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Original article

Synthesis and biochemical evaluation of guanidino-alkyl-ribitol derivatives as nucleoside hydrolase inhibitors

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Abstract

Nucleoside hydrolase (NH) is a key enzyme in the purine salvage pathway. The purine specificity of the IAG-NH from *Trypanosoma vivax* is at least in part due to cation−π-stacking interactions. Guanidinium ions can be involved in cation−π-stacking interactions, therefore a series of guanidino-alkyl-ribitol derivatives were synthesized in order to examine the binding affinity of these compounds towards the target enzyme. The compounds show moderate to good inhibiting activity towards the IAG-NH from *T. vivax*.

Keywords: Nucleoside hydrolase; Inhibitor; Cation−π-stacking interactions; Guanidino-alkyl-ribitol

1. Introduction

Parasitic infections are a continuing health problem, especially in developing countries. Amongst these infections, trypanosomiasis and leishmaniasis are responsible for more than 110 000 deaths a year. Drugs that are currently in use suffer from disadvantages such as toxicity, upcoming resistance, complex administration mode, high costs or are simply not effective in certain stages of the disease. Hence, there is an urgent need for new drugs in the treatment of trypanosomiasis [1].

One approach for the development of new anti-trypanosomal compounds is based on the knowledge that parasitic protozoa lack the ability to synthesize purines *de novo* [2]. Protozoa use the purine salvage pathway to obtain purine bases from nucleosides of the host. A key enzyme in the purine salvage pathway is nucleoside hydrolase (NH). This enzyme hydrolytically cleaves the *N*-glycosidic bond of nucleosides to give a nucleobase and ribose. It plays a central role in purine salvage by purine auxotrophic parasites, since it is the step preceding the phosphoribosyltransferase reaction responsible for the synthesis of purine nucleotides [3].

During cleavage of a nucleoside by nucleoside hydrolase, an oxocarbenium ion-like transition state is formed in which the ribose-oxygen carries a partial positive charge in which N^7 of the purine is protonated [4]. At present, four types of NH's are known, each differing in substrate specificity [5]. The target enzyme of our research is the NH from *Trypanosoma vivax*. This NH shows a high specificity for inosine, adenosine and guanosine (IAG-NH). The purine specificity is imposed by parallel aromatic stacking of the purine ring between Trp83 and Trp260 (Fig. 1) [5,6]. These stacking interactions contribute in raising the pK_a of N^7 of the purine, allowing direct protonation of the base by solvent molecules, hence functioning as an alternative to general acid catalysis [7].

The active site and hence the catalytic mechanism of the IAG-NH is different from that of the mammalian nucleoside phosphorylase, the enzyme that cleaves nucleosides in mammalian cells. Therefore selective inhibition of the former enzyme could be an effective way to kill the parasite without causing toxicity towards the host [2].

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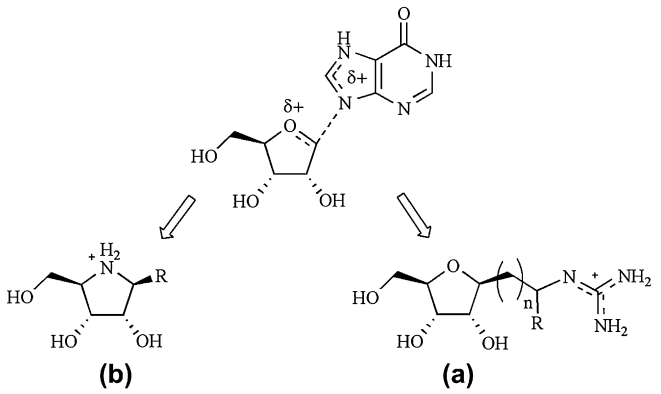


Fig. 1. Rational design of the inhibitors, based on the structure of the transition state (top drawing). (a) Guanidinium ions can mimic the partial positive charge in the purine ring. (b) Iminoribitol derivatives can mimic the partial positive charge in the ribose ring.

2. Results and discussion

2.1. Design of the inhibitors

In our search for new inhibitors against NH we wanted to mimic the partial positive charge on the purine ring by other functional groups that would also be able to participate in cation–π-stacking interactions (Fig. 1a). Guanidinium ions are candidates to get involved in this type of interactions [8] (Fig. 2).

Docking results confirm the cation–π-stacking interaction potential involving the guanidinium fragment. This moiety is situated between the Trp83 and Trp260 side chains, both responsible for stacking with the purine ring. The docking study points out that the length of the alkyl chain separating the ribitol and the guanidine moieties could be relevant for correct stacking. The best stacking interaction is observed with a linker of two methylene units (**8**).

A closer vicinity of the guanidinium to the ribitol moiety (**3**) increases the inhibitor's tendency to adopt unfavourable conformations stabilized by intramolecular H-bonding. Inhibitors containing a three-carbon atom spacer (**10**) are slightly hindered in the NH active pocket.

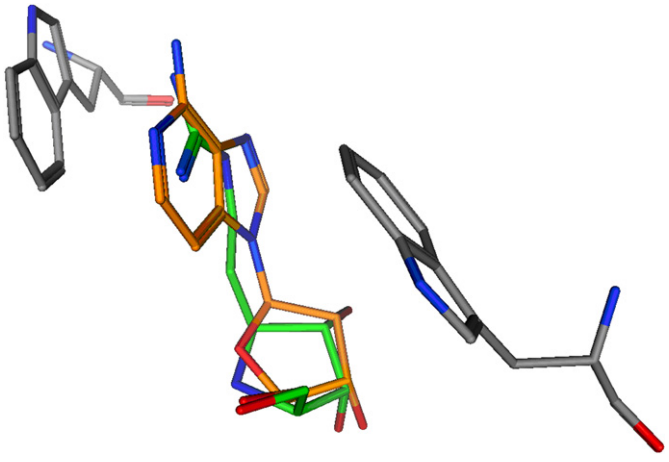


Fig. 2. Aromatic stacking of the purine ring of 3-deaza-adenosine (pdb code 1HP0) (carbons in orange) and the guanidino-alkyl-iminoribitol **20** (carbons in green) with Trp83 and Trp260 (carbons in grey) (For interpretation of the references to colour in figure legends, the reader is refered to the web version of this article).

As these docking studies suggested that guanidino-alkyl-ribitol derivatives could be able to fit into the active site of the target enzyme, we synthesized guanidino-alkyl-ribitol derivatives (**3** and **8**–**10**). Concurrently we synthesized compounds lacking a basic group (**1** and **7**) and a quaternary ammonium salt (**2**), to prove that the guanidino function contributes favourably to the binding interactions of the compounds with the enzyme.

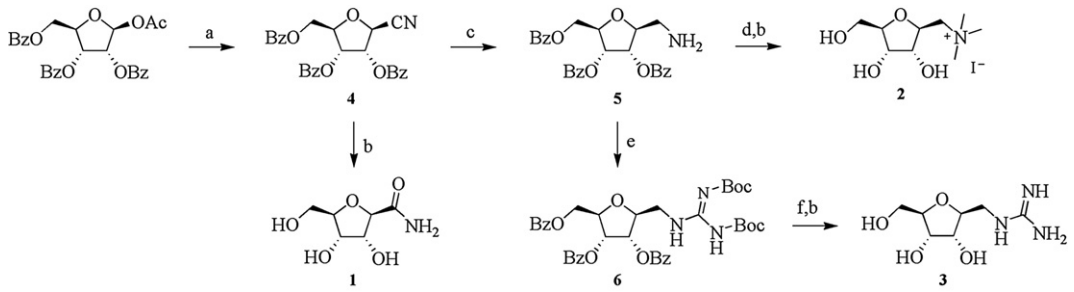
A further objective was to apply the transition-state-analogue hypothesis proposed by Schramm and Horenstein [9]. They proposed that the partial positive charge that develops in the ribose ring during hydrolysis can be mimicked by substituting the ribose-oxygen for a protonated nitrogen and developed nM active inhibitors, characterized by a deaza-purine substituted iminoribitol moiety. (Fig. 1b) [10]. The best docking pose obtained for compound **20**, containing a guanidino-ethyl arm, confirms the cation–π-stacking interaction with the Trp83 and Trp260 side chains (Fig. 2). As revealed by Versées et al. [11] the iminoribitol moiety is stabilized in the active pocket by an additional interaction of the imino group with Asn186. The same stabilization occurs when the guanidine-alkyl chain was introduced on the imino group (**26** and **27**). The length of the alkyl chain determines the correct position of the guanidine group for stacking. The spacer arm separating the iminoribitol and guanidine moieties should minimally consist of three methylene units (**27**) for a right assignment of both groups.

To enhance the aromatic stacking interactions, we also developed the aromatic guanidine and amidine compounds **32** and **33**. The aromatic side chains occupy the same location as the purine ring of ImmH [(1*S*)-1-(9-deazahypoxanthin-9-yl)-1,4-dideoxy-1,4-imino-D-ribitol] [11].

2.2. Synthesis

Synthesis of compounds **1**–**3** (Scheme 1) started from commercially available β-D-ribofuranose-1-acetate-2,3,5-tribenzoate. Reaction with Me_3SiCN selectively afforded the β-cyanide **4** [12]. Stirring **4** in NH_3 saturated MeOH resulted in the amide **1**. Reduction of nitrile **4** was performed with $NaBH_4$/TFA [13]. The resulting amine **5** underwent methylation to yield, after deprotection, the quaternary ammonium salt **2**. Introduction of the guanidino group was achieved by coupling amine **5** with *N*,*N*′-bis(*tert*-butoxycarbonyl)-1*H*-pyrazole-1-carboxamidine as the guanylating reagent [14]. Deprotection of **6** gave the guanidino-methyl-ribitol **3**.

