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N-Arylmethyl substituted iminoribitol derivatives as inhibitors of a purine specific nucleoside hydrolase

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ABSTRACT

A key enzyme within the purine salvage pathway of parasites, nucleoside hydrolase, is proposed as a good target for new antiparasitic drugs. We have developed *N*-arylmethyl-iminoribitol derivatives as a novel class of inhibitors against a purine specific nucleoside hydrolase from *Trypanosoma vivax*. Several of our inhibitors exhibited low nanomolar activity, with 1,4-dideoxy-1,4-imino-*N*-(8-quinolinyl)methyl-D-ribitol (UAMC-00115, K_i 10.8 nM), *N*-(9-deaza-adenin-9-yl)methyl-1,4-dideoxy-1,4-imino-D-ribitol (K_i 4.1 nM), and *N*-(9-deazahypoxanthin-9-yl)methyl-1,4-dideoxy-1,4-imino-D-ribitol (K_i 4.4 nM) being the three most active compounds. Docking studies of the most active inhibitors revealed several important interactions with the enzyme. Among these interactions are aromatic stacking of the nucleobase mimic with two Trp-residues, and hydrogen bonds between the hydroxyl groups of the inhibitors and amino acid residues in the active site. During the course of these docking studies we also identified a strong interaction between the Asp40 residue from the enzyme and the inhibitor. This is an interaction which has not previously been considered as being important.

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1. Introduction

Parasitic infections remain an important health issue in developing countries. Among the parasites, different species from the Trypanosomatidae family are pathogenic to humans and cause African trypanosomiasis (sleeping sickness, *Trypanosoma brucei*), American trypanosomiasis (Chagas' disease, *T. cruzi*) or leishmaniasis (*Leishmania* spp.). Together these three infections are responsible for over one hundred thousand deaths annually and severe disabilities in surviving patients.[1] Other parasites from the same family can cause disease in animals and are therefore a threat to livestock. Drugs that are currently used to treat these parasitic infections are old and often toxic. Moreover, resistance is also a growing problem.

In the search for new antiparasitic drugs, it has been proposed that the purine metabolism of the parasite could provide a good drug target.[2] In contrast to mammals, parasites are unable to synthesize purines *de novo*, and rely instead on the purine salvage pathway to obtain purines which are essential for their survival. Nucleoside hydrolase (NH) is an enzyme from the purine pathway which hydrolyses nucleosides obtained from the host, with subsequent release of the purine bases. Four types of NH are currently known.[3] Our chosen target enzyme is a purine specific NH (IAG-NH), preferring inosine, adenosine, and guanosine as substrates, and is isolated from *Trypanosoma vivax*, a parasite that causes a sleeping-sickness like disease in cattle.[4] It should be noted that NH-activity is not present in mammalian cells. The active site and hence the catalytic mechanism of the IAG-NH is different from that of the mammalian purine nucleoside phosphorylase (PNP), the enzyme that cleaves nucleosides in mammalian cells. Substrate specificity is also different between the two enzymes, with IAG-NH cleaving inosine, adenosine, and guanosine, whereas the physiological substrates for mammalian PNP are inosine, guanosine and deoxyguanosine, with no significant substrate activity for adenosine.[5] Based on this information, selective inhibition of IAG-NH seems possible, and would be an effective way to kill the parasite without causing toxicity toward the host.[2]

2. Inhibitor design

The catalytic mechanism of NH proceeds via an oxocarbenium ion-like transition state.[6] Transition state analogs are generally very good inhibitors, with the iminoribitol (1,4-dideoxy-1,4-imino-D-ribitol) scaffold providing a good transition state mimic for NH.[7,8] The purine substrate specificity of IAG-NH from *T. vivax* (*Tv*NH) is imposed by parallel aromatic stacking interactions

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between the purine ring of the substrate and two Trp-residues (Trp83 and Trp260) in the active site.[9] These stacking interactions contribute to raising the pK_a of N^7 of the purine substrate, allowing direct protonation by solvent molecules. Hence these stacking interactions function as an alternative to general acid catalysis.[10]

In the natural substrates, as well as in the known inhibitors of IAG-NH (Chart 1: 3-deazaadenosine,[4] Immucillin-H[11,12] and Immucillin-A[11,12]) the purine is attached to the C1 position of the ribose or iminoribitol moiety. From preliminary modeling studies we observed that the purine analogs could also be placed on the iminoribitol-N, and the correct distance between the sugar and substituent could be provided by a methylene group. Such structures would provide a novel class of NH-inhibitors, which are synthetically easier to access than *C*1-substituted iminoribitols. That the inhibitory activity is conserved or even improved with *N*-substitution of iminoribitol was confirmed by earlier results from our group, where iminoribitol was substituted with an alkylguanidine group or a phenyl, both on C1 (Chart 1, structure **1**[13] and **3**[14]) or *via* a methylene-linker on the iminoribitol-N (Chart 1, structure **2**[13] and **4**[13]). Other *N*-substituted iminoribitols developed by our group also showed promising results as inhibitors of *Tv*NH.[13] During the time of our research, Evans et al. reported the synthesis of the *N*-substituted analog of Immucillin-H as an inhibitor of PNP.[15] Here we describe a different synthetic approach toward this compound and report for the first time its inhibitory activity against IAG-NH.

As substituents for the iminoribitol-N, a wide range of aromatic moieties were chosen with preference for nitrogen-containing aromatics. Commercial available aromatic derivatives were preferred in order to allow rapid generation of a library of inhibitors. This enabled us to confirm our hypothesis on substitution of the iminoribitol-N. Three main types of aromatic derivatives were used in the synthesis of our target compounds: arylmethylhalides (usually –bromide), arylcarboxaldehydes, and indole derivatives, which were 'coupled' to the iminoribitol moiety via three different methods: (1) *N*-Alkylation; (2) Reductive amination; and (3) Mannich reaction.

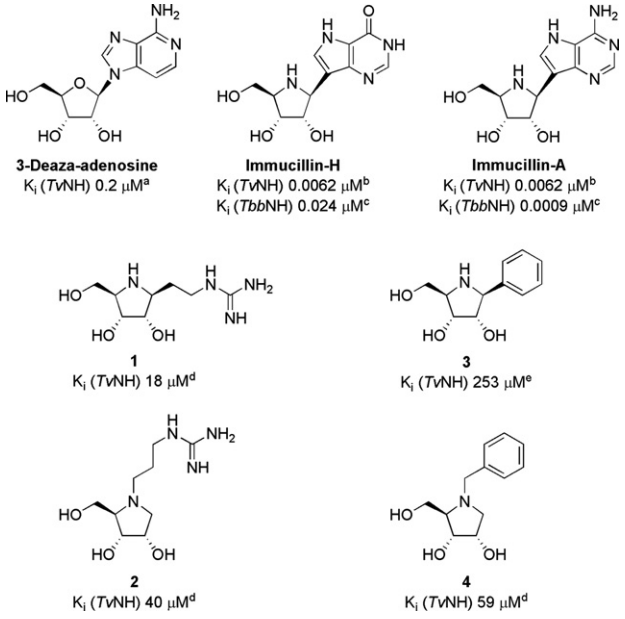


Chart 1. Inhibitors against purine specific NH (*Tv*NH = IAG-NH from *T. vivax*; *Tbb*NH = IAG-NH from *T. brucei brucei*). Data taken from [a]*J. Mol. Biol.* **2001**, *307*, 1363–1379, [b] *J. Mol. Biol.* **2006**, *359*, 331–346, [c]*Biochemistry* **1999**, *38*, 13147–13154, [d]*Eur. J. Med. Chem.* **2008**, *43*, 315–326, [e]unpublished results from our group.

3. Results and discussion

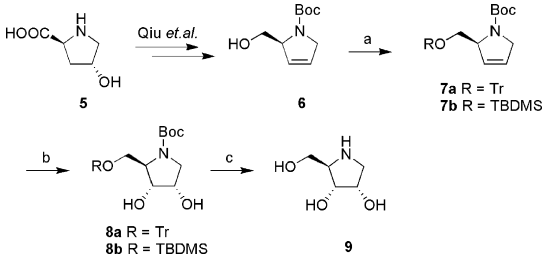
3.1. Chemistry

The iminoribitol scaffold was synthesized first and subsequently derivatized to obtain the target compounds. Two methods were applied for the synthesis of the iminoribitol scaffold. The first method was reported by Fleet et al. and starts from D-gulonolactone. An important intermediate in this synthesis is 5-*O*-*tert*-butyldimethylsilyl-1,4-dideoxy-1,4-imino-2,3-*O*-isopropylidene-D-ribitol (Scheme 2, compound **10**), which can be treated with aqueous acid to obtain 1,4-dideoxy-1,4-imino-D-ribitol (Scheme 1, compound **9**).[16,17] Due to the lengthy character and variable yields experienced for several steps of this synthesis, an alternative method, based on a publication of Qiu et al., was investigated (Scheme 1).[18] In the synthesis reported by Qui et al., *trans*-4-hydroxy-L-proline is used as the starting material. Compound **6**[18] is obtained as an intermediate, and Qiu et al. suggested to protect the 2′-hydroxyl group as a TBDPS ether.[18] However, for our purposes, we wanted a protecting group which could be easily cleaved under acidic conditions. As the protecting group needs bulk to ensure stereoselective dihydroxylation in the subsequent reaction, two possibilities were investigated (Scheme 1, step a): protection as a triphenylmethylether (**7a**) or as a TBDMS ether (**7b**). Comparison of the NMR spectra of iminoribitol **9** obtained after deprotection of both intermediate **8a** and **8b** with iminoribitol obtained from D-gulonolactone, confirmed that both protecting groups furnished the desired ribitol stereochemistry after stereoselective dihydroxylation.

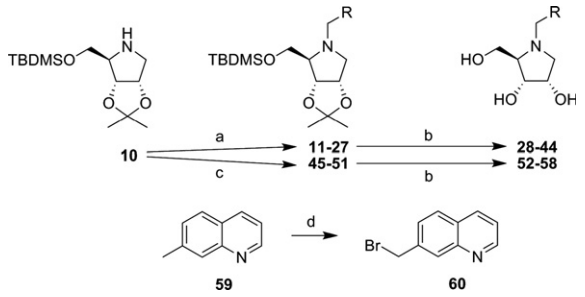
Both methods applied for the synthesis of the iminoribitol scaffold are rather lengthy and time consuming, with comparable overall yields in the range of 15–20%. For the majority of our synthetic purposes, the TBDMS and isopropylidene protected iminoribitol (**10**) were required. The synthesis starting from D-gulonolactone appeared to be the most convenient method to obtain this compound. However, unprotected iminoribitol (**9**) was synthesized from *trans*-4-hydroxy-L-proline.

Three strategies were used to substitute the iminoribitol nitrogen. A first strategy was *N*-alkylation. For this, protected iminoribitol **10** was reacted with the appropriate arylmethylbromide, followed by deprotection with aqueous trifluoracetic acid (Scheme 2). For the synthesis of compound **44**, 7-methylquinoline (**59**) was brominated in the benzylic position and the resulting arylmethylbromide **60** was used in a similar *N*-alkylation procedure (Scheme 2). Protected iminoribitol **10** was also employed for the second strategy, namely reductive aminations, in which it was reacted with the appropriate arylcarboxaldehyde in the presence of polymer supported cyanoborohydride, followed by deprotection with aqueous trifluoroacetic acid (Scheme 2).

