Published in final edited form as:
J Med Chem. 2009 February 26; 52(4): 1126–1143. doi:10.1021/jm801421q.

Third-Generation Immucillins: Syntheses and Bio-Activities of Acyclic Immucillin Inhibitors of Human Purine Nucleoside Phosphorylase

Keith Clinch[a], **Gary B. Evans**[a,*], **Richard F.G. Fröhlich**[a], **Richard H. Furneaux**[a], **Peter M. Kelly**[a], **Laurent Legentil**[a], **Andrew S. Murkin**[b], **Lei Li**[b], **Vern L. Schramm**[b], **Peter C. Tyler**[a], and **Anthony D. Woolhouse**[a]

[a]*Carbohydrate Chemistry Team, Industrial Research Limited, P. O. Box 31310, Lower Hutt 5040, New Zealand* [b]*Department of Biochemistry, Albert Einstein College of Medicine of Yeshiva University, 1300 Morris Park Avenue, Bronx, NY 10461*

Abstract

ImmH (**1**) and DADMe-ImmH (**2**) are potent inhibitors of human purine nucleoside phoshorylase (PNP), developed by us and currently in clinical trials for the treatment of a variety of T-cell related diseases. Compounds **1** and **2** were used as templates for the design and synthesis of a series of acyclic immucillin analogues (**8–38**) in order to identify simplified alternatives to **1** and **2**. SerMe-ImmG (**8**) and DATMe-ImmG (**9**) displayed the lowest inhibition constants of 2.1 and 3.4 pM, respectively, $_{vs}$ PNP. It was postulated that the flexible natures of **8** and **9** enabled them to adopt conformations resembling those of **1** and **2** within the active site of PNP and that the positioning of two hydroxyl groups was critical for picomolar activity. SerMe-ImmH (**10**, K_d = 5.2 pM) was shown to be orally available in mice with a long biological residence time on blood PNP.

Introduction

The application of transition state theory and use of KIEs in the rational design of inhibitors of PNPs has been recently validated.[1–4] This has resulted in the design and synthesis of a series of putative drug candidates, two of which, D-immucillin-H (ImmH[a], **1**)[5–7] (Figure 1) and D-DADMe-immucillin-H (DADMe-ImmH, **2**)[8–10] are currently in human clinical trials for the treatment of T- and B-cell cancers and a variety of autoimmune diseases.[11–15] Compound **1**, a first-generation immucillin and **2**, a second-generation immucillin, exert their effects on human T- and B-cells by inhibiting the human form of PNP, an enzyme involved in recycling deoxyguanosine.[16] The interest of medicinal chemists in developing inhibitors of PNP was piqued by the observation that a genetic deficiency of PNP in some humans caused a specific T-cell immune deficiency syndrome as its primary phenotype.[17] Despite the considerable efforts of several pharmaceutical companies to find suitable small molecule PNP inhibitors to mimic this phenotype, to date only the work of our group has afforded inhibitors with dissociation constants low enough to observe clinical effects in vivo in humans.[11]

*Corresponding author. Fax 4-9313055; e-mail E-mail: g.evans@irl.cri.nz.

[a]Abbreviations: DADMe-ImmH, 4′-deaza-1′-aza-2′-deoxy-1′-(9-methylene)-ImmH; DATMe-ImmH, 2′-deoxy-2′-amino-tetritol-*N*-(9-methylene)-ImmH; ImmH, Immucillin-H; SerMe-ImmH, serinol-*N*-(9-methylene)-ImmH; KIE, kinetic isotope effect; PNP, purine nucleoside phosphorylase; ADME, absorption, distribution, metabolism, and excretion; PK, pharmacokinetic; TRIS, tris(hydroxymethyl) aminomethane.

The selective binding preference of PNP for **1** and **2** was borne out by the observation that the corresponding L-enantiomers[18,19] **5** and **6**, respectively, were much less active inhibitors. On the other hand, substitution of the deazahypoxanthine moiety present in **1** and **2** with deazaguanine led to analogues **3**[5] and **4**[20], respectively, together with a corresponding increase in the potency of enzyme inhibition. After taking into account the mandatory requirements of efficacy and appropriate ADME as well as a favorable PK profile, the only other major consideration in the selection of a putative drug candidate over and above **1** and **2** is the "cost of goods" of that candidate. The practicalities of synthesizing deazaguanine immucillins vs deazahypoxanthine immucillins suggested the latter were preferred on cost grounds and ease of synthesis. To extend the range of cost effective alternatives to **1** and **2** we have prepared the achiral azetidine derivative **7**[21] but despite being simpler to make than **1** or **2** compound **7** was not deemed a potent enough PNP inhibitor to pursue as a drug candidate. Other workers have also reported their approaches to finding a suitable drug contender by synthesizing some carbocyclic[22] and acyclic[23] analogues of **3**.

Since the discovery of the antiviral drug **9**-[(2-hydroxyethoxy)methyl]guanine (acyclovir) some 30 years ago,[24, 25] there has been considerable interest and research into acyclic nucleoside analogues and their efficacy as antiviral and anti-cancer drugs.[26–28] We recently communicated the biological activities of several acyclic derivatives[1, 29] of which DATMe-ImmH (**11**) was found to be a surprisingly potent inhibitor. Prompted by this exciting discovery we elected to explore the SAR of acyclic immucillin derivatives in more detail. In order to provide a systematic basis for identifying the target acyclic analogues required for synthesis we used the structures of our two clinical candidates, **1** and **2** as starting points, concentrating on alterations to the pyrrolidine rings.[30] Central in the selection of targets arising out of these analyses was the incorporation of a secondary or tertiary nitrogen atom, which after protonation, could mimic an oxacarbenium ion, postulated to be an important transition state feature in our original enzyme inhibitor models.[31–33] Attachment to these nitrogen atoms of a variety of branched and linear alkyl chains, both chiral and achiral, bearing pendant hydroxyl groups completed the simple target structures (Figure 1). A good number of these target compounds were readily revealed through disconnecting individual carbon-carbon bonds of the pyrrolidine ring component in **1** or **2**, although the final list of targets was not restricted to this approach.

In this paper we present full details on the syntheses and human PNP inhibitory activity of compounds **8 – 38**. Among the 31 acyclic inhibitors described herein, several putative drug candidates, readily available on-scale from commercially available starting materials, were revealed. In particular SerMe-immucillin-G (SerMe-ImmG, **8**) exhibited the lowest dissociation constant so far reported against human PNP. Readily synthesized from achiral, commercially available starting materials, **8** is also the simplest structure to display such potent enzyme inhibitory activity. Importantly, SerMe-ImmH (**10**) (K_d = 5.2 pM), the 9-deazahypoxanthinyl analogue of **8** was shown to be orally available in mice with a long biological residence time on blood PNP.

Results and Discussion

Commercially available serinol (**39**) and TRIS (**40**) were both treated with protected 9-deazaguanine derivative **41**[23] under reductive amination/alkylation conditions with sodium cyanoborohydride to yield compounds **42** and **43** in good to low yields, respectively. Global deprotection of compounds **42** and **43** afforded **8** and the TRIS derivative **13**, respectively (Scheme 1). Similarly, **39** and **40** were treated under the same conditions with aldehyde **44**[8] to yield products **45** and **47** in good to low yields, respectively. Compound **45** was readily deprotected with refluxing concentrated aqueous hydrochloric acid to afford the acyclic immucillin, **10** in good yield and this was readily converted to **15** by reductive alkylation with the masked hydroxy aldehyde, 1,4-dioxane-2,5-diol, using sodium cyanoborohydride. Likewise, reductive alkylation of **45** with paraformaldehyde afforded the *N*-methyl derivative **46** which was deprotected under acidic conditions to afford compound **14**. On the other hand the TRIS derivative, compound **47** (vide supra), was deprotected using boron tribromide in dichloromethane to afford the target compound **16** in a low yield.

Attempts were also made to synthesize compounds **10** and **16** by applying the more elegant Mannich reaction of 9-deazahypoxanthine (**75**) with **39** and **40**, respectively, under conditions previously employed for the syntheses of our second generation immucillins.[10] No products were observed under these conditions, possibly due to the formation of unreactive 1*H*-oxazolo [3,4-*c*]oxazoles arising from the reaction of the pendant hydroxyls and amino groups with formaldehyde.[34]

Intriguingly, when a racemic mixture consisting of **11** and its enantiomer **19** was screened for enzyme inhibition activity, it exhibited a relatively low inhibition constant against human PNP. We therefore synthesized all four possible stereoisomers **11, 19, 24**, and **25** (Scheme 2). The starting points were the previously described[35–37] chiral diethyl 2-amino-3-hydroxysuccinates **48 – 51**, readily available from D- and L-diethyl tartrates which were each reductively alkylated with aldehyde **44**[8] in either methanol or 1,2-dichloroethane using sodium cyanoborohydride or sodium triacetoxyborohydride, respectively, to afford coupled products **52 – 55** in good yields. Compounds **52 – 55** were then reduced using the reagent combination of lithium borohydride-methanol[38] to afford the triols **56 – 59** in moderate to good yields. Global deprotection of **56 – 59** by treatment with either boron tribromide at room temperature or concentrated hydrochloric acid at reflux gave the acyclic immucillins **11, 19, 24**, and **25**.

Compound **11** proved to be a highly potent inhibitor of human PNP and its inhibition activity has been reported previously by us.[1] Reductive alkylation of **11** using paraformaldehyde and sodium cyanoborohydride in methanol afforded the *N*-methyl derivative **26** in good yield.

Compound **9**, the 9-deazaguanine analogue of **11** in this series, was also synthesized. To this end aldehyde **41**[23] was reductively aminated with (5*S*,6*R*)-6-amino-2,2-dimethyl-1,3-dioxepan-5-ol[39] using sodium triacetoxyborohydride in 1,2-dichloroethane to afford the protected immucillin **60** which was globally deprotected to afford **9**. Compound **9** was 2 – 3 times more active an inhibitor of human PNP than its 9-deazahypoxanthine analogue **11**, a result consistent with our previous observations to date when comparing the 9-deazaguanine immucillin series with the corresponding 9- deazahypoxanthine series.[26]

Another expedient route to an acyclic immucillin was accessed from the first generation immucillin, **1.HCl** (Scheme 3). Protection of the pyrrolidine nitrogen of **1** followed by periodate oxidation of the contiguous secondary alcohols and then reduction of the resulting dialdehyde intermediate afforded the triol **61** in excellent yield for the three steps. Removal of the *tert*-butoxycarbonyl protecting group by acid catalyzed hydrolysis gave compound **12.HCl** in good yield.

The synthesis of an analogue of **2** bearing an extra hydroxyl at C-2′ was considered of interest, but as it was thought that the hemi-aminal structure so created would be hydrolytically unstable hence, the acyclic analogue (±)-**17** became of interest. *cis*-But-2-ene-1,4-diol (**62**) was reacted with *N*-benzylhydroxylamine and formaldehyde to afford the racemic isoxazolidine (±)-**64** in quantitative yield (Scheme 4). Reductive cleavage of the N-O bond gave amino alcohol (±)-**66**, in moderate yield, which was reductively alkylated with aldehyde **44**[8] under standard conditions to afford (±)-**68**, again in moderate yield. Deprotection of (±)-**68** via acid catalyzed hydrolysis followed by hydrogenolysis gave the acyclic immucillin (±)-**17** in an unoptimized yield for the two steps. *Trans*-but-2-ene-1,4-diol (**63**) underwent a similar cycloaddition reaction, although in a much poorer yield, to afford isoxazolidine (±)-**65**. This isoxazolidine was then converted, as in the previous case, through (±)-**67** and (±)-**69** to the target compound (±)-**38** in moderate overall yield.

In order to survey a non-exhaustive series of simple achiral acyclic immucillins we investigated the conversion of three primary amino-alcohols, ethanolamine (**70**), 3-amino-propan-1-ol and 4-amino-butan-1-ol, and two secondary amino-alcohols diethanolamine (**73**) and 3-(2-hydroxyethylamino)propan-1-ol (**74**) to their corresponding immucillins (Scheme 5). Compounds **70, 73** and **74** underwent the Mannich reaction with **75**[10] and formaldehyde to afford compounds **20, 21** and **33**, respectively, where **33** represents the acyclic analogue of **2** with the pyrrolidine C3′-C4′ bond lysed.

Both 3-amino-propan-1-ol and 4-amino-butan-1-ol required *N*-benzylation to their secondary amines **71** and **72**, respectively, before they would undergo the Mannich reaction with **75**[10] to afford compounds **76** and **77**, respectively. Subsequent hydrogenolysis of the *N*-benzyl groups in compounds **76** and **77** with hydrogen and a palladium catalyst afforded the target compounds **18** and **29**, respectively, in good overall yields. It is interesting to note that amines **71 – 74** underwent the Mannich reaction despite their potential to form, through reaction with formaldehyde, unreactive tertiary amine species in the form of *N*-alkylated oxazolidines, 1,3-oxazinanes or 1,3-oxazepanes. This contrasts with **39** and **40** mentioned earlier which failed to undergo similar Mannich reactions with **75**.[10]

Disconnecting the N-C2′ bond or the C2′-C3′/N-C2′ bonds in the pyrrolidine ring of **2** provided immucillin targets **22** and **27**, respectively. The protected mesylate **78** was readily prepared from the commercially available 2-(hydroxymethyl)propane-1,3-diol (Scheme 6).[40] Displacement of the mesylate with either methylamine or benzylamine afforded compounds **79** and **81**, respectively, in moderate to good yields. The Mannich reactions of **79** and **81** with **75**[10] proceeded smoothly, although in poor yield for **81**, to rovide compounds **80** and **82**, respectively. Deprotection of **80** was achieved in a single step through acid treatment at room temperature to afford **22** in good yield, whereas deprotection of **82** was achieved in two steps via acid catalyzed removal of the acetonide followed by hydrogenolysis of the benzyl group to afford **27** in unoptimized yield for the two steps.

Naturally occurring carbohydrates are an important and convenient source by which to obtain functionalized polyol derivatives. Those derived from trioses, tetroses, and pentoses were considered to be the most important in our investigations. We therefore investigated a series of acyclic amino alcohols containing only a single chiral centre and available from the commercial chiral acetonides **83** and **84** as examples in the triose series (Scheme 7). Mesylation of **83** and **84** afforded the known corresponding mesylates **85** and **86** which were treated with benzylamine in refluxing acetonitrile to provide amines **87** and **88**, respectively, in good yields.[41,42]

At this point two different routes were pursued utilizing compounds **87** and **88**. Firstly, we investigated the direct reductive amination of aldehyde **44**[8] with **87** and **88**, followed by acid catalyzed hydrolysis of the resulting adducts with refluxing concentrated hydrochloric acid to provide coupled products **89** and **37** in moderate to good yields, respectively. Compound **37** was screened as an inhibitor in order to observe the effect on the inhibition constant of a large hydrophobic group. Hydrogenolysis of **89** in water under a hydrogen atmosphere using 10% Pd/C as a catalyst afforded the immucillin **23** in moderate yield. Similarly, hydrogenolysis of **37** under the same conditions as **89** gave the immucillin **28** in near quantitative yield. Secondly, the treatment of amines **87** and **88** with 2-bromoethanol in refluxing acetonitrile, with potassium carbonate as base, afforded the corresponding tertiary ethanolamines which, after hydrogenolysis were reductively alkylated with aldehyde **44**[8] in the presence of sodium triacetoxyborohydride to afford compounds **90** and **91** in poor to moderate yields, respectively, for the three steps. Acid catalyzed hydrolysis of **90** and **91** afforded the immucillins **32** and **34** in good yields.

Following this we investigated a tetrose derived series of immucillins for screening. The D-erythronamide **92**[43] was readily reduced using LAH in THF at room temperature to afford the amino alcohol **93** in excellent yield (Scheme 8). Compound **93** was then reductively alkylated with aldehyde **44**[8] and sodium triacetoxyborohydride in 1,2-dichloroethane to afford the protected immucillin **94** in moderate yield. Standard acid catalyzed deprotection of **94** gave immucillin **30** in unoptimized yield.

Immucillin (±)-**31**, as a racemic mixture of stereoisomers was synthesized as shown (Scheme 9). The D,L-threo-acetonide (±)-**95** was prepared in good yield from (±)-diethyl tartrate using dimethoxypropane and TsOH in refluxing benzene, conditions which led to a partial transesterification. Treatment of (±)-**95** with 1 mole equivalent of 7M methanolic ammonia solution at room temperature provided amido ester (±)-**96** in moderate yield. Reduction of (±)-**96** with LAH gave the amino alcohol (±)-**97** in excellent yield and this product was reductively alkylated with aldehyde **44**[8] under standard conditions to afford the protected immucillin (±)-**98** in good yield. Deprotection of the racemic mixture (±)-**98** with refluxing hydrochloric acid afforded immucillin (±)-**31** in unoptimized yield.

By way of an example of a pentose derived immucillin the D-ribonamide (**99**),[43] the 1-C homologue of D-erythronamide (**92**), was reduced with LAH in THF to afford the amine **100** in moderate yield and then reductively aminated with alkylated **44**[8] again under standard conditions to afford the protected immucillin **101** in poor yield (Scheme 10). Deprotection of **101** in refluxing concentrated hydrochloric acid afforded immucillin **35**.

Finally, we investigated the synthesis from commercially available (±)-1,2,4-butanetriol (**102**), of (±)-**36**, the *N*-Me, 3′-deoxy variant of (±)-**30** and (±)-**31**. Acetalization of (±)-**102** with benzaldehyde gave benzylidene acetal (±)-**103** and this compound in turn was sulfonated with methane sulfonyl chloride and the resulting mesylate treated with aqueous methylamine to afford methylamino acetal (±)-**104** in good yield for the three steps (Scheme 11). Compound (±)-**104** underwent a Mannich reaction with **75**[10] under standard conditions and in good yield to afford adduct (±)-**105** which was readily deprotected to provide immucillin (±)-**36**.

Inhibition Studies

The inhibition of the phosphorolysis of inosine catalyzed by human PNP was tested with third-generation compounds **8 – 38** and compared to the first-generation inhibitors **1** and **3** and the second-generation inhibitors **2** and **4** (Figure 1). Among the acyclic analogues evaluated were those that could be conceptually formed by a carboncarbon bond cleavage in the pyrrolidine rings of the parent compounds. Though weaker than the 56 pM dissociation constant for **1**, the ImmH-based analogues **24** and **12** maintained moderate to strong inhibition at 4,300 and 210 pM, respectively. The DADMe-ImmH-based analogues (±)-**17, 22, 33**, and (±)-**36** yielded widely varying inhibition becoming progressively weaker as the cleavage site was moved around the pyrrolidine ring, ranging from 780 pM with (±)-**17** to 227,000 pM with (±)-**36**. Comparing the two series of seco-Immucillins, it therefore seems that human PNP tolerates a loss in binding to the 2′-position much better than at other positions in the ring.

Intriguingly, inversion of configuration of the 3′-hydroxyl group found in **24** to that present in **11** yielded a K_i* of 8.6 pM, corresponding to a 500-fold improvement in inhibition.[1] It is possible that the more flexible nature of the third-generation inhibitors enables **11** to adopt a conformation resembling **1** and **2** within the active site of PNP. Differential binding affinity due to increased rotational freedom has also been observed with the L-enantiomers of **1** and **2**, where protein structures showed the more flexible LDADMe-ImmH (**6**; K_i = 380 pM) was bound in an orientation similar to its D-enantiomer, but L-ImmH (**5**; K_i* = 12,000 pM) was not.[19]

The potent inhibition observed with **11** prompted further exploration of acyclic analogues possessing variations in the amino alcohol moiety. The most potent human PNP inhibitor in the 9-deazahypoxanthine series with a K_i* of only 5.2 pM, was found to be **10**, which differs from **11** by the removal of a hydroxymethyl moiety. Further simplification was evaluated with **20**, which with a K_i of 1,100 pM bound weaker to human PNP by a factor of 500. Lengthening of the alkyl chain found in **18** to that in **29** resulted in poorer binding (K_i = 25,000 pM). Compound **27**, which contains a methylene group inserted into **10**, making it a closer mimic of **2**, gave similarly weaker affinity (K_i = 14,100 pM). Thus, it is apparent that the positioning of the two hydroxyl groups is critical to picomolar inhibition. Reintroduction of hydroxymethyl groups into **10**, though significantly less potent, resulted in good (compounds **12 – 16**; K_i = 210 – 620 pM) to moderate (compound **24**; K_i = 4,300 pM) levels of inhibition. Like **10**, four of these compounds, **13 – 16**, are achiral, making them particularly attractive for synthetic development as inhibitors of PNP.

Bioavailability

SerMe-ImmH (**10**) was administrated to mice orally or by intraperitoneal (i.p.) injection with a single dosage of 50 nmol. Complete inhibition of PNP catalytic activity was observed in the i.p. injection group within 20 min, yielding a $t_{1/2}$ of 10 min for the onset of inhibition (Figure 2). For the orally treated group, the $t_{1/2}$ of onset was 18 min. Thus, SerMe-ImmH (**10**) is orally available and only modestly less effective than direct i.p. injection. Both i.p. and oral groups regained 50% of the initial PNP catalytic activity after 25 days, yielding a $t_{1/2}$ of offset greater than the lifetime of mouse erythrocytes (25 days). Therefore, it can be concluded that after the inhibited erythrocyte is degraded, some of the released inhibitor becomes recycled by newer cells, prolonging the period of inhibition.

Conclusions

Expanded chemical scaffolds were designed to simplify chemical approaches to transition state analogue inhibitors of human PNP. Inhibitors have been obtained with dissociation constants as low as 2.1 pM. Compounds **8** (K_d = 2.1 pM) and **10** (K_d = 5.2 pM) are achiral molecules and thereby simplify synthesis and lower “the cost of goods” relative to compounds **1** and **2**, both currently in clinical trials. Remarkably, compound **10** showed equivalent bioavailability to mouse blood PNP by oral or intraperitoneal administration. A single oral dose inhibited >50% of blood PNP catalytic activity for a period of 25 days. Closely related compounds were weaker inhibitors. Thus, a new family of powerful PNP inhibitors is described with favourable pharmacokinetic properties in mice.

Experimental Section

Anhydrous solvents were obtained commercially. Air sensitive reactions were carried out under argon. Organic solutions were dried over $MgSO_4$. TLC was performed on glass or aluminum sheets coated with 60 F254 silica gel. Organic compounds were visualized under UV light or by use of a dip of cerium(IV) sulfate (0.2%, w/v) and ammonium molybdate (5%) in sulfuric acid (2M), or one of I_2 (0.2%) and KI (7%) in H_2SO_4 (1M) or one of *p*-(*N*,*N*-dimethylamino) benzaldehyde in cHCl-MeOH (Ehrlich’s reagent). Chromatography (flash column) was performed on Scharlau or Merck silica gel 60 (40–60 µm). Melting points are uncorrected. Optical rotations were recorded with a path length of 1 dm and are in units of 10^{-1} deg cm^2 g^{-1}; concentrations are in g/100 ml. NMR spectra were recorded on a Bruker AC-300 instrument at 300 MHz (^1H) or 75.5 MHz (^{13}C) unless otherwise stated. Analytical and preparative HPLC were carried out on Phenomenex Synergi Polar RP 80A columns eluting with 3–50% MeOH in 0.1% aqueous TFA with detection of products at 230 nm. Microanalyses were carried out by the Campbell Microanalytical Laboratory, University of Otago, New Zealand. Positive electrospray high resolution mass spectra (ESI-HRMS) were recorded on a Waters QTOF Premier Tandem Mass Spectrometer.