Compounds **4**–**7** were synthesized from protected D-ribose (Scheme 2) [15]. The protected ribose **11** underwent a Moffat–Wittig reaction with the appropriate phosphorylide reagent [16]. After chromatographic separation of the α- and β-isomer (1:3 ratio), the β-isomer was carried into the next step: reduction of the ester **12a** resulted in the primary alcohol which was transformed into the corresponding mesylate **13**. Substitution of the mesyl group of **13** by sodium azide and deprotection afforded azide **7**. Reduction of the protected azide **14**, reaction with the guanylating reagent and subsequent deprotection



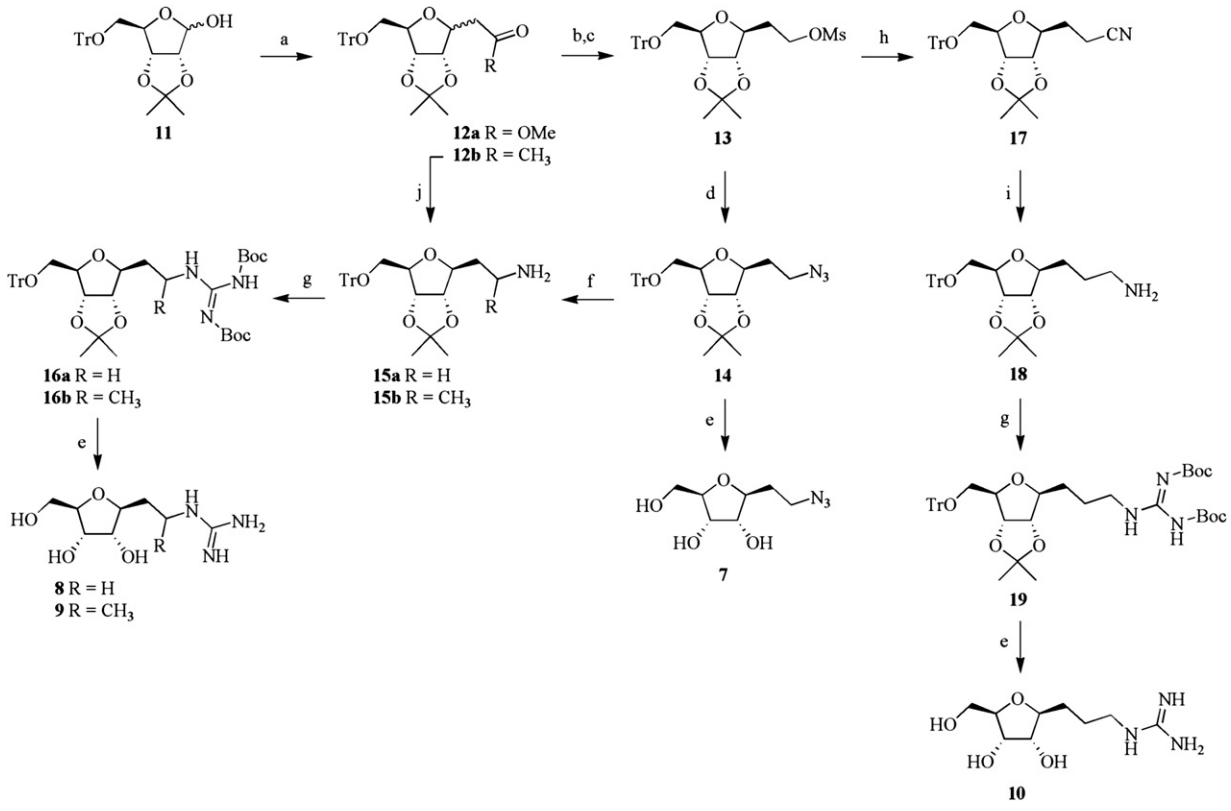
Scheme 1. Reagents and conditions: (a) Ref. [12]: Me_3SiCN, $SnCl_4$, ACN, rt, 10 min; (b) NH_3, MeOH, rt, 4 days; (c) Ref. [13]: $NaBH_4$, TFA, rt, 18 h; (d) CH_3I, DMF, rt, 20 h; (e) *N*,*N*′-bis(*tert*-butoxycarbonyl)-1*H*-pyrazole-1-carboxamidine, $CHCl_3$, rt, 24 h; (f) TFA:DCM 1:1, rt, 18 h.

resulted in the guanidino-ethyl-ribitol **8**. Guanidino-methyl-ethyl-ribitol **9** was synthesized from ketone **12b** via a reductive amination with ammonium formate as the amine source, followed by reaction with the guanylating agent and subsequent deprotection. Substitution of mesylate **13** with NaCN afforded nitrile **17**. Reduction, reaction with the guanylating reagent and deprotection resulted in the guanidino-propyl-ribitol **10**.

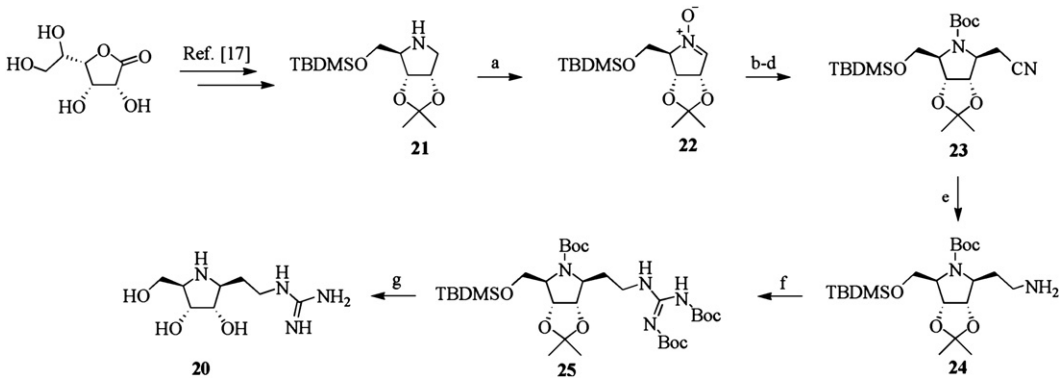
For the synthesis of compound **20** (Scheme 3), protected iminoribitol **21**, synthesized from D-gulonolactone by the method described by Fleet and Son [17], was oxidized to nitrone **22** and addition of lithiated acetonitrile selectively afforded β-cyanide **23** [18]. Compound **23** was reduced, treated with the guanylating reagent and deprotected to give the guanidino-iminoribitol target compound **20**.

Compounds **26** and **27** were synthesized starting from 2-bromoethylamine hydrobromide and 3-bromopropylamine hydrobromide, respectively (Scheme 4). After Boc-protection of the amines, the bromides were substituted by the protected iminoribitol **21** [17]. The guanidino group was introduced as before and deprotection afforded guanidine iminoribitols **26** and **27**.

For the synthesis of compounds **32** and **33**, the protected iminoribitol **21** [17] was reacted with the appropriate benzylbromide (Scheme 5). The nitrobenzyl derivative **35a** was reduced with $SnCl_2$ in ethanol and the resulting amine was treated with the guanylating reagent. Deprotection yielded the target compound **32**. Nitrile derivative **35b** was converted into an amidine with lithium hexamethyldisilazide [19]. Deprotection



Scheme 2. Reagents and conditions: (a) Ref. [16]: $Ph_3P{=}CHCOOMe$ or $Ph_3P{=}CHCOCH_3$, ACN, reflux, 18 h; (b) $LiAlH_4$, Et_2O, 0 °C, 15 min; (c) MsCl, pyridine, DMAP, rt, 2 h; (d) NaN_3, DMF, 100 °C, 7 h; (e) TFA:H_2O 1:1, rt, 18 h; (f) $LiAlH_4$, Et_2O, 0 °C, 3 h; (g) *N*,*N*′-bis(*tert*-butoxycarbonyl)-1*H*-pyrazole-1-carboxamidine, $CHCl_3$, rt, 24 h; (h) NaCN, NaI, DMF, 100 °C, 7 h; (i) $LiAlH_4$, Et_2O, rt, 18 h; (j) $NH_4^+HCOO^-$, $NaCNBH_3$, 3 Å MS, MeOH, rt, 18 h.



Scheme 3. Reagents and conditions: (a) Ref. [18]: SeO_2, H_2O_2, acetone, <4 °C, 3–4 h; (b) Ref. [18]: *n*-BuLi, ACN, −78 °C, 0.5 h; (c) Ref. [18]: Zn dust, AcOH, rt, 6 h; (d) Ref. [18]: $(Boc)_2O$, $CHCl_3$, rt, 18 h; (e) $LiAlH_4$, THF, rt, 3 h; (f) *N*,*N*′-bis(*tert*-butoxycarbonyl)-1*H*-pyrazole-1-carboxamidine, $CHCl_3$, rt, 24 h; (g) TFA:H_2O 1:1, rt, 18 h.

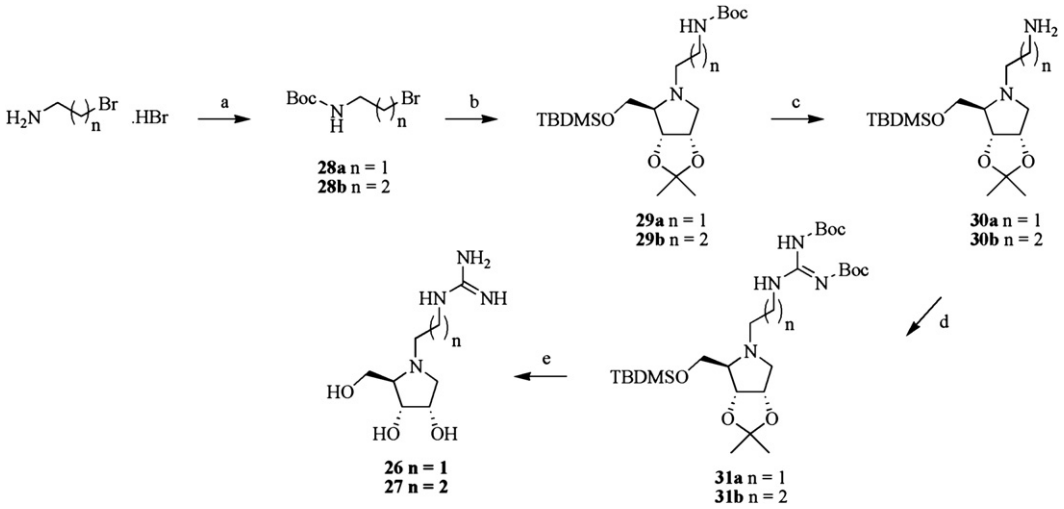
resulted in the target compound **33**. Deprotection of **21** [17] yielded iminoribitol **34**. An intermediate in the synthesis of iminoribitol **21** is the protected benzyl derivative **37** (Scheme 6), which, after deprotection, led to benzyl iminoribitol **38**.