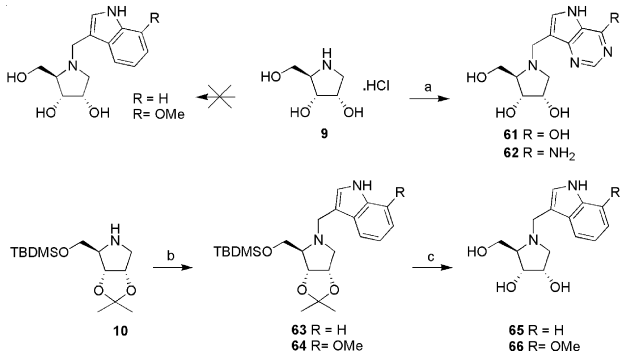
Mannich reactions on similar azasugar scaffolds have been reported using the unprotected azasugar and a purine derivative.[19,20] We applied this Mannich reaction to the synthesis of the *N*-substi-



Scheme 1. Reagents and conditions: (a) TrCl, pyridine, rt, 24 h, or TBDMSCl, imidazol, Et_3N, CH_2Cl_2, rt, 18 h; (b) $K_2OsO_4.2H_2O$, 4-methylmorpholine *N*-oxide, acetone, H_2O, rt, 18 h; (c) TFA/H_2O 1:1, rt, 18 h.



Scheme 2. Reagents and conditions: (a) Procedure A: arylmethylhalide, Na_2CO_3, CH_2Cl_2, H_2O, reflux, 3–20 h, Procedure B: arylmethylhalide, K_2CO_3 or $KHCO_3$, DMF, 40 °C, 1.5–4 h; (b) TFA/H_2O 1:1, rt, 18 h; (c) Procedure for the synthesis of **52**: 4-pyridinecarboxaldehyde, $NaCNBH_3$, MeOH, rt, 24 h; Procedure for the synthesis of target compounds **53**–**58**: arylcarboxaldehyde, CH_2Cl_2/AcOH 10/1, (polystyrylmethyl)trimethylammonium cyanoborohydride, rt, 28 h; (d) *N*-bromosuccinimide, dibenzoylperoxide, CCl_4, reflux, 18 h.



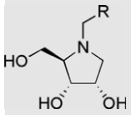
Scheme 3. Reagents and conditions: (a) 9-deazahypoxanthine or Boc-protected 9-deaza-adenine, aq CH_2O, NaOAc, H_2O, 95 °C, 17 h; (b) indole or 7-methoxy-indole, aq CH_2O, H_2O/dioxane 1:1, 95 °C, 20 h; (c) TFA/H_2O 1:1, rt, 18 h.

tuted analog of Immucillin-H, compound **61** (Scheme 3). The hydrochloric acid salt of iminoribitol **9** was treated with aqueous formaldehyde in the presence of 9-deaza-hypoxanthine. The latter compound was synthesized from isoxazole following a method published by Furneaux et al.[21] This synthesis of compound **61** is an alternative to the reductive amination approach reported by Evans *et al.*[15] The same approach (Mannich reaction) was used for the synthesis of the novel *N*-substituted analog of Immucillin-A, compound **62**. The Mannich reaction procedure as described for compounds **61**and **62** was also applied to the synthesis of indole derivatives **65** and **66**. However, we were unable to obtain the desired compounds. Similar reaction conditions were more successful when applied to the TBDMS and isopropylidene protected iminoribitol derivative **10** (Scheme 3).

3.2. Biochemical results

All target compounds were tested as inhibitors of the IAG-NH isolated from *T. vivax* (*Tv*NH). The target compounds can be divided into three groups, based on their general structure, with each group showing interesting structure–activity relationships. A first group of compounds includes iminoribitol derivatives substituted with a single aromatic ring and non-fused biaryl compounds. The biochemical results of group I compounds are shown in Table 1. Group II includes naphthalene and (iso)quinoline derivatives (Table 2). Group III includes iminoribitols with indole, benzimidazole, benzotriazole, and 9-deaza-hypoxanthine substituents (Table 3). For group I compounds, a first observation is that compounds with only one aromatic ring as substituent, are less active inhibitors compared to the biaryl compounds. Comparing the

Table 1
Inhibition constants (K_i) against *Tv*NH of group I target compounds

Compound	R=	K_i (μM)
		
4[a]		$5.9 \times 10^1 \pm 1.8 \times 10^1$
28		4.3 ± 1.2
29		7.5 ± 2.2
30		$2.6 \times 10^1 \pm 0.3 \times 10^1$
31		$2.3 \times 10^1 \pm 0.1 \times 10^1$
32		3.8 ± 1.7
33		3.4 ± 0.2
34		1.0 ± 0.1
35		1.2 ± 0.1
36		1.2 ± 0.1
37		0.063 ± 0.007
52		$2.0 \times 10^1 \pm 0.7 \times 10^1$
53		$1.2 \times 10^2 \pm 0.1 \times 10^2$
54		$3.2 \times 10^1 \pm 0.3 \times 10^1$
55		$4.3 \times 10^1 \pm 0.6 \times 10^1$

[a] *Eur. J. Med. Chem.* **2008**, *43*, 315–326).

biochemical results for the non-fused biaryl compounds of group I with the fused aromatics of groups II and III, it appears that the latter are in general more active. It is known that stacking interactions are more favorable with aromatics containing multiple rings than with monocyclics.[22]

The series of naphthalene and (iso)quinoline derivatives in group II were developed to investigate the role of a nitrogen in the nucleobase mimic. From the biochemical results in Table 2 it

Table 2
Inhibition constants (K_i) against *Tv*NH of group II target compounds

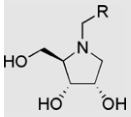
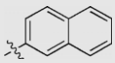
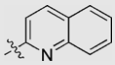
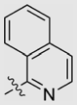
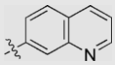
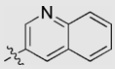
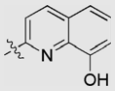
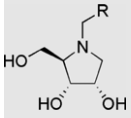
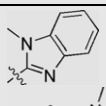
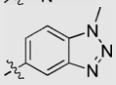
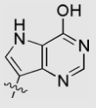
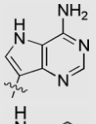
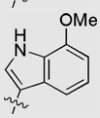
Compound	R=	K_i (μM)
		
40		2.0 ± 0.6
41		0.18 ± 0.05
42		0.0108 ± 0.0012
43		0.75 ± 0.09
44		0.72 ± 0.13
56		$2.0 \times 10^1 \pm 0.2 \times 10^1$
57		$2.9 \times 10^1 \pm 0.3 \times 10^1$
58		1.0 ± 0.1

Table 3
Inhibition constants (K_i) against *Tv*NH of group III target compounds

Compound	R=	K_i (μM)
		
38		2.1 ± 0.3
39		0.72 ± 0.07
61		0.0044 ± 0.0005
62		0.0041 ± 0.0007
65		$1.2 \times 10^1 \pm 0.1 \times 10^1$
66		2.3 ± 0.3

appears that there is a significant difference in activity between these compounds, with the 8-methylquinolinyl compound **42** (UAMC-00115) being the strongest inhibitor developed in this group, showing a K_i of 10.8 nM. The results from group II compounds suggest that there is a correlation between the inhibitory activity of the (iso)quinoline derivatives and the position of the (iso)quinoline-nitrogen in these compounds. This hypothesis was further investigated in the molecular modeling study discussed below.

In group III (Table 3), Immucillin-H analog **61** and Immucillin-A analog **62** are by far the strongest inhibitors, with K_i's of, respectively, 4.4 and 4.1 nM. Comparing the K_i of compound **61** with the K_i of 6.2 nM for both 'lead-compounds', Immucillin-H and Immucillin-A, confirms once again our hypothesis that placing the nucleobase on the iminoribitol-N via a methylene linker results in very good inhibitors of nucleoside hydrolase. It appears that the other compounds of group III do not have enough favorable features to bind tightly in the active site of the target enzyme.

3.3. Molecular modeling study

Docking studies were performed in an attempt to understand the difference in biochemical activity observed with the naphthalene and (iso)quinoline derivatives and to further investigate the structure–activity-relationship. In particular, the possible role of the nitrogen in the (iso)quinoline nucleobase mimic was investigated. The compounds in this study included **39**, **40**, **41**, **42**, **43**, **44**, **56**, **57**, **61**, and **62**, ranging in activity from 4.1 nM to 29 μM. They were docked in the active site of the target enzyme, which was obtained from the pdb structure of *Tv*NH co-crystallized with Immucillin-H (pdb code 2FF2).[11] The structure of this complex is illustrated in Figure 1.

Immucillin-H interacts with different active site residues of the enzyme through the hydroxyl groups and the ring-N of its iminoribitol moiety. The 9-deazahypoxanthine ring of Immucillin-H is orientated almost parallel between two Trp-residues (Trp83 and

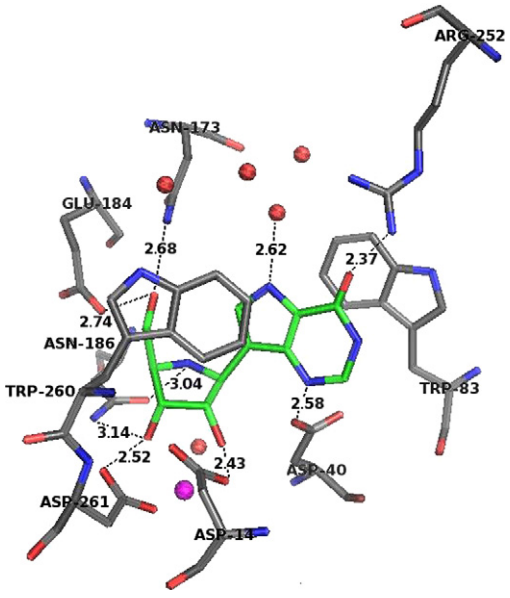


Figure 1. The active site of *Tv* NH co-crystallized with Immucillin-H (pdb code 2FF2).[11] Interaction distances are given in Å. The active site residues are depicted in grey (carbons), blue (nitrogens), and red (oxygens). The carbons of the inhibitor are depicted in green, the color codes for nitrogens and oxygens are the same as for the active site residues. Water molecules are depicted as red spheres, the Ca^{2+} ion is depicted as a magenta sphere. The image was made with PyMOL (PyMOL Molecular Graphics System, DeLano Scientific, San Carlos, CA).

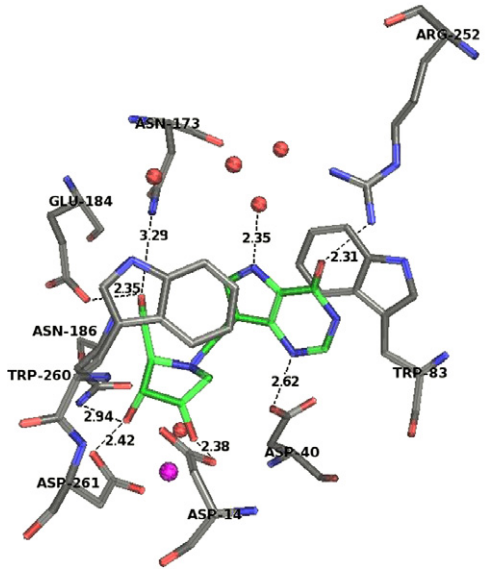


Figure 2. Inhibitor **61** in the active site of the target enzyme. Interaction distances are given in Å. Color codes are the same as for Figure 1. The images were made with PyMOL (PyMOL Molecular Graphics System, DeLano Scientific, San Carlos, CA).