Chemistry

2-Amino-7-{[(1,3-dihydroxypropan-2-yl)amino]methyl}-3,5-dihydro-4*H*-pyrrolo[3,2-*d*] pyrimidin-4-one (SerMe-ImmG, 8) and its hydrochloride salt (8.HCl)

Acetyl chloride (0.072 ml, 1.01 mmol) was added to stirred MeOH (5 ml) followed by serinol (**39**) (180 mg, 2.02 mmol), aldehyde **41**[23] (140 mg, 0.403 mmol) and sodium cyanoborohydride (38 mg, 0.61 mmol). After 4 h, the solvent was evaporated and the residue chromatographed on silica gel ($CHCl_3$/7M NH_3-MeOH, 95:5 → 9:1) to give **42** as a yellow solid (65 mg). Compound **42** was stirred in a 1:1 mixture of MeOH : 1M NaOH (4 ml) at room temperature for 48 h. The mixture was acidified with 5% aqueous HCl and concentrated in vacuo. Chromatography (*i*PrOH/H_2O/28% aq NH_4OH = 80:15:0.5) of the resulting residue gave **8** as a colorless solid which was triturated with 7M NH_3-MeOH and the colorless solid filtered off, dried, and converted to its HCl salt with 5% aqueous HCl to afford **8.HCl** (14 mg 11%) as a white solid. ^1H NMR (D_2O, internal CH3CN at δ 2.06): δ 7.63 (s, 1H), 4.45 (s, 2H), 3.95 (dd, *J* = 12.6, 4.5 Hz, 2H), 3.86 (dd, *J* = 12.6, 5.9 Hz, 2H), 3.48 (m, 1H). ^{13}C NMR (D_2O, internal CH_3CN at δ 1.47): δ 154.3, 151.1, 133.1, 132.2, 112.5, 101.6, 60.2, 58.4, 58.4, 39.3. ESI-HRMS for $C_{10}H_{16}N_5O_3$ [MH^+] calcd, 254.1253; found, 254.1253.

{7-[(1,3-Dihydroxypropan-2-ylamino)methyl]-2-[(dimethylamino)methyleneamino]-4-oxo-4,5-dihydro-3*H*-pyrrolo[3,2-*d*]pyrimidin-3-yl}methyl 2,2-dimethylpropanoate (43)

To a mixture of TRIS (**40**) (128 mg, 1.06 mmol) and **41**[23] (64 mg, 0.18 mmol) in MeOH (5 ml) was added sodium cyanoborohydride (20 mg, 0.30 mmol) and acetyl chloride (32 µl, 0.45 µmol) and the resulting bright yellow heterogeneous mixture was stirred overnight at room temperature. The reaction mixture was concentrated in vacuo onto silica gel (~1 g) and purified by two chromatography columns, eluting the first one with EtOAc/MeOH/28% aq NH_4OH = 80:20:1 and the second one with CHCl3/MeOH/28% aq NH_4OH = 80:20:1 to afford **43** (41 mg, 49%) as an amorphous white solid. ^1H NMR (500 MHz, CD_3OD): δ 8.55 (s, 1H), 7.24 (s, 1H), 6.31 (s, 2H), 3.88 (s, 2H), 3.66 (s, 6H), 3.18 (s, 3H), 3.06 (s, 3H), 1.15 (s, 9H). ^{13}C NMR (125.7 MHz, CD_3OD): δ 179.0, 158.7, 156.4, 155.6, 144.5, 128.4, 115.5, 115.3, 67.1, 62.5, 62.5, 62.5, 61.9, 41.1, 39.8, 36.4, 35.1, 27.4; ESI-HRMS for $C_{20}H_{33}N_6O_6$ [MH^+] calcd, 473.2462; found, 473.2458.

2-Amino-7-({[1,3-dihydroxy-2-(hydroxymethyl)propan-2-yl]amino}methyl)-3,5-dihydro-4*H*pyrrolo[3,2-*d*]pyrimidin-4-one hydrochloride salt (13.HCl)

Compound **43** (38 mg, 84 µmol) was dissolved in THF (2 ml) and aqueous sodium hydroxide (1 ml, 1 mmol, 1.0 M) was added and the resulting mixture was stirred overnight. After 28 h water (5 ml) was added and the resulting solution concentrated in vacuo. Purification of the residue by chromatography (*i*PrOH/28% aq NH_4OH = 4:1) afforded an *N*-CHO derivative of **13** which was treated with 5% aqueous HCl to afford **13.HCl** (22 mg, 92%) as a white foam. ^1H-NMR (500 MHz, D_2O, internal 1,4-dioxane at δ 3.75): δ 7.64 (s, 1H), 4.44 (s, 2H), 3.88 (s, 6H). ^{13}C-NMR (125.7 MHz, D_2O, internal 1,4-dioxane at 67.19): δ 154.2, 151.1, 132.8, 132.3, 112.3, 102.0, 66.8, 58.7, 58.7, 58.7, 35.8. ESI-HRMS for $C_{11}H_{18}N_5O_4$ [MH^+] calcd, 284.1359; found, 284.1365. Anal. ($C_{11}H_{17}N_5O_4$.3HCl) C, H, N.

5-(Benzyloxymethyl)-7-{[(1,3-dihydroxypropan-2-yl)amino]methyl}-4-methoxy-5*H*pyrrolo [3,2-*d*]pyrimidine (45)

Acetyl chloride (0.117 ml, 1.65 mmol) was added to a stirred solution of serinol (**39**) (300 mg, 3.29 mmol) and aldehyde **44**[8] (196 mg, 0.66 mmol) in MeOH (5 ml). Sodium cyanoborohydride (62 mg, 0.99 mmol) was added and the resulting reaction mixture was stirred at room temperature for 16 h. The mixture was then concentrated in vacuo and the residue purified by chromatography (CH_2Cl_2/MeOH/28% aq NH_4OH = 95:5:0.5 → 9:1:0.05) to afford **45** (188 mg, 77 %) as a colorless solid. ^{1}H NMR (CD_3OD): δ 8.42, (s, 1H), 7.65 (s, 1H), 7.25-7.16 (m, 5H), 5.75 (s, 2H), 4.50 (s, 2H), 4.10 (s, 3H), 4.03 (s, 2H), 3.68 (dd, *J* = 11.2, 5.4 Hz, 2H), 3.58 (dd, *J* = 11.2, 5.9 Hz, 2H), 2.81 (pentet, *J* = 5.6 Hz, 1H). ^{13}C NMR (CD_3OD, center line at δ 49.0): δ 157.9, 150.8, 150.6, 138.7, 134.1, 129.3, 128.8, 128.6, 117.0, 116.2, 78.5, 71.5, 62.5, 62.5, 61.3, 54.3, 41.4. ESI-HRMS for $C_{19}H_{25}N_4O_4$ [MH^+] calcd, 373.1876; found, 373.1865.

7-{[(1,3-Dihydroxypropan-2-yl)amino]methyl]}-3,5-dihydro-4*H*-pyrrolo[3,2-*d*]pyrimidin-4-one (SerMe-ImmH, 10) and its hydrochloride salt (10.HCl)

Compound **45** (180 mg, 0.48 mmol) was heated under reflux in concentrated aqueous HCl (2 ml) for 1.5 h. The reaction mixture was concentrated in vacuo and the residue dissolved in a 1:1 mixture of MeOH:H_2O, neutralized with Amberlyst A-21 resin, filtered and the filtrate concentrated in vacuo. The residue was purified by chromatography (*i*PrOH/H_2O/28% aq NH_4OH = 92:4:4) to afford **10** which was treated with 5% aqueous HCl to afford **10.HCl** (85 mg, 64 %) as a colorless hygroscopic solid. An analytical sample was recrystallized from H_2O/EtOH. M.p. 217–219 °C. ^{1}H NMR (500 MHz, D_2O + drop DCl, internal CH_3CN at δ 2.06): δ 9.07 (s, 1H), 7.92 (s, 1H), 4.60 (s, 2H), 3.97 (dd, *J* = 12.6, 4.4 Hz, 2H), 3.89 (dd, *J* = 12.6, 5.9 Hz, 2H), 3.54 (m, 1H). ^{13}C NMR (125.7 MHz, D_2O + drop DCl, internal CH_3CN at δ 1.47): δ 152.8, 145.3, 133.4, 132.0, 118.7, 103.5, 60.4, 58.3, 58.3, 39.2. ESI-HRMS for $C_{10}H_{14}N_4O_3Na$ [MNa^+] calcd, 261.0964; found, 261.0964. Anal. ($C_{10}H_{15}ClN_4O_3$ 0.5H_2O) C, H, N.

5-(Benzyloxymethyl)-7-{[(1,3-dihydroxypropan-2-yl)(methyl)amino]methyl}-4-methoxy-5*H*pyrrolo[3,2-*d*]pyrimidine (46)

Acetyl chloride (8.91 µl, 0.125 mmol) was added to MeOH (5 ml) with stirring and then **45** (155 mg, 0.42 mmol), paraformaldehyde (62 mg, 2.08 mmol) and sodium cyanoborohydride (39 mg, 0.62 mmol) added. After 16 h the reaction mixture was concentrated in vacuo and the residue purified by chromatography ($CHCl_3$/MeOH/28% aq NH_4OH = 95:5:0.5) to afford **46** (115 mg, 72%) as a colorless gum. ^{1}H NMR (500 MHz, CD_3OD): δ 8.40 (s, 1H), 7.61 (s, 1H), 7.26-7.18 (m, 5H), 5.74 (s, 2H), 4.50 (s, 2H), 4.10 (s, 3H), 3.95 (s, 2H), 3.77 (dd, *J* = 11.4, 7.3 Hz, 2H), 3.65 (dd, *J* = 11.4, 5.6 Hz, 2H), 2.93 (m, 1H), 2.37 (s, 3H). ^{13}C NMR (125.7 MHz, CD_3OD, center line at δ 49.0): δ 157.9, 151.1, 150.6, 138.8, 134.6, 129.3, 128.7, 128.6, 117.2, 115.8, 78.5, 71.5, 66.3, 60.3, 60.3, 54.3, 48.1, 38.2. ESI-HRMS for $C_{20}H_{27}N_4O_4$ [MH^+] calcd, 387.2032; found, 387.2034.

7-{[(1,3-Dihydroxypropan-2-yl)(methyl)amino]methyl]}-3,5-dihydro-4*H*-pyrrolo[3,2-*d*] pyrimidin-4-one (14) and its hydrochloride salt (14.HCl)

Compound **46** (110 mg, 0.285 mmol) was heated under reflux in concentrated aqueous HCl (4 ml) for 1.5 h. The solution was concentrated in vacuo and the residue dissolved in MeOH/H_2O (1:1, 3 ml) and neutralized with Amberlyst A-21 resin. The resin was removed by filtration and the filtrate concentrated in vacuo and the resulting residue purified by chromatography ($CHCl_3$/7M NH_3-MeOH = 9:1 → 85:15) to afford **14** as a colorless solid. Conversion of **14** to the HCl salt with 5% aqueous HCl afforded **14.HCl** (54 mg, 66%). ^{1}H NMR (D_2O, pH ~ 1, internal CH_3CN at δ 2.06): δ 8.73 (s, 1H), 7.87 (s, 1H), 4.64, 4.59 (2 partially coalescing br s, 2H), 4.10-3.86 (m, 4H), 3.65 (pentet, *J* = 5.9 Hz, 1H), 2.96 (s, 3H). ^{1}H NMR (D_2O + NaOD pH ~ 10, internal CH_3CN at δ 2.06) 7.99 (s, 1H), 7.48 (s, 1H), 3.88 (s, 2H), 3.82 (dd, *J* = 11.8, 6.1 Hz, 2H), 3.72 (dd, *J* = 11.8, 5.7 Hz,2 H), 2.89 (pentet, *J* = 5.9 Hz, 1H), 2.33 (s, 3H). ^{13}C NMR (D_2O, pH ~ 1, internal CH_3CN at δ 1.47) 153.9, 145.0, 136.8, 133.6, 118.8, 103.4, 65.7, 57.3, 57.1, 49.1, 37.0. ESI-HRMS for $C_{11}H_{17}N_4O_3$ [MH^+] calcd 253.1301; found, 253.1292.

7-{[(1,3-Dihydroxypropan-2-yl)(2-hydroxyethyl)amino]methyl}-3,5-dihydro-4*H*pyrrolo[3,2-*d*] pyrimidin-4-one (15) and its hydrochloride salt (15.HCl)

Sodium cyanoborohydride (34 mg, 0.54 mmol) was added to a solution of compound **10** (105 mg, 0.38 mmol) and 1,4-dioxane-2,5-diol (69 mg, 0.57 mmol) in MeOH (5 ml) and the mixture stirred at room temperature for 16 h. The solution was concentrated in vacuo and the residue purified by chromatography ($CHCl_3$/MeOH/28% aq NH_4OH = 85:15:0.1) to afford **15** as a colorless solid. Conversion of the free base to the HCl salt using 5% aqueous HCl afforded **15.HCl** (43 mg, 35%) as a colorless solid. ^{1}H NMR (D_2O, internal CH_3CN at δ 2.06): δ 8.56 (s, 1H), 7.85 (s, 1H), 4.79 (s, partly coalesced with HOD, 2H), 4.00-3.89 (m, 6H), 3.79 (pentet, *J* = 6.2 Hz, 1H), 3.59 (br s, 1H), 3.50 (m, 1H). ^{13}C NMR (D_2O, internal CH_3CN at δ 1.47): δ 154.3, 144.7, 138.6, 133.1, 118.8, 104.0, 64.5, 56.9, 55.9, 55.6, 52.8, 47.2. ESI-HRMS for $C_{12}H_{19}N_4O_4$ [MH^+] calcd, 283.1406; found, 283.1400.

5-Benzyloxymethyl-7-({[1,3-dihydroxy-2-(hydroxymethyl)propan-2-yl]amino}methyl)-4-methoxy-5*H*-pyrrolo[3,2-*d*]pyrimidine (47)

Sodium cyanoborohydride (18 mg, 0.28 mmol) was added to a suspension of compound **44**[8] (50 mg, 0.17 mmol) and TRIS (**40**) (20 mg, 0.17 mmol) in MeOH (5 ml) and the resulting reaction mixture stirred for 16 h at room temperature. The crude reaction mixture was absorbed onto silica gel and concentrated in vacuo and then the residue purified by chromatography (MeOH/EtOAc = 1:4) to afford **47** (24 mg, 36 %) as a syrup. ^{1}H NMR (CD_3OD): δ 8.42 (s, 1H), 7.65 (s, 1H), 7.23 (m, 5H), 5.74 (s, 2H), 4.51 (s, 2H), 4.11 (s, 3H), 4.04 (s, 2H), 3.68 (s, 6H). ^{13}C NMR (CD_3OD): δ 158.4, 151.2, 150.9, 139.1, 134.4, 129.7, 129.2, 129.0, 117.5, 116.4, 78.9, 71.9, 62.9, 62.9, 62.9, 62.6, 54.8, 36.6. ESI-HRMS for $C_{20}H_{27}N_4O_5$ [MH^+] calcd, 403.1981; found, 403.1985.

7-({[1,3-Dihydroxy-2-(hydroxymethyl)propan-2-yl]amino}methyl)-3,5-dihydro-4*H*pyrrolo[3,2-*d*]pyrimidin-4-one (16) and its hydrochloride salt (16.HCl)

Boron tribromide (1 ml, 1.0 mmol, 1.0 M CH_2Cl_2) was added dropwise to a solution of compound **47** (30 mg, 74.5 μmol) in CH_2Cl_2 (5 ml) and stirred at room temperature. A white solid precipitated from the reaction after 1 h and the reaction mixture was then quenched with MeOH, concentrated in vacuo and co-distilled with MeOH (3 × 25 ml) to afford crude product. The residue was dissolved in MeOH, concentrated in vacuo onto silica gel and purified by chromatography (CH_2Cl_2/MeOH/28% aq NH_4OH = 5:4.5:0.5) to afford **16** (7 mg, 35%) as a white solid and converted with 5% aqueous HCl to **16.HCl**. M.p. 223–224 °C (plates from EtOH). ^{1}H NMR (D_2O, internal acetone at 2.225 ppm): δ 9.06, (s, 1H), 7.92 (s, 1H), 4.59 (s, 2H), 3.91 (s, 6H). ^{13}C NMR (D_2O, internal acetone at δ 31.5): δ 153.7, 146.0, 134.2, 133.0, 119.4, 104.7, 67.6, 59.3, 59.3, 59.3, 36.4. ESI-HRMS for $C_{11}H_{17}N_4O_4$ [MH^+] calcd, 269.1250; found, 269.1263.

5-Benzyloxymethyl-4-methoxy-7-({[(2*R*,3*S*)-1,3,4-trihydroxybutan-2-yl]amino} methyl)-5*H*pyrrolo[3,2-*d*]pyrimidine (56)

A mixture of diethyl (2*S*,3*S*)-2-amino-3-hydroxysuccinate (**48**)[36] (0.87 g, 4.24mmol, prepared from diethyl-D-tartrate according to literature methods[35,36]), sodium cyanoborohydride (0.44 g, 7.07 mmol) and **44**[8] (1.05 g, 3.54 mmol) were evaporated from MeOH (3×). The residue was dissolved in MeOH (20 ml) and acetic acid added (10 drops). The reaction mixture was stirred at room temperature for 18 h and then silica gel added and the mixture concentrated in vacuo. Purification of the resulting residue by chromatography ($Et_3N/MeOH/CH_2Cl_2$ = 1:3:99) afforded crude **52** (2.58 g, 150%) as a colorless oil. To a stirred solution of crude **52** (1.73 g, 2.33 mmol, 66%) in diethyl ether (30 ml) was added MeOH (1.43 ml, 35.3 mmol) and then lithium borohydride[38] (8.83 ml, 17.7 mmol, 2.0 M in THF). After 1 h MeOH (1.43 ml, 35.3mmol) was added to the reaction mixture and stirring continued. After 1h further the reaction mixture was diluted with MeOH and then concentrated in vacuo and the residue dissolved in MeOH (20 ml), diluted with HCl (20 ml, 1M) and concentrated in vacuo. The resulting residue was purified by chromatography (7M NH_3-MeOH/CH_2Cl_2 = 15:85) to afford **56** (0.940g, 98% based on crude **52**) as a white solid. ^{1}H NMR (CD_3OD): δ 8.43 (s, 1H), 7.69 (s, 1H), 7.28-7.15 (m, 5H), 5.77 (s, 2H), 4.52 (s, 2H), 4.12 (m, 2H), 4.11 (s, 3H), 3.80 (dd, *J* = 11.7, 5.3 Hz, 1H), 3.80-3.60 (m, 3H), 3.59 (dd, *J* = 11.0, 4.9 Hz, 1H), 2.90 (q, *J* = 4.9 Hz, 1H). ^{13}C NMR (CD_3OD): δ 158.4, 151.4, 151.0, 139.1, 135.0, 129.7, 129.2, 129.1, 117.5, 115.3, 79.0, 72.5, 72.0, 65.6, 62.0, 61.6, 54.8, 42.1.

7-({[(2*R*,3*S*)-1,3,4-Trihydroxybutan-2-yl]amino}methyl)-3,5-dihydro-4*H*-pyrrolo[3,2-*d*] pyrimidin-4-one (DATMe-ImmH, 11) and its trifluoroacetate salt (11.TFA) and hydrochloride salt (11.HCl)

To a stirred solution of compound **56** (0.940 g, 2.34 mmol) in CH_2Cl_2 (30 ml) at −78 °C was added boron tribromide (23.4 ml, 23.4 mmol, 1.0M in CH_2Cl_2). After 15 mins the reaction mixture was warmed to room temperature and co-evaporated with MeOH (3×). The residue was stirred in 7M NH_3 MeOH solution for 10 min after which time silica gel was added and the resulting mixture concentrated in vacuo. The resulting residue was purified by chromatography ($CHCl_3$/MeOH/28% aq NH_4OH = 10:9:1) to afford **11** and then further purified by preparative HPLC to afford **11.TFA** (0.234 g, 26%) as a crystalline white solid. A small portion of the product was evaporated from 5% aqueous HCl to afford **11.HCl**. ^{1}H NMR (D_2O + drop DCl, internal CH_3CN at δ 2.06): δ 8.95 (s, 1H), 7.89 (s, 1H), 4.66 (d, *J* = 14.3 Hz, 1H), 4.57 (d, *J* = 14.3 Hz, 1H), 4.05-3.94 (m, 2H), 3.91 (dd, *J* = 13.0, 5.4 Hz, 1H), 3.78 (dd, *J* = 12.4, 3.3 Hz, 1H), 3.66 (dd, *J* = 12.4, 4.4 Hz, 1H), 3.47 (m, 1H). ^{13}CNMR (D_2O + drop DCl, internal CH_3CN at δ 1.47) 153.4, 145.2, 133.7, 133.3, 118.9, 104.0, 68.4, 63.4, 60.6, 57.7, 39.6. ESI-HRMS for $C_{11}H_{16}N_4O_4Na$ [MNa^+] calcd, 291.1069; found, 291.1071.

7-({Methyl[(2*R*,3*S*)-1,3,4-trihydroxybutan-2-yl]amino}methyl)-3,5-dihydro-4*H*-pyrrolo[3,2-*d*] pyrimidin-4-one (26) and its hydrochloride salt (26.HCl)

To a suspension of compound **11** (30 mg, 0.11 mmol) in MeOH (1 ml) was added paraformaldehyde (3.4 mg, 0.11 mmol). The mixture was stirred at 50 °C for 30 min and then sodium cyanoborohydride (7.4 mg, 0.12 mmol) was added and the resulting reaction mixture stirred at room temperature for 16 h. The mixture was concentrated in vacuo and the resulting residue purified by chromatography (CH_2Cl_2/MeOH/28% aq NH_4OH = 8:2:0.1) to afford **26** (22 mg, 70%) as a white solid. M.p. 193 °C. $[\alpha]_D^{21}$ −5.3 (c 1, H_2O). ^{1}H NMR (D_2O + 0.1% DCl, internal CH_3CN at 2.06): δ 8.04 (s, 1H), 7.68 (s, 1H), 4.61 (d, *J* = 13.7 Hz, 1H), 4.53 (d, *J* = 13.7 Hz, 1H), 4.06-3.99 (m, 2H), 3.94 (dd, *J* = 13.5, 6.9 Hz, 1H), 3.75 (d, *J* = 12.3 Hz, 1H), 3.57 (dd, *J* = 12.3, 4.2 Hz, 1H), 3.50-3.43 (m, 1H), 2.94 (s, 3H). ^{13}C NMR (D_2O + 0.1% DCl, internal CH_3CN at 1.47): δ 155.7, 144.6, 143.6, 132.0, 118.4, 106.5, 67.6, 64.8, 63.3, 56.6, 49.8, 37.8. ESI-HRMS for $C_{12}H_{19}N_4O_4$ [MH^+] calcd, 283.1406; found, 283.1404.

5-Benzyloxymethyl-4-methoxy-7-({[(2*S*,3*R*)-1,3,4-trihydroxybutan-2-yl]amino} methyl)-5*H*pyrrolo[3,2-*d*]pyrimidine (57)

A mixture of diethyl (2*R*,3*R*)-2-amino-3-hydroxysuccinate (**49**)[36] (83 mg, 0.40 mmol, prepared from diethyl-L-tartrate by a known literature method[36]), sodium cyanoborohydride (42 mg, 0.67 mmol) and **44**[8] (100 mg, 0.34 mmol) were evaporated from MeOH (3×) and then dissolved in MeOH (10 ml) and acetic acid added (5 drops). After stirring at room temperature for 16 h silica gel was added and the mixture concentrated in vacuo and the resulting residue purified by chromatography (Et_3N/EtOAc/hexanes = 1:66:33) to afford crude **53** (114 mg, 70%) as a colorless oil. ^{1}H NMR revealed the product was slightly contaminated with (co-polar) starting amine. To a stirred solution of crude **53** (114 mg, 0.23mmol) in diethyl ether (10 ml) was added MeOH (0.10 ml, 2.34mmol) and then lithium borohydride[38] (0.59 ml, 1.17 mmol, 2.0 M in THF). After 30 min the reaction mixture was diluted with MeOH, silica gel was added and the mixture concentrated in vacuo. The resulting residue was purified by chromatography (28% aq NH_4OH/MeOH/CH_2Cl_2 = 0.5:5:95 → 0.5:15:85) to afford **57** (56 mg, 59%) as a colorless gum. The ^{1}H and ^{13}C NMRs were identical to those of the enantiomer, **56**.

