Structure-based design, synthesis and preliminary evaluation of selective inhibitors of dihydrofolate reductase from *Mycobacterium tuberculosis*

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**Abstract**

Tuberculosis is an increasing threat, owing to the spread of AIDS and to the development of resistance of the causative organism, *Mycobacterium tuberculosis*, to the currently available drugs. Dihydrofolate reductase (DHFR) is an important enzyme of the folate cycle; inhibition of DHFR inhibits growth and causes cell death. The crystal structure of *M. tuberculosis* DHFR revealed a glycerol tightly bound close to the binding site for the substrate dihydrofolate; this glycerol-binding motif is absent from the human enzyme. A series of pyrimidine-2,4-diamines was designed with a two-carbon tether between a glycerol-mimicking triol and the 6-position of the heterocycle; these compounds also carried aryl substituents at the 5-position. These, their diastereoisomers, analogues lacking two hydroxy groups and analogues lacking the two-carbon spacing linker were synthesised by acylation of the anions derived from phenylacetonitriles with ethyl (4S,5R)-4-benzyloxymethyl-2,2-dimethyl-1,3-dioxolane-4-propanoate, ethyl (4S,5S)-4-benzyloxymethyl-2,2-dimethyl-1,3-dioxolane-4-propanoate, tetrahydrooxepin-2-one and 2,3-O-isopropylidene-D-erythronolactone, respectively, to give the corresponding α-acylphenylacetonitriles. Formation of the methyl enol ethers, condensation with guanidine and deprotection gave the pyrimidine-2,4-diamines. Preliminary assay of the abilities of these compounds to inhibit the growth of TB5 *Saccharomyces cerevisiae* carrying the DHFR genes from *M. tuberculosis*, human and yeast indicated that 5-phenyl-6-((3R,4S)-3,4,5-trihydroxypentyl)pyrimidine-2,4-diamine selectively inhibited *M. tuberculosis* DHFR and had little effect on the human or yeast enzymes.

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1. Introduction

Tuberculosis (TB) is responsible for the highest number of deaths of all infectious diseases. Rates of TB continue to rise, leading to an estimated eight million new cases every year and an annual death toll of two million. Several factors have contributed to this increase, such as the HIV pandemic. Current therapy (DOTS) consists of an initial phase with four drugs, isoniazid, rifampin, pyrazinamide and ethambutol daily for two months, followed by a continuation phase of treatment with isoniazid and rifampin thrice weekly for a further four months, and has a cure rate of up to 95%, given patient compliance. Poor patient compliance with this prolonged regimen, together with other factors, has led to the emergence of multidrug-resistant tuberculosis (MDR-TB), against which DOTS is relatively ineffective. In view of this, DOTS-Plus (DOTS plus second-line TB drugs) is now recommended for treating MDR-TB and TB in areas with high incidence of MDR-TB. However, DOTS-Plus is expensive, takes longer to administer and has significant side-effects.

Dihydrofolate reductase (DHFR) is an important enzyme in the folate cycle which supplies one-carbon units derived from the action of serine hydroxymethyltransferase on L-serine for the biosynthesis of deoxythymidine monophosphate (dTMP). Inhibition of the folate cycle leads to interruption of the supply of thymidine and thus to inhibition of DNA biosynthesis and inhibition of proliferation of cells. Inhibition of proliferation is a useful goal in the therapy of cancer and of bacterial and protozoal infections. Highly potent inhibition of DHFR.
has been achieved with analogues of the substrate, dihydrofolate 1 (Figure 1). Methotrexate 2 is a highly potent inhibitor of mammalian DHFR and mammalian tumour DHFR (IC$_{50}$ = 2.5 nM vs. rat liver DHFR)$^{14}$ and is one of the most widely used anticancer antimetabolite drugs. It has ca. seven-fold selectivity for inhibition of human DHFR vs. M. tuberculosis DHFR.$^{15}$

The biological activities of pyrimidine-2,4-diamines have shown that it is not necessary to have the full pteridinediamine structure. These “non-classical” inhibitors have advantages in that they are more lipophilic than 2 and can enter cells by passive diffusion, not requiring the folate carrier. Pyrimethamine 3 was developed over 50 years ago as a DHFR-inhibiting anti-malarial drug,$^{16}$ it has selectivity for inhibition of Plasmodium falciparum DHFR activity of ca. forty-fold vs. human DHFR.$^{17}$ It is several orders of magnitude less potent than 2 against human DHFR.$^{17-19}$ Sulphadoxine / pyrimethamine plus isoniazid has some utility as prophylaxis against tuberculosis in HIV-positive patients$^{20}$ but isoniazid itself has been implicated in inhibition of M. tuberculosis DHFR after metabolism.$^{21}$ DDMP / metoprine 4a is a close analogue of 3 which shows a similar profile of inhibition of DHFRs, showing some activity as an antitumour agent in clinical trial.$^{22}$ However, this compound is also a highly potent inhibitor of histamine N-methyltransferase,$^{23,24}$ leading to neurological complications with its use. The 6-ethyl analogue etoprine 4b shows similar antileukaemic activity;$^{25}$ its inhibition of testicular DHFR causes infertility in male rats.$^{26}$ Methylbenzoprim 5 was designed as a non-classical DHFR inhibitor which lacks the full pteridine ring structure of methotrexate 2 but remains extremely potent against mammalian DHFRs (IC$_{50}$ vs. rat liver DHFR 3.2 pM) with some antitumour activity.$^{14}$ Interestingly, this compound is markedly less active against Pneumocystis carinii, Toxoplasma gondii and Escherichia coli DHFRs;$^{14,18}$ these activities have been rationalised in a crystallographic and modelling study.$^{18}$ Trimethoprim 6, in which the 5-aryl substituent is linked through a methylene bridge for increased flexibility, is often cited as an inhibitor of M. tuberculosis DHFR and other bacterial DHFRs, yet it is reported to lack potency (IC$_{50}$ 16.5 μM) and to be only five-fold selective for inhibition of M. tuberculosis DHFR vs. the human enzyme.$^{15}$ There is thus a great need for rationally designed selective inhibitors of M. tuberculosis DHFR for treatment of this widespread and often fatal disease.

### 2. Structure-based design

Several groups have pointed to structural differences between M. tuberculosis DHFR and human DHFR as possible opportunities for the design of selective inhibitors$^{15,27-29}$ but few studies have exploited these differences successfully in rational drug design for TB.$^{30}$ Da
Cunha et al.\textsuperscript{30} have suggested that addition of hydrophobic groups to 5-deazapteridines should increase selectivity, based on six examples. Suling et al.\textsuperscript{31} have achieved >100-fold selectivity for inhibition of the \textit{M. avium} DHFR vs. human DHFR using similar 5-methyl-5-deazapteridine-2,4-diamines but have not published results for \textit{M. tuberculosis} DHFR. Thus the way is open for rational structure-based design of selective inhibitors of \textit{M. tuberculosis} DHFR exploiting a major difference between human and \textit{M. tuberculosis} enzyme structures.

Li et al.\textsuperscript{ reported crystal structures of \textit{M. tuberculosis} DHFR. One structure contains methotrexate \textbf{2} bound at the dihydrofolate-binding site and NADP\textsuperscript+ at the NADP\textsuperscript+-binding site but also contains a glycerol tightly bound in an adjacent pocket where it forms H-bonds with Asp\textsuperscript{27}, Gln\textsuperscript{28} and Leu\textsuperscript{24} (Figure 2A).\textsuperscript{28} This glycerol is also present in the structure of \textit{M. tuberculosis} DHFR with the 1,3,5-triazine-2,4-diamine inhibitor Br-WR99210 bound but is absent from the crystal of \textit{M. tuberculosis} DHFR containing \textbf{6}, probably owing to the fact that the trimethoxyphenyl unit causes the trimethoprim to bind in a different manner, causing the Gln\textsuperscript{28} side-chain to be disordered.\textsuperscript{28} A more detailed examination of the environment of the glycerol reveals additional H-bonds (Figure 2B), as indicated by O—O and O—N distances and appropriate orientations. O(1)—H makes a H-bond with the side-chain amide carbonyl oxygen of Asp\textsuperscript{27}; O(1) is also involved as an acceptor in a H-bond with the indole N—H of
Trp\textsuperscript{22}. O(3) is also held in a two-H-bond clamp; O(3)—H makes a H-bond with the carbonyl oxygen of Leu\textsuperscript{24} and is also an acceptor in a H-bond with the N—H of the same amino-acid. O(2) accepts a single H-bond from the side-chain amide N—H of Gln\textsuperscript{28}. The glycerol carbon chain is in hydrophobic contact with Leu\textsuperscript{20}.\textsuperscript{28} In contrast, in the structures of human DHFR complexes containing dihydrofolate or 2, this site is well packed with hydrophobic side-chains.\textsuperscript{32,33} Since this glycerol is clearly tightly and specifically bound in a fixed conformation close to N(8) of 2, we designed series of molecules in which contain a 1,2,3-triol joined to a head group which would mimic the binding of 2 deep in the dihydrofolate-binding pocket.

