosides and their hydrates using Jaguar v.4.2.[25] The crystal structure of murine ADA (pdb id:2ADA) was used as a template for docking calculations. Docking of each analogue was performed by the use of 1000 steps of the mixed Monte Carlo/Low mode search algorithm as it is implemented in Macromodel v.7 and the GB/SA implicit solvent model.[26] The Zn atom was attributed a total charge of 2+ and was included in all calculations. It was restrained in its crystallographic position using harmonic distance potentials of 500 kcal/mol connecting it to the N_ε imidazole nitrogens of His15, His17 and His214 as well as to the side chain carboxylate of Asp295.

4.3. Biological assays

Adenosine deaminase (ADA, EC 3.5.4.4), adenosine and bovine serum albumin (BSA) were purchased from Sigma. The target compounds were screened against calf spleen ADA in vitro. The total volume of the reaction in 50 mM phosphate buffer (pH 7.0) was 1 mL containing substrate concentration 60 μM, 0.003% BSA and 0.02 unit of ADA. The compounds were dissolved in DMSO final concentration 2%. The reaction was performed at 25 °C. The conversion of adenosine to inosine was monitored by following the absorbance decrease at 265 nm using a thermostatted Spectrophotometer (Analytikjena, Specord 200) at 234 nm (room temperature) and compared to the appropriate solution, which did not contain the extracts. Absorbances were below 1 absorbance unit in a 1 cm path length cuvette. The inhibitor concentration ranged from 0.01 to 1 mM. The inhibitors were used both with 5 min pre-incubation with enzyme or without pre-incubation with enzyme, and the reactions were initiated by addition of enzyme or the mixture enzyme-inhibitor. Assays were performed in triplicate and the standard deviation in absorbance determinations was less than±10%.

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Supplementary data

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.tet.2010.10.005.

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22. Data for *N*-(2-methylpyridin-3-yl)trifluoroacetamide (**18**). Mp 120–1 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.49 (s, 3H, CH_3), 7.21 (dd, 1H, H-5, J_{5-6}=4.70 Hz, J_{5-4}=7.83 Hz), 8.02 (d, 1H, H-4, J_{4-5}=7.83 Hz), 8.38 (d, 1H, H-6, J_{6-5}=4.70 Hz), 8.47 (br s, 1H, NH, D_2O exch.). ^{13}C NMR (50 MHz, $CDCl_3$) δ 20.08 (CH_3), 107.26, 112.99, 118.71, 124.44 (CF_3), 122.15 (C-5), 129.82 (C-3), 132.78 (C-4), 147.26 (C-6), 152.19 (C-2), 155.05, 155.81, 156.56, 157.25 ($COCF_3$).
23. Data for *tert*-butyl-*N*-(3-trifluoroacetamido-2-methylpyridin-6-yl) carbamate (**19**). Mp 118–9 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.50 (s, 9H, $(CH_3)_3$), 2.55 (s, 3H, CH_3), 7.97 (d, 1H, H-5, J_{5-4}=9.00Hz), 8.10 (d, 1H, H-4, J_{4-5}=9.00 Hz), 10.46 (br s, 1H, $NHCOCF_3$, D_2O exch.), 11.21 (br s, 1H, NHBoc, D_2O exch.). ^{13}C NMR (50 MHz, $CDCl_3$) δ 16.69 (CH_3), 27.88 [$(CH_3)_3$], 84.90 [$(CH_3)_3$–*C*], 107.15, 112.86, 118.57, 124.29 (CF_3), 113.33 (C-5), 125.92 (C-3), 143.58 (C-4), 146.65 (C-2), 148.65 (C-6), 152.20 (OCONH), 155.62, 156.40, 157.17, 157.94 ($COCF_3$).
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