7-({[(2*S*,3*R*)-1,3,4-Trihydroxybutan-2-yl]amino}methyl)-3,5-dihydro-4*H*-pyrrolo[3,2-*d*]pyrimidin-4-one (19) and its hydrochloride salt (19.HCl)

Boron tribromide (1.39 ml, 1.39 mmol, 1.0M in CH_2Cl_2) was added dropwise to a stirred solution of compound **57** (56 mg, 0.14 mmol) in CH_2Cl_2 (7 ml) at −78°C. After 15 min the reaction mixture was warmed to room temperature and coevaporated with MeOH (3×). The residue was stirred in 7M NH_3 - MeOH for 10 min and then silica gel was added and the resulting mixture concentrated in vacuo. The residue was purified by chromatography ($CHCl_3$/MeOH/28% aq NH_4OH = 10:9:1) to afford **19** (17 mg, 46%) as a white solid. A small portion was purified by preparative HPLC and the product evaporated from 5% aqueous HCl to afford **19.HCl**. The ^{1}H and ^{13}C NMR data were as for **11.HCl**. The minor differences in chemical shift between 11.HCl and **19.HCl** were likely due to concentration effects. ESI-HRMS for $C_{11}H_{16}N_4O_4Na$ [MNa+] calcd, 291.1069; found, 291.1065.

Diethyl (2*S*,3*R*)-2-hydroxy-3-({[5-benzyloxymethyl-4-methoxy-5*H*-pyrrolo[3,2-*d*]pyrimidin-7-yl]methyl}amino)butanedioate (54)

Sodium triacetoxyborohydride (0.545 g, 2.57 mmol) was added to a solution of diethyl (2*S*, 3*R*)-2-amino-3-hydroxysuccinate (**50**)[36] (0.53 g, 2.57 mmol, prepared from diethyl-L-tartrate by known literature methods[36,37]) and compound **44**[8] (0.588 g, 1.98 mmol) in 1,2-dichloroethane (30 ml) and the resulting reaction mixture stirred at room temperature for 4 h. The mixture was then diluted with CH_2Cl_2 and washed with aqueous saturated $NaHCO_3$, dried and concentrated in vacuo. The residue was purified by chromatography (EtOAc/hexanes = 9:1 → EtOAc) to afford **54** (0.67 g, 69%) as a pale yellow gum. $[\alpha]_D^{21}$ −5.9 (c 0.54, EtOH). ^{1}H NMR ($CDCl_3$): δ, 8.55 (s, 1H), 7.34 (s, 1H), 7.33-7.23 (m, 5H), 5.70 (s, 2H), 4.62 (d, *J* = 3.4 Hz, 1H), 4.48 (s, 2H), 4.23-4.15 (m, 5H), 4.10 (s, 3H), 3.99 (d, *J* = 13.8 Hz, 1H), 3.85 (d, *J* = 3.3 Hz, 1H), 2.25 (br s, exchanged to D_2O, 2H), 1.26 (t, *J* = 7.1 Hz, 3H), 1.25 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR ($CDCl_3$, center line at δ 77.0): δ 171.9, 171.1, 156.2, 150.0, 149.8, 136.9, 130.9, 128.4, 127.9, 127.6, 116.1, 77.0, 72.0, 70.2, 63.8, 61.4, 61.3, 53.5, 42.6, 14.1, 14.1. ESI-HRMS for $C_{24}H_{31}N_4O_7$ [MH^+] calcd, 487.2193; found, 487.2174.

5-Benzyloxymethyl-4-methoxy-7-({[(2*R*,3*R*)-1,3,4-trihydroxybutan-2-yl]amino}methyl)-5*H*pyrrolo[3,2-*d*]pyrimidine (58)

Solid lithium borohydride[38] (268 mg, 12.3 mmol) was added portionwise to a refluxing solution of compound **54** (600 mg, 1.23 mmol) in THF (10 ml) and MeOH (1.0 ml, 24.6 mmol) over 2 h. After cooling, the solution was concentrated in vacuo and the residue purified by chromatography (CH_2Cl_2/MeOH/28% aq NH_4OH = 95:5:0.5 → 85:15:0.5) to afford **58** (219 mg, 44%) as a colorless gum which crystallized on standing. The product was of sufficient purity to proceed to the next step but a small quantity was recrystallized for characterization purposes from ethyl acetate. M.p. 108–109 °C. $[\alpha]_D^{18}$ −6.1 (c 0.59, MeOH). ^{1}H NMR (CD_3OD): δ 8.42 (s, 1H), 7.64 (s, 1H), 7.28-7.16 (m, 5H), 5.75 (s, 2H), 4.50 (s, 2H), 4.10 (s, 3H), 4.07

(d, J = 13.5 Hz, 1H), 4.00 (d, J = 13.5 Hz, 1H), 3.83-3.68 (m, 3H), 3.63 (d, J = 5.5 Hz, 2H), 3.31 (pentet, J = 1.6 Hz, 1H). ^{13}C NMR (CD_3OD, center line δ 49.0): δ 158.0, 150.8, 150.7, 138.8, 134.2, 129.3, 128.8, 128.6, 117.0, 116.1, 78.5, 72.1, 71.5, 65.6, 62.3, 61.0, 54.3, 41.6. ESI-HRMS for $C_{20}H_{27}N_4O_5$ [MH^+] calcd 403.1981; found, 403.1980.

7-({[(2*R*,3*R*)-1,3,4-Trihydroxybutan-2-yl]amino}methyl)-3,5-dihydro-4*H*-pyrrolo[3,2- *d*] pyrimidin-4-one (24) and its hydrochloride salt (24.HCl)

Compound **58** (100 mg, 0.25 mmol) was heated under reflux in concentrated HCl (4 ml) for 2 h. After cooling the solution was concentrated in vacuo and the residue dissolved in MeOH and neutralized with Amberlyst A-21 resin. The mixture was filtered to remove the resin and the filtrate concentrated in vacuo. Purification of the resulting residue by chromatography (CH_2Cl_2/MeOH/28% aq NH_4OH = 7:3:0.3 → 5:4.5:0.5) gave **24** which was then converted with 5% aqueous HCl to **24.HCl** (55 mg, 73 % yield) as a colorless solid. A small portion was further purified by preparative HPLC and the product evaporated from 5% aqueous HCl to afford **24.HCl**. ^{1}H NMR (D_2O + drop DCl, internal CH_3CN at δ 2.06): δ 9.03 (s, 1H), 7.90 (s, 1H), 4.61 (s, 2H), 4.16 (m, 1H), 4.02-3.89 (m, 2H), 3.69 (d, J = 5.5 Hz, 2H), 3.55 (m, 1H). 13CNMR (D_2O + drop DCl, internal CH_3CN at δ 1.47): δ 153.1, 145.4, 133.5, 132.6, 118.9, 103.5, 68.8, 62.7, 60.9, 57.4, 39.7. ESI-HRMS for $C_{11}H_{16}N_4O_4Na$ [MNa^+] calcd, 291.1069; found, 291.1067.

Diethyl (2*R*,3*S*)-2-hydroxy-3-({[5-benzyloxymethyl-4-methoxy-5*H*-pyrrolo[3,2-*d*]pyrimidin-7-yl]methyl}amino)butanedioate (55)

Diethyl (2*R*,3*S*)-2-amino-3-hydroxysuccinate (**51**)[36] (109 mg, 0.53 mmol, prepared from diethyl-D-tartrate by known literature methods[36,37]), sodium cyanoborohydride (55 mg, 0.88 mmol) and compound **44**[8] (131 mg, 0.44 mmol) were evaporated from MeOH (3×) and then dissolved in MeOH (10 ml) and acetic acid added (5 drops). The reaction mixture was stirred at room temperature for 2 h and then silica gel was added and concentrated in vacuo. The resulting residue was purified by chromatography (EtOAc/hexanes = 2:1 → EtOAc/Et_3N = 1:99) to afford **55** (166 mg, 77%) as a colorless oil. The ^{1}H NMR was identical to its enantiomer, **54**.

7-({[(2*S*,3*S*)-1,3,4-Trihydroxybutan-2-yl]amino}methyl)-3,5-dihydro-4*H*-pyrrolo[3,2-*d*] pyrimidin-4-one (25) and its TFA salt (25.TFA) and hydrochloride salt (25.HCl)

To a stirred solution of compound **55** (166 mg, 0.34mmol) in diethyl ether (10 ml) was added MeOH (0.14 ml, 3.41 mmol) and then lithium borohydride[38] (0.85 ml, 1.71 mmol, 2.0 M in THF). After 30 min the reaction mixture was diluted with MeOH and concentrated in vacuo. The residue was dissolved in MeOH, diluted with concentrated aqueous ammonia (1 ml), and concentrated in vacuo onto silica gel. Purification of the resulting residue by chromatography (CH_2Cl_2/MeOH/28% aq NH_4OH = 85:15:2 → 70:30:2 → 50:50:4) afforded crude **59** (103 mg, 0.26 mmol, 75%) as a colorless oil which was dissolved in CH_2Cl_2 (10 ml), cooled to −78° C and boron tribromide (2.56 ml, 2.56mmol, 1.0 M in CH_2Cl_2) added. After 15min the reaction mixture was warmed to room temperature and coevaporated with MeOH (3×). The residue was stirred in methanolic ammonia solution (7 M) for 10 min, silica gel was added and the mixture concentrated in vacuo. The resulting residue was purified by chromatography ($CHCl_3$/MeOH/28% aq NH_4OH = 10:9:1) to give **25** and then further purified by preparative HPLC to afford **25.TFA** (18 mg, 26 %) as a white solid. A small portion was evaporated from 5% aqueous HCl to afford **25.HCl**. The ^{1}H and ^{13}C NMR data were the same as for compound **24.HCl**, its enantiomer. The minor differences in chemical shift between **24** and **25** were likely due to concentration effects. ESI-HRMS for $C_{11}H_{16}N_4O_4Na$ [MNa^+] calcd, 291.1069; found, 291.1066.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

(2-[(Dimethylamino)methyleneamino]-7-{[(5*R*,6*S*)-6-hydroxy-2,2-dimethyl-1,3-dioxepan-5-ylamino]methyl}-4-oxo-4,5-dihydro-3*H*-pyrrolo[3,2-*d*]pyrimidin-3-yl)methyl pivalate (60)

To a mixture of compound **41**[23] (66 mg, 0.19 μmol) and (5*S*,6*R*)-6-amino-2,2-dimethyl-1,3-dioxepan-5-ol[39] (44 mg, 0.27 mmol) in 1,2-dichloroethane (6.5 ml) was added $MgSO_4$ (400 mg, 3.3 mmol) and sodium triacetoxyborohydride (61 mg, 0.29 mmol). The resulting mixture was stirred overnight at room temperature and then filtered and the solids were rinsed with CH_2Cl_2 (20 ml). The filtrate was washed with saturated aqueous $NaHCO_3$ and brine (20 ml each), dried, filtered, and then silica gel (1 g) was added and the mixture concentrated in vacuo. The resulting residue was purified by chromatography ($CHCl_3$/MeOH/28% aq NH_4OH = 90:10:1) to afford **60** (86 mg, 92%) as an oil. $[\alpha]_D^{20}$ +2.4 (c 2.37, $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$): δ 10.60 (br s, D_2O-exchangeable, 1H), 8.56 (s, 1H), 7.09 (s, 1H), 6.35 (s, 2H), 4.02 (d, *J* = 13.4 Hz, 1H), 3.89 (d, *J* = 13.4 Hz, 1H), 3.78 (dd, *J* = 12.4, 2.8 Hz, 1H), 3.75 (dd, *J* = 11.3, 1.8 Hz, 1H), 3.65-3.57 (m, 2H), 3.56 (dd, *J* = 12.4, 7.6 Hz, 1H), 3.15 (s, 3H), 3.04 (s, 3H), 2.71 (ddd, *J* = 7.3, 6.8, 2.6 Hz, 1H), 2.32 (br s, 1H, D_2O exchangeable), 1.33 (s, 3H), 1.32 (s, 3H), 1.15 (s, 9H). ^{13}C NMR (125.7 MHz, $CDCl_3$): δ 178.0, 157.3, 155.7, 154.0, 143.5, 127.0, 115.2, 115.0, 101.5, 73.0, 66.3, 63.1, 62.7, 60.5, 41.7, 41.2, 39.2, 35.3, 27.4, 25.1, 25.0. ESI-HRMS for $C_{23}H_{37}N_6O_6$ $[MH^+]$ calcd, 493.2764; found, 493.2775.

2-Amino-7-({[(2*R*,3*S*)-1,3,4-trihydroxybutan-2-yl]amino}methyl)-3,5-dihydro-4*H*pyrrolo[3,2-*d*]pyrimidin-4-one (DATMe-ImmG, 9)

A mixture of acetyl chloride (70 μL, 0.98 mmol) in MeOH (5 ml) was added to **60** (49 mg, 99 μmol) and stirred at room temperature for 16 h. The resulting solution was concentrated in vacuo and the residue was treated with water (2 ml) and concentrated HCl (2 ml) at 100 °C for 75 min. The solution was again concentrated in vacuo, dissolved in MeOH and concentrated in vacuo onto silica gel (~1g). Purification of the residue by chromatography ($CHCl_3$/MeOH/28% aq NH4OH = 6:4:1) afforded **9** (20 mg, 71%) as an amorphous white solid. $[\alpha]_D^{20}$ −15 (c 0.21, MeOH). ^1H NMR (500 MHz, D_2O, internal 1,4-dioxane at δ 3.75): δ 7.30 (s, 1H), 3.95 (d, *J* = 13.7 Hz, 1H), 3.85 (d, *J* = 13.7 Hz, 1H), 3.83-3.77 (m, 2H), 3.73-3.66 (m, 2H), 3.58 (dd, *J* = 12.0, 6.2 Hz, 1H), 2.93-2.87 (m, 1H). ^{13}C NMR (125.7 MHz, D_2O, internal 1,4-dioxane at 67.2) δ 158.0, 152.6, 143.9, 129.5, 113.0, 109.6, 71.1, 63.7, 59.8, 59.8, 40.5. ESI-HRMS for $C_{11}H_{18}N_5O$ $[MH^+]$ calcd, 284.1359; found, 284.1352.

tert-Butyl (1,3-dihydroxypropan-2-yl)[(1*S*)-2-hydroxy-1-(4-hydroxy-5*H*-pyrrolo[3,2-*d*]pyrimidin-7-yl)ethyl]carbamate (61)

Di-*tert*-butyl dicarbonate (91 mg, 0.42 mmol) was added to a solution of ImmH (**1.HCl**)[7] (70 mg, 0.23 mmol) and Et_3N (65 μl, 0.46 mmol) in a mixture of water (1 ml) and MeOH (3 ml). The solution was stirred for 30 min and then concentrated in vacuo to afford 110 mg of a colorless solid which consisted, as estimated by ^1H NMR, of about 78 mg, 0.21 mmol of (2*S*,3*S*,4*R*,5*R*)-*tert*-butyl 3,4-dihydroxy-2-(4-hydroxy-5*H*pyrrolo[3,2-*d*]pyrimidin-7-yl)-5-(hydroxymethyl)pyrrolidine-1-carboxylate with the rest being triethylamine hydrochloride. This mixture was dissolved in MeOH (4 ml) and water (3 ml) and sodium periodate (55 mg, 0.26 mmol) added. After stirring for 15 min a precipitate formed and sodium borohydride (24 mg, 0.64 mmol) was added and the mixture stirred for an additional 15 min, filtered through Celite® and the mixture concentrated in vacuo. The residue was purified by chromatography (CH_2Cl_2/MeOH = 85:15) to afford **61** (72 mg, 92 %) as a colorless solid. $[\alpha]_D^{20}$ +35.8 (c 0.505, MeOH). ^1H NMR (CD_3OD): δ 7.89 (s, 1H), 7.50 (br s 1H), 5.32 (br s, 0.5 H), 5.08 (br s, 0.5H), 4.28 (br s 1H), 4.17-3.50 (br m, 6H), 1.39 (br d, 9H). ESI-HRMS for $C_{16}H_{25}N_4O_6$ $[MH^+]$ calcd, 369.1774; found, 369.1760.

7-{(1*S*)-1-[(1,3-Dihydroxypropan-2-yl)amino]-2-hydroxyethyl}-3,5-dihydro-4*H*-pyrrolo[3,2-*d*]pyrimidin-4-one hydrochloride (12)

Compound **61** (68 mg, 0.19 mmol) was dissolved in a mixture of MeOH (2 ml) and concentrated HCl (0.2 ml). After a few min the solution was concentrated in vacuo to give a yellow foam which was crystallized from EtOH to afford **12** (42 mg, 75%) as a colorless solid. M.p. >300 °C. $[\alpha]_D^{20}$ +24.1 (c 0.435, H_2O + 1drop concentrated HCl). ^1H NMR (D_2O + DCl, internal acetone at δ 2.225): δ 8.95 (s, 1H), 7.96 (s, 1H), 5.09 (t, *J* = 4.7 Hz, 1H), 4.24 (dd, *J* = 12.2, 4.3 Hz, 1H), 4.11 (dd, *J* = 12.2, 5.1 Hz, 1H), 3.97-3.77 (m, 4H), 3.46 (pentet, *J* = 5.3 Hz, 1H). ^{13}C NMR (D_2O + DCl, internal acetone at δ 31.5): δ 154.0, 146.0, 133.4, 132.5, 119.7, 106.6, 62.1, 60.1, 59.3, 59.1, 54.7. ESI-HRMS for $C_{11}H_{17}N_4O_4$ [MH^+] calcd, 269.1250; found, 269.1239.

(4*R*/*S*,5*R*/*S*)-(2-Benzylisoxazolidine-4,5-diyl)dimethanol (64)

A mixture of *N*-benzylhydroxylamine hydrochloride (13.59 g, 85.15 mmol) and sodium acetate (9.31 g, 114 mmol) were stirred together in ethanol (75 ml) at room temperature for 15 min. Aqueous 37% formaldehyde solution (12.68 ml, 170 mmol) was added and stirring continued for 30 min and then *cis*-but-2-ene-1,4-diol (**62**) (4.67 ml, 56.8 mmol) added and the mixture heated under reflux for 16 h. The solvent was evaporated and the residue dissolved in $CHCl_3$ and washed with aqueous saturated $NaHCO_3$, dried, filtered and then concentrated in vacuo to afford (±)-**64** (12.5 g, 98%) as a brown syrup suitable for use without further purification. An aliquot of (±)-**64** was purified by chromatography (EtOAc → EtOAc/MeOH = 95:5). ^{13}C NMR ($CDCl_3$): δ 136.5, 129.0, 128.4, 127.6, 78.5, 62.4, 61.3, 60.4, 56.8, 45.8.

(2*R*/*S*,3*R*/*S*)-3-[(Benzylamino)methyl]butane-1,2,4-triol (66)

Zinc dust (11.1 g, 170 mmol) was added to a solution of (±)-**64** (12.5 g, 56.1 mmol) in acetic acid (150 ml) - exotherm to 67 °C - and the mixture stirred at room temperature for 1 h. The mixture was filtered, the filtrate concentrated in vacuo and the resulting residue purified by chromatography (CH_2Cl_2/7M NH_3 in MeOH = 9:1 →8:2) to afford (±)-**66** (5.8 g, 45%) as a colorless syrup. ^1H NMR ($CDCl_3$): δ 7.33-7.23 (m, 5H), 4.11 (br s, 4H), 3.76-3.66 (m, 5H), 3.61-3.51 (m, 2H), 2.81-2.70 (m, 2H), 1.82 (sextet, *J* = 5.5 Hz, 1H). ^{13}C NMR ($CDCl_3$): δ 138.8, 128.5, 128.2, 127.3, 73.3, 64.5, 63.1, 54.0, 49.7, 43.3.

5-Benzyloxymethyl-7-({benzyl[(2*R*/*S*,3*R*/*S*)-3,4-dihydroxy-2-(hydroxymethyl)butyl]amino}methyl)-4-methoxy-5*H*-pyrrolo[3,2-*d*]pyrimidine (68)

Acetyl chloride (21 μl, 0.30 mmol), compound **44**[8] (179 mg, 0.60 mmol), compound (±)-**66** (136 mg, 0.60 mmol) and sodium cyanoborohydride (57 mg, 0.90 mmol) were successively added to MeOH (6 ml) and the resulting reaction mixture stirred at room temperature for 64 h. The mixture was concentrated in vacuo and the residue purified by chromatography (CH_2Cl_2/7M NH_3 in MeOH = 96:4) to afford (±)-**68** (159 mg, 52%) as a colorless gum. ^1H NMR (CD_3OD): δ 8.43 (s, 1H), 7.58 (s, 1H), 7.32-7.12 (m, 10H), 5.75 (s, 2H), 4.50 (s, 2H), 4.10 (s, 3H), 3.85 (s, 2H), 3.71 (dd, *J* = 10.9, 4.8 Hz, 1H), 3.65-3.51 (m, 4H), 3.48-3.37 (m, 2H), 2.69-2.57 (m, 2H), 2.19 (m, 1H). ^{13}C NMR (CD_3OD, center line at δ 49.0): δ 157.9, 151.3, 150.9, 139.8, 138.7, 135.1, 130.4, 129.3, 128.7, 128.6, 128.2, 116.8, 114.2, 78.6, 74.1, 71.6, 65.4, 63.7, 59.9, 55.5, 54.3, 48.5, 42.1. ESI-HRMS for $C_{28}H_{35}N_4O_5$ [MH^+] calcd, 507.2607; found, 507.2604.

7-({[(2*R*/*S*,3*R*/*S*)-3,4-dihydroxy-2-(hydroxymethyl)butyl]amino}methyl)-3,5-dihydro-4*H*pyrrolo[3,2-*d*]pyrimidin-4-one (17) and its hydrochloride salt (17.HCl)

Compound (±)-**68** (150 mg, 0.30 mmol) was heated under reflux in concentrated HCl (4 ml) for 1.5 h. The solution was concentrated in vacuo to a cream coloured foam which was dissolved in a 1:1 mixture of MeOH/water (10 ml, v/v) and the solution neutralized with Amberlyst A-21 resin. The resin was filtered off and 10% Pd-C (50 mg) added to the filtrate and the mixture stirred under an atmosphere of hydrogen for 1 h. The suspension was filtered through Celite® and concentrated in vacuo and the resulting residue purified by chromatography (CH_2Cl_2/MeOH/28% aq NH_4OH = 5:4.5:0.5) to afford (±)-**17** as a colorless solid which was further purified by prep. HPLC and the product evaporated from 5% aqueous HCl to afford (±)-**17.HCl** (26 mg, 28%). ^{1}H NMR (D_2O, internal CH_3CN at δ 2.06): δ 8.60 (s, 1H), 7.81 (s, 1H), 4.45 (s, 2H), 3.84-3.78 (m, 2H), 3.69-3.53 (m, 3H), 3.35-3.23 (m, 2H), 2.22 (m, 1H). ^{13}C NMR (D_2O, internal CH_3CN at δ 1.5): δ 154.3, 144.6, 137.9, 132.6, 118.7, 104.8, 71.4, 63.4, 60.6, 47.8, 41.6, 41.0. ESI-HRMS for $C_{12}H_{19}N_4O_4$ [MH^+] calcd, 283.1406; found, 283.1406.

(4*R*/*S*,5*S*/*R*)-(2-Benzylisoxazolidine-4,5-diyl)dimethanol (65)

A mixture of *N*benzylhydroxylamine (4.4 g, 27.7 mmol) and anhydrous NaOAc (3.0 g, 36.7 mmol) in EtOH (35 ml) was stirred at room temperature for 15 min, after which time aqueous 37% formaldehyde (4.1 ml, 55.1 mmol) was added and the mixture stirred for another 30 min. A solution of *trans*-but-2-ene-1,4-diol (**63**) (1.63 g, 18.5 mmol) in ethanol (20 ml) was then introduced to the mixture in a single portion and the resulting solution refluxed for 18 h. After cooling the mixture was concentrated in vacuo and the residue dissolved in CH_2Cl_2 and the solution washed with aqueous saturated $NaHCO_3$ solution, dried, filtered and the filtrate then concentrated in vacuo. The crude product was purified by chromatography (EtOAc) to afford (±)-**65** (1.46 g, 35 %) as an immobile syrup. ^{1}H NMR ($CDCl_3$): δ 7.41-7.22 (m, 5H), 4.10-3.82 (m, 2H), 3.80-3.60 (m, 4H), 3.5-2.3 (m, 5H). ^{13}C NMR ($CDCl_3$): δ 137.0, 129.3, 128.8, 128.0, 81.6, 65.0, 64.1, 63.0, 58.7, 47.7.