Since 3 is a weak inhibitor of M. avium DHFR activity\textsuperscript{34} and many other pyrimidine-2,4-diamines inhibit various DHFRs, we chose pyrimidine-2,4-diamine as the template to which to attach the linker from the triol. Compounds 7 (Scheme 1) were designed directly from modelling the orientation of the glycerol and overlay of the pyrimidine-2,4-diamine unit with the diaminopteridine of 2. This overlay suggested that a two-carbon linker (-CH\textsubscript{2}CH\textsubscript{2}-) would be optimum to join the triol to the pyrimidine 6-position; it also showed the need for R configuration at the C(3) secondary alcohol of the 3,4,5-trihydroxypentyl side-chain (mimicking glycerol O(1)) and S configuration at the C(4) secondary alcohol (mimicking glycerol O(2)), as in 7. The diastereomeric series 8 is S at C(3); this series tests the validity of the drug design, since the linker length is the same as in 7 but the orientation of the triol relative to the
pyrimidine-diamine should not be apposite for binding. In 9, the secondary alcohols are missing, leaving only the primary alcohol of the 6-(5-hydroxypentyl) group to mimic O(3) of the glycerol and H-bond to Leu$^{24}$ in the glycerol-binding pocket, losing the ability to H-bond to Trp$^{22}$, Asp$^{27}$ and Gln$^{28}$, but retaining possible hydrophobic interactions with Leu$^{20}$. The length of the linker between the triol and the pyrimidine-2,4-diamine is tested in the 6-(1,2,3-trihydroxypropyl) compounds 10; these compounds retain the triol motif with the same configuration at the secondary alcohols as in 7 but joined directly to pyrimidine C(6).

In each of the sets of 6-((poly)hydroxyalkyl)pyrimidine-2,4-diamines 7-10, a phenyl is located at position-5 of the pyrimidine, to occupy a (largely) hydrophobic pocket which the hinge region (-CH$_2$NMe-) of 2 occupies in Figure 2A. This phenyl is unsubstituted in 7a-10a, whereas this ring is halogenated in other designed compounds. It carries a 4'-chlorine in 7b-10b (reflecting the 4'-chlorine in 3) and a 4'-bromine in 7c-10c. 3',4'-Dichlorophenyl was incorporated into 7d, 9d and 10d to mimic the dichlorophenyl in 4a,b; the corresponding analogue in the 8 series was planned but was synthetically inaccessible. Compounds 11 and 12 (Scheme 1) were designed as gross tests of the structure-based design of inhibitors, while retaining the essential pyrimidine-2,4-diamine. In 11, the designed triol is replaced by a hydrophobic aromatic benzene ring which should interact unfavourably with the H-bonding environment of the glycerol-binding pocket. In 12, there is no group which may enter this pocket.

3. Chemical synthesis

3.1. Synthetic strategy

The planned synthetic approaches to the series of target pyrimidine-2,4-diamines 7-12 are shown in retrosynthetic format in Scheme 1. In each case, condensation of an appropriately substituted corresponding enol ether 13 with guanidine would furnish the pyrimidinediamine. The enol ethers would be readily prepared by methylation of the $\alpha$-acylphenylacetonitriles 14, which, in turn would be available by acylation of anions derived from (Ar-substituted)phenylacetonitriles 15 with the appropriate esters 16, with or without protection of the side-chain alcohols. Several questions needed to be addressed during the development of the synthetic routes: how should the condensation with guanidine be optimised? how should the acylation be optimised? do the primary and secondary alcohols in the side-chains need to be protected during the acylation or condensation steps? if so, what should the protecting groups be? We elected to use the general synthetic approach, condensation of guanidine with enol ethers
derived from \( \alpha \)-acylphenylacetonitriles, used by Russell and Hitchings \(^{16} \) in their syntheses of pyrimethamine 3, etoprine 4 and related antimalarial compounds carrying simple small-alkyl substituents at the 6-position of the pyrimidine-2,4-diamine core. Tarnchompoo et al. \(^{19} \) have extended this synthetic approach to analogues carrying larger alkyl and \( \alpha \)-arylalkyl groups at this position, in their search for pyrimidine-2,4-diamines which inhibit DHFR activity in *Plasmodium falciparum* which is resistant to 3. The acylation steps and the protection of the OH groups were optimised individually for each series of target compounds.

### 3.2. Synthesis of 5-aryl-6-((3\( \text{R}, 4\text{S} \))\text{-3,4,5-trihydroxypentyl})pyrimidine-2,4-diamines 7

Scheme 2 shows our approach to the 5-aryl-6-((3\( \text{R}, 4\text{S} \))\text{-3,4,5-trihydroxypentyl})pyrimidine-2,4-diamines 7, using protection for the primary alcohol. We rationalised that the ester 21 would provide the required masked triol at the 6-position and could be synthesised by a two-carbon chain extension from a protected L-erythrose 18. Acetonide protection was introduced between the cis 3-OH and 4-OH of L-arabinose 17 by acid-catalysed reaction with 2,2-dimethoxypropane. Oxidative cleavage of the C(1)—C(2) bond with periodate then gave L-erythrose-2,3-acetonide 18. The required two-carbon chain-extension was achieved by base-free Wittig reaction of the latent aldehyde of 18 with pre-formed ethyl triphenyl-phosphoranylidineacetate to afford the stereoisomeric \( \alpha,\beta \)-unsaturated esters 19\( \text{E} \) and 19\( \text{Z} \) in 69% overall yield (ratio of geometrical isomers 3:11, 19\( \text{E} \) and 19\( \text{Z} \), respectively). These geometrical isomers were readily separated chromatographically and were identified on the basis of the \(^1\text{H} \) NMR coupling constants in the –HC=CH- system. Separation of the isomers was unnecessary in the synthetic plan, as catalytic hydrogenation of the mixture of 19\( \text{E} \) and 19\( \text{Z} \) gave the saturated ester 20 quantitatively. \(^1\text{H} \) NMR spectroscopy confirmed the presence of only one diastereoisomer of 20. A variety of protecting groups was investigated for the primary alcohol; we proposed that this alcohol should not be exposed during the reaction of the ester with the carbanion derived from the phenylacetonitriles, to avoid possible quenching of the carbanion and to avoid lactonisation of the hydroxy-ester 20. The primary alcohol of 20 was benzylated by generation of the alkoxide with lithium bis(trimethylsilyl)amide and reaction with benzyl bromide to give the fully protected ester 21. The classical conditions for using esters to acylate phenylacetonitrile carbanions, \(^{16} \) sodium ethoxide in ethanol, failed to effect the required reaction. However, the carbanions were generated from the (halo)phenylacetonitriles under aprotic conditions with lithium bis(trimethylsilyl)amide in diethyl ether at low temperature; these reacted with 21 to afford the \( \alpha \)-acylphenylacetonitriles 22\( \text{a-d} \) in 16-26%
The 1H NMR spectra indicated the presence of varying amounts of the enol tautomers. Methylation with diazomethane gave the enol ethers as inseparable mixtures of geometrical isomers. Condensation of these mixtures with guanidine in boiling 2-methoxy-ethanol then led to the pyrimidine-2,4-diamines in satisfactory yields; similar reactions in the conventional solvent for these condensations, ethanol, gave lower yields.