Trp260) of the active site, allowing for aromatic stacking interactions. The 9-deazahypoxanthine ring is also involved in several hydrogen bonds: a first hydrogen bond is formed between N7 and a water molecule in the active site, a second hydrogen bond is formed between the carbonyl-O6 and amino acid residue Arg252, and a third hydrogen bond is formed between N3 and amino acid residue Asp40. The pK_a of Asp40 was calculated to be approximately 7.2–7.8, making it mostly protonated at our experimental conditions (pH 7.0) and thus allowing a hydrogen bond with N3.[23]

An automatic docking protocol and minimization was used to position our inhibitors into the active site. The interactions for our inhibitors with the target enzyme were compared to the interactions for Immucillin-H. Where possible, the interactions described for Immucillin-H were also observed with our inhibitors.

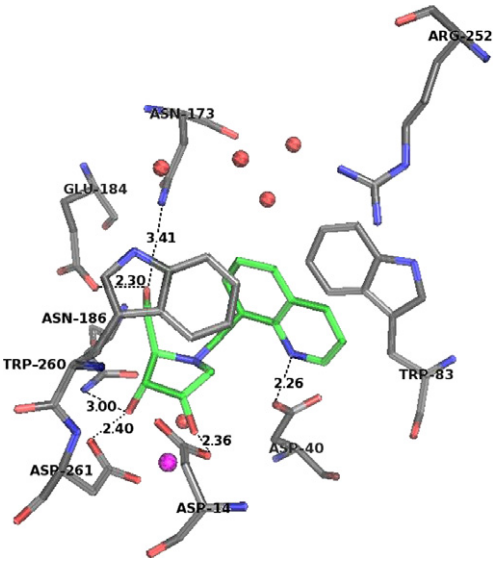


Figure 3. Inhibitor **42** in the active site of the target enzyme. Interaction distances are given in Å. Color codes are the same as for Figure 1. The images were made with PyMOL (PyMOL Molecular Graphics System, DeLano Scientific, San Carlos, CA).

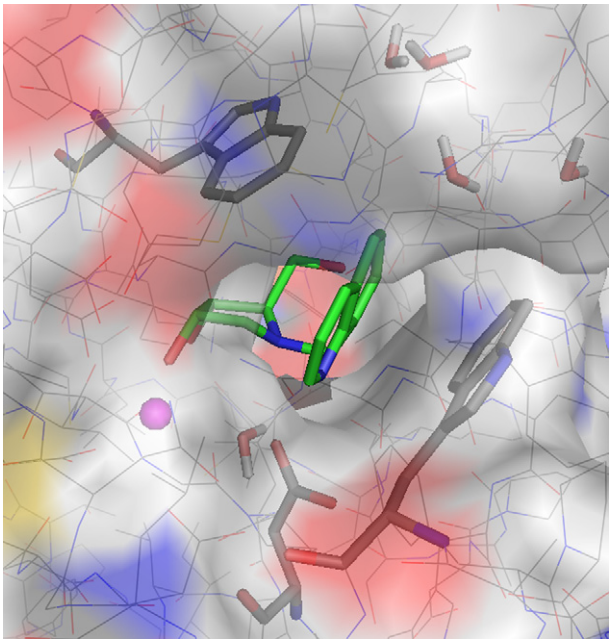


Figure 4. Binding of inhibitor **42** in the active site of the target enzyme. The nucleobase mimic is orientated parallel between Trp83 and Trp260, enabling aromatic stacking interactions. The active site residues are depicted in grey (carbons), blue (nitrogens), and red (oxygens). The carbons of the inhibitor are depicted in green, the color codes for nitrogens and oxygens are the same as for the active site residues. Water molecules are depicted as red spheres, the Ca^{2+} ion is depicted as a magenta sphere. The colors of the enzyme surface are according to the elements. The image was made with PyMOL (PyMOL Molecular Graphics System, DeLano Scientific, San Carlos, CA).

In Figure 2, docking of inhibitor **61** is illustrated. This compound is the *N*-substituted analog of Immucillin-H. Comparison of Figures 1 and 2 clearly demonstrates that the interactions between the inhibitor and the enzyme are very similar.

Our most active inhibitors among the docked compounds are **62**, **61**, **42**, **41**, **44**, **39**, and **43** with inhibition constants of 4.1, 4.4, 10.8, 180, 720, 720, and 750 nM, respectively. All these compounds appear to have a common feature as observed in this modeling study: the preferred conformation obtained by the docking experiments allows an interaction between a nucleobase-N and the Asp40 residue, an interaction that was also described with Immucillin-H.[11] This observation leads us to put forward the hypothesis that Asp40 plays an important role in binding of the inhibitor to the enzyme. Previous results from our group also support this hypothesis.[24] The presence of this N-Asp40 interaction was recently confirmed by a crystallography study of *Tv*NH in complex with inhibitor **42**.[25] Docking of inhibitor **42** is illustrated in Figure 3, clearly showing the N-Asp40 interaction.

A further observation made during the docking study was that the orientation of our inhibitors, as obtained from the docking experiments, always favored aromatic stacking interactions. As illustrated in Figure 4, the nucleobase mimic of the inhibitor is orientated parallel between the aromatic side chains of Trp83 and Trp260, allowing stacking interactions to occur.

4. Conclusions

We have synthesized a small library of novel *N*-arylmethyl substituted iminoribitols, which were screened for inhibitory activity against IAG-NH from *T. vivax*. These tests identified three very potent inhibitors, **62** with K_i = 4.1 nM, **61** with K_i = 4.4 nM,

and **42** with K_i = 10.8 nM. These compounds are among the most active inhibitors of IAG-NH reported. This confirms our hypothesis that *N*-arylmethyl substitution of iminoribitol is allowable for inhibitors of this target enzyme. Docking experiments on a series of the most active inhibitors revealed an interaction between a nitrogen in the nucleobase mimic and the Asp40 residue in the active site of the target enzyme. We propose here that this interaction plays an important role in the binding of an inhibitor in the enzyme. Inhibitor **42** was shown to be the one of the most active inhibitors developed in this series (K_i 10.8 nM) and this compound provides us with a good lead structure for further discovery of more potent NH-inhibitors.

5. Experimental

5.1. General procedures

All starting materials and dry solvents were obtained from Acros Organics or Aldrich. The arylmethylhalides used for *N*-alkylation were obtained from Acros Organics or Maybridge. 7-Methylquinoline was obtained from TCI. (Polystyrylmethyl)trimethylamonium cyanoborohydride and tris-(2-aminoethyl)-amine polystyrene resin were obtained from Novabiochem. Column chromatography was performed on a Flashmaster II (Jones Chromatography) with Isolute® columns pre-packed with silica gel (30–90 μM) for normal phase and C_{18} (30–90 μM) for reversed phase chromatography. Melting points were determined on an Electrothermal® digital melting point apparatus and are uncorrected. NMR-spectra were recorded on a Bruker Avance® DRX-400 spectrometer (400 MHz), coupling constants are reported in Hz. Electrospray Ionization (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 equiped with a C_{18} column (2.1 × 50 mm, 5 μm, Supelco, Sigma–Aldrich) for system I, and a HILIC Silica column (2.1 × 100 mm, 5 μm, Atlantis HILIC, Waters) for system II, both 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 I-A: 5% B to 100% B, 20 min, 0.2 mL/min; gradient I-B: 0% B to 100% B, 20 min, 0.2 mL/min; gradient II-A: 90% B to 50% B, 20 min, 0.2 mL/min; gradient II-B: 90% B to 40% B, 12 min, 0.2 mL/min). HPLC was performed on a Gilson instrument equiped with a C_{18} column (4.6 × 25 cm, 5 μm, Ultrasphere™ ODS) for system I, and a HILIC Silica column (2.1 × 100 mm, 5 μm, Atlantis HILIC, Waters) for system II (system I: solvent A: H_2O with 0.1% trifluoroacetic acid, solvent B: ACN with 1% trifluoroacetic acid; gradient I-A: 10% B to 100% B, 36 min, 1 mL/min; gradient I-B: 5% B to 50% B, 36 min, 1 mL/min; system II: solvent A: H_2O with 0.1% formic acid, solvent B: ACN with 0.1% formic acid; gradient II: 90% B to 50% B, 20 min, 0.4 mL/min).

5.2. Chemistry

5.2.1. (2*S*)-*N*-*tert*-Butoxycarbonyl-2-triphenylmethoxymethyl-3-pyrroline (7a)

Compound **6**[18] (5.4 g, 27 mmol) was dissolved in pyridine (22 mL) and triphenylmethylchloride (9.0 g, 32 mmol) was added. The reaction mixture was stirred at rt for 24 h. The solvent was removed under reduced pressure to give a syrup which was dissolved in EtOAc. This was washed with H_2O and the aqueous phase was extracted with EtOAc. The combined organic layers were dried (Na_2SO_4) and concentrated under reduced pressure. The residue was purified by column chromatography (hexane to hexane/EtOAc 4:1) to yield the product as a syrup (11 g, 92%). MS (ESI) *m*/*z* 463.6 $[M+Na]^+$.

5.2.2. (2*S*)-*N*-*tert*-Butoxycarbonyl-2-tert-butyldimethylsiloxymethyl-3-pyrroline (7b)

A solution of imidazole (0.17 g, 2.5 mmol) and TBDMSCl (0.38 g, 2.5 mmol) in CH_2Cl_2 (15 mL) was added dropwise to a stirred solution of compound **6**[18] and Et_3N (0.35 mL) in CH_2Cl_2 (5 mL). The reaction mixture was stirred at room temperature for 18 h, then it was concentrated under reduced pressure. The residue was dissolved in EtOAc, this was washed with water and brine. The organic layer was dried (Na_2SO_4) and concentrated under reduced pressure. The residue was purified by column chromatography (hexane to hexane/EtOAc 4:1) to yield the product as a syrup (0.32 g, 61%). MS (ESI) *m*/*z* 314.5 $[M+H]^+$.

5.2.3. *N*-*tert*-Butoxycarbonyl-1,4-dideoxy-1,4-imino-5-*O*-triphenylmethyl-D-ribitol (8a)

Compound **7a** (11 g, 25 mmol) and 4-methylmorpholine *N*-oxide (10 g, 74 mmol) were dissolved in acetone (250 mL) and H_2O (60 mL) and this was cooled to 0 °C. $K_2OsO_4.2H_2O$ (50%, 0.12 g, 0.16 mmol) was added and the resulting mixture was allowed to warm to rt and was then stirred at rt for 18 h. $NaHSO_3$ (5 g) was added and after 30 min of stirring, the mixture was extracted three times with EtOAc. The combined organic layers were washed with brine, dried (Na_2SO_4), and the solvent was removed under reduced pressure. The crude product was purified by column chromatography (hexane/EtOAc 1:1) to yield the title compound as a syrup (7.2 g, 62%). MS (ESI) *m*/*z* 475.0 $[M+Na]^+$.