(2*S*/*R*,3*R*/*S*)-[(Benzylamino)methyl]butane-1,2,4-triol (67)

Zinc dust (470 mg, 7.2 mmol) was added to a solution of compound (±)-**65** (320 mg, 1.43 mmol) in acetic acid (6 ml) and the mixture stirred vigorously for 1.5 h. The suspension was filtered through Celite® and the filtrate concentrated in vacuo and the resulting residue dissolved in CH_2Cl_2, silica gel added and the resulting mixture concentrated in vacuo. The residue was purified by chromatography (CH_2Cl_2/7M NH_3-MeOH = 4:1) to afford (±)-**67** (260 mg, 81 %) as an immobile syrup. ^{1}H NMR ($CDCl_3$): δ 7.41-7.21 (m, 5H), 3.90 (q, *J* = 4.0 Hz, 1H), 3.80 (s, 2H), 3.75 (d, *J* = 5.1 Hz, 2H), 3.66 (dq, *J* = 11.7, 3.9 Hz, 2H), 3.18 (br s, 4H), 2.94 (d, *J* = 6.9 Hz, 2H), 1.78 (m, 1H). ^{13}C NMR ($CDCl_3$): δ 138.9, 129.1, 128.7, 127.9, 73.7, 65.4, 65.3, 54.4, 48.9, 43.9.

5-Benzyloxymethyl-7-({benzyl[(2*R*/*S*,3*S*/*R*)-3,4-dihydroxy-2-(hydroxymethyl)butyl]amino}methyl)-4-methoxy-5*H*-pyrrolo[3,2-*d*]pyrimidine (69)

To a solution of (±)-**67** (250 mg, 1.11 mmol) and compound **44**[8] (330 mg, 1.11 mmol) in MeOH (11 ml) was added acetyl chloride (40μL, 0.56 mmol) and this was followed by the addition of sodium cyanoborohydride (105 mg, 1.67 mmol). The mixture was stirred at room temperature for 72 h and then diluted with CH_2Cl_2, washed with aqueous saturated $NaHCO_3$ solution, dried, filtered and the filtrate concentrated in vacuo. The resulting residue was purified by chromatography (7M NH_3 in MeOH/CH_2Cl_2 = 4:96) to afford (±)-**69** (310 mg, 55 %) as an immobile syrup. ^{1}H NMR ($CDCl_3$): δ 8.56 (s, 1H), 7.46-7.17 (m, 11H), 5.74 (s, 2H), 5.33 (s, 2H), 4.14 (s, 3H), 4.05 (d, *J* = 14.2 Hz, 1H), 3.92 (d, *J* = 14.2 Hz, 1H), 3.80-3.41 (m, 7H), 2.90 (dd, *J* = 13.2, 9.0 Hz, 1H), 2.72 (dd, *J* = 12.9, 5.1 Hz, 1H), 2.38 (m, 1H). ^{13}C NMR ($CDCl_3$): δ 156.9, 150.9, 150.7, 138.0, 137.2, 133.5, 129.7, 129.0, 128.9, 128.4, 128.0, 127.9, 112.3, 77.2, 74.3, 70.7, 65.1, 64.5, 59.4, 54.3, 54.1, 46.0, 40.9. ESI-HRMS for $C_{28}H_{35}N_4O_5$ [MH^+] calcd, 507.2607; found, 507.2592.

7-({[(2*R*/*S*,3*S*/*R*)-3,4-Dihydroxy-2-(hydroxymethyl)butyl]amino}methyl)-3,5-dihydro-4*H*pyrrolo[3,2-*d*]pyrimidin-4-one (38) and its hydrochloride salt (38.HCl)

A solution of compound (±)-**69** (310 mg, 0.61 mmol) was refluxed in concentrated HCl (8 ml) for 2.5 h and the solution concentrated in vacuo. The crude residue was dissolved in MeOH (10 ml) and water (2 ml) and neutralized with Amberlyst A-21 resin. The resin was filtered off and the filtrate concentrated in vacuo. The residue was dissolved in MeOH (6 ml) and water (3 ml), and then 10 % Pd/C (0.1 g) added and the mixture stirred under a hydrogen atmosphere for 2 h. The suspension was filtered through Celite® and the filtrate concentrated in vacuo. The resulting residue was purified by chromatography (CH_2Cl_2/MeOH/28% aq NH4OH = 5:4.5:0.5) to afford (±)-**38** (60 mg, 35%) as a colorless solid. ^{1}H NMR (D_2O + DCl): δ 8.64 (s, 1H), 7.76 (s, 1H), 4.40 (s, 2H), 3.75-3.48 (m, 5H), 3.27-3.20 (m, 2H), 2.07 (m, 1H). ^{13}C NMR (D_2O + DCl): δ 153.7, 144.6, 136.3, 132.5, 118.5, 104.3, 71.2, 63.9, 61.4, 47.1, 41.2, 40.2. ESI-HRMS for $C_{12}H_{19}N_4O_4$ [MH^+] calcd 283.1406; found 283.1413.

7-{[(2-Hydroxyethyl)amino]methyl}-3,5-dihydro-4*H*-pyrrolo[3,2-*d*]pyrimidin-4-one (20) and its hydrochloride salt (20.HCl)

A mixture of ethanolamine (**70**) (78 µl, 0.96 mmol), 9-deazahypoxanthine[10] (**75**) (100 mg, 0.74 mmol) and 37% aqueous formaldehyde (72 µl, 0.96 mmol) in water (5 ml) was stirred and heated in a stoppered flask at 85 °C for 16 h. After cooling, the solution was concentrated in vacuo and the residue was purified by chromatography (CH_2Cl_2/MeOH/28% aq NH_4OH = 6:3.5:0.5) to afford **20** (100 mg, 65%) as a pale brown solid. M.p. 214 °C. ^{1}H NMR (D_2O + 0.1% DCl): δ 8.75 (s, 1H), 7.83 (s, 1H), 4.47 (s, 2H), 3.86 (t, *J* = 5.1 Hz, 2H), 3.26 (t, *J* = 5.1 Hz, 2H). ^{13}C NMR (D_2O + 0.1% DCl): δ 154.0, 144.9, 136.3, 132.8, 118.7, 104.5, 57.2, 49.0, 40.9. ESI-HRMS for $C_9H_{13}N_4O_2$ [MH^+] calcd, 209.1039; found, 209.1031.

3-(Benzyl((4-hydroxy-5H-pyrrolo[3,2-*d*]pyrimidin-7-yl)methyl)amino)propanol (76) and its hydrochloride salt (76.HCl)

A mixture of 3-(benzylamine)propanol (**71**) (159 mg, 0.96 mmol), 9-deazahypoxanthine[10] (**75**) (100 mg, 0.74 mmol) and 37% aqueous formaldehyde (72 µl, 0.96 mmol) in water (5 ml) was stirred and heated at 85 °C in a stoppered flask for 16 h. After cooling the solution was concentrated in vacuo and the residue purified by chromatography (CH_2Cl_2/MeOH = 9:1 → 8:2) to afford **76** (37 mg, 16%) as a white solid. M.p. 200–205 °C. ^{1}H NMR (CD_3OD + DCl): δ 9.09 (s, 1H), 7.99 (s, 1H), 7.64-7.59 (m, 2H), 7.45-7.42 (m, 3H), 4.80 (d, *J* = 14.5 Hz, 1H), 4.70 (d, *J* = 14.5 Hz, 1H), 4.65 (d, *J* = 13.5 Hz, 1H), 4.49 (d, *J* = 13.5 Hz, 1H), 3.55 (t, *J* = 5.6 Hz, 2H), 3.32-3.26 (m, 2H), 2.02-1.95 (m, 2H). ^{13}C NMR (CD_3OD + DCl): δ 153.0, 147.1, 134.6, 133.4, 132.7, 131.4, 131.2, 130.7, 120.4, 103.7, 60.65, 58.6, 51.9, 27.8. ESI-HRMS for $C_{17}H_{21}N_4O_2$ [MH^+] calcd, 313.1665; found, 313.1661.

3-((4-Hydroxy-5H-pyrrolo[3,2-*d*]pyrimidin-7-yl)methylamino)propanol (18) and its hydrochloride salt (18.HCl)

To a solution of **76** (35 mg, 0.11 mmol) in *i*PrOH (2 ml) was added 10% Pd-C (20 mg). The mixture was stirred at 50 °C under an atmosphere of hydrogen for 16 h. The resulting suspension was filtered through Celite® and the filtrate concentrated in vacuo and the residue purified by chromatography (CH_2Cl_2/MeOH/28% aq NH_4OH = 5:5:0.1) to afford **18** (18 mg, 74%) as a white solid. M.p. 189 °C. ^{1}H NMR (D_2O + 0.1% DCl, internal acetonitrile at δ 2.06): δ 8.76 (s, 1H), 7.82 (s, 1H), 4.43 (s, 2H), 3.69 (t, *J* = 6 Hz, 2H), 3.21 (t, *J* = 7.5 Hz, 2H), 1.97-1.91 (m, 2H). ^{13}C NMR (D_2O + 0.1% DCl, internal acetonitrile at δ 1.47): δ 153.9, 144.9, 136.0, 132.7, 118.7, 104.6, 59.5, 45.3, 41.0, 28.5. ESI-HRMS for $C_{10}H_{15}N_4O_2$ [MH^+] calcd, 223.1195; found, 223.1194.

7-{[Benzyl(4-hydroxybutyl)amino]methyl}-3,5-dihydro-4*H*-pyrrolo[3,2-*d*]pyrimidin-4-one (77) and its hydrochloride salt (77.HCl)

A mixture of 4-(benzylamino)butan-1-ol (**72**) (172 mg, 0.96 mmol), 9-deazahypoxanthine (**75**)[10] (100 mg, 0.74 mmol) and 37% aqueous formaldehyde (72 µl, 0.96 mmol) in water (5 ml) was stirred and heated in stoppered flask at 85 °C for 16 h. After cooling the solution was concentrated in vacuo and the residue purified by chromatography (CH_2Cl_2/MeOH = 9:1 → 8:2) to afford **77** (130 mg, 55%) as a white solid. M.p. >250 °C. ^{1}H NMR (CD_3OD + DCl): δ 9.10 (s, 1H), 8.00 (s, 1H), 7.63 (m, 2H), 7.45 (m, 3H), 4.79 (d, *J* = 14.1 Hz, 1H), 4.72 (d, *J* = 14.1 Hz, 1H), 4.62 (d, *J* = 13 Hz, 1H), 4.50 (d, *J* = 13 Hz, 1H), 3.55 (t, *J* = 6 Hz, 2H), 3.20 (m, 2H), 1.93 (m, 2H), 1.50 (m, 2H). ^{13}C NMR (CD_3OD + DCl): δ 153.0, 147.1, 134.5, 133.5, 132.7, 131.4, 130.7, 120.4, 103.7, 100.2, 62.3, 58.2, 53.6, 50.0, 30.8, 22.2. ESI-HRMS for $C_{18}H_{23}N_4O_2$ [MH^+] calcd, 327.1821; found, 327.1815.

7-{[(4-Hydroxybutyl)amino]methyl}-3,5-dihydro-4*H*-pyrrolo[3,2-*d*]pyrimidin-4-one (29) and its hydrochloride salt (29.HCl)

To compound **77** (230 mg, 0.70 mmol) in *i*PrOH (3 ml) was added 10% Pd-C (50 mg) and the mixture stirred at 50 °C under an atmosphere of hydrogen for 16 h. Whilst still warm the solution was filtered through Celite® and the filtrate concentrated in vacuo. The resulting residue was purified by chromatography (CH_2Cl_2/MeOH/28% aq NH_4OH = 8:2:0.1) to afford **29** (133 mg, 80%) as a white solid. M.p. >250 °C. ^{1}H NMR (D_2O + 0.1% DCl, internal acetonitrile at δ 2.06): δ 8.32 (s, 1H), 7.73 (s, 1H), 4.39 (s, 2H), 3.60 (t, *J* = 6.3 Hz, 2H), 3.12 (t, *J* = 7.7 Hz, 2H), 1.75 (pentet, *J* = 7.7 Hz, 2H), 1.59 (pentet, *J* = 6.3 Hz, 2H). ^{13}C NMR (D_2O + 0.1% DCl, internal acetonitrile at δ 1.47): δ 155.1, 144.0, 141.2, 131.9, 118.5, 106.0, 61.4, 47.2, 40.9, 29.0, 23.0. ESI-HRMS for $C_{11}H_{17}N_4O_2$ [MH^+] calcd, 237.1352; found, 237.1349.

7-{[Bis(2-hydroxyethyl)amino]methyl}-3,5-dihydro-4*H*-pyrrolo[3,2-*d*]pyrimidin-4-one (21)

Aqueous 37% formaldehyde (160 µl, 2.0 mmol) was added to a solution of diethanolamine hydrochloride (**73**) (283 mg, 2.0 mmol) and sodium acetate (160 mg, 2.0 mmol) in water (2 ml) followed by the addition of 9-deazahypoxanthine (**75**)[10] (300 mg, 2.2 mmol). The mixture was stirred at 95 °C (bath temperature) for 12 h and after cooling, silica gel (1.0 g) added and the suspension concentrated in vacuo. Purification of the resulting residue by chromatography (CH_2Cl_2/MeOH/28% aq NH_4OH = 5:4.8:0.2) afforded **21** (244 mg, 44%) as a syrup, which solidified on standing. M.p. 178–180 °C. ^{1}H NMR (D_2O): δ 8.21 (s, 1H), 7.77 (s, 1H), 4.63 (s, 2H), 3.94 (d, *J* = 3.0 Hz, 4H), 3.37 (d, *J* = 3.0 Hz, 4H). ^{13}C NMR (D_2O): δ 154.8, 143.5, 142.5, 132.4, 118.1, 103.4, 55.4, 55.4, 54.1, 54.1, 47.9. ESI-HRMS for $C_{11}H_{17}N_4O_3$ [MH^+] calcd, 253.1301; found, 253.1301.

7-{[(2-Hydroxyethyl)(3-hydroxypropyl)amino]methyl}-3,5-dihydro-4*H*-pyrrolo[3,2-*d*]pyrimidin-4-one (33)

3-(Hydroxyethylamino)-propanol hydrochloride (**74**) (280 mg, 1.8 mmol) and sodium acetate (150 mg, 1.8 mmol) were dissolved in water (2 ml) and added to the solution were aqueous formaldehyde (150 µl, 1.8 mmol) and 9-deazahypoxanthine (**75**)[10] (250 mg, 1.85 mmol). The reaction mixture was stirred at 95 °C (bath temperature) for 12 h and then silica gel (1.0 g) was added and the mixture concentrated in vacuo. Purification of the resulting residue by chromatography (CH_2Cl_2/MeOH/28% aq NH_4OH = 5:4.8:0.2) afforded **33** (331 mg, 68%) as a syrup, which solidified on standing. M.p. 179–180 °C. ^{1}H NMR (D_2O): δ 7.94 (s, 1H), 7.62 (s, 1H), 4.37 (s, 2H), 3.86 (t, *J* = 5.4 Hz, 2H), 3.61 (t, *J* = 6.0 Hz, 2H), 3.18 (m, 4H), 1.95 (m, 2H). ^{13}C NMR (D_2O): δ 155.3, 144.6, 143.3, 131.9, 118.0, 105.2, 59.6, 56.2, 54.4, 51.2, 47.4, 26.5. ESI-HRMS for $C_{12}H_{19}N_4O_3$ [MH^+] calcd, 267.1457; found, 267.1454.

(2,2-Dimethyl-1,3-dioxan-5-yl)-*N*-methylmethanamine (79)

An aqueous methylamine solution (3 ml, 34.8 mmol) was added to a solution of (2,2-dimethyl-1,3-dioxan-5-yl)methyl methanesulfonate (**78**)[40] (900 mg, 4.0 mmol) in DMSO (7 ml) and stirred at 75 °C for 16 h. After cooling the reaction mixture was diluted with $CHCl_3$ and washed with water (×2), dried, filtered, and the filtrate concentrated in vacuo. The crude material was purified by chromatography (CH_2Cl_2 → MeOH/CH_2Cl_2 = 1:4 → 7M NH_3 in MeOH/CH_2Cl_2 = 1:4) to afford **79** (330 mg, 52 %) as an oil. ^1H NMR (CD_3OD): δ 3.94 (dd, *J* = 12.0, 4.4 Hz, 2H), 3.66 (dd, *J* = 12.0, 7.6 Hz, 2H), 2.53 (d, *J* = 6.9 Hz, 2H), 2.36, (s, 3H), 1.89 (m, 1H), 1.38 (s, 3H), 1.37 (s, 3H). ^{13}C NMR (CD_3OD): δ 99.7, 64.4, 64.4, 52.3, 36.9, 36.0, 26.0, 23.5.

7-({[3-Hydroxy-2-(hydroxymethyl)propyl](methyl)amino}methyl)-3,5-dihydro-4*H*pyrrolo[3,2-*d*]pyrimidin-4-one (22)

Acetic acid (0.180 ml, 3.10 mmol) was added dropwise to a solution of compound **79** (100 mg, 0.63 mmol) in 1,4-dioxane (2 ml) followed by 9-deazahypoxanthine (**75**)[10] (170 mg, 1.3 mmol) and the resulting suspension heated at 95 °C (bath temperature) for 16 h. After cooling, silica gel (1 g) was added to the solution and concentrated in vacuo. The resulting residue was purified by chromatography (7M NH_3 in MeOH/CH_2Cl_2 = 1:9) to afford crude **80** (120 mg, 62 %). Concentrated HCl (1 ml) was added to a solution of crude **80** (100 mg, 0.33 mmol) in MeOH (2 ml) and the resulting reaction mixture left to stand for 30 min. After concentration in vacuo, the resulting residue was dissolved in MeOH, silica gel (1 g) was added and the mixture again concentrated in vacuo. The residue was purified by chromatography (7M NH_3 in MeOH/CH_2Cl_2 = 1:4) to afford **22** (80 mg, 92 %) as a solid. ^1H NMR (D_2O): δ 7.92 (s, 1H), 7.64 (s, 1H), 4.39 (s, 2H), 3.62 (dd, *J* = 11.2, 5.0 Hz, 2H), 3.47 (dd, *J* = 11.2, 7.0 Hz, 2H), 3.19 (d, *J* = 6.5 Hz, 2H), 2.83 (s, 3H), 2.28 (septet, *J* = 6.3 Hz, 1H). ^{13}C NMR (D_2O): δ 155.1, 144.4, 143.3, 132.1, 118.0, 104.4, 61.3, 61.3, 56.7, 50.5, 40.9, 38.3. ESI-HRMS for $C_{12}H_{19}N_4O_3$ [MH^+] calcd, 267.1457; found, 267.1449.

N-Benzyl(2,2-dimethyl-1,3-dioxan-5-yl)methanamine (81)

A solution of (2,2-dimethyl-1,3-dioxan-5-yl)methyl methanesulfonate (**78**)[40] (1.70 g, 7.58 mmol) in neat benzylamine (10 ml, 92 mmol) was stirred at 80 °C for 2 h. After cooling to room temperature the solvent was evaporated, the residue dissolved in toluene (containing a small amount of EtOAc) and washed with water, dried, filtered and the filtrate concentrated in vacuo. The resulting residue was purified by chromatography (EtOAc) to afford **81** (1.51 g, 85 %) as a yellow oil. ^1H NMR (CD_3OD): δ 7.32-7.18 (m, 5H), 3.93 (dd, *J* = 12.0, 6.0 Hz, 2H), 3.72 (s, 2H), 3.64 (dd, *J* = 12.0, 6.0 Hz, 2H), 2.54 (d, *J* = 6.0 Hz, 2H), 1.89 (m, 1H), 1.31 (s, 6H). ^{13}C NMR (CD_3OD): δ 140.8, 129.4, 129.4, 128.1, 99.3, 64.0, 64.0, 54.7, 33.7, 25.6, 23.0.

7-({[3-Hydroxy-2-(hydroxymethyl)propyl]amino}methyl)-3,5-dihydro-4*H*-pyrrolo[3,2-*d*] pyrimidin-4-one (27)

Acetic acid (0.122 ml, 2.13 mmol) was added dropwise to a solution of compound **81** (100 mg, 0.43 mmol) in 1,4-dioxane (2 ml) followed by 9-deazahypoxanthine (**75**)[10] (115 mg, 0.85 mmol) and the resulting suspension heated at 95 °C (bath temperature) for 16 h. After cooling to room temperature silica gel (1.0 g) was added and the mixture concentrated in vacuo. The resulting residue was purified by chromatography (7M NH_3 MeOH/CH_2Cl_2 = 1:19) to afford, presumably, compound **82** (56 mg, 35 %) which was committed to the next step without characterization. Concentrated HCl (1 ml) was added to a stirred solution of compound **82** (50 mg, 0.13 mmol) in MeOH (2 ml). After 0.5 h the solution was concentrated in vacuo and the residue dissolved in MeOH to which silica gel was added and then concentrated in vacuo. The residue was purified by chromatography (7M NH_3 in MeOH/CH_2Cl_2 = 1:4) to afford 9-{[(2,2-

dimethyl-1,3-dioxan-5-yl)methylamino]methyl}-9-deazahypoxanthine (19 mg, 0.56 mmol, 42%) as a white solid which was dissolved in H_2O (2 ml), 10% Pd/C (56 mg) added and the mixture was stirred under an atmosphere of hydrogen for 72 h. After filtering through Celite® the filtrate was concentrated in vacuo and the residue purified by chromatography (CH_2Cl_2/MeOH/28% aq NH_4OH = 5:4.5:0.5) to afford **27** (4 mg, 28%) as a white solid. ^1H NMR (D_2O): δ 8.03 (s, 1H), 7.31 (s, 1H), 4.71 (s, 2H), 3.79 (s, 2H), 3.54 (d, *J* = 6.0 Hz, 2H), 2.59 (d, *J* = 6.6 Hz, 2H), 1.83 (septet, *J* = 6.0 Hz, 1H).^{13}C NMR (D_2O): δ 163.5, 151.6, 144.8, 126.4, 118.9, 112.0, 61.2, 61.2, 47.0, 42.2. ESI-HRMS for $C_{11}H_{17}N_4O_3$ [MH^+] calcd, 253.1301; found, 253.1292.