2.3. Biochemical results

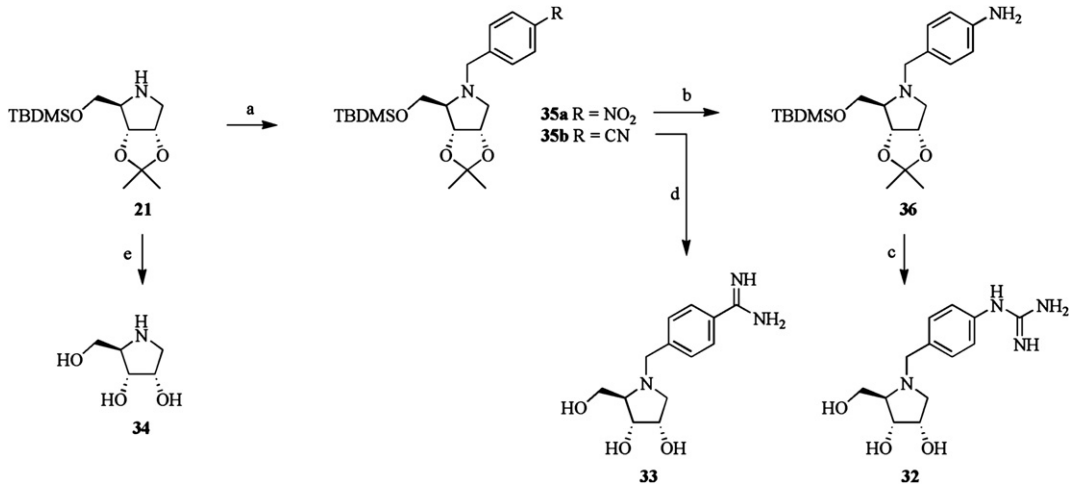
The target compounds were tested as inhibitors against IAG-NH isolated from *T. vivax* and the results are shown in Table 1. The neutral ribitol derivatives **1** and **7**, and the quaternary ammonium **2** showed poor inhibiting activity, with K_i values in the mM range. Compared to this, guanidino-alkyl-ribitol derivatives **3** and **8–10** showed a 10-fold decrease in K_i values, compound **8** with a C2 linker being the most active one confirming the results obtained by the docking studies. It can therefore be concluded that the guanidine function plays a role in the binding of the inhibitor to the enzyme, as the binding of D-ribose in the target enzyme shows a K_{rib} of 148 mM [7]. Guanidino-alkyl-iminoribitol **20** shows a significant increase in potency compared to its ribitol analogue **8**, supporting the transition-state-analogue hypothesis described earlier. *N*-Guanidino-ethyl-iminoribitol **26** is less active than its propyl equivalent **27**, confirming the optimal chain length conclusions of the modeling study. The inhibitory activity of benzylguanidine **32** and benzylamidine **33** appears higher (7.0 and 6.7 μM) and shows that the aromatic ring improves the stacking properties substantially. The guanidine and amidine moieties are clearly important, increasing the activity 10-fold compared to the non-substituted benzyl derivative **38**. The loss in activity of compound **38** compared to unsubstituted iminoribitol **34** could be due to an increase in conformational freedom for compound **38**. Substitution of the benzyl moiety of **38** with a guanidine or amidine group possibly results in an additional interaction with the enzyme, which allows more favourable orientation of the aromatic moiety within the active site.

3. Conclusion

Our hypothesis – adding a guanidino group to a ribitol moiety improves the binding of ribitol derivatives with nucleoside



Scheme 4. Reagents and conditions: (a) di-*tert*-butyl dicarbonate, Et_3N, DCM, rt, 2 h to overnight; (b) **21** [17], K_2CO_3, DMF, 60 °C, 24 h; (c) TFA:DCM 1:1, rt, 30 min; (d) *N*,*N*′-bis(*tert*-butoxycarbonyl)-1*H*-pyrazole-1-carboxamidine, Et_3N, $CHCl_3$, rt, 20 h to 3 days; (e) TFA:H_2O 1:1, rt, 42–60 h.



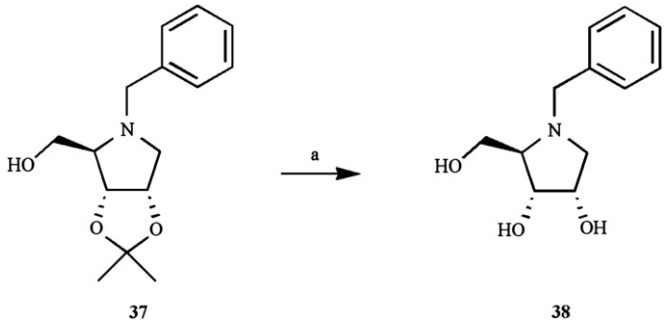
Scheme 5. Reagents and conditions: (a) 4-nitrobenzylbromide, DMF, K_2CO_3, 40 °C, 3 h or α-bromo-*para*-tolunitrile, DCM, H_2O, Na_2CO_3, reflux, 3 h; (b) $SnCl_2$, EtOH, N_2, 70 °C, 30 min; (c) (1) *N*,*N*′-bis(*tert*-butoxycarbonyl)-1*H*-pyrazole-1-carboxamidine, $CHCl_3$, reflux, 48 h; (2) TFA:H_2O 1:1, rt, 18 h; (d) *n*-BuLi, HMDS, Et_2O, N_2, 0 °C to rt, 2 h; (e) aqueous 6 N HCl, MeOH, rt, 20 h.

hydrolase – was confirmed. Although the inhibiting activity of these guanidino-alkyl-ribitol derivatives is rather poor, it was observed that the guanidine function does contribute to the binding of the inhibitor with the enzyme, possibly by cation–π-stacking interactions. Using an iminoribitol scaffold improved the activity drastically and low μM active compounds (**20**, **27**, **32** and **33**) were obtained. These compounds are now being used as leads for further inhibitor design.

4. Experimental protocols

4.1. General procedures

All starting materials were obtained from Acros Organics or Aldrich. Tris-(2-aminoethyl)-amine polystyrene resin was obtained from Novabiochem. NMR spectra were recorded on a Bruker Avance® DRX-400 spectrometer (400 MHz), coupling constants are reported in Hz. Column chromatography was performed on a Flashmaster II (Jones Chromatography) with Isolute® columns pre-packed with silica gel (30–90 μM) for normal phase chromatography and C_{18} (30–90 μM) for reversed phase chromatography. Melting points were determined on an Electrothermal® digital melting point apparatus and are uncorrected. Electrospray Ionisation (ESI) mass spectra were acquired on an ion trap mass spectrometer (Bruker Daltonics® esquire™ 3000^{plus}). LC–MS spectra were recorded on an Agilent 1100 Series HPLC system equipped with a HILIC Silica column (2.1 × 100 mm, 5 μm, Atlantis HILIC, Waters) coupled with a Bruker Daltonics® esquire™ 3000^{plus} mass spectrometer (solvent A: H_2O with 0.1% formic acid, solvent B: ACN with 0.1% formic acid, gradient 2: 90% B to 40% B, 12 min, 0.2 mL/min).



Scheme 6. Reagents and conditions: (a) TFA:H_2O 1:1, rt, 18 h.

4.2. Chemistry

4.2.1. (2R,3S,4R,5R)-3,4-Dihydroxy-5-(hydroxymethyl)-tetrahydro-2-furancarboxamide (*1*)

2,3,5-Tri-*O*-benzoyl-β-D-ribofuranosyl cyanide (**4**) [12] (0.90 g, 1.9 mmol) was dissolved in a solution of 7 N NH_3 in methanol (20 mL) and stirred at rt for 4 days. The reaction mixture was concentrated, the residue dissolved in water and washed with $CHCl_3$. The aqueous layer was concentrated and the residue was purified by column chromatography ($CHCl_3$:MeOH, 4:1) to yield the title compound as a gum (0.11 g, 34%); MS (ESI): *m*/*z* 178.0 (MH^+); LC–MS: rt 2.6 min, *m*/*z* 177.9 (MH^+); ^{1}H NMR (D_2O): δ 3.70 (dd, 1H, $J = 12.6$, $J' = 4.3$), 3.84 (dd, 1H, $J = 12.6$, $J' = 2.8$), 4.01 (m, 1H), 4.06 (dd, 1H, $J = 6.6$, $J' = 4.9$), 4.23 (m, 1H), 4.30 (d, 1H, $J = 3.8$); ^{13}C NMR (D_2O): δ 60.4, 70.3, 74.5, 82.3, 83.2, 175.9.