5.2.4. *N*-*tert*-Butoxycarbonyl-1,4-dideoxy-1,4-imino-5-*O*-*tert*-butyldimethylsilyl-D-ribitol (8b)

Compound **7b** (0.32 g, 1.0 mmol) and 4-methylmorpholine *N*-oxide (0.41 g, 3.1 mmol) were dissolved in acetone (15 mL) and H_2O (3 mL) and this was cooled to 0 °C. OsO_4 (0.006 g, 0.02 mmol) was added and the resulting mixture was allowed to warm to rt and was then stirred at rt for 18 h. $NaHSO_3$ (0.25 g) was added and after 30 min of stirring, the mixture was extracted three times with EtOAc. The combined organic layers were washed with brine, dried (Na_2SO_4), and the solvent was removed under reduced pressure. The crude product was purified by column chromatography (hexane to hexane/EtOAc 1:4) to yield the title compound as a syrup (0.14 g, 39%). MS (ESI) *m*/*z* 348.5 $[M+H]^+$.

5.2.5. 1,4-Dideoxy-1,4-imino-D-ribitol·HCl (9·HCl)

Method A. Compound **8a** (7.2 g, 15 mmol) was dissolved in TFA/H_2O (1:1, 15 mL) and this was stirred at rt for 18 h. The reaction mixture was concentrated under reduced pressure, and the residue was dissolved in aq 2 N HCl. This was extracted twice with EtOAc. The combined organic phases were dried (Na_2SO_4), and the solvent was removed by evaporation under reduced pressure to yield the iminoribitol compound as a crystalline solid (2.5 g, 97%). MS, LC-MS, and NMR analysis showed that the compound was identical to iminoribitol **9** obtained from D-gulonolactone (Method C). *Method B.* Compound **8b** (0.14 g, 0.4 mmol) was dissolved in TFA/H_2O (1:1, 2 mL) and this was stirred at rt for 30 h. The reaction mixture was concentrated under reduced pressure and the residue was dissolved in aq 2 N HCl. This was extracted twice with EtOAc. The combined organic phases were dried (Na_2SO_4) and the solvent was removed by evaporation under reduced pressure to yield the iminoribitol compound as a crystalline solid. MS, LC-MS, and NMR analysis showed that the compound was identical to iminoribitol **9** obtained from D-gulonolactone (Method C). *Method C.* 5-*O*-*tert*-Butyldimethylsilyl-1,4-dideoxy-1,4-imino-2,3-*O*-isopropylidene-D-ribitol **10**[16] (0.22 g, 0.76 mmol) was dissolved in MeOH (1 mL), aq 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 title compound as a crystalline solid (0.13 g, 96%), mp 125–128 °C. MS (ESI) *m*/*z* 134.1 $[M+H]^+$; LC-MS (II-B) R_t 10.2 min, *m*/*z* 134.1 $[M+H]^+$; ^1H NMR (D_2O) δ 3.34 (d, 1H, J = 13.0), 3.47 (dd, 1H, J = 12.9, J' = 3.9), 3.57–3.63 (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), 4.33–4.37 (m, 1H); ^{13}C NMR (D_2O) δ 49.7, 58.1, 61.9, 69.5, 71.2.

5.2.6. General procedures for *N*-alkylation

Procedure A. The protected iminoribitol **10**[16] (1 equiv) was dissolved in CH_2Cl_2 (10 mL per mmol iminoribitol) and a solution of Na_2CO_3 (2 equiv) in H_2O (4 mL per mmol Na_2CO_3) was added. The arylmethylhalide (1 equiv) was added, and the reaction mixture was stirred under reflux for 3 h. The organic layer was separated, and the aqueous layer was extracted twice with CH_2Cl_2. The combined organic extracts were dried (Na_2SO_4), and the solvent was evaporated under reduced pressure. The crude product was dissolved in TFA/H_2O (1:1, 10 mL per mmol product), and this was stirred at rt overnight. H_2O was added, and this was extracted twice with CH_2Cl_2. The aqueous layer was concentrated under reduced pressure and the residue was purified by column chromatography ($CHCl_3$/MeOH 4:1) to yield the final product. *Procedure B.* The protected iminoribitol **10**[16] (1 equiv) was dissolved in DMF (2 mL per 0.3 mmol iminoribitol). K_2CO_3 (2 equiv.) and the arylmethylhalide (1.1 equiv) were added and this was stirred at 40 °C for 3–4 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 reduced pressure. The crude product was dissolved in TFA/H_2O (1:1, 10 mL per mmol product) and this was stirred at rt overnight. H_2O was added and this was extracted twice with CH_2Cl_2. The aqueous layer was concentrated under reduced pressure and redissolved in a mixture of H_2O and methanol (usually 1:1, depending on the solubility of the product) and stirred with Amberlyst A26(OH) ion exchange resin until the pH was neutral. The resin was removed by filtration and rinsed extensively with H_2O and methanol. The solvents were evaporated under reduced pressure and the residue was purified by column chromatography (CH_2Cl_2/MeOH 4:1 or 3:1) to yield the final product.

5.2.7. 1,4-Dideoxy-1,4-imino-*N*-(4-iodobenzyl)-D-ribitol·TFA (28·TFA)

4-Iodobenzyl bromide was used in procedure A to obtain the title compound as a brown amorphous solid (0.099 g, 45%). MS (ESI) *m*/*z* 350.0 $[M+H]^+$ LC-MS (I-A) R_t 9.5 min, *m*/*z* 350.1 $[M+H]^+$; HPLC (I-A, λ = 254 nm) R_t 11.7 min (100%); ^1H NMR (CD_3OD) δ 3.33 (d, 1 H, J = 15.5), 3.53 (dd, 1H, J = 12.6, J' = 4.1), 3.59–3.62 (m, 2H), 3.74–3.80 (m, 1H), 4.13 (dd, 1H, J = 6.7, J' = 4.2), 4.27 (d, 1H, J = 3.4), 4.41 (d, 1H, J = 12.9), 4.58 (d, 1H, J = 12.8), 7.32 (d, 2H, J = 8.1), 7.84 (d, 2H, J = 8.1); ^{13}C NMR (CD_3OD) δ 58.6, 58.7, 61.0, 70.5, 72.4, 72.9, 97.1, 118.1 (q, CF_3COOH), 131.1, 133.9, 139.6, 163.0 (q, CF_3COOH).

5.2.8. 1,4-Dideoxy-1,4-imino-*N*-(4-nitrobenzyl)-D-ribitol·TFA (29·TFA)

4-Nitrobenzyl bromide was used in procedure A (reaction time 20 h) to obtain the title compound as a brownish syrup (0.039 mg, 94%). MS (ESI) *m*/*z* 269.1 $[M+H]^+$; LC-MS (II-B) R_t 8.9 min, *m*/*z* 268.9 $[M+H]^+$; HPLC (I-A, λ = 254 nm) R_t 6.9 min (100%); ^1H NMR (CD_3OD) δ 3.19–3.22 (m, 1H), 3.47–3.51 (m, 2H), 3.63–3.78 (m, 2H), 4.07–4.12 (m, 1H), 4.22–4.26 (m, 1H), 4.43 (d, 1H, J = 11.0), 4.67 (d, 1H, J = 13.0), 7.78 (d, 2H, J = 8.6), 8.30 (d, 2H, J = 8.7); ^{13}C NMR (CD_3OD) δ 59.1, 59.6, 61.1, 70.7, 72.8, 73.2, 125.1, 133.0, 140.2, 150.1, 163.0 (br signal, CF_3COOH).

5.2.9. 1,4-Dideoxy-1,4-imino-*N*-(4-cyanobenzyl)-D-ribitol (30)

α-Bromo-*p*-tolunitrile was used in procedure A to obtain, after a second purification by column chromatography, the title compound as a yellow sticky gum (0.0061 g, 28%). MS (ESI) *m*/*z* 271.1 $[M+Na]^+$; LC-MS (I-B) R_t 3.1 min, *m*/*z* 249.1 $[M+H]^+$; HPLC (I-A, λ = 254 nm) R_t 4.1 min (100%); ^1H NMR (CD_3OD) δ 2.37–2.43 (m, 1 H), 2.71–2.76 (m, 1H), 3.01–3.07 (m, 1H), 3.54–3.65 (m, 3H), 3.88 (dd, 1H, J = 9.7, J' = 4.8), 3.99–4.05 (m, 1H), 4.13 (dd, 1H, J = 13.8, J' = 4.0), 7.52–7.56 (m, 2H), 7.64–7.68 (m, 2H); ^{13}C NMR (CD_3OD) δ 59.2, 60.4, 63.0, 71.2, 72.5, 74.3, 111.8, 119.8, 130.8, 133.2, 146.8.

5.2.10. 1,4-Dideoxy-1,4-imino-*N*-(3-nitrobenzyl)-D-ribitol (31)

3-Nitrobenzyl bromide was used in procedure A. The crude product was dissolved in a mixture of H_2O and methanol (1:1) and stirred with Amberlyst A26(OH) ion exchange resin until the pH was neutral. The resin was removed by filtration and rinsed extensively with H_2O and methanol. The solvents were evaporated under reduced pressure, and the residue was purified by column chromatography (CH_2Cl_2/methanol 4:1) to obtain the title compound as a brown sticky gum (0.0082 g, 11%). MS (ESI) *m*/*z* 291.1 $[M+Na]^+$; LC-MS (I-B) R_t 3.5 min, *m*/*z* 269.0 $[M+H]^+$; HPLC (I-A, λ = 254 nm) R_t 7.3 min (100%); ^1H NMR (CD_3OD) δ 2.43 (dd, 1H, J = 9.5, J' = 6.8), 2.75 (dd, 1H, J = 9.1, J' = 4.6), 3.06 (dd, 1H, J = 9.5, J' = 5.8), 3.57–3.68 (m, 3H), 3.92 (t, 1H, J = J' = 5.0), 4.04 (dd, 1H, J = 12.2, J' = 5.7), 4.18 (d, 1H, J = 13.6), 7.56 (t, 1H, J = J' = 7.9), 7.77 (d, 1H, J = 7.6), 8.12 (dd, 1H, J = 8.1, J' = 1.6), 8.25 (s, 1H); ^{13}C NMR (CD_3OD) δ 59.1, 59.9, 63.1, 71.1, 72.4, 74.3, 123.0, 124.5, 130.4, 136.2, 143.2, 149.8.

5.2.11. 1,4-Dideoxy-1,4-imino-*N*-(3-cyanobenzyl)-D-ribitol (32)

α-Bromo-*m*-tolunitrile was used in procedure A. The crude product was dissolved in a mixture of H_2O and methanol (1:1) and stirred with Amberlyst A26(OH) ion exchange resin until the pH was neutral. The resin was removed by filtration and rinsed extensively with H_2O and methanol. The solvents were evaporated under reduced pressure and the residue was purified by column chromatography (CH_2Cl_2/methanol 4:1) to obtain the title compound as a yellow sticky gum (0.0046 g, 6%). MS (ESI) *m*/*z* 249.1 $[M+H]^+$; LC-MS (II-B) R_t 8.9 min, *m*/*z* 249.0 $[M+H]^+$; HPLC (I-B, λ = 254 nm) R_t 11.2 min (100%), (I-B, λ = 214 nm) R_t 10.0 min (88%); ^1H NMR (CD_3OD) δ 2.40 (dd, 1H, J = 9.5, J' = 6.8), 2.73 (dd, 1H, J = 8.9, J' = 4.5), 3.03 (dd, 1H, J = 9.5, J' = 5.8), 3.56–3.65 (m, 3H), 3.91 (t, 1H, J = J' = 5.0), 4.03 (dd, 1H, J = 12.2, J' = 5.8), 4.07 (d, 1H, J = 13.6), 7.49 (t, 1H, J = J' = 7.7), 7.60 (d, 1H, J = 7.7), 7.66 (d, 1H, J = 7.8), 8.75 (s, 1H); ^{13}C NMR (CD_3OD) δ 59.0, 59.9, 62.9, 71.1, 72.4, 74.2, 113.3, 119.8, 130.4, 131.9, 133.5, 134.7, 142.6.