(*R*)-(2,2-Dimethyl-1,3-dioxolan-4-yl)methyl methanesulfonate (85)

The title compound was prepared from (*S*)-(2,2-dimethyl-1,3-dioxolan-4-yl)methanol (**83**) (Sigma-Aldrich, 99% ee) by known literature procedures.[41,42] $[\alpha]_D^{21}$ −3.1 (c 0.83, $CHCl_3$). $[\alpha]_J^{21}$ −3.3 (c 0.83, $CHCl_3$). Lit.[41] $[\alpha]_J^{25}$ −3 (c 0.028, $CHCl_3$). The ^1H and ^{13}C NMRs were in agreement with those reported in the literature.[41, 42]

(*S*)-*N*-Benzyl-1-(2,2-dimethyl-1,3-dioxolan-4-yl)methanamine (87)

The title compound was prepared according to the literature method[41] from compound **85**. $[\alpha]_D^{21}$ +4.0 (c 0.69, $CHCl_3$). Lit.[45] $[\alpha]_D^{20}$ +5.8 (c 1.04, $CHCl_3$). $[\alpha]_J^{21}$ +4.3 (c 0.69, $CHCl_3$). Lit.[41] $[\alpha]_J^{25}$ +5.5 (c 0.054, $CHCl_3$). The ^1H and ^{13}C NMRs were in agreement with those reported in the literature.[41, 42, 45]

7-({Benzyl[(2*S*)-2,3-dihydroxypropyl]amino}methyl)-3,5-dihydro-4*H*-pyrrolo[3,2-*d*]pyrimidin-4-one (89)

To a solution of compound **87** (0.67 g, 3.03 mmol) and compound **44**[8] (0.9 g, 3.03 mmol) in 1,2-dichloroethane (30 ml) was added sodium triacetoxyborohydride (0.834 g, 3.94 mmol) and anhydrous $MgSO_4$ (2 g) and the mixture stirred for 5 h. The reaction mixture was then diluted with CH_2Cl_2, washed with aqueous saturated $NaHCO_3$, brine, dried, filtered and the filtrate concentrated in vacuo. The residue was purified by chromatography (EtOAc/hexanes = 1:1) to afford 7-[(benzyl{[(4*S*)-2,2-dimethyl-1,3-dioxolan-4-yl]methyl}amino)methyl]-3,5-dihydro-4*H*-pyrrolo[3,2-*d*]pyrimidin-4-one (1.18 g, 78 %) as a pale yellow gum. $[\alpha]_D^{21}$ +21.6 (c 0.92, MeOH). 1H NMR ($CDCl_3$): δ 8.55 (s, 1H), 7.41 (s, 1H), 7.37-7.18 (m, 10H), 5.73 (s, 2H), 4.46 (s, 2H), 4.38-4.29 (m, 1H), 4.10, (s, 3H), 4.05-3.98 (m, 2H), 3.92 (d, *J* = 14.4 Hz, 1H), 3.79 (d, *J* = 13.9 Hz, 1H), 3.63 (d, *J* = 13.9 Hz, 1H), 3.53, (t, *J* = 7.9 Hz, 1H), 2.73 (dd, *J* = 13.3, 5.9 Hz, 1H), 2.64 (dd, *J* = 13.3, 5.9 Hz, 1H), 1.32 (s, 6H). ^{13}C NMR ($CDCl^3$, center line at δ 77.0): δ 156.2, 150.7, 150.0, 139.6, 136.9, 132.2, 128.8, 128.4, 128.2, 128.0, 127.7, 126.9, 115.7, 114.7, 109.0, 77.0, 74.7, 70.1, 68.4, 59.2, 56.2, 53.5, 47.8, 26.8, 25.6. ESI-HRMS for $C_{29}H_{35}N_4O_4$ [MH^+] calcd, 503.2658; found, 503.2635. 7-[(Benzyl{[(4*S*)-2,2-dimethyl-1,3-dioxolan-4-yl]methyl}amino)methyl]-3,5-dihydro-4*H*-pyrrolo[3,2-*d*]pyrimidin-4-one (1.1 g, 2.189 mmol) was heated to 100 °C in concentrated HCl (15 ml) for 3 h. After cooling to room temperature the solution was evaporated in vacuo and the residue dissolved in MeOH, neutralized with Amberlyst A-21 resin, filtered and the filtrate concentrated in vacuo. The residue was purified by chromatography (CH_2Cl_2/7M NH_3 MeOH = 9:1 → 85:15) to afford **89** (0.427 g, 59%) as a colorless solid. $[\alpha]_D^{20}$ −12.7 (c 0.715, MeOH). ^1H NMR (CD_3OD): δ 7.90 (s, 1H), 7.37 (s, 1H), 7.31-7.16 (m, 5H), 3.97-3.78 (m, 3H), 3.69 (d, *J* = 13.8 Hz, 1H), 3.63 (d, *J* = 13.8 Hz, 1H), 3.50 (dd, *J* = 11.2, 4.8 Hz, 1H), 3.42 (dd, *J* = 11.2, 5.7 Hz, 1H), 2.60 (d, *J* = 6.4 Hz, 2H). ^{13}C NMR (CD_3OD, center line at δ 49.0): δ 156.1, 145.5, 142.5, 140.2, 130.2, 129.4, 129.2, 128.1, 119.3, 115.0, 70.6, 66.3, 60.0, 57.6, 49.0. ESI-HRMS for $C_{17}H_{21}N_4O_3$ [MH^+] calcd, 329.1614; found, 329.1618.

7-({[(2*S*)-2,3-Dihydroxypropyl]amino}methyl)-3,5-dihydro-4*H*-pyrrolo[3,2-*d*]pyrimidin-4-one (23) and its hydrochloride salt (23.HCl)

Compound **89** (100 mg, 0.305 mmol) was dissolved in MeOH (10 ml), diluted with water (10 ml) and 10% Pd-C (50 mg) added and the resulting mixture stirred under an atmosphere of hydrogen at room temperature for 45 min. The reaction mixture was filtered through Celite® and the filtrate concentrated in vacuo. The crude residue was purified by chromatography (CH_2Cl_2/MeOH/28% aq NH_4OH = 5:4.5:0.5) to give **23** as a colorless solid which was converted with 5% aqueous HCl to **23.HCl** as a colorless foam and crystallized from MeOH (35 mg, 42%) as a white solid. M.p. 241–242 °C. $[\alpha]_D^{20}$ −12.9 (c 0.535, H_2O). ^{1}H NMR (D_2O + DCl): δ 9.07 (s, 1H), 7.91 (s, 1H), 4.53 (s, 2H), 4.06 (m, 1H), 3.69-3.57 (m, 2H), 3.33 (dd, *J* = 12.9, 2.9 Hz, 1H), 3.17 (dd, *J* = 12.9, 9.8 Hz, 1H). ^{13}C NMR (D_2O + DCl): δ 153.8, 146.1, 134.1, 133.1, 119.6, 104.1, 68.5, 64.5, 50.2, 41.8. ESI-HRMS for $C_{10}H_{15}N_4O_3$ [MH^+] calcd, 239.1144; found, 239.1136.

(*S*)-(2,2-Dimethyl-1,3-dioxolan-4-yl)methyl methanesulfonate (86)

Compound **86** was prepared from (*R*)-(2,2-dimethyl-1,3-dioxolan-4-yl)methanol (**84**) (Sigma-Aldrich, 99% ee) as described for its enantiomer, **85**. $[\alpha]_D^{25}$ +3.1 (c 0.72, $CHCl_3$). The ^{1}H and $_{13}$C NMRs were identical to those of the enantiomer, compound **85**.

(*R*)-*N*-Benzyl-1-(2,2-dimethyl-1,3-dioxolan-4-yl)methanamine (88)

A solution of compound **86** (3.0 g, 14.27 mmol) and benzylamine (6.23 ml, 57.1 mmol) were refluxed together in CH_3CN (38 ml) for 48 h. After cooling to room temperature the solvent was evaporated and the residue dissolved in EtOAc and washed with aqueous saturated $NaHCO_3$, dried, filtered and the filtrate concentrated in vacuo. The resulting residue was purified by chromatography (EtOAc/hexanes = 6:4 → 8:2) to afford **88** (2.56 g, 81%) as a yellow oil. $[\alpha]_D^{21}$ −3.7 (c 0.885, $CHCl_3$). The ^{1}H and ^{13}C NMRs were identical to those of the enantiomer, compound **87**.

7-({Benzyl[(2*R*)-2,3-dihydroxypropyl]amino}methyl)-3,5-dihydro-4*H*-pyrrolo[3,2-*d*] pyrimidin-4-one (37)

Compound **88** (372 mg, 1.68 mmol) was converted into 7-[(benzyl{[(4*R*)-2,2-dimethyl-1,3-dioxolan-4-yl]methyl}amino)methyl]-3,5-dihydro-4*H*pyrrolo[3,2-*d*]pyrimidin-4-one (631 mg, 75%) as a colorless gum as described for its enantiomer in the preparation of **89**. $[\alpha]_D^{21}$ −21.9 (c 0.905, MeOH). The ^{1}H and ^{13}C NMRs were identical to those of the *S* enantiomer. ESI-HRMS for $C_{29}H_{35}N_4O$ [MH^+] calcd, 503.2658; found, 503.2643. 7-[(Benzyl{[(4*R*)-2,2-dimethyl-1,3-dioxolan-4-yl]methyl}amino)methyl]-3,5-dihydro-4*H*pyrrolo[3,2-*d*] pyrimidin-4-one (600 mg, 1.19 mmol) was converted, as described for the Senantiomer in the preparation of **89**, into **37** (360 mg, 92 %) as a colorless solid. $[\alpha]_D^{22}$ +13.0 (c 0.715, MeOH). The ^{1}H and ^{13}C NMRs were identical to those of the enantiomer, compound **89**. ESI-HRMS for $C_{17}H_{21}N_4O_3$ [MH^+] calcd, 329.1614; found, 329.1600.

7-({[(2*R*)-2,3-Dihydroxypropyl]amino}methyl)-3,5-dihydro-4*H*-pyrrolo[3,2-*d*]pyrimidin-4-one (28) and its hydrochloride salt (28.HCl)

Compound **37** (100 mg, 0.31 mmol) was dissolved in hot water (20 ml), cooled to room temperature and 10% Pd-C (50 mg) added and the resulting mixture stirred under an atmosphere of hydrogen at room temperature for 4 h. The hydrogen was replaced with Ar and the mixture heated to 80 °C to ensure product was in solution and then the mixture was filtered through Celite® and the filtrate concentrated in vacuo to afford **28** (72 mg, 99%) as a colorless solid. Compound **28** was treated with 5% aqueous HCl to afford **28.HCl**. $[\alpha]_D^{18}$ +12.6 (c 0.565,

H_2O). The ^1H and ^{13}C NMRs were identical to those of the enantiomer, compound **23**. ESI-HRMS for $C_{10}H_{14}N_4O_3Na$ [MNa$^+$] calcd, 261.0964; found, 261.0952.

5-(Benzyloxymethyl)-7-({[(4*S*)-2,2-dimethyl-1,3-dioxolan-4-yl](2-hydroxyethyl)amino}methyl)-4-methoxy-5*H*-pyrrolo[3,2-*d*]pyrimidine (90)

Potassium carbonate (125 mg, 0.904 mmol) was added to a solution of compound **87** (200 mg, 0.90 mmol) and 2-bromoethanol (96 µl, 1.36 mmol) in CH_3CN (4 ml) and the mixture heated under reflux for 64 h. After cooling to room temperature the mixture was diluted with EtOAc and washed with saturated $NaHCO_3$, dried, filtered and the filtrate concentrated in vacuo. The resulting residue was purified by chromatography (EtOAc/hexanes = 1:1 → 7:3) to afford (*S*)-2-[benzyl(2,2-dimethyl-1,3-dioxolan-4-yl)methylamino]ethanol (172 mg, 72 %) which was dissolved in MeOH (5 ml), 10% Pd-C (50 mg) was added and the mixture stirred under an atmosphere of hydrogen for 1 h. The mixture was then filtered through Celite® and the filtrate concentrated in vacuo. The resulting residue was purified by chromatography (CH_2Cl_2/MeOH/28% aq NH_4OH = 97:3:0.5 → 9:1:0.1) and the product obtained distilled on a kugelrohr apparatus at 120 ° C/0.05 mmHg to afford (*S*)-2-[(2,2-dimethyl-1,3-dioxolan-4-yl)methylamino]ethanol (70 mg, 62%) as a colorless gum. $[\alpha]_D^{20}$ −9.6 (c 0.555, MeOH). ^1H NMR (CD_3OD): δ 4.24 (pentet, *J* = 6.2 Hz, 1H), 4.06 (dd, *J* = 8.2, 6.3 Hz, 1H), 3.71-3.59 (m, 3H), 2.76-2.66 (m, 4H), 1.39 (s, 3H), 1.33 (s, 3H). ^{13}C NMR (CD_3OD, centre line at δ 49.0): δ 110.4, 76.4, 68.6, 61.6, 53.4, 52.5, 27.3, 25.7. ESI-HRMS for $C_8H_{18}NO_3$ [MH$^+$] calcd, 176.1287; found, 176.1274. (*S*)-2-[(2,2-Dimethyl-1,3-dioxolan-4-yl)methylamino]ethanol (48 mg, 0.27 mmol), $MgSO_4$ (500 mg), aldehyde **44**[8] (81 mg, 0.27 mmol) and sodium triacetoxyborohydride (75 mg, 0.36 mmol) were stirred together at room temperature in 1,2-dichloroethane (3 ml) for 16 h. The mixture was then diluted with CH_2Cl_2 and washed with saturated aqueous $NaHCO_3$ solution, dried, filtered and the filtrate concentrated in vacuo. The resulting residue was purified by chromatography (EtOAc → EtOAc/MeOH/28% aq NH_4OH = 97:3:0.01) to afford **90** (100 mg, 80%) as a colorless gum which turned pale yellow on standing. ^1H NMR ($CDCl_3$): δ 8.53 (s, 1H), 7.36-7.23 (s, 6H), 5.71 (s, 2H), 4.60 (br s, exchanged to D_2O, 1H), 4.46 (s, 2H), 4.20 (pentet, *J* = 6.3 Hz, 1H), 4.10 (s, 3H), 4.02 (d, *J* = 14.1 Hz, 1H), 3.88-3.67 (m, 4H), 3.26 (dd, *J* = 8.0, 6.9 Hz, 1H), 2.86-2.70 (m, 3H), 2.61 (dd, *J* = 13.2, 6.7 Hz, 1H), 1.32 (s, 3H), 1.28 (s, 3H). ^{13}C NMR ($CDCl_3$, center line at δ 77.0): δ 156.3, 150.2, 149.9, 136.8, 131.5, 128.5, 128.0, 127.7, 116.2, 115.1, 108.9, 76.9, 74.2, 70.1, 68.4, 59.9, 57.2, 56.5, 53.6, 47.8, 26.8, 25.4. ESI-HRMS for $C_{24}H_{33}N_4O_5$ [MH$^+$] calcd, 457.2451; found, 457.2469.

NIH-PA Author Manuscript

7-({[(2*S*)-2,3-Dihydroxypropyl](2-hydroxyethyl)amino}methyl)-3,5-dihydro-4*H*-pyrrolo[3,2- *d*]pyrimidin-4-one (32)

Compound **90** (100 mg, 0.22 mmol) was dissolved in concentrated HCl (4 ml) and heated at 100 °C for 1.5 h and then concentrated in vacuo. The residue was dissolved in MeOH and a little water and the solution neutralized with Amberlyst A-21 resin. The mixture was filtered, the filtrate concentrated in vacuo, and the residue purified by chromatography (*i*PrOH/H_2O/28 % aq NH_4OH = 9:0.5:0.5 → 8:1.5:0.5) to afford **32** (42 mg, 68%) as a colorless solid. $[\alpha]_D^{20}$ −17.9 (c 0.545, H_2O). ^1H NMR (D_2O + NaOD, internal acetonitrile at δ 2.06): δ 8.07 (s, 1H), 7.35 (s, 1H), 3.94-3.65 (m, 3H), 3.68 (t, *J* = 6.1 Hz, 2H), 3.50 (dd, *J* = 11.7, 4.2 Hz, 1H), 3.40 (dd, *J* = 11.6, 6.3 Hz, 1H), 2.75-2.48 (m, 4H). ^{13}C NMR (D_2O, internal acetone at δ 31.5) δ 156.3, 145.4, 143.7, 132.0, 118.6, 109.2, 68.9, 65.1, 58.5, 56.7, 56.3, 48.8. ESI-HRMS for $C_{12}H_{19}N_4O_4$ [MH$^+$] calcd, 283.1406; found, 283.1404.

NIH-PA Author Manuscript

5-(Benzyloxymethyl)-7-({[(4*R*)-2,2-dimethyl-1,3-dioxolan-4-yl](2- hydroxyethyl)amino} methyl)-4-methoxy-5H-pyrrolo[3,2-*d*]pyrimidine (91)

In the same way as that described for the preparation of **90**, compound **88** (200 mg, 0.94 mmol) was converted first into intermediate (R)-2-[(2,2-dimethyl-1,3-dioxolan-4-yl)methylamino] ethanol (103 mg, 78 %) $[\alpha]_D^{20}$ +9.5 (c, 0.525, MeOH). The ^{1}H and ^{13}C NMRs were identical to those of the enantiomer. ESI-HRMS for $C_8H_{18}NO_3$ [MH$^+$] calcd, 176.1287; found, 176.1278. Reductive alkylation of this intermediate then afforded **91** (180 mg, 86 %) as a colorless gum. ^{1}H and ^{13}C NMRs were identical to those of **90**, its enantiomer. ESI-HRMS for $C_{24}H_{33}N_4O_5$ [MH$^+$] calcd, 457.2451; found, 457.2467.

7-({[(2R)-2,3-Dihydroxypropyl](2-hydroxyethyl)amino}methyl)-3,5-dihydro-4*H*-pyrrolo[3,2-*d*] pyrimidin-4-one (34)

Compound **91** (165 mg, 0.36 mmol) was converted into **34** (74 mg, 73 %) as a colorless solid as described for the preparation of **32**. $[\alpha]_D^{20}$ +18.4 (c, 0.56, H_2O). The ^{1}H and ^{13}C NMR data were identical to those of **32** its enantiomer. ESI-HRMS for $C_{12}H_{19}N_4O_4$ [MH$^+$] calcd, 283.1406; found, 283.1398.

(2*S*,3*R*)-*O*-Isopropylidene-4-hydroxybutylamine (93)

A solution of 2,3-*O*-isopropylidene-D-erythronamide (**92**)[43] (2.80 g, 16.0 mmol) in anhydrous THF (45 ml) was added dropwise over 30 min to a stirred suspension of LAH (2.43 g, 64.0 mmol) in THF (40 ml) at such a rate as to maintain the reaction at room temperature. The resulting mixture was then heated under reflux for 16 h and then cooled to room temperature and worked up by the cautious addition of water (2.5 ml), 15% aqueous NaOH (2.5 ml), and more water (7.5 ml). The resulting suspension was filtered through Celite® and the filtrate concentrated in vacuo to afford **92** (2.17 g, 84 %) as a mobile syrup. ^{1}H NMR ($CDCl_3$): δ 4.31 (td, *J* = 6.7, 4.2 Hz, 1H), 4.23 (td, *J* = 6.7, 3.9 Hz, 1H), 3.76 (dd, *J* = 12.0, 7.0 Hz, 1H), 3.69 (dd, *J* = 12.0 Hz, 4.1 Hz, 1H), 3.06 (dd, *J* = 12.6, 7.1 Hz, 1H), 2.95 (dd, *J* = 12.6, 3.9 Hz, 1H), 2.72 (brs, 3H), 1.45 (s, 3H), 1.36 (s, 3H). ^{13}C NMR ($CDCl_3$); δ 108.3, 77.6, 77.5, 60.8, 41.2, 27.8, 25.3.

5-Benzyloxymethyl-7-[({[(4*S*,5*R*)-5-(hydroxymethyl)-2,2-dimethyl-1,3-dioxolan-4- yl]methyl} amino)methyl]-4-methoxy-5*H*-pyrrolo[3,2-*d*]pyrimidine (94)

To a mixture of compound **44**[8] (390 mg, 1.30 mmol) and compound **93** (210 mg, 1.30 mmol) in 1,2-dichloroethane (8 ml) containing anhydrous $MgSO_4$ (500 mg) was added, in a single portion, sodium triacetoxyborohydride (360 mg, 1.70 mmol) and the resulting reaction stirred overnight at room temperature. The mixture was diluted with CH_2Cl_2 and washed with saturated aqueous $NaHCO_3$ before being dried, filtered and concentrated to dryness. The resulting residue was purified by chromatography (MeOH/EtOAc = 2:98 → 1:19) to afford **94** (230 mg, 40%) as a syrup. ^{1}H ($CDCl_3$): δ 8.45 (s, 1H), 7.28 (s, 1H), 7.26-7.13 (m, 5H), 5.62 (s, 2H), 4.41 (s, 2H), 4.30-4.21 (m, 2H), 4.03 (s, 3H), 3.95 (s, 2H), 3.88 (br s, 2H), 3.67 (dd, *J* = 12.0, 7.4 Hz, 1H), 3.59 (dd, *J* = 12.0, 3.7 Hz, 1H), 2.93 (dd, *J* = 12.2, 7.6 Hz, 1H), 2.83 (dd, *J* = 12.2, 3.1 Hz, 1H), 1.34 (s, 3H), 1.26 (s, 3H). ^{13}C NMR ($CDCl_3$): δ 156.7, 150.4, 150.3, 137.3, 131.5, 128.8, 128.3, 128.0, 116.3, 115.1, 108.3, 77.9, 77.4, 76.2, 70.6, 60.8, 53.9, 48.4, 43.5, 27.8, 25.3. ESI-HRMS for $C_{23}H_{31}N_4O_5$ [MH$^+$] calcd, 443.2294; found, 443.2327.

7-({[(2*S*,3*R*)-2,3,4-Trihydroxybutyl]amino}methyl)-3,5-dihydro-4*H*-pyrrolo[3,2-*d*]pyrimidin-4-one (30) and its hydrochloride (30.HCl)

Concentrated HCl (10 ml) was added to compound **94** (480 mg, 1.08 mmol) and the resulting solution heated at reflux for 2 h. After cooling to room temperature the solvent was concentrated in vacuo and the residue dissolved in MeOH (10 ml) and water (2 ml). The solution was neutralized with Amberlyst A-21 resin and then filtered and concentrated in vacuo onto silica (1 g). The residue was purified by chromatography (CH_2Cl_2/MeOH/28% aq NH_4OH = 5:4:1) to afford **30** (61 mg, 21%) as a white solid. ^1H NMR (500 MHz, D_2O/DCl): δ 8.87 (s, 1H), 7.71 (s, 1H), 4.32 (s, 2H), 3.77 (m, 1H), 3.54 (dd, *J* = 11.6, 3.4 Hz, 1H), 3.49 (m, 1H), 3.42 (dd, *J* = 11.6, 5.9 Hz, 1H), 3.24 (dd, *J* = 13.0, 3.1 Hz, 1H), 3.01 (dd, *J* = 13.0, 9.6 Hz, 1H). ^{13}C NMR (D_2O/DCl): δ 152.5, 144.9, 132.9, 131.8, 118.3, 102.8, 73.0, 67.1, 62.1, 49.0, 40.6. ESI-HRMS for $C_{11}H_{17}N_4O_4$ [MH^+] calcd, 269.1250; found, 269.1242. Anal. ($C_{11}H_{16}N_4O_4$) C, H, N.

Ethyl methyl (*2R/S*,3*R/S*)-2,2-dimethyl-1,3-dioxolane-4,5-dicarboxylate (95)

To a solution containing (±)-diethyl tartrate (2.0 g, 9.7 mmol) and dimethoxypropane (2.0 g, 19.4 mmol) in benzene (30 ml) was added TsOH (0.1 g) and the mixture refluxed for 3 h. After cooling to room temperature the solution was diluted with EtOAc (200 ml) and washed with a saturated brine/sodium bicarbonate solution, and then dried, filtered and the filtrate concentrated in vacuo to afford (±)-**95** (1.78 g, 79%) as a mobile liquid, a sample of which was purified by Kugelrohr distillation (120–140 °C/18 mmHg). 1H NMR ($CDCl_3$): δ 4.79-4.74 (m, 2H), 4.26 (q, *J* = 7.1 Hz, 2H), 3.81 (s, 3H) 1.48 (s, 6H), 1.32 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR ($CDCl_3$): δ 170.4, 170.0, 114.1, 77.6, 77.4, 62.3, 53.1, 26.7, 14.4.

Methyl (2*R/S*,3*R/S*)-5-carbamoyl-2,2-dimethyl-1,3-dioxolane-4-carboxylate (96)

Compound (±)-**95** (1.78 g, 7.66 mmol) was dissolved in MeOH (10 ml) and stirred at room temperature as methanolic ammonia (1.09 ml, 7.66 mmol, 7M) was added. The solution was stirred for two days at room temperature before silica gel (10 g) was added the mixture concentrated in vacuo. The resulting residue was purified by chromatography (EtOAc/hexanes = 1:1) to afford (±)-**96** (0.67 g, 43%) as an oil that solidified on standing. 1H NMR ($CDCl_3$): δ 6.52 (br s, 1H), 6.34 (br s, 1H), 4.73 (s, 2H), 3.84 (s, 3H), 1.52 (s, 3H), 1.48 (s, 3H). ^{13}C NMR ($CDCl_3$): δ 173.0, 170.9, 113.9, 77.8, 77.4, 53.2, 27.0, 26.5. ESI-HRMS for $C_8H_{13}NO_5Na$ [MNa^+] calcd, 226.0691; found 226.0696.