Removal of the acetonide protection from with aq. trifluoroacetic acid revealed the secondary alcohols in excellent yields but subsequent removal of the benzyl protection from the primary alcohol was more challenging. Catalytic hydrogenolysis (H₂, Pd/C, various solvents) failed to remove the benzyl group from even in the presence of catalytic per-

Scheme 2. Synthetic routes to 5-aryl-6-((3R,4S)-3,4,5-trihydroxypentyl)pyrimidine-2,4-diamines 7, using Bn protection for the primary OH and omitting protection for the primary OH. Reagents: i, Me₂C(OMe)₂, TsOH, DMF; ii, NaIO₄, H₂O, hexane; iii, EtO₂CCH=PPh₃, CH₂Cl₂; iv, H₂, Pd/C, EtOH; v, LiN(SiMe₃)₂, BnBr, THF, DMF; vi, LiN(SiMe₃)₂, ArCH₂CN, Et₂O; vii, CH₂N₂, Et₂O; viii, guanidine.HCl, NaOMe, MeO(CH₂)₂OH; ix, aq. CF₃CO₂H; x, Na, liquid NH₃, xi, FeCl₃, CH₂Cl₂.
chloric acid. However, addition of a catalytic amount of chloroform\textsuperscript{35} to the hydrogenolysis reaction mixture in methanol facilitated the deprotection to give triol \textit{7a}. This method could not be extended to debenzylation of the halogen-bearing analogues \textit{26b-d}, as hydrogenolysis of the carbon—halogen bonds occurred; \textit{26b} and \textit{26c} gave \textit{7a} only, whereas \textit{26d} gave an inseparable mixture of \textit{7a}, \textit{7b} and the \textit{meta}-monochloro analogue. Attempted debenzylation with hydrogen bromide in acetic acid, another common method, gave regioisomeric mixtures of bromo- and acetoxy-pentylpyrimidine-2,4-diamines. The most effective method for preparation of the Ar-unsubstituted analogue \textit{7a} was reductive cleavage of the O—benzyl protecting group with sodium in liquid ammonia. This method could not be extended to preparation of the halogenated congeners \textit{7b-d}, as reduction of the carbon—halogen bonds led to exclusive formation of the phenyl analogue \textit{7a} from \textit{26b-d}. The most generally applicable debenzylation for this series was the use of the Lewis acid anhydrous iron(III) chloride in dichloromethane, as developed by Park \textit{et al.}\textsuperscript{36} By this method, \textit{26b-d} were converted in high yields into the required triols \textit{7b-d}. Moreover, the Lewis acidity of this reagent could be exploited also in removal of the acetonides, in that both acetonide and benzyl ether protecting groups could be removed from \textit{25a-d} in one pot to furnish \textit{7a-d} directly, albeit in lower overall yields than in the two-step processes. TLC analysis suggested that, in this one-pot process, the acetonide was cleaved within 5 min and the debenzylation was essentially complete within 80 min.

In view of these challenges, the assembly of the pyrimidine ring was attempted with a free primary alcohol in the side chain. As shown in Scheme 2, the phenylacetonitriles were deprotonated with lithium bis(trimethylsilyl)amide and the anions were quenched with the ester \textit{20}. Use of two equivalents of base was necessary to achieve condensation to obtain the \textit{α}-acyl-phenylacetonitriles \textit{27} in a maximum yield of 10\%, indicating that protection of the primary alcohol is beneficial for this acylation to proceed efficiently. Methylation of the tautomeric enols \textit{28} with diazomethane and condensation of the enol ethers \textit{29} with guanidine gave the pyrimidine-2,4-diamines \textit{30}. Again, the yields were significantly lower with the exposed primary alcohol (\textit{30a}: 42\%, \textit{30b}: 12\%, \textit{30c}: 20\%, \textit{30d}: 9\%). Deprotection was straightforward to furnish the target triols \textit{7}.

### 3.3. Synthesis of 5-aryl-6-((3S,4S)-3,4,5-trihydroxypentyl)pyrimidine-2,4-diamines \textit{8}

The approach to the diastereomeric 5-aryl-6-((3S,4S)-3,4,5-trihydroxypentyl)pyrimidine-2,4-diamines \textit{8} was broadly similar to that for \textit{7}, using the benzyl protection method. In this series (Scheme 3), the key intermediate was the \textit{trans} dioxolane \textit{38}, a diastereomer of the \textit{cis}
dioxolane ester 21 above. The approach to 38 started with protection of the secondary alcohols of diethyl R,R-tartrate 31 as the acetonide 32; these secondary alcohols will become the secondary alcohols of the targets 8 with the appropriate configurations. Reduction with lithium aluminium hydride furnished the C2-symmetric diol 33. Mono-protection of this diol was essential for developing the chain-extension of only one arm. The optimum conditions were found to be deprotonation with one equivalent of sodium hydride in DMF, followed by alkylation with benzyl chloride, giving the required monoether 35, with a trace of diether 34. Pyridinium chlorochromate oxidation converted the exposed alcohol to the aldehyde 36, which was immediately condensed with ethyl triphenylphosphoranylidineacetate in a Wittig reaction to give the chain-extended α,β-unsaturated esters 37E and 37Z. In contrast to the analogous uncatalysed formation of 19E and 19Z (which carry free primary alcohols) at ambient temper-
ature, this reaction required prolonged heating at 110°C and catalysis with benzoic acid. In this case, the mixture of the separable geometrical isomers 37E and 37Z was approximately equimolar. Careful control of the hydrogenation conditions was required to reduce the alkene of the 37E / 37Z mixture to form key intermediate 38 without causing loss of the benzyl protecting group through hydrogenolysis. The fully protected ester 38 was then used, as for the diastereomer 21, to alkylate the carbanions derived from the (halo)phenylacetonitriles to afford the α-acylphenylacetonitriles 39a-c; 3,4-dichlorophenylacetonitrile failed to react. Methylation of the enols 40 and condensation of the enol ethers 41 with guanidine led to the pyrimidine-2,4-diamines 42, in much higher yields (42a: 67%, 42b: 48%, 42c: 53%) than in the R,S series. The side-chain alcohols were deprotected in two steps. Acid-hydrolysis of the acetonide rapidly gave the diols 43. As in the diastereomeric series, hydrogenolysis removed the benzyl group from 43a to afford 8a in high yield; debenzylation with iron(III) chloride converted 43b and 43c to the triols 8b and 8c, respectively, avoiding the dehalogenations associated with other debenzylation procedures.

3.4. Synthesis of 5-aryl-6-(5-hydroxypentyl)pyrimidine-2,4-diamines 9

Although a similar approach of protection of the primary alcohol could have been used in the syntheses of 6-(5-hydroxypentyl)pyrimidine-2,4-diamines 9, a strategy was devised to use a lactone to provide the necessary acylating ester, simultaneously masking the primary alcohol (Scheme 4). The carbanions of the (halo)phenylacetonitriles were generated in the usual way with lithium bis(trimethylsilyl)amide; the yields of the reactions with lactone 44 to give the α-(6-hydroxyhexanoyl)phenylacetonitriles 45 were low but provided sufficient material for further methylation of the enols 46 and condensation of 47 with guanidine to give the required 6-(5-hydroxypentyl)pyrimidines 9 in moderate yields. No deprotection steps were required in this series as the primary alcohols had been revealed during the reaction of the lactone with the phenylacetonitrile anions.
3.5. Synthesis of 5-aryl-6-((1S,2R)-1,2,3-trihydroxypropyl)pyrimidine-2,4-diamines 10

The lactone strategy was also used for the chain-shortened triols 10 (Scheme 5). 2,3-\textit{O}-Isopropylidene-\textit{D}-erythronolactone 48 reacted with the phenylacetonitriles anions to afford 49 in 21-38% yields. In the usual way, methylation of the enols 50, condensations of the enol ethers 51 with guanidine and aqueous acid deprotection of 52 gave the pyrimidine-2,4-diamines 10 carrying the 6-((1S,2R)-1,2,3-trihydroxypropyl) side-chains.

The dioxolanylpyrimidine intermediates 52 carry two bulky groups in close proximity in the 5- and 6-positions of the pyrimidine. MM2 energy minimisation suggests that this twists the 5-(4-halo)phenyl group in 52a-c out of the pyrimidine plane by \textit{ca.} 60° (Figure 3). The restricted rotation about the pyrimidine—Ph bond is evident in the NMR spectra of these compounds. The benzene ring is held close to the dioxolane, which bears two chiral centres. Thus the Ph 2-H and 6-H become diastereotopic, as do the Ph 3-H and 5-H. For example, in the $^1$H NMR spectrum of 52a, the Ph 2-H signal is separated from the Ph 6-H signal by 0.21 ppm, whereas the 3-H and 5-H signals are coincident. In the spectrum of the 4-chloro compound 52b, the Ph 2-H and 6-H signals are separated by 0.22 ppm and the 3-H and 5-H signals are separated by 0.04 ppm. In the spectrum of the 4-bromo compound 52c, the separations are 0.05 ppm and 0.02 ppm, respectively. In 52a-c, the substituents, if present, are in the 4-position of the benzene ring and are therefore coaxial with the pyrimidine—benzene bond. However, 52d carries a chlorine atom in position-3 of the benzene ring, which is off the axis of this bond. Therefore, two different conformers 52dA and 52dB can exist, as shown in Figure 3 in stick and space-filling representations. Conformers 52dA and 52dB are diastereoisomers of very similar energy,
according to MM2 calculations. The $^1$H NMR spectrum of 52d shows the presence of both conformers in 1:1 ratio; the sharpness of the signals indicates that, as could be predicted from the severe steric crowding, interconversion is slow. The $^1$H signals for 2-H for the diastereomeric conformers are separated by 0.10 ppm, the signals for 5-H by 0.02 ppm and the signals for 6-H by 0.10 ppm. Other $^1$H NMR signals are co-incident for the two conformers, as are all the peaks in the $^{13}$C NMR spectrum. The latter was assigned by analogy with the spectra for 3 and related compounds examined in detail earlier.\textsuperscript{37} This effect was not observed for the triols 10a-d and only one set of signals could be seen for each compound, with 2-H and 6-H being magnetically equivalent. This probably reflects the greater flexibility in the triol side-chain. The effects were also not observed for the homologues 7 and 8, also owing to increased flexibility and the remoteness of the chiral dioxolane from the benzene ring in these structures.