5.2.12. 1,4-Dideoxy-1,4-imino-*N*-[3-(1*H*-pyrrol-1-yl)benzyl]-D-ribitol (33)

1-[3-(Bromomethyl)phenyl]-1*H*-pyrrole was used in procedure B to obtain the title compound as a yellow sticky gum (0.0051 g, 6%). MS (ESI) *m*/*z* 289.0 $[M+H]^+$; LC-MS (I-B) R_t 8.7 min, *m*/*z* 289.0 $[M+H]^+$; HPLC (I-B, λ = 254 nm) R_t 19.6 min (100%), (I-B, λ = 214 nm) R_t 21.3 min (95%); ^1H NMR (CD_3OD) δ 2.60 (m, 1H), 2.86 (d, 1H, J = 4.0), 3.16 (dd, 1H, J = 9.9, J' = 5.6), 3.61 (d, 2H, J = 4.3), 3.71 (d, 1H, J = 13.3), 3.95 (t, 1H, J = J' = 5.1), 4.07 (dd, 1H, J = 11.3, J' = 5.6), 4.17 (d, 1H, J = 13.0), 6.27 (s, 2H), 7.19 (s, 2H), 7.24 (d, 1H, J = 4.5), 7.38–7.41 (m, 2H), 7.51 (s, 1H); ^{13}C NMR (CD_3OD) δ 59.2, 60.9, 62.3, 71.0, 72.5, 74.2, 111.4, 120.1, 120.2, 121.9, 127.4, 129.9, 130.8, 132.4, 140.9, 142.4.

5.2.13. 1,4-Dideoxy-1,4-imino-*N*-[4-(1*H*-pyrrol-1-yl)benzyl]-D-ribitol (34)

1-[4-(Bromomethyl)phenyl]-1*H*-pyrrole was used in procedure B to obtain the title compound as an amorphous solid (0.0044 g,

6%). MS (ESI) *m*/*z* 289.1 $[M+H]^+$; LC-MS (I-B) R_t 7.5 min, *m*/*z* 289.0 $[M+H]^+$; HPLC (I-A, λ = 254 nm) R_t 12.6 min (100%); ^{1}H NMR (CD_3OD) δ 2.47 (dd, 1 H, J = 9.5, J' = 7.1), 2.73–2.75 (m, 1H), 3.09 (dd, 1H; J = 9.6, J' = 5.9), 3.55–3.61 (m, 3H), 3.92 (t, 1H, $J = J'$ = 5.0), 4.01–4.08 (m, 2H), 6.27 (t, 2H, $J = J'$ = 2.1), 7.16 (t, 2H, $J = J'$ = 2.1), 7.42 (s, 4H); ^{13}C NMR (CD_3OD) δ 59.2, 60.3, 63.0, 71.1, 72.6, 74.5, 111.4, 120.2, 121.0, 131.6, 137,3, 141.4.

5.2.14. 1,4-Dideoxy-1,4-imino-*N*-[4-(1*H*-pyrazol-1-yl)benzyl]-D-ribitol (35)

1-[4-(Bromomethyl)phenyl]-1*H*-pyrazole was used in procedure B to obtain the title compound as a green gum (0.069 g, 85%). MS (ESI) *m*/*z* 290.0 $[M+H]^+$; LC-MS (I-B) R_t 9.0 min, *m*/*z* 290.0 $[M+H]^+$; HPLC (I-B, λ = 254 nm) R_t 15.4 min (100%), (I-B, λ = 214 nm) R_t 14.6 min (100%); ^{1}H NMR (CD_3OD) δ 2.48 (dd, 1H, J = 9.5, J' = 7.0), 2.75 (dd, 1H, J = 8.9, J' = 4.5), 3.09 (dd, 1H, J = 9.5, J' = 5.9), 3.56–3.65 (m, 3H), 3.95 (t, 1H, $J = J'$ = 5.0), 4.03–4.09 (m, 2H), 6.51 (t, 1H, $J = J'$ = 2.1), 7.46 (d, 2H, J = 8.4), 7.67 (d, 2H, J = 8.4), 7.70 (d, 1H, J = 1.7), 8.17 (d, 1H, J = 2.4); ^{13}C NMR (CD_3OD) δ 59.1, 60.0, 62.8, 71.0, 72.4, 74.4, 108.6, 120.3, 129.0, 131.3, 138.8, 140.4, 142.0.

5.2.15. 1,4-Dideoxy-1,4-imino-*N*-[4-(1*H*-1,2,4-triazol-1-yl) benzyl]-D-ribitol (36)

1-[4-(Bromomethyl)phenyl]-1*H*-1,2,4-triazole was used in procedure B to obtain the title compound as a white amorphous solid (0.072 g, 89%). MS (ESI) *m*/*z* 291.0 $[M+H]^+$; LC-MS (II-B) R_t 10.6 min, *m*/*z* 290.9 $[M+H]^+$; HPLC (I-B, λ = 254 nm) R_t 11.8 min (100%), (I-B, λ = 214 nm) R_t 11.0 min (100%); ^{1}H NMR (CD_3OD) δ 2.46 (dd, 1 H, J = 9.5, J' = 7.0), 2.75 (dd, 1H, J = 9.0, J' = 4.5), 3.08 (dd, 1H, J = 9.5, J' = 5.9), 3.58–3.66 (m, 3H), 3.94 (t, 1H, $J = J'$ = 5.0), 4.05 (dd, 1H, J = 12.3, J' = 5.9), 4.10 (d, 1H, J = 13.3), 7.53 (d, 2H, J = 8.3), 7.75 (d, 2H, J = 8.4), 8.15 (s, 1H), 9.04 (s, 1H); ^{13}C NMR (CD_3OD) δ 59.1, 60.0, 62.9, 71.1, 72.4, 74.3, 120.9, 131.4, 137.3, 140.9, 142.9, 152.8.

5.2.16. 1,4-Dideoxy-1,4-imino-*N*-(5-methyl-2-phenyl-2*H*-1,2, 3-triazol-4-yl)methyl-D-ribitol (37)

4-(Bromomethyl)-5-methyl-2-phenyl-2*H*-1,2,3-triazole was used in procedure B to obtain the title compound as a white amorphous solid (0.055 g, 65%). MS (ESI) *m*/*z* 305.1 $[M+H]^+$; LC-MS (I-B) R_t 9.6 min, *m*/*z* 305.1 $[M+H]^+$; HPLC (I-B, λ = 254 nm) R_t 20.3 min (100%), (I-B, λ = 214 nm) R_t 19.6 min (100%); ^{1}H NMR (CD_3OD) δ 2.38 (s, 3H), 2.61 (dd, 1H, J = 9.6, J' = 7.0), 2.80 (dd, 1H, J = 9.2, J' = 4.6), 3.15 (dd, 1H, J = 9.5, J' = 5.9), 3.60–3.70 (m, 2H), 3.77 (d, 1H, J = 13.9), 3.92 (t, 1H, $J = J'$ = 5.0), 4.05 (dd, 1H, J = 12.4, J' = 5.8), 4.10 (d, 1H, J = 13.9), 7.32 (t, 1H, $J = J'$ = 7.4), 7.47 (m, 2H), 7.97 (d, 2H, J = 7.7); ^{13}C NMR (CD_3OD) δ 10.1, 49.4, 59.1, 62.9, 71.0, 71.9, 74.3, 119.3, 128.1, 130.3, 141.0, 145.9, 146.0.

5.2.17. 1,4-Dideoxy-1,4-imino-*N*-(1-methyl-1*H*-benzimidazol-2-yl)methyl-D-ribitol (38)

2-(Bromomethyl)-1-methyl-1*H*-benzimidazole was used in procedure A to obtain the title compound as a yellow amorphous solid (0.013 g, 15%). MS (ESI) *m*/*z* 278.2 $[M+H]^+$ and *m*/*z* 300.2 $[M+Na]^+$; LC-MS (I-B) R_t 7.8 min, *m*/*z* 278.1 $[M+H]^+$; HPLC (I-A, λ = 254 nm) R_t 6.5 min (100%); ^{1}H NMR (CD_3OD) δ 2.67 (dd, 1 H, J = 9.5, J' = 6.6), 2.87 (dd, 1H, J = 8.8, J' = 4.4), 3.08 (dd, 1H, J = 9.4, J' = 5.7), 3.52–3.64 (m, 2H), 3.88 (s, 3H), 3.91 (t, 1H, $J = J'$ = 4.9), 3.96 (d, 1H, J = 14.2), 4.04 (dd, 1H, J = 11.2, J' = 5.5), 4.34 (d, 1H, J = 14.3), 7.21–7.31 (m, 2H), 7.47 (d, 1H, J = 8.0), 7.59 (d, 1H, J = 7.9); ^{13}C NMR (CD_3OD) δ 30.4, 52.4, 59.4, 63.2, 71.5, 72.3, 74.5, 110.8, 119.4, 123.3, 124.0, 137.2, 142.4, 153.8.

5.2.18. 1,4-Dideoxy-1,4-imino-*N*-(1-methyl-1*H*-1,2,3-benzotriazol-5-yl)methyl-D-ribitol (39)

5-(Bromomethyl)-1-methyl-1*H*-1,2,3-benzotriazole was used in procedure B to obtain the title compound as a pale yellow amorphous solid (0.066 g, 85%). MS (ESI) *m*/*z* 279.0 $[M+H]^+$; LC-MS (II-B) R_t 13.3 min, *m*/*z* 278.9 $[M+H]^+$; HPLC (I-B, λ = 254 nm) R_t 10.0 min (100%), (I-B, λ = 214 nm) R_t 9.6 min (100%); ^{1}H NMR (CD_3OD) δ 2.48 (dd, 1H, J = 9.5, J' = 7.0), 2.78 (dd, 1H, J = 8.9, J' = 4.5), 3.05 (dd, 1H, J = 9.5, J' = 5.8), 3.57–3.64 (m, 2H), 3.69 (d, 1H, J = 13.1), 3.95 (t, 1H, $J = J'$ = 4.9), 4.05 (dd, 1H, J = 12.2, J' = 5.9), 4.19 (d, 1H, J = 13.1), 4.29 (s, 3H), 7.58 (d, 1H, J = 8.6), 7.66 (d, 1H, J = 8.6), 7.89 (s, 1H); ^{13}C NMR (CD_3OD) δ 34.7, 59.0, 60.5, 62.9, 71.1, 72.4, 74.3, 110.9, 119.3, 130.5, 134.4, 137.3, 146.7.