4.2.2. 2,5-Anhydro-1-deoxy-1-(trimethylammonio)-D-allitol iodide (*2*)

A solution of 1-amino-2,5-anhydro-3,4,6-tri-*O*-benzoyl-1-deoxy-D-allitol (**5**) [12,13] (0.13 g, 0.27 mmol) and MeI (1.5 mL, 24 mmol) in dry DMF was stirred under a N_2 atmosphere for 24 h. Subsequently the reaction mixture was co-evaporated with hexane, EtOAc was added to the residue and the precipitate that formed was filtered off. The filtrate was evaporated and the residue purified by column chromatography ($CHCl_3$:MeOH, 4:1) to yield 3,4,6-*O*-tribenzoyl-2,5-anhydro-1-deoxy-1-(trimethylammonio)-D-allitol iodide as

Table 1
Inhibition of IAG-nucleoside hydrolase isolated from *T. vivax* by the target compounds (K_i = inhibition constant)

Compds	K_i (μM)
1	4500 ± 1307
2	2283 ± 357
3	545 ± 63
7	5640 ± 1725
8	309 ± 32
9	685 ± 74
10	677 ± 107
20	18 ± 5
26	286 ± 58
27	40 ± 3
32	7.0 ± 1.4
33	6.7 ± 0.4
34	6.1 ± 0.6
38	59 ± 18

a syrup (0.10 g, 70%). 3,4,6-*O*-Tribenzoyl-2,5-anhydro-1-deoxy-1-(trimethylammonio)-D-allitol iodide (0.10 g, 1.19 mmol) was dissolved in a solution of 7 N NH_3 in methanol (5 mL). The reaction mixture was stirred at rt for 4 days, concentrated under reduced pressure, and the residue dissolved in water and washed with $CHCl_3$. The aqueous layer was concentrated and the residue was recrystallized from hot EtOH to yield the title product as a white crystalline powder (22 mg, 58%); mp 156–158 °C; MS (ESI): *m/z* 206.3 (M^+); LC–MS: rt 11.6 min, *m/z* 205.9 (M^+); ^{1}H NMR (D_2O): δ 3.19 (s, 9H), 3.59 (m, 3H), 3.70 (m, 1H), 3.87 (m, 1H), 4.01 (dd, 2H, $J = 13.5$, $J' = 3.4$), 4.21 (m, 1H); ^{13}C NMR (D_2O): δ 56.7, 64.3, 71.4, 73.0, 75.9, 78.2, 88.4.

4.2.3. *N,N′-Bis(tert-butoxycarbonyl)-1-guanidino-2,5-anhydro-3,4,6-tri-O-benzoyl-1-deoxy-D-allitol (**6**)*

1-Amino-2,5-anhydro-3,4,6-tri-*O*-benzoyl-1-deoxy-D-allitol (**5**) [12,13] (2.21 g, 4.65 mmol) was dissolved in $CHCl_3$ and *N,N′*-bis(*tert*-butoxycarbonyl)-1*H*-pyrazole-1-carboxamidine (1.49 g, 4.80 mmol) was added. After stirring at rt for 24 h, the solvent was removed under reduced pressure and the residue was redissolved in DCM and tris-(2-aminoethyl)-amine polystyrene resin was added to scavenge the excess of *N,N′*-bis(*tert*-butoxycarbonyl)-1*H*-pyrazole-1-carboxamidine. After 3 h of stirring at rt, the resin was filtered off and the solvent was evaporated. The residue was purified by reversed phase column chromatography (H_2O:ACN, 1:1) to obtain **6** as a syrup (0.73 g, 22%); MS (ESI): *m/z* 718.4 (MH^+); ^{1}H NMR ($CDCl_3$): δ 1.40 (s, 9H), 1.42 (s, 9H), 3.85 (m, 2H), 4.51 (m, 1H), 4.55–4.61 (m, 2H), 4.67 (dd, 1H, $J = 13.5$, $J' = 5.0$), 5.48 (dd, 1H, $J = 7.2$, $J' = 5.7$), 5.73 (dd, 1H, $J = 5.6$, $J' = 3.7$), 7.35 (m, 4H), 7.41 (t, 2H, $J = 7.5$), 7.55 (m, 3H), 7.90, 7.97, 8.09 (3dd, 3 × 2H, $J = 8.4$, $J' = 1.4$).

4.2.4. *1-Guanidino-2,5-anhydro-1-deoxy-D-allitol (**3**)*

A solution of **6** (0.27 g, 0.38 mmol) in DCM:TFA (1:1, 2 mL) was stirred at rt overnight. The solvents were evaporated to yield, without further purification, 3,4,6-*O*-tribenzoyl-1-guanidino-2,5-anhydro-1-deoxy-D-allitol as a TFA salt (quantitative yield). The TFA salt of 3,4,6-*O*-tribenzoyl-1-guanidino-2,5-anhydro-1-deoxy-D-allitol was dissolved in a solution of 7 N NH_3 in methanol (10 mL) and stirred at rt for 4 days. The reaction mixture was concentrated, the residue dissolved in water and washed with $CHCl_3$. The aqueous layer was concentrated and the residue was purified by reversed phase column chromatography (H_2O:ACN, 9:1) to yield the title compound as a gum (80 mg, 98%); MS (ESI): *m/z* 206.0 (MH^+); LC–MS: rt 10.3 min, *m/z* 205.9 (MH^+); ^{1}H NMR (CD_3OD): δ 3.32 (dd, 1H, $J = 14.5$, $J' = 6.7$), 3.48 (dd, 1H, $J = 14.5$, $J' = 2.9$), 3.61 (dd, 1H, $J = 11.9$, $J' = 4.2$), 3.70 (dd, 1H, $J = 11.9$, $J' = 3.4$), 3.82–3.91 (m, 3H), 4.01 (t, 1H, $J = 5.0$); ^{13}C NMR (CD_3OD): δ 44.9, 62.8, 72.6, 73.7, 82.9, 86.5, 159.6.

4.2.5. *3,6-Anhydro-2-deoxy-4,5-O-isopropylidene-1-O-(methylsulfonyl)-7-O-trityl-D-allo-heptitol (**13**)*

To a suspension of $LiAlH_4$ (0.17 g, 4.6 mmol) in dry Et_2O (10 mL) at 0 °C, a solution of **12a** [16] (0.74 g, 1.5 mmol) in dry Et_2O (7.5 mL) was added dropwise. The reaction mixture was stirred at rt for 18 h, cooled to 0 °C and the excess of $LiAlH_4$ was destroyed by the dropwise addition of water (2 mL). After the reaction mixture was washed with water and brine, the Et_2O layer was dried (Na_2SO_4) and concentrated to yield the alcohol derivative as a sticky white gum (0.52 g, 74%) which was used without further purification. The alcohol derivative (0.52 g, 1.1 mmol) was dissolved in pyridine (2.5 mL), and mesylchloride (259 mL, 2.26 mmol) together with a catalytic amount of DMAP was added while stirring. The reaction mixture was stirred at rt for 2 h. The pyridine was evaporated and the residue dissolved in $CHCl_3$, washed with water, dried (Na_2SO_4) and concentrated. Purification by column chromatography (Hex:EtOAc, 1:1) yielded the title compound as a syrup (0.45 g, 74%); MS (ESI): *m/z* 561.2 (MNa^+); ^{1}H NMR ($CDCl_3$): δ 1.32 (s, 3H), 1.52 (s, 3H), 1.99 (m, 1H), 2.15 (m, 1H), 2.97 (s, 3H), 3.18 (dd, 1H, $J = 10.1$, $J' = 4.8$), 3.29 (dd, 1H, $J = 10.1$, $J' = 3.7$), 4.01 (m, 1H), 4.12 (m, 1H), 4.34–4.42 (m, 3H), 5.59 (dd, 1H, $J = 6.7$, $J' = 3.8$), 7.21–7.32 (m, 9H), 7.42–7.45 (m, 6H).

4.2.6. *3,6-Anhydro-1-azido-1,2-dideoxy-4,5-O-isopropylidene-7-O-trityl-D-allo-heptitol (**14**)*

To **13** (0.44 g, 0.82 mmol) in dry DMF (8 mL) was added NaN_3 (0.27 g, 4.1 mmol). The reaction mixture was stirred at 100 °C for 7 h, cooled to rt and diluted with EtOAc. The organic solution was washed with brine and the aqueous layer was extracted twice with EtOAc. The combined organic phases were dried (Na_2SO_4) and concentrated. Purification by column chromatography (Hex:EtOAc, 4:1) yielded the desired product **14** as a sticky yellow gum (0.33 g, 83%); MS (ESI): *m/z* 508.2 (MNa^+); ^{1}H NMR ($CDCl_3$): δ 1.81–2.00 (m, 2H), 3.17 (dd, 1H, $J = 10.1$, $J' = 4.7$), 3.27 (dd, 1H, $J = 10.1$, $J' = 3.9$), 3.44 (m, 1H), 3.96 (m, 1H), 4.12 (m, 1H), 4.38 (dd, 1H, $J = 6.6$, $J' = 5.2$), 4.58 (dd, 1H, $J = 6.7$, $J' = 3.7$), 7.21–7.32 (m, 9H), 7.41–7.46 (m, 6H).

*4.2.7. 3,6-Anhydro-1-azido-1,2-dideoxy-D-allo-heptitol (**7**)*

Azide **14a** (0.46 g, 0.95 mmol) was dissolved in TFA:H_2O (1:1, 10 mL) and stirred at rt for 18 h. The solvent was evaporated and the residue was purified by column chromatography ($CHCl_3$:MeOH, 9:1) to furnish the title compound as a gum (0.18 g, 91%); MS (ESI): *m/z* 226.0 (MNa^+); LC–MS: rt 1.7 min, *m/z* 225.8 (MNa^+); ^{1}H NMR (CD_3OD): δ 1.78 (m, 1H), 1.91 (m, 1H), 3.46 (m, 2H), 3.57 (dd, 1H, $J = 11.9$, $J' = 4.7$), 3.67 (dd, 1H, $J = 11.9$, $J' = 3.5$), 3.73 (m, 1H), 3.80 (m, 2H), 3.95 (m, 1H); ^{13}C NMR (CD_3OD): δ 33.8, 49.2, 63.4, 72.7, 76.3, 81.0, 85.9.