5-Benzyloxymethyl-7-[({[(4*R/S*,5*R/S*)-5-(hydroxymethyl)-2,2-dimethyl-1,3-dioxolan-4- yl]methyl}amino)methyl]-4-methoxy-5*H*-pyrrolo[3,2-*d*]pyrimidine (98)

Compound (±)-**96** (830 mg, 4.08 mmol) was dissolved in anhydrous THF (10 ml) and added dropwise to a suspension of LAH (608 mg, 16.0 mmol) in THF (60 ml) at room temperature. The resulting suspension was heated under reflux for 4 h and after cooling to 0 °C quenched cautiously with water (0.7 ml), 15% aqueous NaOH (0.7 ml), and water (2 ml) again. The resulting suspension was filtered through Celite® and the filtrate concentrated in vacuo to afford (±)-**97** (600 mg, 91%) as a colorless syrup that was used in the next step without purification. To a mixture of compound **44**[8] (590 mg, 1.98 mmol) and compound (±)-**97** (410 mg, 2.54 mmol) in 1,2- dichloroethane (10 ml) containing anhydrous $MgSO_4$ (1.0 g) was added, in a single portion, sodium triacetoxyborohydride (700 mg, 3.31 mmol) and the resulting mixture stirred for 16 h at room temperature. The mixture was then diluted with CH_2Cl_2 and washed with saturated aqueous $NaHCO_3$ and then dried, filtered and the filtrate concentrated in vacuo. The crude residue was purified by chromatography (MeOH/EtOAc = 2:98 → 1:19) to afford (±)-**98** (650 mg, 58%) as an immobile, colorless syrup. ^1H NMR ($CDCl_3$): δ 8.45 (s, 1H), 7.28 (s, 1H), 7.27-7.13 (m, 5H), 5.63 (s, 2H), 4.41 (s, 2H), 4.03 (s, 3H), 3.95 (s, 2H), 3.80-3.69 (m, 3H), 3.49 (m, 1H), 3.21 (br s, 2H), 3.07 (dd *J* = 12.1, 3.0 Hz, 1H), 2.63 (m, 1H), 1.32 (s, 3H), 1.31 (s, 3H). ^{13}C ($CDCl_3$): δ 156.7, 150.5, 150.3, 137.3, 131.5, 128.8, 128.4, 128.1, 116.3, 115.2, 108.9, 82.2, 80.1, 77.4, 70.6, 62.8, 54.0, 50.6, 43.7, 27.3, 27.1. ESI-HRMS for $C_{23}H_{30}N_4O_5Na$ [MNa^+] calcd, 465.2114; found, 465.2144.

7-({[(2*R*/*S*,3*R*/*S*)-2,3,4-Trihydroxybutyl]amino}methyl)-3,5-dihydro-4*H*-pyrrolo[3,2- *d*]pyrimidin-4-one (31) and its trifluoroacetate salt (31.TFA)

A solution of compound (±)-**98** (480 mg, 1.09 mmol) in concentrated HCl (10 ml) was heated at reflux for 3 h. After cooling to room temperature the solution was evaporated and the crude residue dissolved in MeOH (10 ml) containing water (3 ml) and neutralized with Amberlyst A-21 resin. The resin was removed by filtration and the filtrate concentrated in vacuo to afford (±)-**31** (110 mg, 38%) as an immobile syrup, a portion of which was purified by preparative HPLC to afford (±)-**31.TFA**. ^{1}H NMR (CD_3OD): δ 8.08 (s, 1H), 7.55 (s, 1H), 4.32 (s, 2H), 3.93 (septet, *J* = 2.8 Hz, 1H), 3.59-3.44 (m, 1H), 3.53 (s, 2H), 3.14 (d, *J* = 5.9 Hz, 2H). ^{13}C NMR (CD_3OD): δ 161.5 (q, *J* = 38 Hz), 161.2, 160.7, 155.6, 144.8, 143.3, 131.6, 120.1, 117.6 (q, *J* = 283 Hz), 107.7, 74.3, 71.1, 63.9, 51.0, 42.3. ESI-HRMS for $C_{11}H_{17}N_4O_4$ [MH^+] calcd, 269.1250; found, 269.1241.

(1*R*)-1-[(4*R*,5*S*)-5-(Aminomethyl)-2,2-dimethyl-1,3-dioxolan-4-yl]ethane-1,2-diol (100)

To a stirred suspension of LAH (7.1 g, 0.187 mol) in anhydrous THF (80 ml) was added, dropwise at room temperature, a solution of 2,3-*O*-isopropylidene D-ribonamide (**99**)[43] (3.3 g, 16.1 mmol) in THF (90 ml). After the addition was complete the mixture was heated under reflux and maintained as such for 20 h. After cooling to 0 °C the reaction was quenched cautiously with water (7 ml), 15% aqueous NaOH (7 ml), and water (21 ml) again. The resulting suspension was filtered through Celite® and the inorganics washed successively with small volumes of warm ethyl acetate. The combined filtrates were concentrated in vacuo to afford **100** (1.45 g, 47 %) as an immobile syrup. ^{1}H NMR ($CDCl_3$): δ 4.17 (m, 2H), 3.83 (m, 3H), 3.77 (m, 1H), 3.24 (br d, 3H), 3.02 (m, 2H), 1.40 (s, 3H), 1.33 (s, 3H). ^{13}C NMR ($CDCl_3$): δ 108.8, 78.2, 78.0, 69.6, 65.0, 41.7, 28.3, 25.7. ESI-HRMS for $C_8H_{18}NO_4$ [MH^+] calcd, 192.1236; found, 192.1237.

7-({[(2*S*,3*S*,4*R*)-2,3,4,5-Tetrahydroxypentyl]amino}methyl)-3,5-dihydro-4*H*-pyrrolo[3,2-*d*]pyrimidin-4-one (35) and its hydrochloride salt (35.HCl)

To a mixture containing **100** (210 mg, 1.08 mmol), **44**[8] (320 mg, 1.08 mmol), and anhydrous $MgSO_4$ (600 mg) in 1,2-dichloroethane (8 ml) was added, in a single portion, sodium triacetoxyborohydride (310 mg, 1.40 mmol) and the resulting reaction mixture was then stirred for 16 h at room temperature.After dilution with CH_2Cl_2 (50 ml) and a small volume of water, the solution was washed with saturated aqueous $NaHCO_3$ solution and then dried, filtered and the filtrate concentrated in vacuo. The residue was purified by chromatography (MeOH/EtOAc = 7:93) to afford crude **101** (160 mg, 32%) as an immobile syrup. A solution of **101** (160 mg, 0.30 mmol) in concentrated HCl was heated under reflux for 2 h. After cooling to room temperature the solvent was evaporated and the residue dissolved in MeOH (2 ml) and H_2O (10 ml) and then neutralized with Amberlyst A-21 resin. The resin was removed by filtration and silica added to the filtrate and the resulting mixture concentrated in vacuo. The resulting residue was purified by chromatography (CH_2Cl_2/MeOH/28% aq NH_4OH = 5:4:1) to afford **35** (12 mg, 12 %) as a colorless powder. ^{1}H NMR (500 MHz, D_2O/DCl): ^{1}H NMR (500 MHz, D_2O/DCl): δ 8.90 (s, 1H), 7.81 (s, 1H), 4.44 (s, 2H), 4.08 (m, 1H), 3.72-3.59 (m, 3H), 3.55 (dd, *J* = 11.5, 6.1 Hz, 1H), 3.32 (dd, *J* = 12.7, 1.8 Hz, 1H), 3.18 (dd, *J* = 12.7, 9.6 Hz, 1H). ^{13}C NMR (125.7 MHz, D^2O/DCl): δ 155.4, 147.3, 135.6, 135.3, 120.9, 105.7, 75.4, 74.4, 69.8, 65.1, 50.7, 43.1. ESI-HRMS for $C_{12}H_{19}N_4O_5$ [MH^+] calcd, 299.1355; found, 299.1347.

(2*R*/*S*,4*R*/*S*)-(2-Phenyl-1,3-dioxan-4-yl)methanol (103)

A mixture of (±)-1,2,4-butanetriol (**102**) (3.0 g, 28.3 mmol) and benzaldehyde (11.48 ml, 113 mmol) in dry toluene (50 ml) with TsOH (0.269 g, 1.413 mmol) was heated under reflux in a Dean-Stark apparatus. After 1 h, the solution was washed with saturated aqueous NaHCO3, and then dried, filtered and the filtrate concentrated in vacuo. The resulting residue was purified by chromatography to afford (±)-**103** (2.88 g, 53%) as a syrup. The ^{1}H and ^{13}C NMR spectra were the same as those previously reported.[46]

(2*R/S*,4*R/S*)-*N*-Methyl-1-(2-phenyl-1,3-dioxan-4-yl)methanamine (104)

To a solution of (±)-**103** (0.80 g, 4.12 mmol) in CH_2Cl_2 (20 ml) was added *N*,*N*-diisopropylethylamine (1.702 ml, 10.30 mmol) and the solution was cooled in an ice bath. Methanesulfonyl chloride (0.414 ml, 5.35 mmol) was added and the solution was allowed to warm to room temperature. After 1 h, the solution was washed with 2M aqueous HCl, saturated aqueous NaHCO3, dried, filtered and the filtrate concentrated in vacuo to a syrup (1.15 g). A solution of this syrup (0.9 g, 3.30 mmol) in DMSO (8 ml) containing 40% aqueous methylamine (2.85 ml, 33.0 mmol) was stoppered and heated at ~ 75–80 °C for 24 h. After cooling the solution to room temperature $CHCl_3$ was added and the mixture washed twice with water, dried, filtered and the filtrate concentrated in vacuo. The resulting residue was purified by chromatography to afford (±)-**104** (0.465 g, 68%) as a syrup. ^{1}H NMR ($CDCl_3$): δ 7.50-7.47 (m, 2H), 7.39-7.30 (m, 3H), 5.52 (s, 1H), 4.78 (m, 1H), 4.09-3.93 (m, 2H), 2.80 (dd, *J* = 12.3, 8.0 Hz, 1H), 2.67 (dd, *J* = 12.3, 3.4 Hz, 1H), 2.45 (s, 3H), 1.96-1.83 (m, 2H), 1.49 (dd, *J* = 13.2, 1.2 Hz, 1H). ^{13}C NMR (CDCl3): δ 139.0, 129.2, 128.6, 126.5, 101.6, 76.8, 67.2, 57.0, 36.8, 29.5.

(2*R/S*,4*R/S*)-7-({Methyl[(2-phenyl-1,3-dioxan-4-yl)methyl]amino}methyl)-3,5-dihydro-4*H*pyrrolo[3,2-*d*]pyrimidin-4-one (105)

A mixture of (±)-**104** (368 mg, 1.78 mmol), 9- deazahypoxanthine (**75**)[10] (288 mg, 2.13 mmol), acetic acid (0.508 ml, 8.88 mmol) and 37% aqueous formaldehyde (0.396 ml, 5.33 mmol) in 1,4-dioxane (10 ml) was stirred and heated in a stoppered flask at 80 °C for 24 h. After cooling to room temperature the solution was concentrated in vacuo and the resulting residue purified by chromatography (7M NH_3 in MeOH/CH_2Cl_2 = 1:9) to afford the product and 9-deazahypoxanthine (**75**)[10] as an inseparable mixture. Further chromatography ($CHCl_3$/EtOAc/MeOH = 5:2:1 → 4:2:3 → 7M NH_3 in MeOH/CH_2Cl_2 = 1:4) of this material afforded (±)-**105** (406 mg, 65%) as a white solid. ^{1}H NMR ($CDCl_3$/CD_3OD): δ 7.84 (s, 1H), 7.48-7.45 (m, 2H), 7.39 (s, 1H), 7.37-7.28 (m, 3H), 5.56 (s, 1H), 4.30-4.11 (m, 2H), 4.02 (dt, *J* = 11.9, 2.5 Hz, 1H), 3.91 (d, *J* = 13.8 Hz, 1H), 3.83 (d, *J* = 13.8 Hz, 1H), 2.73 (dd, *J* = 13.5, 7.5 Hz, 1H), 2.57 (dd, *J* = 13.5, 3.4 Hz, 1H), 2.39 (s, 3H), 1.74 (dd, *J* = 12.5, 4.9 Hz, 1H), 1.53 (d, *J* = 12.4 Hz, 1H). ^{13}C NMR ($CDCl_3$/CD_3OD): δ 156.8, 146.2, 143.2, 140.6, 130.6, 130.5, 129.8, 128.0, 119.7, 113.9, 103.0, 77.4, 68.7, 62.5, 52.0, 44.3, 31.5.

7-({[(2*R/S*)-2,4-Dihydroxybutyl](methyl)amino}methyl)-3,5-dihydro-4*H*-pyrrolo[3,2-*d*]pyrimidin-4-one (36)

A solution of (±)-**105** (70 mg, 0.20 mmol) in MeOH (2 ml) and concentrated aqueous HCl (2 ml) was allowed to stand for 2 h at room temperature. The solution was diluted with water, extracted with $CHCl_3$ (×2), and the aqueous phase concentrated in vacuo. The resulting residue was purified by chromatography (CH_2Cl_2/MeOH/28% aq NH_4OH = 5:4:1) to afford (±)-**36** (40 mg, 76%) as a white solid. ^{1}H NMR (D_2O): δ 8.00 (s, 1H), 7.71 (s, 1H), 4.51 (d, *J* = 14.0 Hz, 1H), 4.44 (d, *J* = 14.0 Hz, 1H), 4.19 (br s, 1H), 3.68 (t, *J* = 6.4 Hz, 2H), 3.17 (br s, 2H), 2.86 (s, 3H), 1.71-1.61 (m, 2H). ^{13}C NMR (D_2O): δ 155.3, 144.6, 143.4, 132.4, 118.2, 104.2, 63.1, 60.0, 58.0, 50.0, 40.2, 36.8. ESI-HRMS for $C_{12}H_{19}N_4O_3$ [MH^+] calcd, 267.1457; found, 267.1449.

Inhibition Assays

Human PNP was expressed as an N-terminal histidine-tagged fusion protein, as previously described.[32] PNP activity assays in the presence of inhibitors were monitored by a xanthine-oxidase coupled assay, as previously described.[5] Inosine (K_m = 40 μM), inhibitor, and protein concentrations were determined spectrophotometrically using an ε_{248} of 12.3 mM^{-1} cm^{-1} (pH 6),[47] an ε_{261} of 9.54 mM^{-1} cm^{-1} (pH 7),[48] and an ε_{280} of 31.65 mM^{-1} cm^{-1},[49] respectively. In most cases, the inhibitor concentration was at least tenfold greater than the enzyme concentration, as required for simple analysis of slow-onset tight-binding inhibition. In the few cases of particularly tight binding where the inhibitor-enzyme ratio was less than ten, the concentration of free inhibitor, [*I*], was corrected by Equation 1:

$$[I]=[I]_{\mathrm{total}}-(1-v_{\mathrm{i}}/v_{\mathrm{o}})\times[E]_{\mathrm{total}} \quad (1)$$

where $[I]_{\mathrm{total}}$ and $[E]_{\mathrm{total}}$ are the total concentrations of inhibitor and enzyme, respectively, and v_{i} and v_{o} are the reaction rates in the presence and absence of inhibitor, respectively. Data were fitted to the equation (2) for competitive inhibition:

$$v_{\mathrm{i}}=v_{\mathrm{o}}[S]/([S]+K_{\mathrm{m}}(1+[I]/K_{\mathrm{i}})) \quad (2)$$

where [*S*] is the concentration of substrate (usually fixed at 1 mM).

Bioavailability of SerMe-ImmH (10)

Male Balb/c mice (~25 grams) were purchased from the National Cancer Institute (NCI). The mice were fasted overnight before drug administration. A single dose of 50 nmol of SerMe-ImmH (**10**) was administrated to the mice orally or by intraperitoneal (i.p.) injection (three mice in each treatment). Blood samples (5 µl) were taken at different times, mixed with the same volume of 1% heparin and 0.3% Triton X-100 in PBS, and stored at 4 °C before assays. The erythrocyte PNP catalytic activity was monitored spectrophotometrically by adding 1 µL of the blood sample to the reaction mixture containing 50 mM potassium phosphate (pH 7.4), 1 mM inosine, and 60 mU of xanthine oxidase. The PNP catalytic activity of each sample was normalized to protein concentration. The normalized rate at each time point was divided by the normalized rate at time zero and then plotted against time. The time ($t_{1/2}$) required for 50% of the PNP catalytic activity to be lost and the time ($t_{1/2}$) required for a 50% regain of the PNP catalytic activity were interpolated from the plot.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by research grant GM41916 from the NIH and by a contract from the New Zealand Foundation for Research, Science & Technology. The authors are grateful to Ms Jennifer Mason for analytical HPLC analysis and Dr Herbert Wong for NMR analysis.

References

1. Taylor EA, Clinch K, Kelly PM, Li L, Evans GB, Tyler PC, Schramm VL. Acyclic ribooxacarbenium ion mimics as transition state analogues of human and malarial purine nucleoside phosphorylases. J. Am. Chem. Soc 2007;129:6984–6985. [PubMed: 17497780]
2. Rinaldo-Matthis A, Wing C, Ghanem M, Deng H, Wu P, Gupta A, Tyler PC, Evans GB, Furneaux RH, Almo SC, Wang CC, Schramm VL. Inhibition and structure of Trichomonas vaginalis purine nucleoside phosphorylase with picomolar transition state analogues. Biochemistry 2007;46:659–668. [PubMed: 17223688]

3. Murkin AS, Birck MR, Rinaldo-Matthis A, Shi W, Taylor EA, Almo SC, Schramm VL. Neighboring group participation in the transition state of human purine nucleoside phosphorylase. Biochemistry 2007;46:5038–5049. [PubMed: 17407325]
4. Taylor Ringia EA, Tyler PC, Evans GB, Furneaux RH, Murkin AS, Schramm VL. Transition state analogue discrimination by related purine nucleoside phosphorylases. J. Am. Chem. Soc 2006;128:7126–7127. [PubMed: 16734442]
5. Miles RW, Tyler PC, Furneaux RH, Bagdassarian CK, Schramm VL. One-third-the-sites transition-state inhibitors for purine nucleoside phosphorylase. Biochemistry 1998;37:8615–8621. [PubMed: 9628722]
6. Kicska GA, Long L, Horig H, Fairchild C, Tyler PC, Furneaux RH, Schramm VL, Kaufman HL, Immucillin H. a powerful transition-state analog inhibitor of purine nucleoside phosphorylase, selectively inhibits human T lymphocytes. Proc. Natl. Acad. Sci. USA 2001;98:4593–4598. [PubMed: 11287638]
7. Evans GB, Furneaux RH, Gainsford GJ, Schramm VL, Tyler PC. Synthesis of Transition State Analogue Inhibitors for Purine Nucleoside Phosphorylase and *N*-Riboside Hydrolases. Tetrahedron 2000;56:3053–3062.
8. Evans GB, Furneaux RH, Lewandowicz A, Schramm VL, Tyler PC. Exploring structure-activity relationships of transition state analogues of human purine nucleoside phosphorylase. J. Med. Chem 2003;46:3412–3423. [PubMed: 12852771]
9. Lewandowicz A, Tyler PC, Evans GB, Furneaux RH, Schramm VL. Achieving the ultimate physiological goal in transition state analogue inhibitors for purine nucleoside phosphorylase. J. Biol. Chem 2003;278:31465–31468. [PubMed: 12842889]
10. Evans GB, Furneaux RH, Schramm VL, Tyler PC. Synthesis of a transition state analogue inhibitor of purine nucleoside phosphorylase via the Mannich reaction. Org. Letters 2003;5:3639–3640.
11. Galmarini CM. Drug evaluation: forodesine - PNP inhibitor for the treatment of leukemia, lymphoma and solid tumor. IDrugs 2006;9:712–722. [PubMed: 17016779]
12. Duvic M. Systemic monotherapy vs combination therapy for CTCL: rationale and future strategies. Oncology 2007;21:33–40. [PubMed: 17474358]
13. Robak T, Lech-Maranda E, Koerycka A, Robak E. Purine nucleoside analogs as immunosuppressive and antineoplastic agents: mechanism of action and clinical activity. Curr. Med. Chem 2006;13:3165–3189. [PubMed: 17168705]
14. Ravandi F, Gandhi V. Novel purine nucleoside analogues for T-cell-lineage acute lymphoblastic leukaemia and lymphoma. Expert Opin. Investig. Drugs 2006;15:1601–1613.
15. Balakrishnan K, Nimmanpalli R, Ravandi F, Keating MJ, Gandhi V. Forodesine, an inhibitor of purine nucleoside phosphorylase, induces apoptosis in chronic lymphocytic leukemia cells. Blood 2006;108:2392–2398. [PubMed: 16778146]
16. Taylor Ringia EA, Schramm VL. Transition states and inhibitors of the purine nucleoside phosphorylase family. Curr. Top. Med. Chem 2005;5:1237–1258. [PubMed: 16305529]
17. Giblett ER, Ammann AJ, Wara DW, Sandman R, Diamond LK. Nucleoside-phosphorylase deficiency in a child with severely defective T-cell immunity and normal B-cell immunity. Lancet 1975;1:1010–1013. [PubMed: 48676]
18. Clinch K, Evans GB, Fleet GW, Furneaux RH, Johnson SW, Lenz DH, Mee SP, Rands PR, Schramm VL, Taylor Ringia EA, Tyler PC. Syntheses and bio-activities of the L-enantiomers of two potent transition state analogue inhibitors of purine nucleoside phosphorylases. Org. Biomol. Chem 2006;4:1131–1139. [PubMed: 16525558]
19. Rinaldo-Matthis A, Murkin AS, Ramagopal UA, Clinch K, Mee SPH, Evans GB, Tyler PC, Furneaux RH, Almo SC, Schramm VL. l-Enantiomers of Transition State Analogue Inhibitors Bound to Human Purine Nucleoside Phosphorylase. J. Am. Chem. Soc 2008;130:842–844. [PubMed: 18154341]
20. Evans GB, Furneaux RH, Lewandowicz A, Schramm VL, Tyler PC. Synthesis of second-generation transition state analogues of human purine nucleoside phosphorylase. J. Med. Chem 2003;46:5271–5276. [PubMed: 14613329]
21. Evans GB, Furneaux RH, Greatrex B, Murkin AS, Schramm VL, Tyler PC. Azetidine Based Transition State Analogue Inhibitors of N-Ribosyl Hydrolases and Phosphorylases. J. Med. Chem 2008;51:948–956. [PubMed: 18251493]