\textbf{Figure 3.} MM2-minimised structures of pyrimidine-2,4-diamines 52a-d, showing the steric interactions between the 5-(halo)phenyl group and the 6-(2,2-dimethyl-5-hydroxymethyl-1,3-dioxolan-4-yl) substituent. As a result of this steric crowding, the (halo)phenyl group is twisted to \textit{ca.} 60° from the plane of the pyrimidine. Compound 52d exists as two diastereomeric conformers, which are evident in the $^1$H and $^{13}$C NMR spectra.
3.5. Synthesis of pyrimidine-2,4-diamines 11, 12 and 3, lacking OH in the 6-substituent

Three pyrimidine-2,4-diamines 11, 12 and 3, lacking alcohols in the 6-substituent, were required as controls in the biological evaluation. The synthetic approaches followed the general sequence (Scheme 6). Acylation of phenylacetonitrile anion with ethyl 2-phenylpropanoate 53, methylation of 55 and condensation of 56 with guanidine gave 6-(2-phenylethyl)pyrimidine-2,4-diamine 11. The minimal analogue 12 was prepared similarly, through acylation of phenylacetonitrile anion with ethyl acetate 57, methylation of 59 and condensation of 60 with guanidine. Finally, 3 was produced in a new route starting with generation of the anion from 4-chloroacetonitrile with LiN(SiMe3)2 and reaction with ethyl propanoate 61 to give 62. Enol 63 was methylated, giving 64; condensation with guanidine in hot 2-methoxyethanol provided 3 in good yield.

4. Biological evaluation

4.1. Inhibition of DHFR activities

Direct screening of candidate drugs with *M. tuberculosis* is slow and requires biosafety Level 3 facilities and procedures. The slow growth of *M. tuberculosis* has been frustrating, with most public health laboratories still employing cultivation techniques that require 3-6 weeks to achieve growth. This mainly reflects the slow generation time inherent in the organism. *M. smegmatis* and *M. avium* have often been used as surrogates for assessment of activity of candidate drugs, as they grow rapidly and are less pathogenic to humans. However, drug screening in wild-type *M. smegmatis* has not always been an accurate predictor of activity or of mechanism of action in *M. tuberculosis*.43
A new approach to screening compounds for selective inhibition of DHFR from *M. tuberculosis* has been developed by Gerum *et al.* In this, the TH5 strain of the yeast *Saccharomyces cerevisiae*, which lacks endogenous expression of DHFR, was engineered to contain a vector p414CYC1 carrying a single copy of the *dfra* gene from *M. tuberculosis*. This gene codes for the protein with DHFR activity in *M. tuberculosis*. The native TH5 strain of *S. cerevisiae* requires supplementation with dTMP, uracil, adenine and a full complement of amino acids to grow, whereas the engineered strain containing the *dfra* gene can grow normally. Thus inhibition of the expressed *M. tuberculosis* DHFR activity would be manifest as inhibition of growth of the yeast. Two engineered TH5-derived strains of *S. cerevisiae* were also engineered to carry yeast or human DHFR genes. Inhibition of the growth of these yeasts by test compounds would indicate that these eukaryotic DHFRs are inhibited and would point to lack of selectivity for the prokaryotic *M. tuberculosis* enzyme. These three engineered yeasts were kindly supplied by Dr. Carol Hopkins Sibley (Department of Genome Sciences, University of Washington, Seattle, Washington, USA). Thus, in the present work, the test compounds were evaluated for their ability to inhibit selectively the growth of yeast carrying *M. tuberculosis* DHFR, while having less inhibition of yeast bearing either the yeast or the human enzyme. This assay, performed on a spoke assay plate, is semi-quantitative; comparison of the diameters of the zones of inhibition of the three yeasts by a particular test compound gives an indication of the selectivity of inhibition of the *M. tuberculosis* DHFR by that compound. Compounds can also be ranked approximately for potency of inhibition, although no quantitative IC₅₀ data can be derived.

Table 1 shows the mean diameters of the zones of inhibition of growth of the three yeasts by the pyrimidine-2,4-diamines 7-10 carrying one or more alcohols in the side-chain, by the pyrimidine-2,4-diamines 11 and 12 with simple lipophilic side-chains and by the known DHFR-inhibiting pyrimidine-2,4-diamines 3 and 6. Data for the negative control, DMSO without drug, are also given. Trimethoprim 6 has been reported to have a broad spectrum of activity against gram-positive bacteria, including methicillin-sensitive (MSSA) and methicillin-resistant (MRSA) *S. aureus*, and gram negative bacteria, including *E. coli*, but less activity or no activity against *Mycobacterium* spp., *Ps. aeruginosa* and *Chlamydia pneumoniae*.44

**Figure 4.** Typical plate (DMSO only control) showing orthogonal streaks of yeast after 3 d of incubation.
At the enzymic level, 6 is only a weak inhibitor of *M. avium* DHFR and of eukaryotic DHFR but is potent in inhibiting DHFR activity in susceptible bacteria.\textsuperscript{41} In line with these reports, 6 was found to be inactive against all three DHFRs in this yeast assay. Pyrimethamine 3 was very poorly active, even against the human enzyme, despite being reported to have $K_i = 58$ nM against human DHFR.\textsuperscript{17} This observation suggests that, despite being only semi-quantitative, the assay is a stringent test of inhibitory activity. The pyrimidine-2,4-diamine 11, which lacks hydroxy groups and carries only lipophilic substituents was also inactive against all the DHFRs. Interestingly, the minimal lipophilic pyrimidine-2,4-diamine 12, which bears only a methyl group at position-6, showed some inhibitory activity, although it was unselective.

Pyrimidine-2,4-diamines 7a-d, which carry the (3R,4S)-3,4,5-trihydroxypentyl side-chain at the 6-position were designed to mimic directly the pteridine of the dihydrofolate and the glycerol, with the configuration of each chiral centre being as predicted by the structure-based design; the –CH$_2$CH$_2$- linker is also of the length indicated by the modelling studies to be apposite. Within this set, the 5-phenyl compound 7a showed notable selectivity for inhibition of the growth of the yeast containing the *M. tuberculosis* DHFR, with only very modest inhibition of the growth of the yeasts containing the *H. sapiens* enzyme or the *S. cerevisiae* enzyme. The 4’-chlorophenyl analogue 7b also showed some selectivity for inhibition of the *M. tuberculosis* enzyme, whereas the 4’-bromophenyl and 3’,4’-dichlorophenyl compounds 7c and 7d had modest and equivalent activity against each DHFR.

The diastereomeric series 8a-c showed modest activity but little evidence of selectivity. Removal of the secondary alcohols from the 6-position side-chain, in 9, led to compounds with increased potency but completely lacking selectivity. In contrast, shortening the side-chain by removal of the –CH$_2$CH$_2$- linker but retaining the configuration of the secondary alcohols effectively abolished inhibitory activity in 9a-c but the 3’,4’-dichlorophenyl compound 9d showed modest but non-selective inhibition of all the DHFRs.

Several trends are noticeable in the structure-activity relationships for these pyrimidine-2,4-diamines. Firstly, comparison of the results for 7a,b with those for the diastereoisomers 8a,b indicates that the configuration of the hydroxy groups is critical for selective inhibition of *M. tuberculosis* DHFR, as predicted by the model. Secondly, the secondary alcohols appear to be necessary to use the binding contacts of one primary and the secondary alcohol of the glycerol, in that the 6-(5-hydroxypentyl) compounds 9 are not selective for the *M. tuberculosis* enz-
yme. Thirdly, the length of the linker joining the dihydrofolate mimic (the pyrimidine-2,4-diamine) to the glycerol mimic is critical; shortening the distance in 10 abolishes activity.