5.2.19. 1,4-Dideoxy-1,4-imino-*N*-(2-naphtyl)methyl-D-ribitol·TFA (40·TFA)

2-(Bromomethyl)naphtalene was used in procedure A to obtain the title compound as a light brown gum (0.077 g, 80%). MS (ESI) *m*/*z* 274.1 $[M+H]^+$; LC-MS (I-B) R_t 10.1 min, *m*/*z* 274.1 $[M+H]^+$; HPLC (I-A, λ = 254 nm) R_t 12.3 min (100%); ^{1}H NMR (CD_3OD) δ 2.78 (br s, 1H), 3.04 (br s, 1H), 3.21 (dd, 1H, J = 10.3, J' = 5.4), 3.56–3.66 (m, 2H), 3.93–4.01 (m, 2H), 4.10 (dd, 1H, J = 10.8, J' = 5.4), 4.35 (d, 1H, J = 12.8), 7.45–7.51 (m, 2H), 7.53 (dd, 1H, J = 8.5, J' = 1.5), 7.83–7.87 (m, 4H); ^{13}C NMR (CD_3OD) δ 58.9, 60.0, 62.3, 70.7, 72.4, 73.4, 118.0 (q, CF_3COOH), 128.8, 129.1, 129.8, 131.1, 132.3, 134.6, 134.9, 163.1 (br, CF_3COOH).

5.2.20. 1,4-Dideoxy-1,4-imino-*N*-(2-quinolinyl)methyl-D-ribitol·TFA (41·TFA)

2-(Chloromethyl)quinoline was used in procedure A (reaction time 16 h, addition of 2 equiv NaI) to obtain the title compound as a gum (0.0020 g, 3%). MS (ESI) *m*/*z* 275.2 $[M+H]^+$; LC-MS (I-B) R_t 10.2 min, *m*/*z* 275.2 $[M+H]^+$; HPLC (I-A, λ = 214 nm) R_t 9.3 min (100%); ^{1}H NMR (CD_3OD) δ 2.60–2.64 (m, 1H), 2.89 (s, 1H), 3.17–3.20 (m, 1H), 3.58–3.73 (m, 2H), 3.93–4.00 (m, 2H), 4.06–4.09 (m, 1H), 4.36 (d, 1H, J = 14), 7.59 (t, 1H, $J = J'$ = 7.5), 7.64 (d, 1H, J = 8.5), 7.76 (t, 1H, $J = J'$ = 7.1), 7.92 (d, 1H, J = 7.9), 8.02 (d, 1H, J = 8.6), 8.31 (d, 1H, J = 8.6); ^{13}C NMR (CD_3OD) δ 59.5, 61.7, 62.4, 71.3, 72.4, 74.1, 122.6, 127.6, 128.6, 128.9, 129.7, 131.0, 132.4, 138.5, 148.2.

5.2.21. 1,4-Dideoxy-1,4-imino-*N*-(8-quinolinyl)methyl-D-ribitol·TFA (42·TFA)

8-(Bromomethyl)quinoline was used in procedure B to obtain the title compound as a white amorphous solid (0.062 g, 72%). MS (ESI) *m*/*z* 275.0 $[M+H]^+$ and *m*/*z* 297.0 $[M+Na]^+$; LC-MS (I-B) R_t 4.5 min, *m*/*z* 275.0 $[M+H]^+$; HPLC (I-B, λ = 254 nm) R_t 14.8 min (100%); ^{1}H NMR (CD_3OD) δ 2.70 (*br* s, 1H), 3.02–3.10 (m, 2H), 3.71 (dd, 1H, J = 11.7, J' = 4.7), 3.89–4.03 (m, 4H), 4.96 (d, 1H, J = 12.7), 7.51–7.58 (m, 2H), 7.73 (d, 1H, J = 6.9), 7.89 (d, 1H, J = 8.2), 8.34 (d, 1H, J = 8.3), 8.84–8.86 (m, 1H); ^{13}C NMR (CD_3OD) δ 57.3, 59.4, 62.2, 71.0, 71.5, 73.7, 118.0 (q, CF_3COOH), 122.6, 127.5, 129.5, 130.2, 131.9, 138.6, 147.5, 150.4, 150.6, 163.3 (d, CF_3COOH).

5.2.22. 1,4-Dideoxy-1,4-imino-*N*-(1-isoquinolinyl)methyl-D-ribitol (43)

1-(Bromomethyl)isoquinoline was used in procedure A to obtain the title compound as a yellow amorphous solid (0.018 g, 31%). MS (ESI) *m*/*z* 297.2 $[M+Na]^+$; LC-MS (I-B) R_t 8.8 min, *m*/*z* 275.1 $[M+H]^+$; HPLC (I-A, λ = 254 nm) R_t 8.2 min (100%); ^{1}H NMR (CD_3OD) δ 2.66 (dd, 1H, J = 9.7, J' = 6.5), 2.93 (dd, 1H, J = 8.9, J' = 4.4), 3.04 (dd, 1H, J = 9.6, J' = 5.7), 3.58–3.70 (m, 2H), 3.93 (t, 1H, $J = J'$ = 5.1), 4.01 (dd, 1H, J = 10.7, J' = 5.8), 4.26 (d, 1H, J = 13.8), 4.72 (d, 1H, J = 13.8), 7.65–7.70 (m, 1H), 7.73–7.79 (m, 2H), 7.93 (d, 1H, J = 8.1), 8.36 (d, 1H, J = 5.8), 8.51 (d, 1H, J = 8.5); ^{13}C NMR (CD_3OD) δ 59.2, 59.5, 63.0, 71.4, 72.7, 74.3, 122.2, 126.9, 128.3, 128.6, 128.8, 131.9, 138.0, 141.4, 159.7.

5.2.23. *N*-(7-Quinolinyl)-5-*O*-*tert*-butyldimethylsilyl-1,4-dideoxy-1,4-imino-2,3-*O*-isopropylidene-D-ribitol (27)

7-Methylquinoline **59** (0.10 g, 0.70 mmol), *N*-bromosuccinimide (0.13 g, 0.73 mmol) and a catalytic amount of dibenzoylperoxide were dissolved in carbontetrachloride (10 mL). The reaction mixture was stirred under reflux for 18h. Protected iminoribitol compound **10**[16] (0.14 g, 0.49 mmol) was dissolved in dimethylformamide (3 mL) and together with $KHCO_3$ (0.10 g, 0.97 mmol) added to the cooled (40 °C) reaction mixture containing 7-bromomethylquinoline **60**. This was stirred at 40 °C for 1.5 h. The reaction mixture was filtered and the filtrate was concentrated under reduced pressure. The residue was dissolved in EtOAc and washed with H_2O, dried (Na_2SO_4), and concentrated under reduced pressure. The residue was purified by column chromatography (hexane/EtOAc 85:15) to yield the protected intermediate product (0.15 g, 49%). MS (ESI) *m*/*z* 429.3 $[M+H]^+$; ^1H NMR ($CDCl_3$) δ 0.04 (s, 3H), 0.07 (s, 3H), 0.88 (s, 9H), 1.33 (s, 3H), 1.58 (s, 3H), 2.80 (dd, J = 10.4, J' = 2.5, 1H), 3.10 (dd, J = 5.8, J' = 3.9, 1H), 3.18 (dd, J = 10.4,J = 5.5, 1H), 3.67 (dd, J = 10.7, J' = 4.2, 1H), 3.84 (dd,J = 10.7, J' = 4.1, 1H), 3.98 (d, J = 13.7, 1H), 4.24 (d, J = 13.7, 1H), 4.58 (dd, J = 6.5, J' = 2.0, 1H), 4.66–4.69 (m, 1H), 7.34–7.37 (m, 1H), 7.45 (d, J = 8.6, 1H), 7.65 (d, J = 8.4, 1H), 7.76 (d, J = 8.4, 1H), 8.03 (s, 1H), 8.11–8.17 (m, 1H).

5.2.24. 1,4-Dideoxy-1,4-imino-*N*-(7-quinolinyl)methyl-D-ribitol·TFA (44·TFA)

Compound **27** (0.15 g, 0.34 mmol) was dissolved in TFA/water (1:1, 2 ml). This was stirred at room temperature overnight. The solvents were evaporated under reduced pressure. After purification by column chromatography (DCM/MeOH 85:15) the TFA-salt of the title compound was obtained as a white amorphous solid (0.065 g, 49%). MS (ESI) *m*/*z* 275.1 $[M+H]^+$; LC-MS (II-B) R_t 11.1 min, *m*/*z* 275.1 $[M+H]^+$; HPLC (II, λ = 214 nm) R_t 6.6 min (100%); ^1H NMR (MeOD) δ 3.18 (d, J = 9.3, 1H), 3.40–3.47 (m, 2H), 3.59 (dd, J = 12,5, J' = 3.5, 1H), 3.70 (dd, J = 12.0, J' = 5.1, 1H), 4.07 (t, J = J' = 6.8, 1H), 4.18–4.21 (m, 1H), 4.44 (d, J = 12.9, 1H), 4.69 (d, J = 13.0, 1H), 7.51–7.55 (m, 1H), 7.70 (d, J = 8.4, 1H), 7.98 (d, J = 8.4, 1H), 8.11 (s, 1H), 8.34 (d, J = 8.3, 1H), 8.86 (*br* s, 1H); ^{13}C NMR (MeOD) δ 59.0, 59.7, 62.0, 70.7, 72.7, 73.3, 116.1, 118.5 (q, *C*F_3COOH), 123.5, 129.5, 130.3, 131.4, 135.4, 138.2, 148.5, 152.2, 164.5 (q, CF_3COOH).

5.2.25. 1,4-Dideoxy-1,4-imino-*N*-(4-pyridinyl)methyl-D-ribitol (52)

The protected iminoribitol **10**[16] (0.084 g, 0.29 mmol) and 4-pyridinecarboxaldehyde (0.03 mL, 0.31 mmol) were dissolved in MeOH (2 mL). $NaCNBH_3$ (0.13 g, 2.1 mmol) was added, and the reaction mixture was stirred at rt for 24 h. The solvent was removed under reduced pressure, and the residue was redissolved in EtOAc. This was filtered through a pad of Silica. The solvent was removed by evaporation under reduced pressure and the residue was dissolved in TFA/H_2O (1:1, 4 mL). This was stirred at rt overnight. The reaction mixture was concentrated under reduced pressure and the residue was purified by column chromatography ($CHCl_3$/MeOH 4:1) to yield the title compound as a gum (0.033 g, 48%). MS (ESI) 225.1 $[M+H]^+$; LC-MS (II-B) R_t 11.6 min, *m*/*z* 225.1 $[M+H]^+$; HPLC (II-B, λ = 254 nm) R_t 8.7 min (88%); ^1H NMR (CD_3OD) δ 3.04–3.08 (m, 1H), 3.37–3.42 (m, 1H), 3.47 (dd, 1H, J = 11.6, J' = 4.7), 3.68–3.79 (m, 2H), 4.09 (dd, 1H, J = 6.8, J' = 4.6), 4.21–4.28 (m, 2H), 4.56 (d, 1H, J = 13.7), 7.61 (d, 2H, J = 5.4), 8.58–8.64 (*br* s, 2H); ^{13}C NMR (CD_3OD) δ 59.2, 60.3, 60.6, 70.7, 73.0, 73.3, 118.2 (q, *C*F_3COOH), 126.7, 145.1, 150.3, 163.2 (q, CF_3COOH).

5.2.26. General procedure used for the reductive aminations (except for compound 52)

The protected iminoribitol **10**[16](1 equiv) was dissolved in a mixture of CH_2Cl_2/acetic acid (10:1, 2 mL per 0.3 mmol protected iminoribitol). The aldehyde (1.2 equiv) and (polystyrylmethyl)trimethylammonium cyanoborohydride (2 equiv) were added. This was stirred at rt for 24–48 h. The reaction mixture was filtered to remove the polymer and the solvent was evaporated under reduced pressure. The residue was dissolved in TFA/H_2O (1:1, 2 mL per 0.3 mmol protected iminoribitol), and this was stirred at rt for 18 h. The TFA and H_2O were evaporated under reduced pressure, and the residue was dissolved in methanol and H_2O. Amberlyst A26(OH) ion exchange resin was added and the mixture was stirred at rt until the pH was neutral or slightly basic. Then the reaction mixture was filtered to remove the resin, which was rinsed extensively with methanol and H_2O. The solvents were evaporated under reduced pressure to yield the crude product.