22. Chun BK, Song GY, Chu CK. Stereocontrolled syntheses of carbocyclic C-nucleosides and related compounds. J. Org. Chem 2001;66:4852–4858. [PubMed: 11442416]
23. Semeraro T, Lossani A, Botta M, Ghiron C, Alvarez R, Manetti F, Mugnaini C, Valensin S, Focher F, Corelli F. Simplified analogues of immucillin-G retain potent human purine nucleoside phosphorylase inhibitory activity. J. Med. Chem 2006;49:6037–6045. [PubMed: 17004717]
24. Elion GB, Furman PA, Fyfe JA, de Miranda P, Beauchamp L, Schaeffer J. Selectivity of action of an antiherpetic agent, 9-(2-hydroxyethoxymethyl)guanine. Proc. Natl. Acad. Sci. U.S.A 1977;74:5716–5720. [PubMed: 202961]
25. Elion GB. Acyclovir: Discovery, Mechanism of Action and Selectivity. J. Vir. Med. Supp 1993;1:2–6.
26. De Clercq E. The acyclic nucleoside phosphonates from inception to clinical use: Historical perspective. Antiviral Res 2007;75:1–13. [PubMed: 17116336]
27. Khandazhinskaya A, Yasko M, Shirokova E. The synthesis and anti-viral properties of acyclic nucleoside analogues with a phosphonomethoxy fragment in the side chain. Curr. Med. Chem 2006;13:2953–2980. [PubMed: 17073640]
28. Horton D, Thomas S, Gallucci J. A synthetic route to 9-(polyhydroxyalkyl)purines. Carbohydr. Res 2006;341:2211–2218. [PubMed: 16824497]
29. Lewandowicz A, Ringia EA, Ting LM, Kim K, Tyler PC, Evans GB, Zubkova OV, Mee S, Painter GF, Lenz DH, Furneaux RH, Schramm VL. Energetic mapping of transition state analogue interactions with human and Plasmodium falciparum purine nucleoside phosphorylases. J. Biol. Chem 2005;280:30320–30328. [PubMed: 15961383]
30. Schramm VL, Tyler PC. Imino-sugar-based nucleosides. Curr. Top. Med. Chem 2003;3:525–540. [PubMed: 12570864]
31. Schramm VL. Enzymatic transition state theory and transition state analogue design. J. Biol. Chem 2007;282:28297–28300. [PubMed: 17690091]
32. Lewandowicz A, Schramm VL. Transition state analysis for human and Plasmodium falciparum purine nucleoside phosphorylases. Biochemistry 2004;43:1458–1468. [PubMed: 14769022]
33. Schramm VL. Enzymatic transition states: thermodynamics, dynamics and analogue design. Arch. Biochem. Biophys 2005;433:13–26. [PubMed: 15581562]
34. Banphavichit V, Bhanthumnavin W, Vilaivan T. Synthesis of new tridentate chiral aminoalcohols by a multicomponent reaction and their evaluation as ligands for catalytic asymmetric Strecker reaction. Tetrahedron 2007;63:8727–8734.
35. Chehade KAH, Baruch A, Verhelst SHL, Bogyo M. An improved preparation of the activity-based probe JPM-OET and in situ applications. Synthesis 2005:240–244.
36. Tang Z, Yang Z-H, Chen X-H, Cun L-F, Mi A-Q, Jiang Y-Z, Gong L-Z. A highly efficient organocatalyst for direct aldol reactions of ketones with aldehydes. J. Am. Chem. Soc 2005;127:9285–9289. [PubMed: 15969611]
37. Breuning A, Vicik R, Schirmeister T. An improved synthesis of aziridine-2,3-dicarboxylates via azido alcohols-epimerisation studies. Tetrahedron: Asymm 2003;14:3301–3312.
38. Soai K, Ookawa A. Mixed Solvents Containing Methanol as Useful Reaction Media for Unique Chemoselective Reductions with Lithium Borohydride. J. Org. Chem 1986;51:4000–4005.
39. Inaba T, Birchler AG, Yamada Y, Sagawa S, Yokata K, Ando K, Uchida I. A Practical Synthesis of Nelfinavir, an HIV-Protease Inhibitor, Using a Novel Chiral C4 Building Block: (5*R*,6*S*)-2,2-Dimethyl-5-hydroxy-1,3-dioxepan-6-ylammonium Acetate. J. Org. Chem 1998;63:7582–7583.
40. Harnden MR, Parkin A, Parratt MJ, Perkins RM. Novel Acyclonucleotides: Synthesis and Antiviral Activity of Alkenylphosphonic Acid Derivatives of Purines and a Pyrimidine. J. Med. Chem 1993;36:1343–1355. [PubMed: 8496903]
41. Lemaire M, Posada F, Gourcy J-G, Jeminet G. Formal Synthesis of a 2- and 8-Functionalized 1,4,7-Trioxa-10-azaspiro[5.5]undecane. Synthesis 1995:627–629.
42. Kim HS, Barak D, Harden TK, Boyer JL, Jacobson KA. Acyclic and Cyclopropyl Analogues of Adenosine Bisphosphate Antagonists of the P2Y1 Receptor: Structure-Activity Relationships and Receptor Docking. J. Med. Chem 2001;44:3092–3108. [PubMed: 11543678]
43. Mitchell DL. Degradation of d-ribono-1→4-lactone to d-erythro-1→4-lactone. Can. J. Chem 1963;41:214–221.

44. Xu B, Stephens A, Kirschenheuter G, Greslin AF, Cheng X, Sennelo J, Cattaneo M, Zighetti ML, Chen A, Kim S-A, Kim HS, Bischofberger N, Cook G, Jacobson KA. Acyclic Analogues of Adenosine Bisphosphates as P2Y Receptor Antagonists: Phosphate Substitution Leads to Multiple Pathways of Inhibition of Platelet Aggregation. J. Med. Chem 2002;45:5694–5709. [PubMed: 12477353]

45. Janqueta F, Merchán FL, Merino P, Tejero T. A Straightforward Synthesis of l-isoserinal. Tetrahedron 1996;52:7045–7052.

46. Sánchez-Sancho F, Valverde S, Herradón B. Stereoselective syntheses and reactions of chiral oxygenated α,β-unsaturated-γ- and δ-lactones. Tetrahedron: Asymm 1996;7:3209–3246.

47. Dawson, RMC.; Elliott, DC.; Elliott, WH.; Jones, KM. Data for Biochemical Research. Vol. 3rd ed.. Oxford, U.K.: Clarendon Press; 1986.

48. Lim M-I, Ren Y-Y, Otter BA, Klein RS. Synthesis of “9-deazaguanosine” and other new pyrrolo[3,2-*d*]pyrimidine C-nucleosides. J. Org. Chem 1983;48:780–788.

49. The protein extinction coefficient was calculated from the primary sequence using the Protparam tool Gasteiger E, Hoogland C, Gattiker A, Duvaud S, Wilkins MR, Appel RD, Bairoch A. Protein Identification and Analysis Tools on the ExPASy Server. The Proteomics Protocols Handbook 2005Humana Press:571–607.607 The value differs from that previously reported (28.83 mM^{-1} cm^{-1}) due to a recent modification in the calculation algorithm; see also http://www.expasy.org/tools/protparam.html.

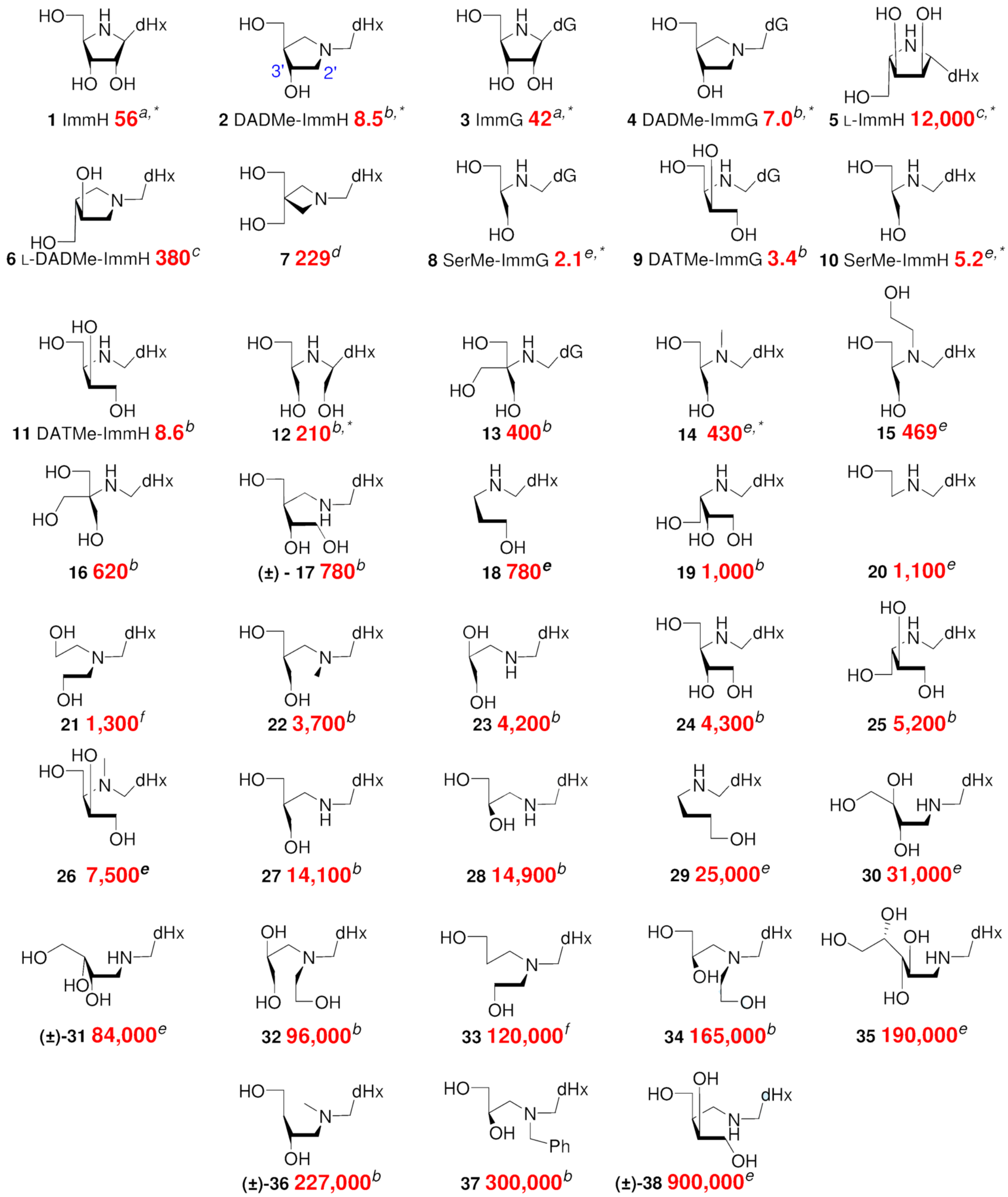


Figure 1.

Dissociation constants for human PNP with acyclic immucillins. Compounds marked with an asterisk exhibited slow-onset inhibition kinetics, whereby a slow protein conformational change following initial binding of the inhibitor resulted in a tighter complex. All values indicated were final, equilibrium dissociation constants in Pm following formation of the tight complex if applicable. Relative errors were typically ±15% or less. Source of K_i value: [a]Miles et. al.,[5] [b]Taylor et. al.,[1] [c]Rinaldo-Matthis et. al.,[2] [d]Evans et. al.,[21] [e]this manuscript, [f]Lewandowicz et. al.[29] Abbreviations; dG, 9-deazaguanine; dHx, 9-deazahypoxanthine.

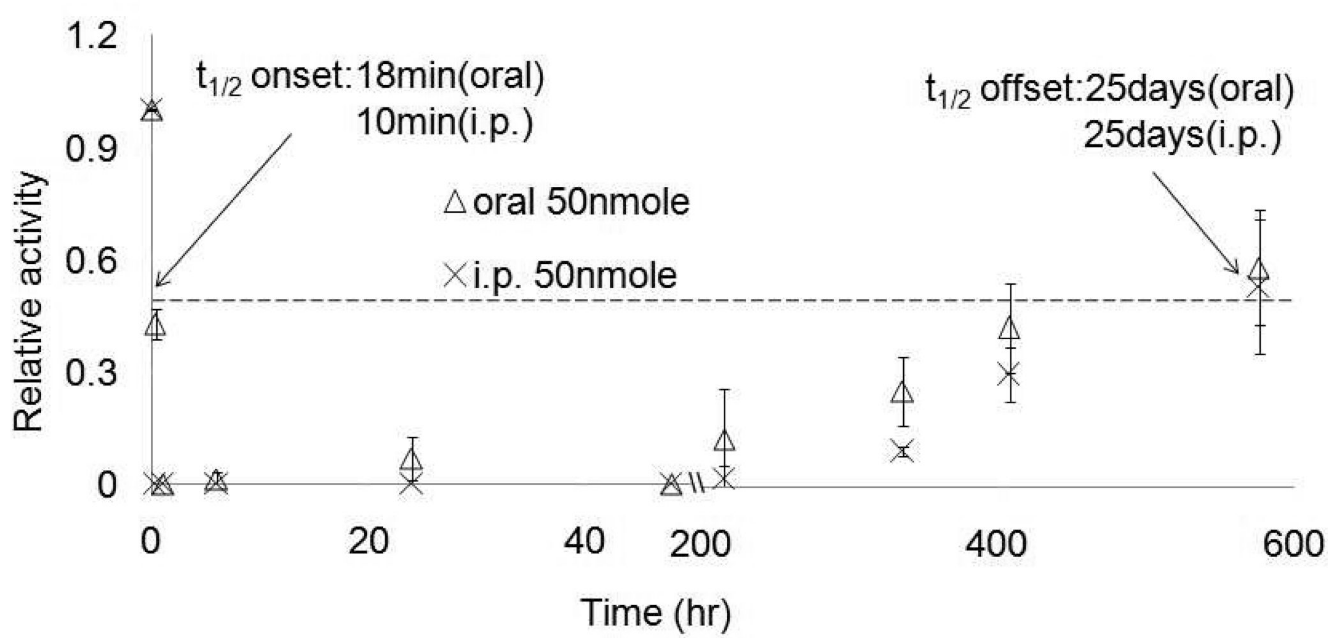
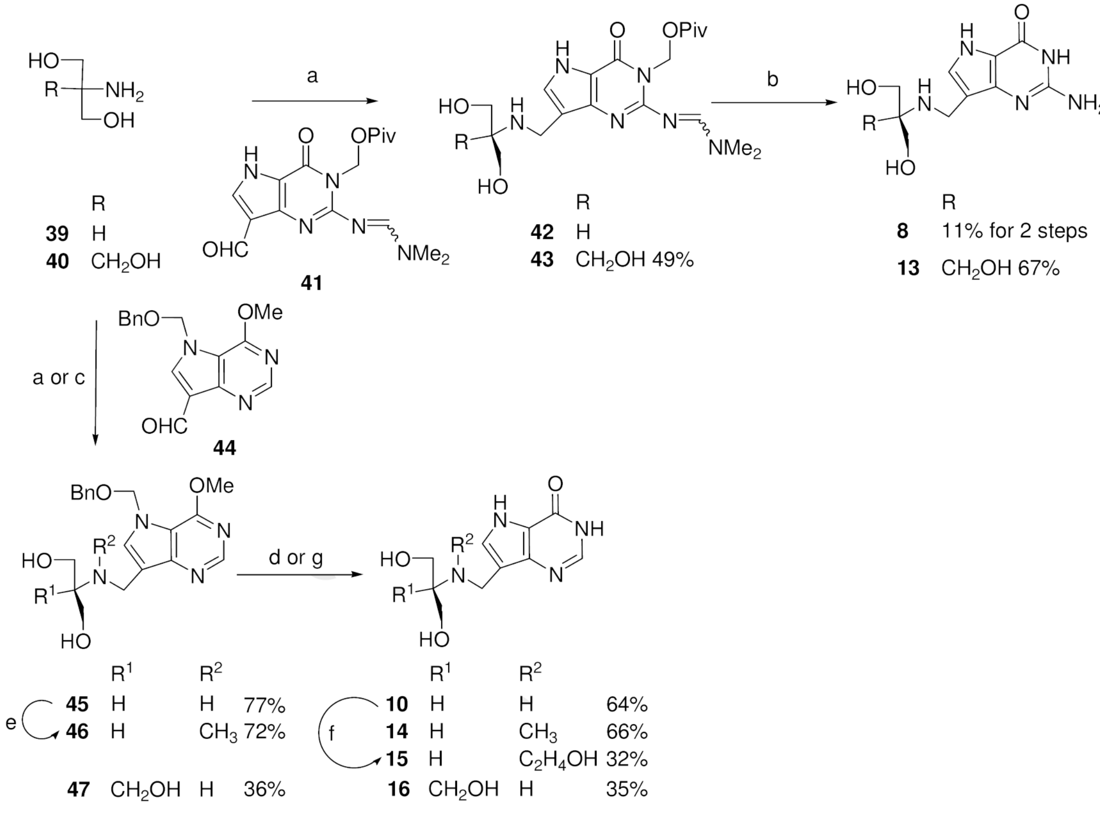


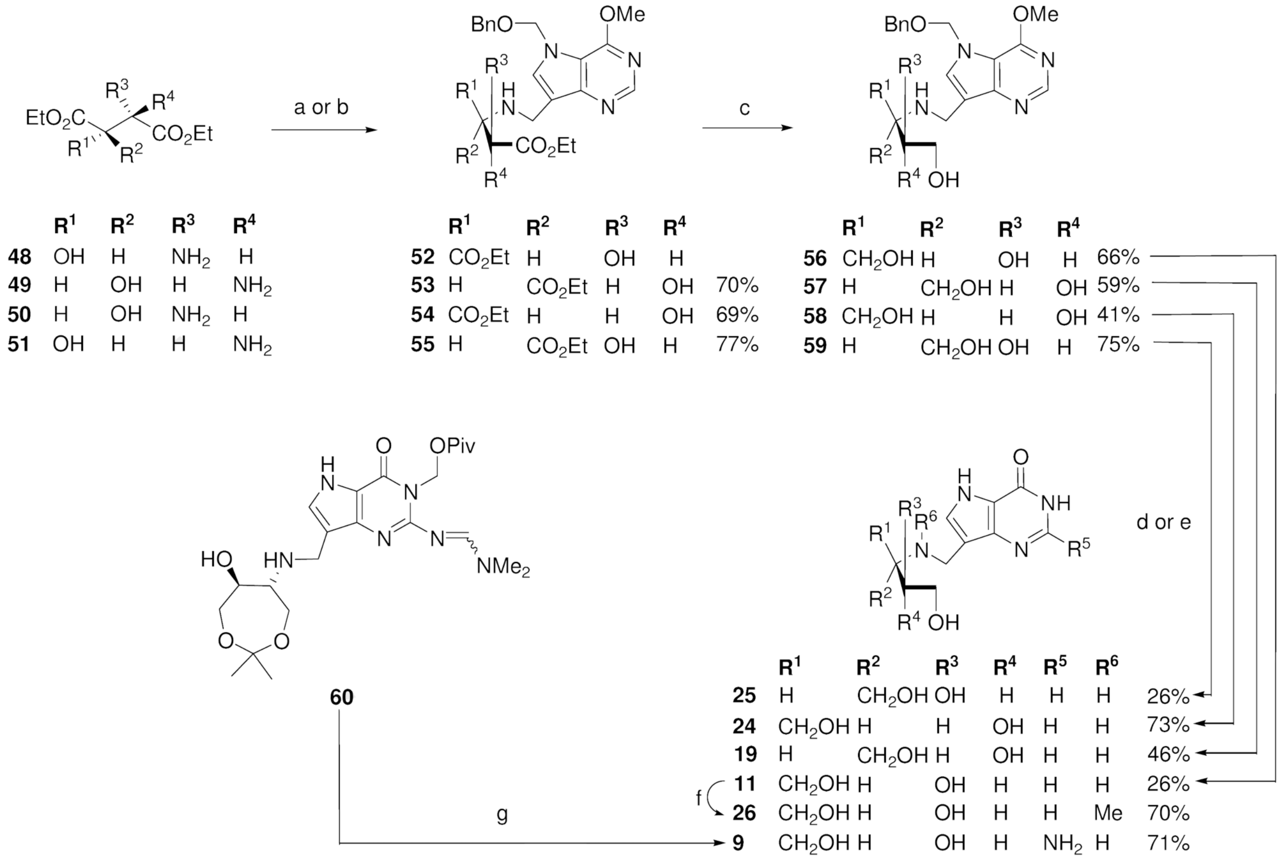
Figure 2.

Bioavailability of SerMe-ImmH (**10**) in mice. Enzyme activity was assayed following administration of 50 nmol by oral treatment (Δ) or intraperitoneal injection (×). Activities were normalized for protein concentration and are reported relative to uninhibited samples assayed prior to dose administration. The $t_{1/2}$ for onset is the time following treatment required to achieve a relative activity of 0.5. The $t_{1/2}$ for offset is the time following treatment required to achieve a relative activity recovery of 0.5. Oral treatment lags only marginally behind i.p. injection for onset of inhibition but remains nearly as effective with a similarly long $t_{1/2}$ offset of 25 days.



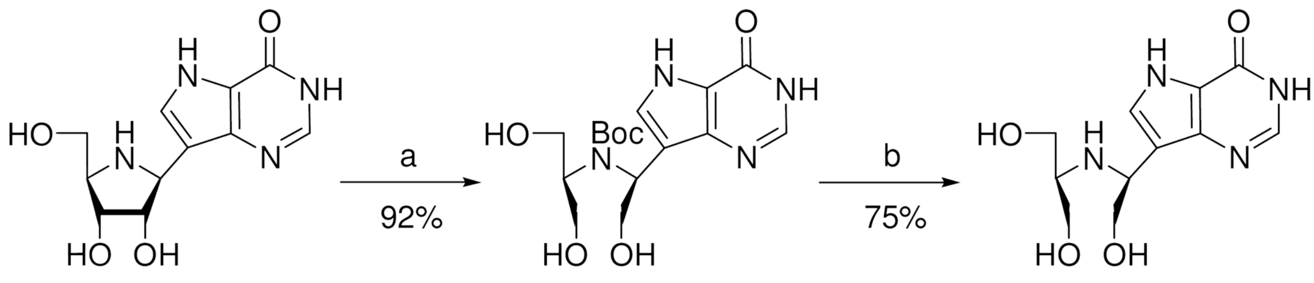
Scheme 1a.

[a]Reagents: (a) $NaCNBH_3$, AcCl, MeOH, room temperature. (b) (i) 1M NaOH, MeOH, room temperature; (ii) HCl, room temperature. (c) $NaCNBH_3$, MeOH, room temperature. (d) cHCl, reflux. (e) Paraformaldehyde, $NaCNBH_3$, AcCl, MeOH, room temperature. (f) 1,4-Dioxane-2,5-diol, $NaCNBH_3$, MeOH, room temperature. (g) BBr_3, CH_2Cl_2, room temperature.



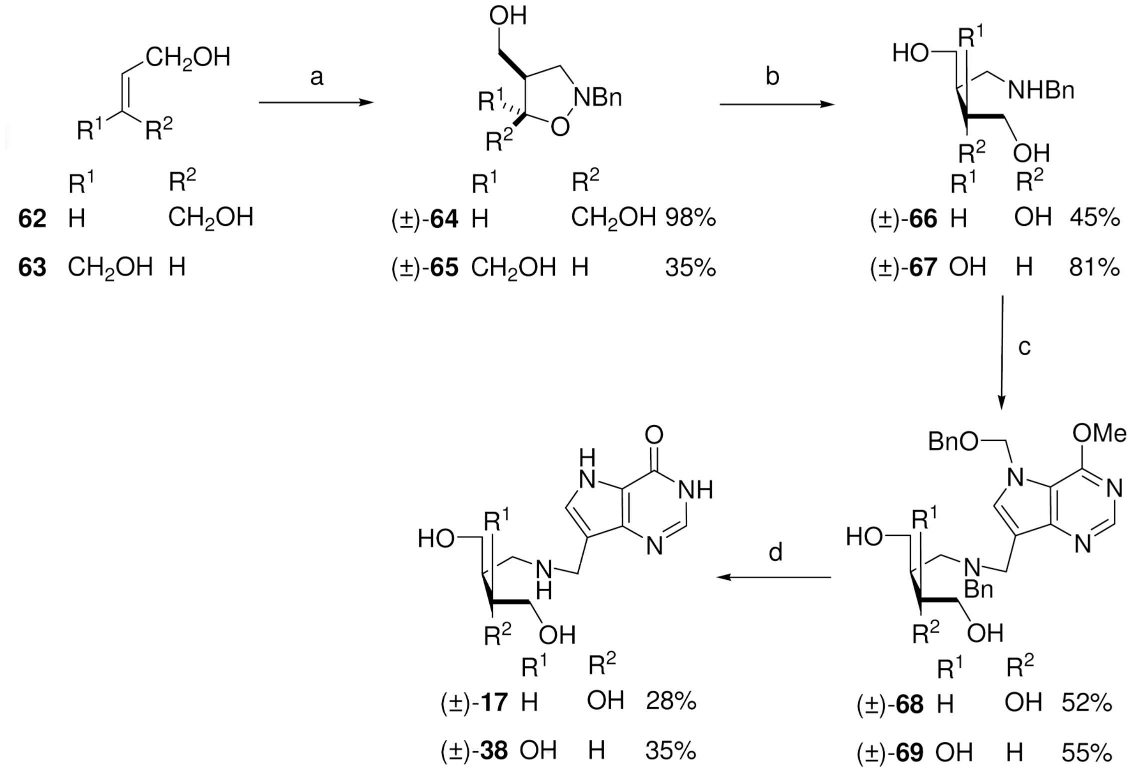
Scheme 2a.