4.2. Modelling of the selective inhibitor 7a in the dihydrofolate- and glycerol-binding sites of *M. tuberculosis* DHFR

The structures of selected pyrimidines from the series were modelled into the dihydrofolate-binding site and the glycerol pocket, to attempt to rationalise the structure-activity observations and thus to validate the design process. The compounds were bound into the dihydrofolate and glycerol binding pockets using the H-bonds from the pyrimidine-2,4-diamine ring to establish an orientation similar to the observed binding conformation of methotrexate 2.28 The triol section was then docked using the H-bonds established from the bound glycerol in the X-ray structure (as distance restraints). Molecular dynamics calculations were then performed on the bound ligand using the H-bonds (X-ray observed) as distance restraints between the bound ligand and the pocket. The ligand was ramped to 300 K over a period of 10 ps and then held at 300 K for 20 ps. Observing the conformations over the final 20 ps gave two distinct binding conformers. Throughout the above procedure, the binding pocket was restrained and only the ligand was allowed to change orientation. Average structures were taken (7-13 ps and 15-20 ps) which were then minimised within a restrained binding pocket. The two structures obtained were then freely minimised (ligand and binding pocket to a radius of 15 Å) to give the structures and conformations shown in Figure 5.

Figure 5 shows the occupation of these sites by the two conformers of 7a, the most selective inhibitor of *M. tuberculosis* DHFR. As expected, the triol makes H-bonds with Asp27, Gln28, Leu24 and Trp22, following the pattern shown by the glycerol in the crystal structure.28 With the glycerol-mimicking triol held by the hydrogen-bonding network, the pyrimidine-2,4-diamine is perfectly located for its own hydrogen-bonding interactions deep in the dihydrofolate-binding site. These constraints place the 5-phenyl substituent of 7a in a pocket of limited size. Indeed, this pocket cannot accommodate halogens in the 4’-position of the phenyl, as this position is tight against the surface of the enzyme; thus the observations that the 4’-bromo- and 3’,4’-dichloro- analogues (7c and 7d, respectively) not selective inhibitors are rationalised in the model. The 4’-chloro- analogue 7b, however, does show slight selective inhibition of *M. tuberculosis* DHFR and it may be possible to accommodate the chlorine, albeit with a significant penalty in displacing the other binding contacts from their ideal positions.
The active lead compound 7a can adopt two different conformations. As with all 5-(substituted)phenyl-6-substituted-pyrimidine-2,4-diamines, the 5-phenyl ring of 7a has to be twisted out of the plane of the aromatic heterocycle to accommodate the adverse steric interactions between the phenyl ortho-hydrogens and the adjacent 4-NH₂ and 6-substituent. This rotation about the Ph—pyrimidine bond can be either clockwise or anticlockwise to achieve the same relief of steric strain. In conformer 7aA, the phenyl is rotated anticlockwise from coplanarity, whereas clockwise rotation produces 7aB; these conformers are almost identical in energy in free space. However, 7aA fits well into the pocket in the M. tuberculosis DHFR (Figure 5A), whereas the forward edge of the 5-phenyl of 7aB is located tightly pressed against the top of the enzyme pocket (Figure 5B). Thus the calculated energy of the complex of M. tuberculosis DHFR with conformer 7aA is of consistently higher energy than than of the complex of M. tuberculosis DHFR with conformer 7aB; indicating that 7a binds in conformer 7aA.

5. Conclusions

In this paper, we have reported our exploitation of a major difference in the local structure in the region of the dihydrofolate-binding sites of human and M. tuberculosis DHFR to design a compound 7a which shows notable selectivity for inhibition of the latter. In the crystal structure of a M. tuberculosis DHFR ternary complex with methotrexate 2 and glycerol, the glycerol is held tightly in its binding pocket by a network of five H-bonds. This glycerol-binding pocket is close to the site of the methotrexate. This glycerol-binding pocket is absent from the structure of human DHFR. In the structures of 7, the two-carbon link suggested by the crystal structure joins a triol (mimicking the glycerol) to the 6-position of a pyrimidine-
2,4-diamine core which binds into the dihydrofolate-binding site. The configurations of the secondary alcohols match the orientation of the glycerol relative to the methotrexate in the crystal structure. Three series of analogues were also designed to test the hypotheses of the design of 7. Compounds 8 tested the assignment of the configuration of the point of attachment of the triol to the linker and hence to the pyrimidine-2,4-diamine. Mono-hydroxy compounds 9 tested the need to take up the H-bonds from all three alcohols of the glycerol in binding selectively to the mycobacterial DHFR. Compounds 10 tested the length of the linker between the triol moiety and the pyrimidine-2,4-diamine.

The target compounds were synthesised by acylation of the anions derived from phenylacetonitriles with appropriately functionalised and protected esters and lactones, followed by methylation, condensation with guanidine and deprotection, if appropriate. The acylation step was optimised as generation of the phenylacetonitrile anion with lithium bis(trimethylsilyl)-amide at -78°C, followed by addition of the ester or lactone. Yields under these optimised conditions ranged from 6% to 41%, with the lower yields being obtained with substrates containing unprotected alcohols. The condensations with guanidine were generally uneventful and high yielding. Removal of benzyl groups presented a particular challenge, as many reductive methods also effected dehalogenation in some analogues.

Evaluation of the test 6-substituted pyrimidine-2,4-diamines for their inhibition of the growth of yeasts containing active DHFR from human, M. tuberculosis and yeast indicated that one compound, 7a, was selective for inhibition of M. tuberculosis DHFR and did not inhibit human DHFR or yeast DHFR significantly in the assay. Other compounds were inactive or less active. Modelling the structure of 7a into the dihydrofolate- and glycerol-binding pockets of M. tuberculosis DHFR rationalised the inhibition data, validating the original design of selective inhibitors and explaining the negative effect of halogenation of the 5-phenyl ring on biological activity. These modelling studies also indicated which of two low-energy conformations was required for binding and that there is a requirement for anticlockwise twist of the 5-phenyl ring relative to the pyrimidine. 5-Phenyl-6-((3R,4S)-3,4,5-trihydroxypentyl)pyrimidine-2,4-diamine 7a is shown here to be an interesting lead compound for further evaluation and further refinement of design for optimisation of potency and selectivity of inhibition of M. tuberculosis DHFR and, hence, new approaches to treatment of this widespread disease.
6. Experimental Section

6.1. General

NMR spectra were recorded on JEOL/Varian GX270 and EX400 spectrometers of samples in CDCl₃, unless otherwise stated. Mass spectra were obtained using a VG7070E spectrometer. IR spectra were measured as thin films or as KBr discs on a Perkin-Elmer RXI FT-IR spectrometer. Optical rotations were measured in a 10 cm cell on an Optical Activity Ltd. polarimeter; c is expressed in g per 100 mL. The stationary phase for chromatography was silica gel. All reactions were carried out under N₂ at ambient temperature, unless otherwise stated. Solvents were evaporated under reduced pressure. Melting points were determined by using a Reichert-Jung Thermo Galen instrument and are uncorrected.

6.2. 1-(4-Chlorophenyl)-1-cyano-2-methoxybut-1-ene (64) and 5-(4-chlorophenyl)-6-ethylpyrimidine-2,4-diamine (pyrimethamine) (3)

Compound 62/63 was treated with CH₂N₂, as for the synthesis of 24a, to give 64 (88%) as a pale yellow oil: IR νmax 2204, 1606 cm⁻¹; NMR 1.32 (3 H, t, J = 7.6 Hz, CMe), 2.80 (2 H, q, J = 7.6 Hz, CH₂), 3.88 (3 H, s, OMe), 7.31 (2 H, d, J = 8.6 Hz, Ph 3,5-H₂), 7.61 (2 H, d, J = 8.6 Hz, Ph 2,6-H₂). Compound 64 was treated with guanidine, as for the synthesis of 25a, to give 3 (50%) as a white solid: mp 233-235°C (lit.¹⁶ mp 233-234°C); NMR δH 0.97 (3 H, t, J = 7.4 Hz, Me), 2.09 (2 H, q, J = 7.4 Hz, CH₂), 5.64 (2 H, br, NH₂), 5.92 (2 H, br, NH₂), 7.22 (2 H, d, J = 8.2 Hz, Ph 3,5-H₂), 7.49 (2 H, d, J = 8.2 Hz, Ph 2,6-H₂); MS m/z 251.0884 (M + H) (C₁₂H₁₄³⁷ClN₄ requires 251.0877), 249.0909 (M + H) (C₁₂H₁₄³⁵ClN₄ requires 311.0910).