*4.2.8. 1-Amino-3,6-anhydro-1,2-dideoxy-4,5-O-isopropylidene-7-O-trityl-D-allo-heptitol (**15a**)*

A solution of **14a** (0.31 g, 0.64 mmol) in dry Et_2O (3 mL) was added at 0 °C to a suspension of $LiAlH_4$ (0.10 g, 2.6 mmol) in dry Et_2O (10 mL). The mixture was stirred for 3 h at 0 °C under a N_2 atmosphere, diluted with Et_2O and saturated aqueous Na_2SO_4 was gradually added at 0 °C. After being stirred at rt for 30 min, the mixture was extracted twice with Et_2O. The combined organic layers were washed with brine, dried (Na_2SO_4) and concentrated to yield the title compound as a syrup (0.27 g, 92%); MS (ESI): *m/z* 460.1 (MH^+), 482.2 (MNa^+); ^{1}H NMR ($CDCl_3$): δ 1.32 (s, 3H), 1.52 (s, 3H), 1.80 (m, 2H), 2.17 (s, 2H), 2.89 (dt, 2H, $J = 6.7$, $J' = 1.8$), 3.17 (dd, 1H, $J = 10.0$, $J' = 4.8$), 3.26 (dd, 1H, $J = 10.0$, $J' = 4.0$), 3.96 (m, 1H), 4.10 (m, 1H), 4.37 (dd, 1H, $J = 6.6$, $J' = 5.4$), 4.56 (dd, 1H, $J = 6.8$, $J' = 3.8$), 7.20–7.33 (m, 9H), 7.44–7.47 (m, 6H).

*4.2.9. 2-Amino-4,7-anhydro-1,2,3-trideoxy-5,6-O-isopropylidene-8-O-trityl-D-allo-octitol (**15b**)*

A mixture of **12b** [16] (0.85 g, 1.8 mmol), ammonium formate (1.25 g, 19.9 mmol), sodium cyanoborohydride (0.62 g, 9.9 mmol) and 3 Å molecular sieves (1 g) in MeOH (20 mL) was stirred at rt for 18 h. The solution was filtered through Celite and evaporated. The residue was taken up in EtOAc, washed with brine and dried (Na_2SO_4). Evaporation of the solvent yielded the product as an inseparable mixture of diastereoisomers which was used without further purification (0.84 g, 98%); MS (ESI): *m/z* 474.2 (MH^+).

*4.2.10. N,N′-Bis(tert-butoxycarbonyl)-1-guanidino-3,6-anhydro-1,2-dideoxy-4,5-O-(1-methylethylidene)-7-O-trityl-D-allo-heptitol (**16a**)*

Amine **15a** (0.25 g, 0.54 mmol) was dissolved in $CHCl_3$ and *N*,*N*′-bis(*tert*-butoxycarbonyl)-1*H*-pyrazole-1-carboxamidine (0.18 g, 0.54 mmol) was added. The reaction mixture was stirred at rt for 24 h. The solvent was removed under reduced pressure and the residue was purified by column chromatography (Hex:EtOAc, 1:1). The product obtained was dissolved in DCM and tris-(2-aminoethyl)-amine polystyrene resin was added to scavenge the remaining excess of *N*,*N*′-bis(*tert*-butoxycarbonyl)-1*H*-pyrazole-1-carboxamidine. After 3 h of stirring at rt, the resin was filtered off and the solvent was evaporated to yield the title compound **16a** as a gum (0.36 g, 94%); MS (ESI): *m/z* 702.3 (MH^+), 724.4 (MNa^+); ^{1}H NMR ($CDCl_3$): δ 1.32 (s, 3H), 1.42 (s, 9H), 1.50 (s, 9H), 1.52 (s, 3H), 1.83 (m, 1H), 1.94 (m, 1H), 3.24 (d, 2H, $J = 4.4$), 3.49 (m, 1H), 3.68 (m, 1H), 3.93 (m, 1H), 4.14 (m, 1H), 4.36 (m, 1H), 4.56 (dd, 1H, $J = 6.6$, $J' = 3.5$), 7.20–7.31 (m, 9H), 7.40–7.46 (m, 6H).

*4.2.11. N,N′-Bis(tert-butoxycarbonyl)-2-guanidino-4,7-anhydro-1,2,3-trideoxy-5,6-O-(1-methylethylidene)-8-O-trityl-D-allo-octitol (**16b**)*

Starting from **15b** (0.84 g, 1.8 mmol), the same procedure was followed as described for **16a**, to yield **16b** as an inseparable mixture of diastereoisomers (0.26 g, 20%); MS (ESI): *m/z* 716.2 (MH^+), 738.2 (MNa^+).

*4.2.12. 1-Guanidino-3,6-anhydro-1,2-dideoxy-D-allo-heptitol·TFA (**8**)*

Starting from **16a** (0.36 g, 0.51 mmol), the same procedure was followed as described for **7**. Purification by reversed phase column chromatography (H_2O:ACN, 9:1) yielded the TFA salt of the title compound as a gum (53 mg, 31%); MS (ESI): *m/z* 220.0 (MH^+); LC–MS: rt 10.3 min, *m/z* 219.9 (MH^+); ^{1}H NMR (CD_3OD): δ 1.75 (m, 1H), 1.96 (m, 1H), 3.34 (m, 2H), 3.58 (dd, 1H, $J = 11.8$, $J' = 5.2$), 3.69 (d, 1H, $J = 3.5$), 3.72 (d, 1H, $J = 5.4$), 3.80 (m, 2H), 3.94 (t, 1H, $J = 5.1$); ^{13}C NMR (CD_3OD): δ 33.6, 39.9, 63.5, 72.7, 76.3, 81.8, 86.3, 158.8.

*4.2.13. 2-Guanidino-4,7-anhydro-1,2,3-trideoxy-D-allo-octitol·TFA (**9**)*

Starting from **16b** (0.23 g, 0.32 mmol), the same procedure was followed as described for **7**. Purification by reversed phase column chromatography (H_2O:ACN, 9:1) yielded the TFA salt of the title compound as an inseparable mixture of diastereoisomers (38 mg, 44%); MS (ESI): *m/z* 234.1 (MH^+); LC–MS: rt 10.2 min, *m/z* 233.9 (MH^+); ^{1}H NMR (CD_3OD): δ 1.27 (d, 3H), 1.66–1.88 (m, 2H), 3.58 (m, 1H), 3.70 (m, 2H), 3.75–3.85 (m, 3H), 3.95 (m, 1H); ^{13}C NMR (CD_3OD), δ isomer A: 20.8, 41.5, 47.2, 63.3, 72.5, 76.7, 81.0, 86.2, 157.7; isomer B: 21.4, 41.2, 46.8, 63.5, 72.6, 76.4, 80.2, 86.1, 158.2.

*4.2.14. 4,7-Anhydro-2,3-dideoxy-5,6-O-isopropylidene-8-O-trityl-D-allo-octononitrile (**17**)*

Mesylate **13a** (0.26 g, 0.48 mmol), sodium cyanide (0.19 g, 3.9 mmol) and sodium iodide (0.063 g, 0.42 mmol) in dry DMF (5 mL) were stirred under a N_2 atmosphere at 100 °C for 7 h. Water was added and extracted with EtOAc. The organic washings were combined, washed with brine, dried (Na_2SO_4), and evaporated. Purification by column chromatography (Hex:EtOAc, 3:1) yielded the product as a gum (0.14 g, 61%); MS (ESI): *m/z* 492.2 (MNa^+); ^{1}H NMR ($CDCl_3$): δ 1.35 (s, 3H), 1.55 (s, 3H), 1.93 (m, 1H), 2.06 (m, 1H), 2.50 (m, 2H), 3.20 (dd, 1H, $J = 10.1$, $J' = 4.6$), 3.30 (dd, 1H, $J = 10.1$, $J' = 3.6$), 3.96 (m, 1H), 4.18 (m, 1H), 4.40 (dd, 1H, $J = 6.5$, $J' = 5.3$), 4.63 (dd, 1H, $J = 6.6$, $J' = 3.5$), 7.24–7.34 (m, 9H), 7.44–7.48 (m, 6H).

*4.2.15. 1-Amino-4,7-anhydro-1,2,3-trideoxy-5,6-O-isopropylidene-8-O-trityl-D-allo-octitol (**18**)*

To nitrile **17** (0.14 g, 0.30 mmol) in dry THF (3 mL) was added $LiAlH_4$ (0.13 g, 3.4 mmol). The reaction mixture was stirred under a N_2 atmosphere at rt for 18 h. Water was added and extracted with EtOAc. The organic washings were combined, washed with brine, dried (Na_2SO_4), and evaporated. Purification by column chromatography (Hex:EtOAc, 1:1) yielded the product as a gum (0.063 g, 45%); MS (ESI): *m/z* 474.1 (MH^+); ^1H NMR ($CDCl_3$): δ 1.34 (s, 3H), 1.54 (s, 3H), 1.66 (m, 2H), 1.86 (m, 2H), 3.20 (m, 1H), 3.27 (m, 1H), 3.76 (m, 2H), 3.92 (m, 1H), 4.13 (m, 1H), 4.35 (m, 1H), 4.57 (m, 1H), 7.20–7.32 (m, 9H), 7.46–7.49 (m, 6H).

*4.2.16. 1-Guanidino-4,7-anhydro-1,2,3-trideoxy-D-allo-octitol·TFA (**10**)*

Starting from **18** (0.063 g, 0.13 mmol), the same procedure was followed as described for **16a**, to yield the fully protected intermediate **19** (0.040 g, 42%); MS (ESI): *m/z* 716.4 (MH^+). A solution of **19** (0.040 g, 0.056 mmol) in TFA:H_2O (1:1, 2 mL) was stirred at rt for 18 h. The solvent was evaporated and the residue was dissolved in EtOH. Filtration and subsequent concentration of the filtrate furnished the TFA salt of the title compound as a gum (17 mg, 59%); MS (ESI): *m/z* 234.1 (MH^+); LC–MS: rt 11.0 min, *m/z* 233.9 (MH^+); ^1H NMR (CD_3OD): δ 1.58 (m, 1H), 1.70–1.79 (m, 3H), 3.22 (t, 2H, $J = 6.7$), 3.57 (dd, 1H, $J = 11.8$, $J' = 5.2$), 3.70–3.74 (m, 3H), 3.80 (dd, 1H, $J = 8.4$, $J' = 4.8$), 3.91 (m, 1H); ^{13}C NMR (CD_3OD): δ 26.5, 31.3, 42.2, 63.5, 72.7, 76.3, 83.6, 86.0, 158.7.