5.2.27. 1,4-Dideoxy-1,4-imino-*N*-(2-aminopyridin-3-yl)methyl-D-ribitol (53)

The general reductive amination procedure was followed with 2-amino-3-pyridinecarboxaldehyde (2 equiv) as the aldehyde. The crude product obtained after deprotection was stirred at rt overnight with tris-(2-aminoethyl)-amine polystyrene resin in CH_2Cl_2/MeOH (3:1) to scavenge the excess of aldehyde. Column purification (CH_2Cl_2/MeOH 3:1) yielded the final compound as a white amorphous solid (0.019 g, 27%). MS (ESI) *m*/*z* 240.0 $[M+H]^+$ and *m*/*z* 262.0 $[M+Na]^+$; LC-MS (II-A) R_t 5.5 min, *m*/*z* 240.1 $[M+H]^+$; HPLC (II, λ = 214 nm) R_t 7.3 min (100%); ^1H NMR (D_2O) δ 2.54 (t, 1H, J = 8.8), 2.84 (m, 1H), 3.09–3.14 (m, 1H), 3.58 (d, 1H, J = 13.2), 3.65–3.78 (m, 2H), 4.01–4.05 (m, 2H), 4.14–4.18 (m, 1H), 6.83–6.87 (m, 1H), 7.63 (d, 1H, J = 7.0), 7.97 (d, 1H, J = 4.1); ^{13}C NMR (D_2O) δ 56.8, 56.9, 61.3, 69.7, 70.2, 72.7, 114.6, 119.0, 139.9, 145.1, 157.3.

5.2.28. 1,4-Dideoxy-1,4-imino-*N*-(6-methoxypyridin-3-yl) methyl-D-ribitol (54)

The general reductive amination procedure was followed with 6-methoxy-3-pyridinecarboxaldehyde as the aldehyde. After purification by column chromatography (CH_2Cl_2/MeOH 3:1) the title compound was obtained as a light yellow gum (0.053 g, 83%). MS (ESI) *m*/*z* 255.2 $[M+H]^+$ and 277.2 $[M+Na]^+$; LC-MS (II-A) R_t 5.1 min, *m*/*z* 255.1 $[M+H]^+$; HPLC (I-B, λ = 254 nm) R_t 7.5 min (100%), and (I-B, λ = 214 nm) R_t 7.4 min (97%); ^1H NMR (CD_3OD) δ 2.62 (s, 1H), 2.88 (s, 1H), 3.11 (dd, 1H, J = 10.0, J' = 5.4), 3.60 (d, 2H, J = 4.5), 3.67–3.71 (m, 1H), 3.95 (t, 1H, J = J' = 5.2), 4.04–4.14 (m, 2H), 6.74–6.78 (m, 1H), 7.68–7.72 (m, 1H), 8.07–8.09 (m, 1H); ^{13}C NMR (CD_3OD) δ 54.3, 57.7, 58.9, 62.1, 71.0, 72.4, 74.2, 111.7, 130.0, 132.5, 142.0, 148.7, 165.6.

5.2.29. 1,4-Dideoxy-1,4-imino-*N*-(4-methyl-2-phenylpyrimidin-5-yl)methyl-D-ribitol (55)

The general reductive amination procedure was followed with 4-methyl-2-phenyl-5-pyridimidinecarboxaldehyde as the aldehyde. After purification by column chromatography (CH_2Cl_2/MeOH 12:1) the title compound was obtained as a white amorphous solid (0.034 g, 35%). MS (ESI) *m*/*z* 316.3 $[M+H]^+$ and *m*/*z* 338.3 $[M+Na]^+$; LC-MS (II-A) R_t 4.9 min, *m*/*z* 316.3 $[M+H]^+$; HPLC (I-B, λ = 254 nm) R_t 18.2 min (100%), and (I-B, λ = 214 nm) 18.1 min (100%); ^1H NMR (CD_3OD) δ 2.47 (dd, 1H, J = 6.9, J' = 2.3), 2.63 (s, 3H), 2.77 (dd, 1H, J = 9.1, J' = 1.5), 3.03 (dd, 1H, J = 5.7, J' = 3.5), 3.57–3.67 (m, 3H), 3.91 (t, 1H, J = J' = 4.9), 4.03 (dd, 1H, J = 11.3, J' = 5.6), 4.15 (d, 1H, J = 13.6), 7.44–7.48 (m, 3H), 8.33–8.36 (m, 2H), 8.62 (s, 1H); ^{13}C NMR (CD_3OD) δ 22.2, 55.7, 59.3, 63.6, 71.4, 73.0, 74.6, 129.3, 129.6, 130.1, 131.7, 139.0, 158.3, 164.4, 168.7.

5.2.30. 1,4-Dideoxy-1,4-imino-*N*-(3-quinolinyl)methyl-D-ribitol (56)

The general reductive amination procedure was followed with 3-quinoline-carboxaldehyde as the aldehyde. After purification

by column chromatography (CH_2Cl_2/MeOH 3:1) the title compound was obtained as a yellow sticky gum (0.023 g, 30%). MS (ESI) *m/z* 275.2 $[M+H]^+$; LC-MS (II-A) R_t 5.3 min, *m/z* 275.2 $[M+H]^+$; HPLC (II-B, $\lambda = 214$ nm) R_t 5.9 min (100%); ^1H NMR (CD_3OD) δ 2.50 (dd, 1H, $J = 9.4$, $J' = 6.8$), 2.80 (dd, 1H, $J = 8.9$, $J' = 4.4$), 3.06 (dd, 1H, $J = 9.5$, $J' = 5.8$), 3.59–3.64 (m, 2H), 3.76 (d, 1H, $J = 13.5$), 3.93 (t, 1H, $J = J' = 5.0$), 4.04 (dd, 1H, $J = 11.9$, $J' = 6.0$), 4.27 (d, 1H, $J = 13.5$), 7.56–7.61 (m, 1H), 7.70–7.75 (m, 1H), 7.91 (d, 1H, $J = 8.1$), 8.00 (d, 1H, $J = 8.5$), 8.27 (s, 1H), 8.85 (s, 1H); ^{13}C NMR (CD_3OD) δ 58.2, 59.2, 63.2, 71.3, 72.6, 74.4, 128.3, 129.0, 129.2, 129.7, 130.9, 133.9, 137.8, 148.1, 153.0.

5.2.31. 1,4-Dideoxy-1,4-imino-*N*-(4-quinolinyl)methyl-D-ribitol (57)

The general reductive amination procedure was followed with 4-quinoline-carboxaldehyde as the aldehyde. After purification by column chromatography (CH_2Cl_2/MeOH 4:1) the title compound was obtained as an white amorphous solid (0.043 g, 57%). MS (ESI) *m/z* 275.1 $[M+H]^+$ and *m/z* 297.1 $[M+Na]^+$; LC-MS (II-A) R_t 5.3 min, *m/z* 275.1 $[M+H]^+$; HPLC (II-B, $\lambda = 214$ nm) R_t 6.0 min (100%); ^1H NMR (D_2O) δ 2.62 (dd, 1H, $J = 9.6$, $J' = 7.6$), 2.98 (dd, 1H, $J = 4.7$, $J' = 4.5$), 3.01 (dd, 1H, $J = 9.8$, $J' = 5.9$), 3.60–3.67 (m, 2H), 3.90–3.93 (m, 2H), 4.02 (dd, 1H, $J = 12.3$, $J' = 6.0$), 4.47 (d, 1H, $J = 13.4$), 7.48 (d, 1H, J = 3.8) 7.60–7.65 (m, 1H), 7.75–7.79 (m, 1H), 7.98 (d, 1H, $J = 8.3$), 8.16 (d, 1H, $J = 8.2$), 8.71 (d, 1H, $J = 4.2$); ^{13}C NMR (CD_3OD) δ 58.0, 59.7, 63.7, 71.5, 73.1, 74.5, 122.6, 125.7, 128.0, 128.9, 129.7, 130.9, 148.1, 148.8, 151.1.

5.2.32. 1,4-Dideoxy-1,4-imino-*N*-(8-hydroxyquinolin-2-yl) methyl-D-ribitol (58)

The general reductive amination procedure was followed with 8-hydroxyquinoline-2-carboxaldehyde as the aldehyde. After purification by column chromatography (CH_2Cl_2/MeOH 3:1) the title compound was obtained as a brown amorphous solid (0.036 g, 44%). MS (ESI) *m/z* 291.2 $[M+H]^+$ and *m/z* 313.1 $[M+Na]^+$; LC-MS (II-A) R_t 5.1 min, *m/z* 291.2 $[M+H]^+$; HPLC (I-B, $\lambda = 254$ nm) R_t 16.1 min (100%), and (I-B, $\lambda = 214$ nm) R_t 15.5 min (100%); ^1H NMR (CD_3OD) δ 2.85 (br s, 1H), 3.18 (br s, 1H), 3.45 (br s, 1H), 3.70–3.80 (m, 2H), 4.00–4.08 (m, 1H), 4.13–4.26 (m, 2H), 4.58 (d, 1H, $J = 14.8$), 7.10 (d, 1H, $J = 5.9$), 7.31–7.44 (m, 2H), 7.51 (d, 1H, $J = 8.5$), 8.24 (d, 1H, $J = 8.3$); ^{13}C NMR (CD_3OD) δ 59.9, 61.5, 62.2, 70.9, 72.1, 73.7, 112.3, 118.7, 122.2, 128.5, 129.5, 138.2, 139.0, 154.1, 156.2.

5.2.33. *N*-(9-Deazahypoxanthin-9-yl)methyl-1,4-dideoxy-1,4-imino-DHCl)-ribitol·HCl (61·HCl)

Iminoribitol hydrochloride **9·HCl** (0.15 g, 0.89 mmol) and NaOAc (0.073 g, 0.89 mmol) were dissolved in H_2O (2 mL) and 37% aq formaldehyde (0.070 mL, 0.89 mmol) and 9-deazahypoxanthine[21] (0.096 g, 0.71 mmol) were added. The reaction mixture was stirred at 95 °C for 17 h. After cooling down to rt, Silica gel was added and this was evaporated to dryness under reduced pressure. Purification by column chromatography (CH_2Cl_2/MeOH/NH_4OH 8:2:1) yielded the product. This was dissolved in methanol and converted into the hydrochloride salt by repeated evaporation of concentrated HCl. The title compound was obtained as an amorphous solid (0.033 g, 12%). MS (ESI) *m/z* 281.0 $[M+H]^+$ and *m/z* 303.0 $[M+Na]^+$; LC-MS (II-B) R_t 11.1 min, *m/z* 280.9 $[M+H]^+$; HPLC (I-B, $\lambda = 254$ nm) R_t 7.0 min (98%), and (II, $\lambda = 214$ nm) R_t 8.0 min (100%); ^1H NMR (MeOD) δ 3.43 (dd, 1H, $J = 12.6$, $J' = 3.1$), 3.64–3.69 (m, 2H), 3.80–3.89 (m, 2H), 4.11–4.16 (m, 1H), 4.27 (dd, 1H, $J = 7.7$, $J' = 4.3$), 4.67 (d, 1H, $J = 14.0$), 4.79 (d, 1H, $J = 14.3$), 7.85 (s, 1H), 8.72 (s, 1H); ^{13}C NMR (MeOD) δ 51.2, 58.5, 58.7, 70.3, 71.7, 72.9, 104.8, 120.0, 133.2, 137.3, 145.9, 153.7.