6.3. 5-Phenyl-6-(((3R,4S)-3,4,5-trihydroxypentyl)pyrimidine-2,4-diamine (7a). Method A

Compound 26a (150 mg, 0.4 mmol) was treated with Na (84 mg, 3.6 mmol) in liquid NH₃ (10 mL) and THF (5 mL) at -33°C for 20 min. Saturated aq. NH₄Cl (2 mL) was added and the mixture was allowed to warm to 20°C. CHCl₃ (14 mL) and MeOH (7 mL) were added and the mixture was filtered. Evaporation and chromatography (CHCl₃ / MeOH 7:3) gave 7a (90 mg, 78%) as a white solid: mp 90-91°C; NMR (D₂O) δH 1.29-1.36 (1H, m, 2-H), 1.49-1.55 (1H, m, 2-H), 2.07 (1H, ddd, J = 13.0, 10.2, 6.2 Hz, 1-H), 2.21 (1H, ddd, J = 13.0, 10.5, 5.3 Hz, 1-H), 3.17-3.21 (1H, m, 3-H), 3.22-3.25 (2H, m, 5-H₂), 3.37 (1H, dt, J = 8.5, 6.1 Hz, 4-H), 7.03 (1H, d, J = 7.5 Hz, Ph 2-H), 7.04 (1H, d, J = 7.5 Hz, Ph 6-H), 7.23 (1H, t, J = 7.5 Hz, Ph 4-H), 7.29 (2H, t, J = 7.5 Hz, Ph 3,5-H₂); NMR (D₂O) δC 30.24 (CH₂), 31.10 (CH₂), 62.25 (5-
6.4. 5-Phenyl-6-((3R,4S)-3,4,5-trihydroxypentyl)pyrimidine-2,4-diamine (7a). Method B

Compound 30a was treated with aq. CF₃CO₂H, as for the synthesis of 25a (reaction time 6 h), to give 7a (85%) as a white solid, with data as above.

6.5. 5-(4-Chlorophenyl)-6-((3R,4S)-3,4,5-trihydroxypentyl)pyrimidine-2,4-diamine (7b).

Method A

Compound 26b (60 mg, 0.14 mmol) was stirred with anhydrous FeCl₃ (68 mg, 0.42 mmol) in dry CH₂Cl₂ (5 mL) under N₂ for 80 min. Water (2 mL) was added. Evaporation and chromatography (CHCl₃ / MeOH 7:3) gave 7b (30 mg, 63%) as a white solid: [α]²⁰ D = -1.0° (c 1.1, MeOH); mp 250-251°C; NMR (D₂O) δH 1.44 (1 H, m, 2'-H), 1.65 (1 H, m, 2'-H), 2.22 (1 H, ddd, J = 13.6, 10.4, 6.0 Hz, 1'-H), 2.36 (1 H, ddd, J = 13.6, 10.0, 5.2 Hz, 1'-H), 3.1 (1 H, m, 3'-H), 3.35-3.38 (2 H, m, 5'-H₂), 3.48-3.53 (1 H, m, 4'-H), 7.18 (2 H, d, J = 8.6 Hz, Ar 2,6-H₂), 7.44 (2 H, d, J = 8.5 Hz, Ar 3,5-H₂); NMR (CD₃OD) δC 30.05, 31.36, 62.70, 71.97, 74.14, 107.03, 129.18, 132.23, 133.13, 133.71, 161.73, 162.08, 163.06; MS m/z 341.1185 (M + H) (C₁₅H₂₀³⁷ClN₄O₃ requires 341.1194), 339.1225 (M + H) (C₁₅H₂₀₃₅ClN₄O₃ requires 339.1223), 308/306 (M - CH₃OH), 249/247 (M – C₃H₇O₃).

6.6. 5-(4-Chlorophenyl)-6-((3R,4S)-3,4,5-trihydroxypentyl)pyrimidine-2,4-diamine (7b). Method B

Compound 30b was treated with aq. CF₃CO₂H, as for the synthesis of 7a, to give 7b (91%) as a white solid, with data as above.

6.7. 5-(4-Bromophenyl)-6-((3R,4S)-3,4,5-trihydroxypentyl)pyrimidine-2,4-diamine (7c).

Method A

Compound 26c was treated with FeCl₃, as for the synthesis of 7b, to give 7c (85%) as a white solid: [α]²⁰ D = -4.2° (c 0.24, MeOH); mp 198-200°C; NMR (D₂O) δH 1.82 (1 H, m, 2’-H), 2.19 (1 H, m, 1’-H), 2.33 (1 H, m, 1’-H), 3.29 (1 H, m, 3’-H), 3.32-3.36 (2 H, m, 5’-H₂), 3.48 (1 H, m, 4’-H), 7.08 (2 H, d, J = 8.0 Hz, Ar 2,6-H₂), 7.55 (2 H, d, J = 8.0 Hz, Ar 3,5-H₂).
Hz, Ar 3,5-H$_2$); MS m/z 385.0683 (M + H) (C$_{15}$H$_{20}^{81}$BrN$_4$O$_3$ requires 385.0698), 383.0714 (M + H) (C$_{15}$H$_{20}^{79}$BrN$_4$O$_3$ requires 383.0718).

6.8. 5-(4-Bromophenyl)-6-((3R,4S)-3,4,5-trihydroxypentyl)pyrimidine-2,4-diamine (7c). Method B

Compound 30c was treated with aq. CF$_3$CO$_2$H, as for the synthesis of 7a, to give 7c (87%) as a pale yellow solid, with data as above.

6.9. 5-(3,4-Dichlorophenyl)-6-((3R,4S)-3,4,5-trihydroxypentyl)pyrimidine-2,4-diamine (7b). Method A

Compound 26d was treated with FeCl$_3$, as for the synthesis of 7b, to give 7d (77%) as a white solid: $[\alpha]_{20}^D = -1.4^\circ$ (c 2.2, MeOH); mp 180-181°C; NMR ((CD$_3$)$_2$SO) 1.43 (1 H, m, 2'-H), 1.63 (1 H, m, 2'-H), 2.08 (1 H, ddd, J = 13.6, 10.4, 5.5 Hz, 1'-H), 2.34 (1 H, ddd, J = 13.6, 10.4, 5.5 Hz, 1'-H), 3.17 (1 H, m, 3'-H), 3.28-3.31 (2 H, m, 5'-H$_2$), 3.48 (1 H, m, 4'-H), 5.79 (2 H, br, NH$_2$), 5.97 (2 H, br, NH$_2$), 7.16 (1 H, dd, J = 8.2, 1.8 Hz, Ar 6-H), 7.42 (1 H, d, J = 1.8 Hz, Ar 2-H), 7.66 (1 H, dd, J = 8.2 Hz, Ar 5-H); NMR ((CD$_3$)$_2$SO) $\delta_C$ 31.26, 32.09, 63.94, 71.95, 75.19, 105.22, 131.38, 131.81, 133.16, 133.74, 133.97, 137.51, 162.42, 166.19; MS m/z 377.0794 (M + H) (C$_{15}$H$_{19}^{37}$Cl$_2$N$_4$O$_3$ requires 377.0775), 375.0814 (M + H) (C$_{15}$H$_{19}^{37}$Cl$_{35}$ClN$_4$O$_3$ requires 375.0804), 373.0836 (M + H) (C$_{15}$H$_{19}^{35}$Cl$_2$N$_4$O$_3$ requires 373.0834), 345/343/341 (M - CH$_3$O).

6.10. 5-(3,4-Dichlorophenyl)-6-((3R,4S)-3,4,5-trihydroxypentyl)pyrimidine-2,4-diamine (7d). Method B

Compound 30d was treated with aq. CF$_3$CO$_2$H, as for the synthesis of 7a, to give 7d (77%) as a white solid, with data as above.