*4.2.17. N,N,N-Tris(tert-butoxycarbonyl)-1-guanidino-7-O-tert-butyldimethylsilyl-2,3,6-trideoxy-3,6-imino-4,5-O-isopropylidene-D-allo-heptitol (**25**)*

A solution of *N-tert*-butoxycarbonyl-7-*O-tert*-butyldimethylsilyl-2,3,6-trideoxy-3,6-imino-4,5-*O*-isopropylidene-D-*allo*-heptononitrile **23** [18] (0.19 g, 0.45 mmol) in dry THF (5 mL) was cooled to 0 °C, $LiAlH_4$ (0.080 g, 2.1 mmol) was added, and the reaction mixture was stirred at rt for 3 h. The reaction was quenched with water, diluted with EtOAc, washed with water and brine, dried (Na_2SO_4) and concentrated to yield the intermediate **24** (0.12 g, 62%) which was used without further purification. A solution of **24** (0.12 g, 0.28 mmol) was dissolved in $CHCl_3$ and *N,N′*-bis(*tert*-butoxycarbonyl)-1*H*-pyrazole-1-carboxamidine (0.13 g, 0.42 mmol) was added. This was stirred at rt for 24 h. The solvent was removed under reduced pressure and the residue was redissolved in DCM and tris-(2-aminoethyl)-amine polystyrene resin was added to scavenge the excess of *N,N′*-bis(*tert*-butoxycarbonyl)-1*H*-pyrazole-1-carboxamidine. After 3 h of stirring at rt, the resin was filtered off and the solvent was evaporated. Further purification by column chromatography (Hex:EtOAc, 3:1) yielded the title compound **25** (19 mg, 10%); MS (ESI): *m/z* 673.6 (MH^+), 695.5 (MNa^+); ^1H NMR ($CDCl_3$): δ 0.06 (d, 6H, $J = 5.6$), 0.89 (s, 9H), 1.26 (s, 3H), 1.32 (s, 3H), 1.45–1.50 (m, 27H), 1.65–2.05 (m, 2H), 3.40–3.65 (m, 2H), 3.71 (dd, 1H, $J = 10.3$, $J' = 2.9$), 3.77 (m, 1H), 3.84–4.13 (m, 2H), 4.44 (d, 1H, $J = 5.4$), 4.69 (dd, 1H, $J = 5.6$, $J' = 1.1$).

*4.2.18. (1S)-1,4-Dideoxy-1-(2-guanidinoethyl)-1,4-imino-D-ribitol·TFA (**20**)*

A solution of **25** (19 mg, 0.028 mmol) in TFA:H_2O (1:1, 1 mL) was stirred at rt for 36 h. The solvent was evaporated and the residue was dissolved in MeOH (0.5 mL) and 5 drops of EtOAc were added. Subsequent cooling resulted in the formation of a precipitate, which was filtered and redissolved in MeOH:H_2O (1:1). Evaporation of the MeOH:H_2O mixture yielded the TFA salt of the title compound **20** as a gum (3.6 mg, 59%); MS (ESI): *m/z* 219.1 (MH^+); LC–MS: rt 13.5 min, *m/z* 218.9 (MH^+); ^1H NMR (CD_3OD): δ 2.08 (dd, 2H, $J = 14.4$, $J' = 7.2$), 3.41 (m, 2H), 3.50 (m, 1H), 3.61 (dd, 1H, $J = 8.1$, $J' = 3.9$), 3.81 (m, 2H), 4.03 (dd, 1H, $J = 7.4$, $J' = 4.9$), 4.12 (m, 1H); ^{13}C NMR (CD_3OD): δ 30.8, 39.4, 59.8, 60.9, 67.4, 72.5, 75.8, 158.8.

*4.2.19. tert-Butyl 2-bromoethylcarbamate (**28a**)*

Di-*tert*-butyl dicarbonate (2.9 g, 13 mmol) was added to a stirred solution of 2-bromoethylamine hydrobromide (3.0 g, 15 mmol) in DCM (80 mL). Et_3N (3.0 g, 29 mmol) was added dropwise to the solution. This was stirred at rt for 1.5 h, then aqueous 2 N HCl was added and the layers were separated. The organic layer was washed with aqueous 2 N HCl and brine, dried (Na_2SO_4) and the solvent was evaporated under reduced pressure. The residue was purified by flash chromatography (Hex:EtOAc, 3:2) to yield the title compound as an oil (2.1 g, 72%); MS (ESI): *m/z* 246.0 (MH^+) and 248.0 (MNa^+).

*4.2.20. N-[2-Amino-N-tert-butoxycarbonyl-ethyl]-5-O-tert-butyldimethylsilyl-1,4-dideoxy-1,4-imino-2,3-O-isopropylidene-D-ribitol (**29a**)*

To a solution of the protected iminoribitol **21** [17] (0.50 g, 1.7 mmol) in DMF (15 mL), K_2CO_3 (0.48 g, 3.5 mmol) and *tert*-butyl 2-bromoethylcarbamate (0.56 g, 2.5 mmol) were added. After stirring at 60 °C for 24 h, EtOAc and H_2O were added. The organic layer was separated and washed twice with H_2O and brine. The combined aqueous layers were extracted with EtOAc. The combined organic layers were dried (Na_2SO_4) and evaporated under reduced pressure. The residue was purified by flash chromatography to yield the title compound as a syrup (Hex:EtOAc, 4:1) (0.59 g, 78%); MS (ESI): *m/z* 431.4 (MH^+).

*4.2.21. N-[2-N,N-Bis(tert-butoxycarbonyl)-guanidinopropyl]-5-O-tert-butyldimethylsilyl-1,4-dideoxy-1,4-imino-2,3-O-isopropylidene-D-ribitol (**31a**)*

Compound **29a** (0.59 g, 1.4 mmol) was stirred at rt in TFA:DCM (1:1, 4 mL). After 30 min, the mixture was evaporated and the residue dissolved in $CHCl_3$ (15 mL) and Et_3N (1.2 mL, 4.1 mmol) was added. After 15 min of stirring, *N,N′*-bis(*tert*-butoxycarbonyl)-1*H*-pyrazole-1-carboxamidine (0.51 g, 1.6 mmol) was added. After 3 days of stirring at rt, tris-(2-aminoethyl)-amine polystyrene resin was added to

scavenge the excess of *N*,*N*′-bis(*tert*-butoxycarbonyl)-1*H*-pyrazole-1-carboxamidine, and this was stirred for 2 h at rt. The resin was removed by filtration and the filtrate was evaporated under reduced pressure. The residue was purified by column chromatography (Hex:EtOAc, 4:1) to furnish the title compound as a syrup (0.17 g, 22%); MS (ESI): *m/z* 573.5 (MH^+).

4.2.22. 1,4-Dideoxy-(2-guanidinoethyl)-1,4-imino-D-ribitol·TFA (**26**)

Compound **31a** (0.13 g, 0.23 mmol) was stirred in TFA:H_2O (1:1, 3 mL) for 60 h at rt. The solvent was removed under reduced pressure to yield the TFA salt of the title compound (54 mg, 70%); MS (ESI): *m/z* 219.13 (MH^+); LC–MS: rt 12.6 min, *m/z* 218.7 (MH^+); ^{1}H NMR (CD_3OD): δ 3.31 (m, 4H), 3.45 (m, 1H), 3.57 (m, 1H), 3.76 (m, 1H), 3.83 (dd, 1H, $J = 12.3$, $J' = 6.9$), 3.97 (dd, 1H, $J = 3.3$, $J' = 12.3$), 4.06 (m, 1H), 4.26 (m, 1H); ^{13}C NMR (CD_3OD): δ 38.6, 57.9, 59.7, 59.8, 70.9, 73.3, 73.5, 159.1.

4.2.23. tert-Butyl 3-bromopropylcarbamate (**28b**)

Di-*tert*-butyl dicarbonate (1.8 g, 8.2 mmol) was added to a stirred solution of 3-bromopropylamine hydrobromide (2.0 g, 9.1 mmol) in DCM (60 mL). Et_3N (3.8 mL, 27 mmol) was added dropwise to the solution. After stirring overnight at rt, aqueous 2 N HCl was added and the layers were separated. The organic layer was washed with aqueous 2 N HCl and brine, dried (Na_2SO_4) and concentrated under reduced pressure. The residue was purified by flash chromatography (Hex:EtOAc, 7:3) to yield the title compound as an oil (0.86 g, 44%); MS (ESI): *m/z* 260.0 (MH^+) and 262.0 (MNa^+).

4.2.24. N-[2-Amino-N-tert-butoxycarbonyl-propyl]-5-O-tert-butyldimethylsilyl-1,4-dideoxy-1,4-imino-2,3-O-isopropylidene-D-ribitol (**29b**)

To a solution of the protected iminoribitol **21** [17] (0.50 g, 1.7 mmol) in DMF (15 mL), K_2CO_3 (0.48 g, 3.5 mmol) and *tert*-butyl 3-bromopropylcarbamate (0.45 g, 1.9 mmol) were added. After stirring at 60 °C for 24 h, EtOAc and H_2O were added. The organic layer was separated and washed with H_2O and brine. The combined aqueous layers were washed with EtOAc. The combined organic layers were dried (Na_2SO_4) and evaporated under reduced pressure. The residue was purified by flash chromatography (Hex:EtOAc, 4:1) to yield the title compound as a syrup (0.30 g, 38%); MS (ESI): *m/z* 445.4 (MH^+).