[a]Reagents: (a) Compound **44**,[8] AcOH, $NaCNBH_3$, MeOH, room temperature. (b) Compound **44**,[8] $Na(OAc)_3BH$, 1,2-dichloroethane, room temperature. (c) $LiBH_4$, MeOH, Et_2O, room temperature. (d) BBr_3, CH_2Cl_2, room temperature. (e) cHCl, reflux. (f) paraformaldehyde, $NaCNBH_3$, MeOH, 50 °C → room temperature. (g) (i) AcCl, MeOH, room temperature; (ii) cHCl, reflux.



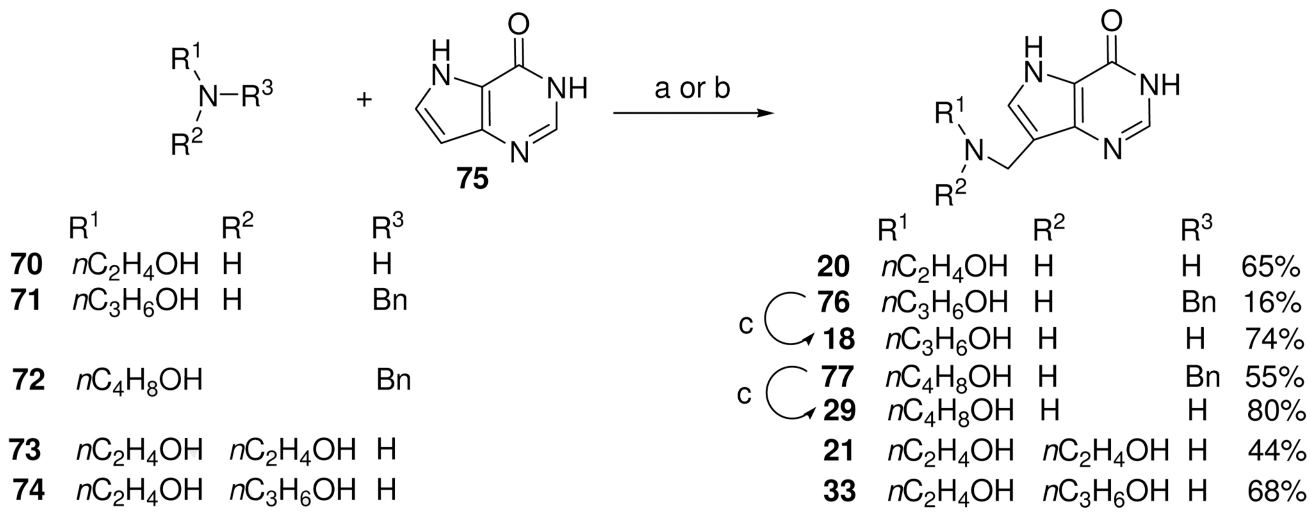
Scheme 3a.

[a]Reagents: (a) (i) Boc_2O, Et_3N, H_2O, MeOH, room temperature. (ii) $NaIO_4$, room temperature. (iii) $NaBH_4$, room temperature. (b) cHCl, MeOH, room temperature.



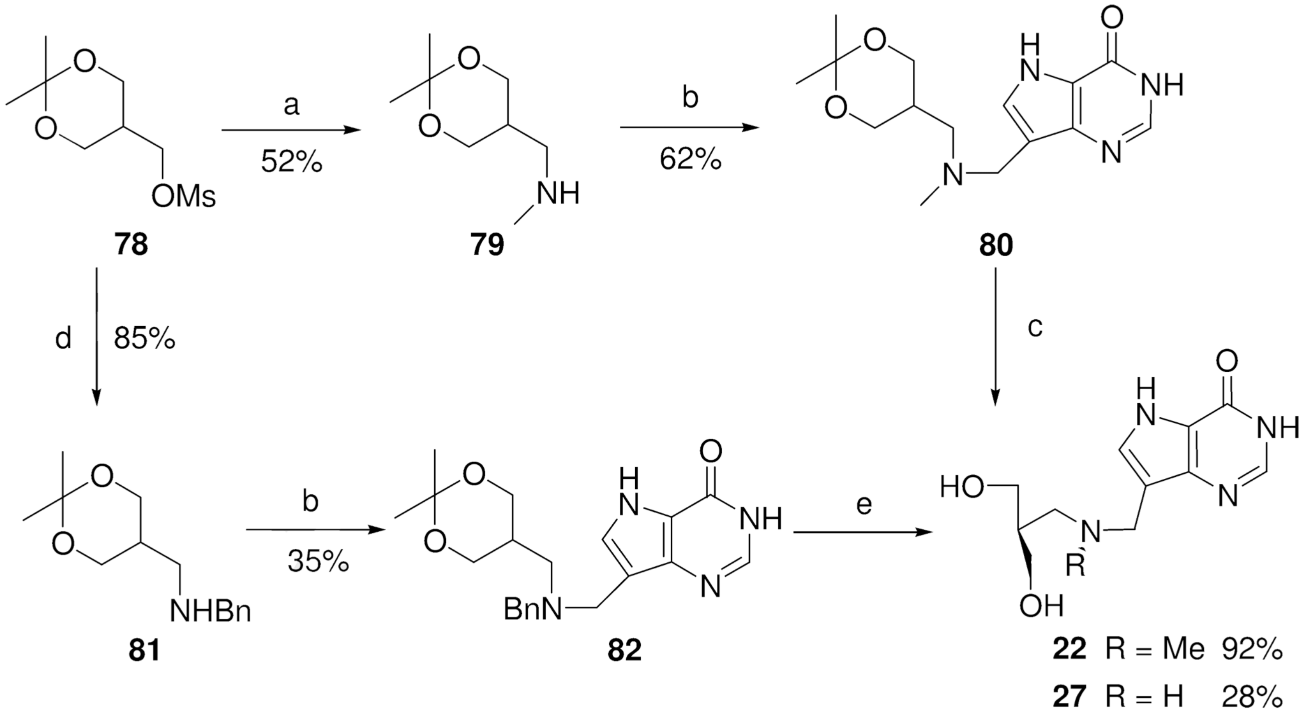
Scheme 4a.

[a]Reagents: (a) BnNHOH.HCl, NaOAc, 37% aq HCHO, EtOH, reflux. (b) Zn, AcOH, room temperature → 67 °C. (c) Compound **44**,[8] AcCl, $NaCNBH_3$, MeOH, room temperature. (d) (i) cHCl, reflux; (ii) H_2, Pd/C, MeOH.



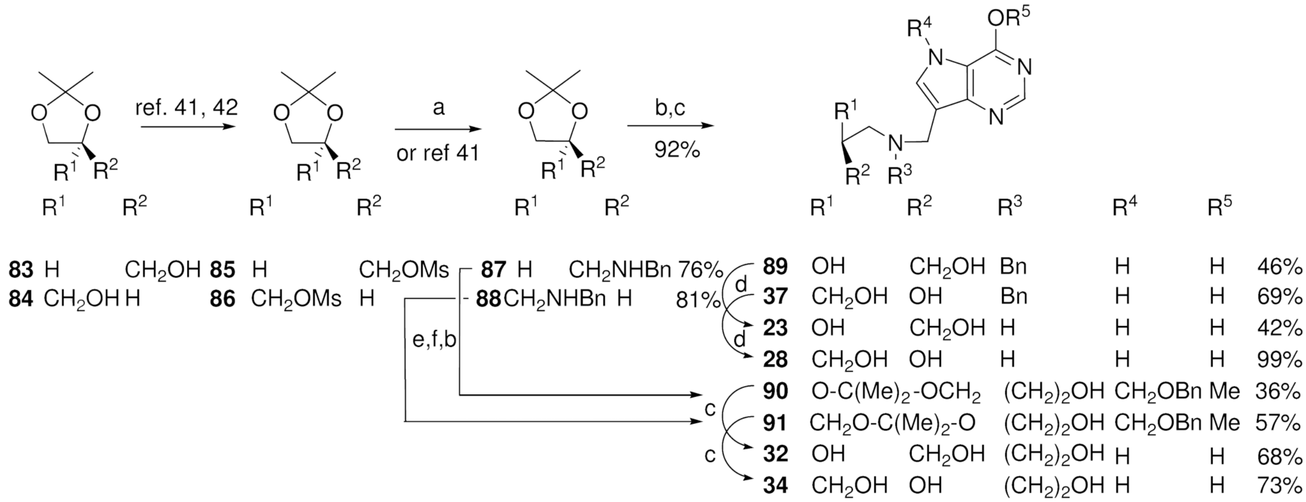
Scheme 5a.

[a]Reagents: (a) 37% aq HCHO, H_2O, 85 °C. (b) 37% aq HCHO, NaOAc, H_2O, 85 °C. (c) Pd/C, H_2, *i*PrOH, 50 °C.



Scheme 6a.

[a]Reagents: (a) Aq methylamine, DMSO, 75 °C. (b) 9-Deazahypoxanthine (**75**),[10] 37% aq HCHO, AcOH, 95 °C. (c) cHCl, MeOH, room temperature. (d) Benzylamine, 80 °C. (e) (i) cHCl, MeOH, room temperature; (ii) 10% Pd/C, H_2, H_2O, room temperature.

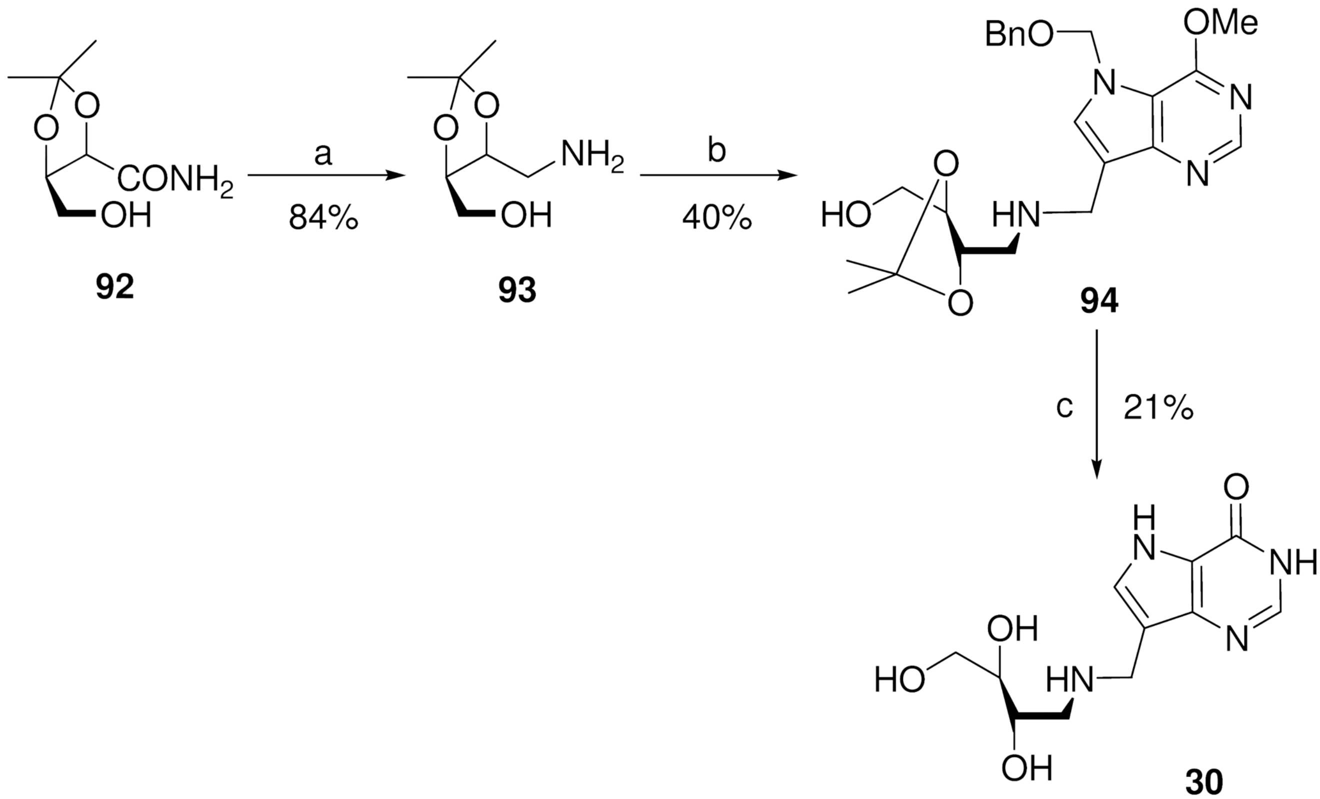


Scheme 7a.

[a]Reagents: (a) Benzylamine, CH_3CN, reflux. (b) Compound **44**,[8] $Na(OAc)_3BH$, 1,2-dichloroethane, $MgSO_4$, room temperature. (c) cHCl, reflux. (d) 10% Pd/C, H_2, H_2O, room temperature. (e) 2-Bromoethanol, K_2CO_3, CH_3CN, reflux. (f) 10% Pd/C, H_2, MeOH, room temperature.

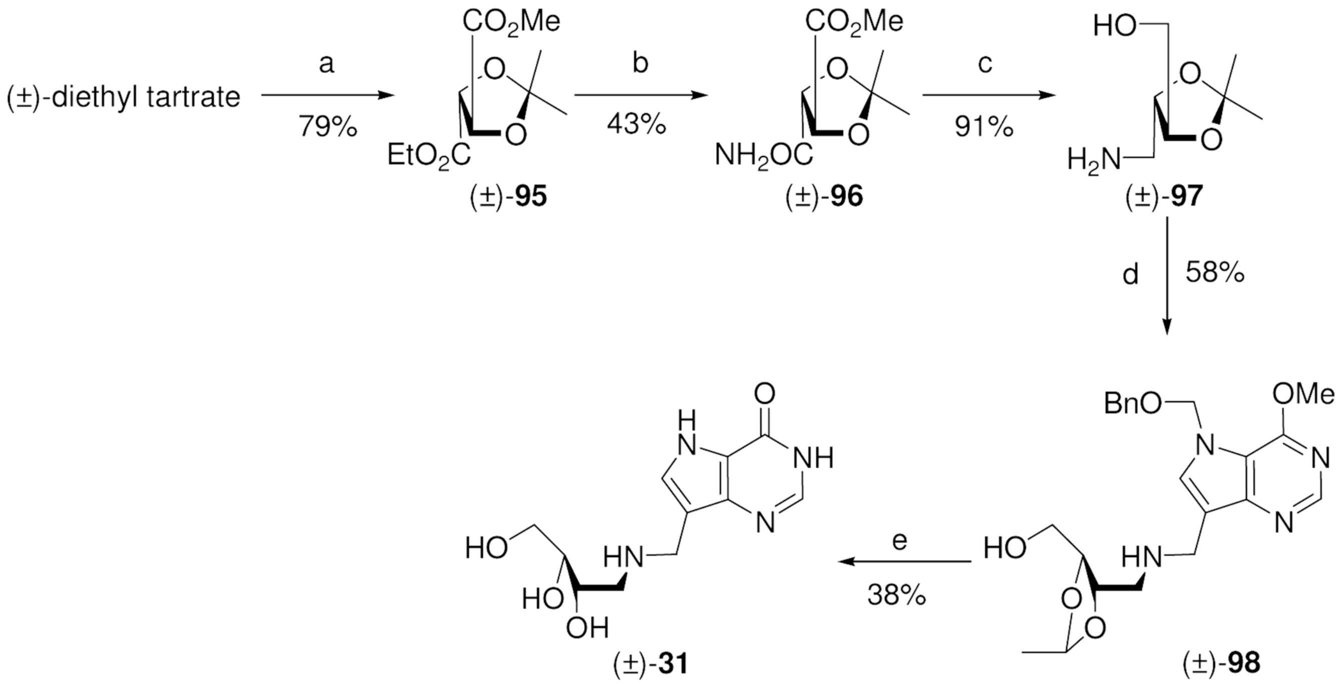
NIH-PA Author Manuscript

NIH-PA Author Manuscript



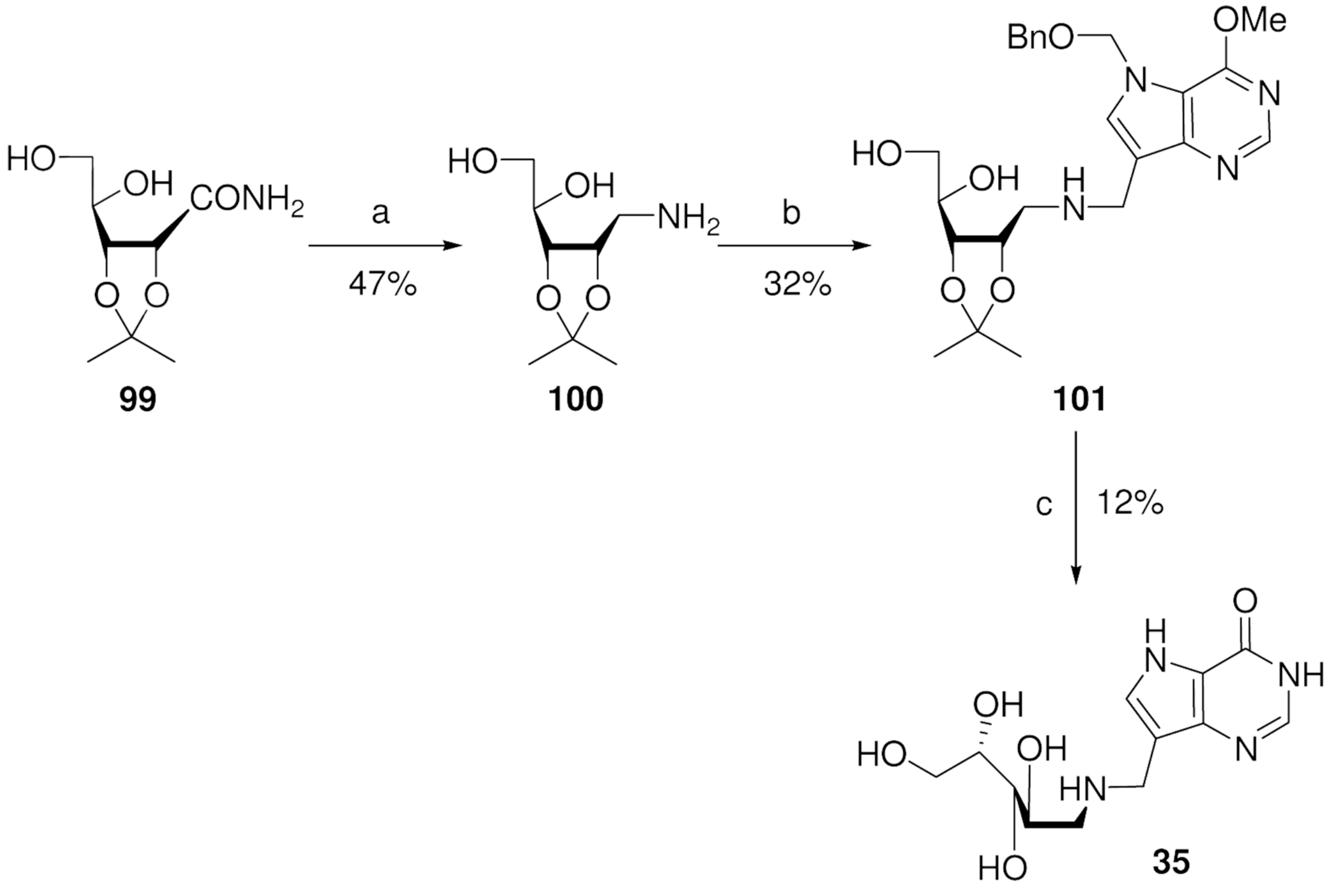
Scheme 8a.

[a]Reagents: (a) LAH, THF, room temperature. (b) Compound **44**,[8] $Na(OAc)_3BH$, 1,2-dichloroethane, $MgSO_4$, room temperature. (c) cHCl, reflux.



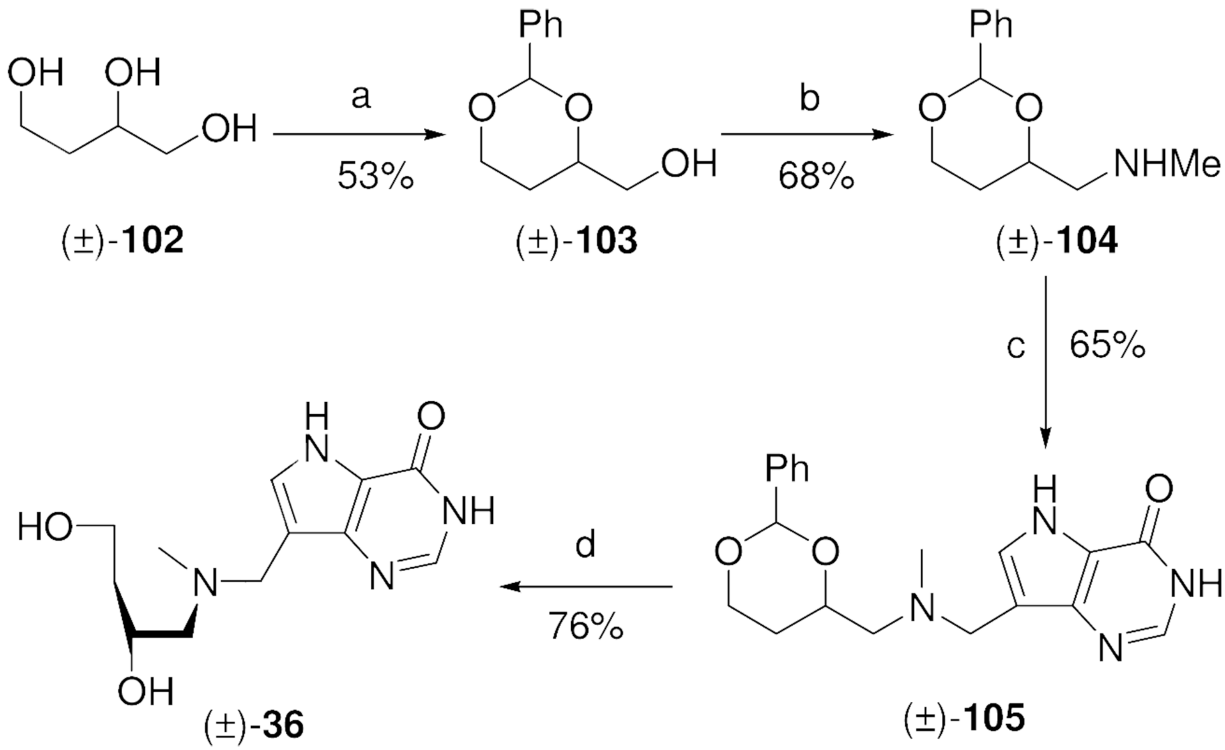
Scheme 9a.

[a]Reagents: (a) DMP, TsOH, benzene, reflux. (b) 7M NH_3 in MeOH, room temperature. (c) LAH, THF, reflux. (d) Compound **44**,[8] $Na(OAc)_3BH$, 1,2-dichloroethane, $MgSO_4$, room temperature. (e) cHCl, reflux.



Scheme 10a.

[a]Reagents: (a) LAH, THF, reflux. (b) Compound **44**,[8] $NaBH(OAc)_3$, 1,2-dichloroethane, room temperature. (c) cHCl, reflux.



Scheme 11a.

[a]Reagents: (a) PhCHO, TsOH, toluene, reflux. (b) (i) *i*Pr_2NEt, MsCl, CH_2Cl_2, 0 °C → room temperature; (ii) 40% aq $MeNH_2$, DMSO, 80 °C. (c) Compound **75**,[10] AcOH, 37% aq HCHO, 1,4-dioxane, 80 °C. (d) cHCl, MeOH, room temperature.