6.11. 5-Phenyl-6-((3S,4S)-3,4,5-trihydroxypentyl)pyrimidine-2,4-diamine (8a)

Compound 43a (200 mg, 0.5 mmol) was stirred in MeOH (20 mL) with Pd/C (5%, 154 mg) and CHCl$_3$ (100 μL) under H$_2$ for 2 h. Filtration (Celite®), evaporation and chromatography (CHCl$_3$ / MeOH 7:3) gave 8a (150 mg, 93%) as a white solid: mp >350 °C; $[\alpha]_{20}^{19}$D = +10.9° (c 0.5, H$_2$O); NMR (D$_2$O) $\delta_H$ 1.50-1.64 (2 H, m, 2'-H$_2$), 2.28 (1 H, ddd, J = 13.9, 10.1, 6.3 Hz, 1'-H), 2.40 (1 H, ddd, J = 13.9, 10.1, 6.3 Hz, 1'-H), 2.31-3.45 (4 H, m, 3',4',5'-H$_4$), 7.25 (2 H, d, J = 7.0 Hz, Ph 2,6-H$_2$), 7.42 (1 H, t, J = 7.0 Hz, Ph 4-H), 7.47 (2 H, t, J = 7.0 Hz, Ph 3,5-
H₂); NMR (D₂O) δC 29.75, 31.57, 62.63, 73.61, 73.64, 109.24, 129.37, 129.37, 130.56, 133.41, 160.29, 161.05, 161.81; MS m/z 305.1618 (M + H) (C₁₅H₂₁N₄O₃ requires 305.1613).

6.12. 5-(4-Chlorophenyl)-6-((3S,4S)-3,4,5-trihydroxypentyl)pyrimidine-2,4-diamine (8b)

Compound 43b was treated with FeCl₃, as for the synthesis of 7b, to give 8b (77%) as a white solid: mp >350 °C; [α]²⁰D = +6.0° (c 0.67, H₂O); NMR (CD₃OD) δH 1.73-1.77 (2 H, m, 2’-H₂), 2.41 (1 H, dt, J = 14.4, 6.7 Hz, 1’-H), 2.54 (1 H, dt, J = 14.4, 6.7 Hz, 1’-H), 3.44-3.61 (4 H, m, 3’,4’,5’-H₄), 7.31 (2 H, d, J = 8.4 Hz, Ar 2,6-H₂), 7.53 (2 H, d, J = 8.4 Hz, Ar 3,5-H₂); MS m/z 341.1180 (M + H) (C₁₅H₂₀₈¹ClN₄O₃ requires 341.1194), 339.1237 (M + H) (C₁₅H₂₀₇₉ClN₄O₃ requires 339.1223).

6.13. 5-(4-Bromophenyl)-6-((3S,4S)-3,4,5-trihydroxypentyl)pyrimidine-2,4-diamine (8c)

Compound 43c was treated with FeCl₃, as for the synthesis of 7b, to give 8c (77%) as a white solid: mp >350 °C; [α]²⁰D = +12.5° (c 0.24, MeOH); IR νmax 3649, 3468, 3418, 1618 cm⁻¹; NMR (D₂O) δH 1.60-1.72 (2 H, m, 2’-H₂), 2.30-2.54 (2 H, m, 1’-H₂), 3.42-3.56 (4 H, m, 3’,4’,5’-H₄), 7.25 (2 H, d, J = 8.7 Hz, Ar 2,6-H₂), 7.72 (2 H, d, J = 8.7 Hz, Ar 3,5-H₂); MS m/z 385.0707 (M + H) (C₁₅H₂₀₈¹BrN₄O₃ requires 385.0698), 383.0717 (M + H) (C₁₅H₂₀₇₉BrN₄O₃ requires 383.0718).

6.14. 1-Cyano-7-hydroxy-2-methoxy-1-phenylhept-1-ene (47a) and 6-(5-hydroxypentyl)-5-phenylpyrimidine-2,4-diamine (9a)

Compound 45a/46a was treated with CH₂N₂, as for the synthesis of 24a (followed by chromatography (EtOAc / hexane 2:1)), to give 47a (78%) as a pale yellow oil: IR νmax 3439, 2204 cm⁻¹; MS m/z 246.1492 (M + H) (C₁₅H₂₀NO₂ requires 246.1494). Compound 47a was treated with guanidine, as for the synthesis of 25a (chromatographic eluant CH₂Cl₂ / MeOH (4:1)), to give 9a (36%) as a white solid: mp 214-216°C; IR νmax 3420, 3331, 3177, 1619 cm⁻¹; NMR δH 1.27 (2 H, qn, J = 7.2 Hz, 3’-H₂), 1.45 (2 H, qn, J = 7.2 Hz, 4’-H₂), 1.55 (2 H, qn, J = 7.2 Hz, 2’-H₂), 2.28 (2 H, t, J = 7.2 Hz, 1’-H₂), 3.56 (2 H, t, J = 6.4 Hz, 5’-H₂), 4.59 (2 H, br, NH₂), 4.98 (2 H, br, NH₂), 7.21 (2 H, d, J = 7.2 Hz, Ph 2,6-H₂), 7.37 (1 H, t, J = 7.2 Hz, Ph 4-H), 7.44 (2 H, t, J = 7.2 Hz, Ph 3,5-H₂); NMR (CD₃OD) δC 25.38, 28.49, 31.81, 33.79, 31.33, 108.29, 127.67, 128.95, 130.51, 134.76, 161.32, 162.98, 165.09; MS m/z 273.1704 (M + H) (C₁₅H₂₀N₄O requires 273.1715), 213 (M – C₃H₇O), 200 (M – C₄H₆O).
6.15. 1-(4-Chlorophenyl)-1-cyano-7-hydroxy-2-methoxyhept-1-ene (47b) and 5-(4-chlorophenyl)-6-(5-hydroxypentyl)pyrimidine-2,4-diamine (9b)

Compound 45b/46b was treated with CH$_2$N$_2$, as for the synthesis of 24a (followed by chromatography (EtOAc / hexane 3:1)), to give 47b (62%) as a pale yellow oil: NMR $\delta$H 1.52-1.80 (6 H, m, 4,5,6-H$_6$), 2.77 (2 H, t, $J = 7.8$ Hz, 3-H$_2$), 3.68 (2 H, t, $J = 6.2$ Hz, 7-H$_2$), 3.85 (3 H, s, Me), 7.29 (2 H, d, $J = 8.6$ Hz, Ar 2,6-H$_2$), 7.54 (2 H, d, $J = 8.6$ Hz, Ar 3,5-H$_2$); MS m/z 282.1077 (M + H) (C$_{15}$H$_{19}$ClNO$_2$ requires 280.1074), 280.1102 (M + H) (C$_{15}$H$_{19}$ClNO$_2$ requires 280.1104), 264/262 (M – OH). Compound 47b was treated with guanidine, as for the synthesis of 9a, to give 9b (59%) as a white solid: mp 165-166°C; IR $\nu_{\text{max}}$ 3407, 3329, 3174, 1631 cm$^{-1}$; NMR ((CD$_3$)$_2$SO) $\delta$H 1.11 (2 H, qn, $J = 7.4$ Hz, 3'-H$_2$), 1.26 (2 H, qn, $J = 7.4$ Hz, 4'-H$_2$), 1.42 (2 H, qn, $J = 7.4$ Hz, 2'-H$_2$), 2.07 (2 H, t, $J = 7.4$ Hz, 1'-H$_2$), 3.29 (2 H, t, $J = 7.4$ Hz, 5'-H$_2$), 7.18 (2 H, d, $J = 8.2$ Hz, Ar 2,6-H$_2$), 7.47 (2 H, d, $J = 8.2$ Hz, Ar 3,5-H$_2$); NMR ((CD$_3$)$_2$SO) $\delta$C 25.88, 28.58, 32.69, 34.63, 61.00, 106.16, 129.34, 132.25, 134.76, 133.10, 135.47, 162.44, 162.47, 165.87; MS m/z 309.1310 (M + H) (C$_{15}$H$_{20}$ClNO$_2$ requires 309.1296), 307.1335 (M + H) (C$_{15}$H$_{20}$ClNO$_2$ requires 307.1325), 236/234 (M - C$_4$H$_8$O).