4.2.25. N-[2-N,N-Bis(tert-butoxycarbonyl)-guanidinopropyl]-5-O-tert-butyldimethylsilyl-1,4-dideoxy-1,4-imino-2,3-O-isopropylidene-D-ribitol (**31b**)

Compound **29b** (0.30 g, 0.67 mmol) was stirred at rt in TFA:DCM (1:1, 3 mL). After 45 min, the mixture was evaporated under reduced pressure and the residue was dissolved in $CHCl_3$ (7 mL) and Et_3N (0.3 mL, 2.0 mmol). This was stirred at rt for 15 min and *N*,*N*′-bis(*tert*-butoxycarbonyl)-1*H*-pyrazole-1-carboxamidine (0.25 g, 0.81 mmol) was added. The reaction mixture was stirred at rt for 20 h, then tris-(2-aminoethyl)-amine polystyrene resin was added to scavenge the excess of *N*,*N*′-bis(*tert*-butoxycarbonyl)-1*H*-pyrazole-1-carboxamidine, this was stirred at rt for 2 h. The resin was removed by filtration and the filtrate was evaporated under reduced pressure. The residue was purified by column chromatography (Hex:EtOAc, 4:1) to yield the title compound as a syrup (0.12 g, 30%); MS (ESI): *m/z* 587.50 (MH^+).

4.2.26. 1,4-Dideoxy-(3-guanidinopropyl)-1,4-imino-D-ribitol·TFA (**27**)

Compound **31b** (0.12 g, 0.2 mmol) was stirred in TFA:H_2O (1:1, 3 mL) for 42 h at rt. The solvent was removed under reduced pressure to obtain the TFA salt of the title compound (44 mg, 63%); MS (ESI): *m/z* 233.1 (MH^+); LC–MS: rt 11.5 min, *m/z* 233.1 (MH^+); ^{1}H NMR (CD_3OD): δ 2.00 (m, 2H), 3.24 (m, 4H), 3.47 (m, 2H), 3.69 (m, 1H), 3.79 (dd, 1H, $J = 12.4$, $J' = 5.7$), 3.92 (dd, 1H, $J = 12.4$, $J' = 3.1$), 4.05 (m, 1H), 4.22 (m, 1H); ^{13}C NMR (CD_3OD): δ 26.0, 39.6, 56.7, 59.0, 59.2, 70.6, 72.9, 73.2, 158.9.

4.2.27. 5-O-tert-Butyldimethylsilyl-1,4-dideoxy-1,4-imino-2,3-O-isopropylidene-N-(4-nitrobenzyl)-D-ribitol (**35a**)

Protected iminoribitol **21** [17] (0.63 g, 2.2 mmol) was dissolved in DMF (5 mL) and K_2CO_3 (0.61 g, 4.4 mmol) was added. 4-Nitrobenzylbromide (0.52 mg, 2.4 mmol) was added and the mixture was stirred at 40 °C for 3 h. EtOAc and H_2O were added and the mixture was separated. The organic layer was washed twice with H_2O and the combined aqueous layers were extracted with EtOAc. The combined organic layers were dried (Na_2SO_4) and concentrated under vacuum. The residue was redissolved in $CHCl_3$ and stirred overnight at rt with tris-(2-aminoethyl)-amine polystyrene resin to scavenge the excess of 4-nitrobenzylbromide. The resin was removed by filtration and the solvent was evaporated to yield the product as a yellowish syrup (0.72 g, 78%); MS (ESI): *m/z* 423.4 (MH^+); ^{1}H NMR ($CDCl_3$): δ 0.06 (d, 6H, $J = 6.0$ Hz), 0.89 (s, 9H), 1.34 (s, 3H), 1.43 (s, 3H), 2.74 (d, 1H, $J = 10.4$), 3.06 (s, 1H), 3.10 (dd, 1H, $J = 10.3$, $J' = 5.4$), 3.65 (dd, 1H, $J = 10.7$, $J' = 4.2$), 3.77 (dd, 1H, $J = 10.7$, $J' = 3.8$), 3.89 (d, 1H, $J = 14.7$), 4.10 (d, 1H, $J = 12.7$), 4.56 (d, 1H, $J = 6.3$), 4.67 (m, 1H), 7.52 (d, 2H, $J = 8.4$), 8.16 (d, 2H, $J = 8.5$); ^{13}C NMR ($CDCl_3$): δ −5.7, −5.3, 18.3, 25.1, 26.0, 27.3, 56.3, 59.4, 63.3, 69.3, 79.8, 83.4, 112.1, 123.7, 129.0, 147.3, 147.8.

4.2.28. 5-O-tert-Butyldimethylsilyl-1,4-dideoxy-1,4-imino-2,3-O-isopropylidene-N-(4-aminobenzyl)-D-ribitol (**36**)

Compound **35a** (0.72 g, 1.7 mmol) was dissolved in EtOH (10 mL) and $SnCl_2$ (1.6 g, 8.5 mmol) was added. This was stirred at 70 °C for 30 min under N_2 atmosphere. The reaction mixture was allowed to cool to rt and poured into ice. The pH was adjusted to pH 7–8 with sat. aq. $NaHCO_3$ before being extracted with EtOAc. The organic phase was washed with brine, dried (Na_2SO_4) and the solvent was removed under reduced pressure. Purification by column chromatography (Hex:EtOAc 1:1) yielded the title compound as a gum (0.12 g, 18%); MS (ESI): *m/z* 393.4 (MH^+); ^{1}H NMR ($CDCl_3$): δ 0.07 (d, 6H, $J = 5.3$), 0.91 (s, 9H), 1.33 (s, 3H), 1.55 (s, 3H), 2.68 (dd, 1H, $J = 10.3$, $J' = 3.0$), 2.97 (d, 1H, $J = 2.1$), 3.10 (dd, 1H,

$J = 10.3$, $J' = 5.6$), 3.59 (d, 1H, $J = 13.0$), 3.65 (dd, 1H, $J = 10.6$, $J' = 4.3$), 3.76 (dd, 1H, $J = 10.6$, $J' = 4.5$), 3.90 (d, 1H, $J = 13.0$), 4.54 (dd, 1H, $J = 6.5$, $J' = 2.2$), 4.64 (m, 1H), 6.62 (d, 2H, $J = 8.4$), 7.11 (d, 2H, $J = 8.3$).

4.2.29. 1,4-Dideoxy-1,4-imino-N-(4-guanidinobenzyl)-D-ribitol·TFA (**32**)

Compound **36** (0.12 g, 0.31 mmol) was dissolved in $CHCl_3$ and *N*,*N*′-bis(*tert*-butoxycarbonyl)-1*H*-pyrazole-1-carboxamidine (0.095 g, 0.31 mmol) was added. The solution was stirred for 48 h under reflux. The solvent was removed under reduced pressure and the residue was redissolved in DCM and tris-(2-aminoethyl)-amine polystyrene resin was added to scavenge the excess of *N*,*N*′-bis(*tert*-butoxycarbonyl)-1*H*-pyrazole-1-carboxamidine. After 18 h of stirring at rt, the resin was removed by filtration and the solvent was evaporated under vacuum. Further purification by column chromatography (Hex:EtOAc, 7:1) yielded the protected intermediate as a syrup (22 mg, 11%); MS (ESI): *m/z* 635.7 (MH^+), 657.6 (MNa^+); ^{1}H NMR ($CDCl_3$): δ 0.07 (d, 6H, $J = 6.0$), 0.91 (s, 9H), 1.34 (s, 3H), 1.54 (s, 3H), 2.71 (dd, 1H, $J = 10.4$, $J' = 2.4$), 3.00 (s, 1H), 3.10 (dd, 1H, $J = 10.3$, $J' = 5.6$), 3.63–3.69 (m, 2H), 3.78 (dd, 1H, $J = 10.6$, $J' = 4.0$), 3.98 (d, 1H, $J = 13.5$), 4.55 (d, 1H, $J = 4.8$), 4.65 (m, 1H), 7.29 (d, 2H, $J = 8.3$), 7.53 (d, 2H, $J = 8.1$). The protected intermediate was dissolved in TFA:H_2O (1:1, 1 mL) and this was stirred at rt overnight. H_2O was added and this was extracted twice with DCM. The H_2O layer was concentrated to obtain the TFA salt of the title compound without further purification (12 mg, 89%); MS (ESI): *m/z* 281.2 (MH^+); LC–MS: rt 13.8 min, *m/z* 280.9 (MH^+); ^{1}H NMR (D_2O): δ 2.50 (dd, 1H, $J = 10.0$, $J' = 7.7$), 2.73 (d, 1H, $J = 5.0$), 3.01 (dd, 1H, $J = 10.0$, $J' = 6.0$), 3.54 (d, 2H, $J = 5.4$), 3.90 (t, 1H, $J = J' = 5.0$), 3.96 (d, 1H, $J = 12.6$), 4.01 (m, 1H), 7.26 (d, 2H, $J = 8.4$), 7.41 (d, 2H, $J = 8.4$); ^{13}C NMR (D_2O): δ 61.1, 61.8, 69.1, 69.9, 70.9, 72.7, 125.2, 126.5, 130.8, 132.2, 133.7, 136.6, 156.4.