5.2.34. *N*-(9-Deaza-adenin-9-yl)methyl-1,4-dideoxy-1,4-imino-D-ribitol·CH_3COOH (62)

6-Chloro-9-deazapurine[26] (0.27 g, 1.7 mmol) was dissolved in a mixture of aqueous ammonia (25% solution, 25 mL) and 1,4-dioxane (25 mL). The reaction was sealed and left at 160 °C for 20 h. After cooling to rt, the reaction mixture was evaporated under reduced pressure and the residue was purified by column chromatography (CH_2Cl_2/MeOH/Et_3N 70:30:1) to obtain 9-deaza-adenine as a light brown amorphous solid (0.19 g, 80%). MS (ESI) *m/z* 135.3 $[M+H]^+$ and *m/z* 156.9 $[M+Na]^+$; LC-MS (II-B) R_t 9.3 min, *m/z* 135.0 $[M+H]^+$; ^1H NMR (DMSO) δ 6.32 (s, 1H), 6.62 (*br* s, 2H), 7.48 (s, 1H), 8.07 (s, 1H), 10.87 (*br* s, 1H). 9-Deaza-adenine (0.090 g, 0.67 mmol) was dissolved in 1,4-dioxane (5 mL). Et_3N (0.039 mL, 0.67 mmol), a catalytic amount of DMAP and Boc_2O (0.15 g, 0.67 mmol) were added, and the reaction mixture was stirred at rt for 24 h. TLC showed that not all starting material was disappeared and another amount of Boc_2O (0.10 g, 0.45 mmol) was added. The reaction mixture was stirred for another 24 h, then concentrated under reduced pressure and the residue was dissolved in EtOAc and washed with water. The organic phase was dried (Na_2SO_4) and evaporated under reduced pressure. The crude product was subjected to column chromatography (CH_2Cl_2/MeOH/Et_3N 80:20:1) to obtain Boc-protected 9-deaza-adenine (0.11 g). This was added to a solution of iminoribitol hydrochloride **9·HCl** (0.073 g, 0.43 mmol), NaOAc (0.050 g, 0.56 mmol) and 37% aq formaldehyde (0.049 mL, 0.56 mmol) in H_2O (2 mL). The reaction mixture was stirred at 95 °C for 3 h. After cooling down to rt, Silica gel was added, and this was evaporated to dryness under reduced pressure. Purification by column chromatography (CH_2Cl_2/MeOH/NH_4OH 5:4:2, two consecutive columns) yielded the product (0.020 g, 14%). MS (ESI) *m/z* 280.1 $[M+H]^+$; LC-MS (II-B) R_t 14.3 min, *m/z* 280.1 $[M+H]^+$; HPLC (II-B, $\lambda = 214$ nm) R_t 12.8 min (98%) and (II-B, $\lambda = 254$ nm) R_t 12.8 min (84%); ^1H NMR (MeOD) δ 3.09 (dd, 1H, $J = 11.6$, $J' = 4.2$), 3.28–3.32 (m, 1H), 3.37 (dd, 1H, $J = 11.6$, $J' = 4.8$), 3.67–3.76 (m, 2H), 3.96–3.99 (m, 1H), 4.10 (dd, 1H, $J = 9.4$, $J' = 4.7$), 4.29 (d, 1H, $J = 13.7$), 4.47 (d, 1H, $J = 13.7$), 7.63 (s, 1H), 8.16 (s, 1H); ^{13}C NMR (MeOD) δ 50.3, 58.6, 59.7, 70.3, 71.0, 73.2, 107.3, 115.5, 132.0, 146.5, 151.4, 152.5.

5.2.35. 1,4-Dideoxy-1,4-imino-*N*-(3-indolyl)methyl-D-ribitol·TFA (65·TFA)

The protected iminoribitol **10**[16] (0.20 g, 0.70 mmol) was dissolved in a mixture of H_2O and 1,4-dioxane (1:1, 4 mL), and 37% aq formaldehyde (0.14 mL, 1.4 mmol) was added. This was stirred at rt for 1 h, then indole (0.090 mg, 0.77 mmol) was added. The reaction mixture was stirred at 95 °C for 20 h. The reaction mixture was concentrated under reduced pressure and the residue dissolved in EtOAc. This was washed with H_2O, dried (Na_2SO_4), and concentrated under reduced pressure. The residue was purified by column chromatography (hexane to EtOAc) to yield the protected intermediate product. MS (ESI) *m/z* 417.4 $[M+H]^+$ and *m/z* 439.4 $[M+Na]^+$; ^1H NMR ($CDCl_3$) δ 0.07 (2s, 6H), 0.91 (s, 9H), 1.32 (s, 3H), 1.50 (s, 3H), 2.83 (dd, 1H, $J = 10.9$, $J' = 2.8$), 3.18–3.22 (m, 2H), 3.66 (dd, 1H, $J = 10.6$, $J' = 5.5$), 3.75 (dd, 1H, $J = 10.6$, $J' = 4.3$), 4.49 (dd, 1H, $J = 6.4$, $J' = 2.4$), 4.64–4.68 (m, 1H), 5.12 (d, 1H, $J = 12.5$), 5.21 (d, 1H, $J = 12.5$), 6.56–6.58 (m, 1H), 7.19–7.26 (m, 2H), 7.61 (d, 1H, $J = 7.8$), 7.67 (d, 1H, $J = 8.0$); The protected intermediate was dissolved in TFA/H_2O (1:1, 2 mL) and this was stirred at rt overnight. The reaction mixture was concentrated and the residue was purified by column chromatography (CH_2Cl_2 to CH_2Cl_2/methanol 4:1) to yield the TFA-salt of the title compound as an amorphous solid (0.039 g, 15% calculated over both steps). MS (ESI) *m/z* 263.2 $[M+H]^+$ and *m/z* 285.1 $[M+Na]^+$; LC-MS (II-B) R_t 9.6 min, *m/z* 262.9 $[M+H]^+$; HPLC (II, $\lambda = 214$ nm) R_t 4.6 min (100%); ^1H NMR (D_2O) δ 3.38 (dd, 1H, $J = 12.9$, $J' = 2.7$), 3.51 (dd, 1H, $J = 13.0$, $J' = 4.4$), 3.62–3.73 (m, 3H), 4.12–4.15 (m, 1H), 4.29

(d, 1H, J = 4.4), 4.55 (d, 1H, J = 13.6), 4.60 (d, 1H, J = 13.6), 7.18–7.28 (m, 2H), 7.51–7.55 (m, 2H), 7.70 (d, 1H, J = 7.8); ^{13}C NMR (D_2O) δ 52.3, 56.3, 57.2, 68.7, 69.2, 70.9, 103.1, 112.2, 116.2 (q, CF_3COOH), 117.9, 120.4, 122.6, 126.5, 128.6, 136.0, 163.0 (q, CF_3COOH).

5.2.36. 1,4-Dideoxy-1,4-imino-*N*-(7-methoxy-indol-3-yl)methyl-D-ribitol (66)

The protected iminoribitol **10**[16] (0.20 g, 0.70 mmol) was dissolved in a mixture of H_2O and 1,4-dioxane (1:1, 4 mL) and 37% aq formaldehyde (0.14 mL, 1.4 mmol) was added. This was stirred at rt for 1 h, then 7-methoxyindole (0.10 mL, 0.77 mmol) was added. The reaction mixture was stirred at 95 °C for 20 h. The reaction mixture was concentrated under reduced pressure and the residue dissolved in EtOAc. This was washed with H_2O, dried (Na_2SO_4) and concentrated under reduced pressure. The residue was purified by column chromatography (hexane to EtOAc) to yield the protected intermediate product. MS (ESI) m/z 447.3 $[M+H]^+$ and m/z 469.3 $[M+Na]^+$; ^{1}H NMR ($CDCl_3$) δ 0.01 (2s, 6H), 0.91 (s, 9H), 1.29 (s, 3H), 1.55 (s, 3H), 2.72–2.77 (m, 1H), 3.02 (*br* s,1H), 3.14–3.18 (m, 1H), 3.67–3.72 (m, 1H), 3.78–3.87 (m, 2H), 4.03 (s, 3H), 4.19 (d, 1H, J = 13.2), 4.53–4.55 (m, 1H), 4.61–4.65 (m, 1H), 6.71 (d, 1H, J = 7.8), 7.01–7.07 (m, 1H), 7.25–7.27 (m, 1H), 7.36 (d, 1H, J = 7.7); The protected intermediate was dissolved in TFA/H_2O (1:1, 2 mL) and this was stirred at rt overnight. The reaction mixture was concentrated and the residue was redissolved in H_2O and this was stirred with Amberlyst A26(OH) ion exchange resin until the pH of the solution was neutral. The resin was removed by filtration and rinsed extensively with H_2O and methanol. The solvents were evaporated under reduced pressure and the residue was purified by column chromatography (CH_2Cl_2/MeOH/Et_3N 30:10:1) to yield the title compound as a brown gum (0.026 g, 13% calculated over both steps). MS (ESI) m/z 292.9 $[M+H]^+$ and m/z 314.8 $[M+Na]^+$; LC-MS (II-B) R_t 9.6 min, m/z 292.9 $[M+H]^+$; HPLC (II, λ = 214 nm) R_t 4.6 min (100%); ^{1}H NMR (D_2O) δ 2.61 (*br* s, 1H), 2.79 (s, 1H), 3.04 (d, 1H, J = 5.6), 3.21 (s, 2H), 3.51 (s, 2H), 3.83 (s, 3H), 3.92–3.95 (m, 1H), 4.14 (d, 1H, J = 12.8), 6.54 (d, 1H, J = 7.2), 6.85–6.89 (m, 1H), 7.12–7.18 (m, 2H); ^{13}C NMR (D_2O) δ 51.2, 55.8, 59.0, 62.3, 70.8, 71.9, 74.3, 102.7, 111.6, 112.5, 120.8, 125.7, 128.3, 130.5, 147.9.

5.3. Biochemistry

5.3.1. Protein expression and purification

Expression and purification of the wild type *T. vivax* IAG-NH were performed as described previously.[4] *E. 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 chromatography 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.

5.3.2. Enzyme inhibition studies

Inhibitor dissociation constants were determined by measuring the rate of hydrolysis of a fixed concentration of *p*-nitrophenyl- β-D-ribofuranoside[27] at variable (at least five) inhibitor concentrations, in a 50 mM phosphate buffer 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 initial rates to the equation describing competitive inhibition: $v_i = (k_{cat} \times S)/(K_M \times (1 + I/K_i) + S)$, 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 and S is the substrate concentration.

5.4. Molecular modeling

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 the target enzyme 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 MMFF94x 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 file 2FF2, the structure of NH co-crystallized with (1*S*)-1-(9-deazahypoxanthin-9-yl)-1,4-imino-D-ribitol (Immucillin-H).[11] 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 iminoribitol target compounds were constructed using the iminoribitol moiety of Immucillin-H 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 MMFX94x force field. The docking protocol was validated by 're-docking' of Immucillin-H in 2FF2.

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