4.2.30. 5-O-tert-Butyldimethylsilyl-1,4-dideoxy-1,4-imino-2,3-O-isopropylidene-N-(4-cyanobenzyl)-methyl-D-ribitol (**35b**)

Protected iminoribitol **21** [17] (0.22 g, 0.77 mmol) was dissolved in DCM (2 mL) and a solution of Na_2CO_3 (0.162 g, 1.53 mmol) in H_2O (2 mL) was added. α-Bromo-*para*-tolunitrile (0.15 mg, 0.77 mmol) was added and the mixture was stirred under reflux for 3 h. The organic layer was separated and the aqueous layer was extracted twice with DCM. The combined extracts were dried (Na_2SO_4) and concentrated under vacuum. The residue was redissolved in $CHCl_3$ and stirred overnight with tris-(2-aminoethyl)-amine polystyrene resin to scavenge the remaining excess of α-bromo-*para*-tolunitrile. The resin was filtered off and the solvent was evaporated. The residue was purified further by thin layer chromatography to yield the product as a yellowish syrup (90 mg, 30%); MS (ESI): *m/z* 403.4 (MH^+); ^{1}H NMR ($CDCl_3$): δ 0.06 (d, 6H, $J = 5.4$), 0.90 (s, 9H), 1.34 (s, 3H), 1.56 (s, 3H), 2.73 (dd, 1H, $J = 10.4$, $J' = 2.2$), 3.04 (s, 1H), 3.09 (dd, 1H, $J = 10.4$, $J' = 5.4$), 3.64 (dd, 1H, $J = 10.7$, $J' = 4.3$), 3.76 (dd, 1H, $J = 10.7$, $J' = 4.0$), 3.84 (d, 1H, $J = 14.5$), 4.05 (d, 1H, $J = 14.5$), 4.56 (dd, 1H, $J = 6.4$, $J' = 1.6$), 4.66 (m, 1H), 7.46 (d, 2H, $J = 8.1$), 7.59 (d, 2H, $J = 8.1$).

4.2.31. 1,4-Dideoxy-1,4-imino-N-(4-amidinobenzyl)-D-ribitol·TFA (**33**)

Compound **35b** (90 mg, 0.22 mmol) was dissolved in dry Et_2O (2 mL) and cooled to 0 °C under N_2 atmosphere. To this was added a solution of HMDS (0.10 mL, 0.44 mmol) and *n*-BuLi (0.20 mL, 0.48 mmol) in 1 mL of dry Et_2O. The resulting mixture was allowed to stir at rt for 2 h. Subsequently, 2 mL of H_2O was added and stirred at rt for 15 min. The reaction mixture was diluted with water and the Et_2O layer was separated from the H_2O and evaporated. The residue was dissolved in TFA:H_2O (1:1, 2 mL) and stirred at rt for 18 h. The mixture was extracted with DCM and the H_2O layer was evaporated. The residue was purified by reversed phase column chromatography on silica C_{18} (gradient: H_2O to H_2O:MeOH 1:1) to yield the TFA salt of the title compound as a gum (79 mg, 95%); MS (ESI): *m/z* 266.0 (MH^+); LC–MS: rt 13.7 min, *m/z* 265.9 (MH^+); ^{1}H NMR (D_2O): δ 3.19 (d, 1H, $J = 12.3$), 3.47–3.51 (m, 2H), 3.60 (dd, 1H, $J = 12.7$, $J' = 3.8$), 3.71 (dd, 1H, $J = 12.7$, $J' = 4.8$), 4.12 (dd, 1H, $J = 6.9$, $J' = 4.7$), 4.28 (m, 1H), 4.34 (d, 1H, $J = 12.8$), 4.54 (d, 1H, $J = 12.9$), 7.67 (d, 2H, $J = 8.2$), 7.81 (d, 2H, $J = 8.2$); ^{13}C NMR (D_2O): δ 57.4, 58.0, 60.0, 68.8, 70.6, 71.4, 128.6, 129.2, 131.5, 137.1, 166.4.

4.2.32. 1,4-Dideoxy-1,4-imino-D-ribitol·HCl (**34**)

Protected iminoribitol **21** [17] (0.22 g, 0.76 mmol) was dissolved in MeOH (1 mL), aqueous 6 N HCl (3 mL) was added and the solution was stirred at rt for 20 h. The reaction mixture was separated between H_2O and $CHCl_3$, the aqueous layer was concentrated under vacuum and the residue was recrystallized from hot EtOH yielding the HCl salt of the title compound as a crystalline solid (0.13 g, 96%); mp 125–128 °C; MS (ESI): *m/z* 134.1 (MH^+); LC–MS: rt 10.2 min, *m/z* 134.1 (MH^+); ^{1}H NMR (D_2O): δ 3.34 (d, 1H, $J = 13.0$), 3.47 (d, 1H, $J = 12.9$, $J' = 3.9$), 3.61 (m, 1H), 3.80 (dd, 1H, $J = 12.6$, $J' = 6.0$), 3.94 (dd, 1H, $J = 12.6$, $J' = 3.3$), 4.18 (dd, 1H, $J = 8.5$, $J' = 4.2$), 4.36 (m, 1H); ^{13}C NMR (D_2O): δ 49.7, 58.1, 61.9, 69.5, 71.2.

4.2.33. N-Benzyl-1,4-dideoxy-1,4-imino-D-ribitol·TFA (**38**)

Protected benzyl derivative **37** [17] (0.089 g, 0.32 mmol) was dissolved in TFA:H_2O (1:1, 2 mL) and the solution was stirred at rt for 18 h. The reaction mixture was concentrated under reduced pressure and the residue was purified by column chromatography ($CHCl_3$:MeOH 4:1) to obtain the TFA salt of the title compound as a gum (0.074 g, 97%); MS (ESI): *m/z* 224.1 (MH^+); LC–MS: rt 9.4 min, *m/z* 223.9 (MH^+); ^{1}H NMR (MeOD): δ 3.32–3.36 (m, 1H), 3.50–3.54 (m, 2H), 3.58–3.62 (m, 1H), 3.75 (dd, 1H, $J = 12.0$, $J' = 4.9$), 4.14 (dd, 1H, $J = 7.7$, $J' = 4.2$), 4.25–4.28 (m, 1H), 4.45 (d, 1H, $J = 12.7$), 4.61 (d, 1H, $J = 12.7$), 7.45–7.48 (m, 3H), 7.53–7.56 (m,

2H); ^{13}C NMR (MeOD): δ 58.7, 58.8, 62.8, 70.5, 72.3, 72.9, 130.3, 131.2, 131.5, 131.9.

4.3. Biochemistry

4.3.1. Protein expression and purification

Expression and purification of the wild type *T. vivax* IAG-NH was performed as described previously [20]. *Escherichia coli* cells (WK6) containing the IAG-NH ORF cloned in the pQE-30 expression vector were used to express the protein. The presence of an N-terminal His_6-tag allowed for a two-step purification scheme, consisting of a Ni–NTA affinity chromatographic step (Qiagen) and gel filtration on a Superdex-200 column (Amersham Bioscience). The concentration of pure protein (expressed per monomer) was determined spectrophotometrically using a ε_{280} of 47752 $M^{-1}\,cm^{-1}$. Typically, 80 mg of purified protein was obtained from a 1 L fermentation. SDS–polyacrylamide gel electrophoresis was used to confirm enzyme purity.

4.3.2. Enzyme inhibition studies

Inhibitor dissociation constants were determined by measuring the initial rate of hydrolysis of a fixed concentration of *p*-nitrophenyl-β-D-ribofuranoside at variable (at least five) inhibitor concentrations, in a 50 mM phosphate buffer of pH 7.0 at 35 °C. Hydrolysis of *p*-nitrophenyl-β-D-ribofuranoside was followed by release of the *p*-nitrophenolate anion which has strong absorbency at 400 nm with an extinction coefficient of 12 $mM^{-1}\,cm^{-1}$ under the assay conditions. K_i was determined by fitting of initial rates to the equation describing competitive inhibition using the Dixon linearization ($1/v_i$ *vs* I):

$$1/v_i = \frac{K_M}{K_i \times k_{cat} \times E \times S} \times I + \frac{K_M + S}{k_{cat} \times E \times S}$$

where v_i is the initial reaction rate, k_{cat} is the catalytic turnover number of *p*-nitrophenyl-β-D-ribofuranoside, K_M is the Michaelis constant for *p*-nitrophenyl-β-D-ribofuranoside, K_i is the dissociation constant of enzyme–inhibitor complex, I is the inhibitor concentration, E is the enzyme concentration and S is the substrate concentration.

The k_{cat} and K_M values of the substrate were determined by direct non-linear fitting on the Michaelis–Menten equation using Microsoft Origin version 7.0.

4.4. Docking of inhibitors in IAG-nucleoside hydrolase of T. vivax

The molecular modeling studies were performed using MOE 2006.08 software (Chemical Computing Group). All software necessary to build the compounds, perform minimizations, alignments and superpositions is available in the MOE package and was used with standard settings, unless otherwise mentioned. Crystal structures of IAG-NH from *T. vivax* were downloaded from the Protein Databank (PDB) and the ligands were checked and corrected when necessary. Residues not belonging to the enzyme or ligand were removed, except the Ca^{2+} ion and the water molecules placed within a distance of 6 Å of the ligand. Hydrogen atoms were added and the energy was minimized with MMFF94× force field (standard parameters) keeping all heavy atoms fixed.

The modeling experiments were performed using several NH crystal structures available through PDB. Special attention was paid to PDB structures 1HP0 [20] and 2FF2 [11]. The former is derived from *T. vivax* co-crystallized with the nucleoside analogue 3-deaza-adenosine. The latter corresponds to a structure of NH co-crystallized with (1*S*)-1-(9-deazahypoxanthin-9-yl)-1,4-imino-D-ribitol (Immucillin H). 2FF2 was chosen for all docking studies reported here since it represents a "closed" structure of the enzyme with all loops entirely ordered, possessing a small and narrow active pocket leading to more reliable docking scores.

The ribitol moiety of 3-deaza-adenosine from 1HP0 was used to construct our ribitol models. The contacts involving the ribitol group were conserved as described [20] and the guanidino-alkyl chains of guanidino-alkyl-ribitols **3** and **8–10**, differing in alkyl chain length, were constructed in the active site and bonded to the ribitol moiety after systematic conformational search. The iminoribitol target compounds were constructed using the iminoribitol moiety of ImmH from 2FF2. The contacts involving the iminoribitol group were conserved as described [11].

The automatic docking protocol with default parameters was used to position the ligands in the active site of the enzyme. Subsequently, the top solution was chosen according to the *S* (Score) function and was minimized as flexible ligand in the rigid enzyme with MMFX94× force field. The docking protocol was validated by 're-docking' of ImmH in 2FF2.

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