6.16. 1-(4-Bromophenyl)-1-cyano-7-hydroxy-2-methoxyhept-1-ene (47c) and 5-(4-bromophenyl)-6-(5-hydroxypentyl)pyrimidine-2,4-diamine (9c)

Compound 45c/46c was treated with CH$_2$N$_2$, as for the synthesis of 47b, to give 47c (32%) as a pale yellow oil: NMR $\delta$H 1.53-1.75 (6 H, m, 4,5,6-H$_6$), 2.76 (2 H, t, $J = 7.0$ Hz, 3-H$_2$), 3.69 (2 H, t, $J = 7.0$ Hz, 7-H$_2$), 3.85 (3 H, s, Me), 7.44 (2 H, d, $J = 8.8$ Hz, Ar 2,6-H$_2$), 7.48 (2 H, d, $J = 8.8$ Hz, Ar 3,5-H$_2$); MS m/z 326.0583 (M + H) (C$_{15}$H$_{19}$BrNO$_2$ requires 326.0578), 324.0596 (M + H) (C$_{15}$H$_{20}$BrNO$_2$ requires 324.0599). Compound 47c was treated with guanidine, as for the synthesis of 9a, to give 9c (43%) as a white solid: mp 177-178°C; IR $\nu_{\text{max}}$ 3550, 3468, 3414, 1617 cm$^{-1}$; NMR ((CD$_3$)$_2$SO) $\delta$H 1.11 (2 H, qn, $J = 7.4$ Hz, 3'-H$_2$), 1.25 (2 H, qn, $J = 7.4$ Hz, 4'-H$_2$), 1.42 (2 H, qn, $J = 7.4$ Hz, 2'-H$_2$), 2.07 (2 H, t, $J = 7.4$ Hz, 1'-H$_2$), 3.27 (2 H, t, $J = 6.4$ Hz, 5'-H$_2$), 4.30 (1 H, br, OH), 5.74 (2 H, br, NH$_2$), 6.00 (2 H, br, NH$_2$), 7.11 (2 H, d, $J = 8.4$ Hz, Ar 2,6-H$_2$), 7.57 (2 H, d, $J = 8.4$ Hz, Ar 3,5-H$_2$); NMR (CF$_3$CO$_2$H salt) ((CD$_3$)$_2$SO) $\delta$C 25.33, 27.72, 30.33, 32.21, 60.68, 108.00, 115.78 (q, $J = 289.1$ Hz), 122.75, 130.74, 132.74, 133.24, 153.43, 155.31, 158 (q, $J = 37.6$ Hz), 164.30; MS m/z 353.0807 (M + H) (C$_{15}$H$_{20}$Br$_{79}$NO requires 353.0800), 351.0816 (M + H) (C$_{15}$H$_{20}$Br$_{79}$NO requires 351.0820).
6.17. 1-Cyano-1-(3,4-dichlorophenyl)-7-hydroxy-2-methoxyhept-1-ene (47d) and 5-(3,4-dichlorophenyl)-6-(5-hydroxypentyl)pyrimidine-2,4-diamine (9d)

Compound 45c/46c was treated with CH₂N₂, as for the synthesis of 47b, to give 47d (68%) as a pale yellow oil: NMR δH 1.41-1.50 (4 H, m, 5,6-H₂), 1.62 (2 H, qn, J = 7.6 Hz, 4-H₂), 2.75 (2 H, t, J = 7.6 Hz, 3-H₂), 3.41 (2 H, t, J = 6.0 Hz, 7-H₂), 3.95 (3 H, s, Me), 7.50 (1 H, dd, J = 8.6, 2.0 Hz, Ar 6-H), 7.63 (1 H, d, J = 8.6 Hz, Ar 5-H), 7.75 (1 H, d, J = 2.0 Hz, Ar 2-H); MS m/z 318.0686 (M + H) (C₁₅H₁₈³⁷Cl₂NO₂ requires 318.0655), 316.0689 (M + H) (C₁₅H₁₈³⁷Cl₃₅ClNO₂ requires 316.0685), 314.0715 (M + H) (C₁₅H₁₈³₅Cl₂NO₂ requires 314.0714), 291/289/287 (M – CN). Compound 47d was treated with guanidine, as for the synthesis of 9a, to give 9d (43%) as a white solid: mp 94-95ºC; IR νmax 3499, 3419, 3333, 1622 cm⁻¹; NMR ((CD₃)₂SO) δH 1.12 (2 H, qn, J = 7.4 Hz, 3'-H₂), 1.25 (2 H, qn, J = 7.4 Hz, 4'-H₂), 1.42 (2 H, qn, J = 7.4 Hz, 2'-H₂), 2.07 (2 H, t, J = 7.4 Hz, 1'-H₂), 3.27 (2 H, q, J = 5.6 Hz, 5'-H₂), 4.28 (1 H, t, J = 5.6 Hz, OH), 5.72 (2 H, br, NH₂), 5.90 (2 H, br, NH₂), 7.11 (1 H, dd, J = 8.2, 2.2 Hz, Ar 6-H), 7.36 (1 H, d, J = 2.2 Hz, Ar 2-H), 7.62 (1 H, d, J = 8.2 Hz, Ar 5-H); NMR ((CD₃)₂SO) δC 25.87, 28.50, 32.70, 34.64, 61.02, 105.23, 130.21, 131.37, 131.74, 131.81, 133.19, 137.66, 162.36, 162.69, 165.96; MS m/z 345.0885 (M + H) (C₁₅H₁₉³⁷Cl₂N₄O requires 345.0876), 343.0901 (M + H) (C₁₅H₁₉³⁷Cl₃₅ClN₄O requires 343.0906), 341.0927 (M + H) (C₁₅H₁₉³₅Cl₂N₄O requires 341.0935), 285/283/281 (M - C₃H₇O), 272/270/268 (M - C₄H₈O).

6.18. 5-Phenyl-6-((1S,2R)-1,2,3-trihydroxypropyl)pyrimidine-2,4-diamine (10a)

Compound 52a was treated with aq. CF₃CO₂H, as for the synthesis of 26a (reaction time 2 h), to give 10a (73%) as a highly hygroscopic white solid: [α]D²₀ = -0.38° (c 4, MeOH); NMR (CD₃CN) δH 3.45 (1 H, dd, J = 11.6, 4.9 Hz, 3'-H), 3.48 (1 H, dd, J = 11.6, 3.9 Hz, 3'-H), 3.72 (1 H, m, 2'-H), 4.46 (1 H, d, J = 6.2 Hz, 1'-H), 4.73 (2 H, br, NH₂), 5.82 (1 H, br, NH), 6.98 (1 H, br, NH), 7.31 (2 H, dd, J = 7.4, 2.0 Hz, Ph 2,6-H₂), 7.45-7.61 (3 H, m, Ph 3,4,5-H₃); MS m/z 299 (M + Na), 277.1308 (M + H) (C₁₅H₁₁N₄O₃ requires 277.1300).

6.19. 5-(4-Chlorophenyl)-6-((1S,2R)-1,2,3-trihydroxypropyl)pyrimidine-2,4-diamine (10b)

Compound 52b was treated with aq. CF₃CO₂H, as for the synthesis of 26a, to give 10b (90%) as a pale yellow solid: mp 196-197 ºC; [α]D²₀ = -41° (c 0.4, MeOH); IR νmax 3550, 3475,
3413, 1617 cm$^{-1}$; NMR ((CD$_3$)$_2$SO) $\delta$H 3.58 (1 H, dd, $J = 11.3$, 4.7 Hz, 3’-H), 3.63 (1 H, dd, $J = 11.3$, 3.5 Hz, 3’-H), 3.89-3.92 (1 H, m, 2’-H), 4.54 (1H, d, $J = 7.0$ Hz, 1’-H), 5.54 (5 H, br) and, 6.62 (1 H, br) (2 × NH$_2$ + 2 × OH), 7.40 (2 H, d, $J = 7.4$ Hz, Ar 2,6-H$_2$), 7.47 (2 H, d, $J = 7.4$ Hz, Ar 3,5-H$_2$), 7.79 (1 H, br, OH); NMR ((CD$_3$)$_2$SO) $\delta$C 62.60, 69.31, 72.47, 108.42, 129.88, 132.39, 133.53, 134.72, 161.21, 161.53, 161.87; MS m/z 313.0877 (M + H) (C$_{13}$H$_{16}$Cl$_6$N$_4$O$_3$ requires 313.0881), 311.0905 (M + H) (C$_{13}$H$_{16}$Cl$_4$N$_4$O$_3$ requires 311.0910).

6.20. 5-(4-Bromophenyl)-6-((1S,2R)-1,2,3-trihydroxypropyl)pyrimidine-2,4-diamine (10c)

Compound 52c was treated with aq. CF$_3$CO$_2$H, as for the synthesis of 26a (reaction time 4 h), to give 10c (95%) as a highly hygroscopic pale yellow solid: $[\alpha]_{D}^{20} = -15^\circ$ (c 0.9, MeOH); IR $\nu_{\text{max}}$ 3550, 3478, 3414, 1618 cm$^{-1}$; NMR (CD$_3$CN) $\delta$H 3.51 (1 H, dd, $J = 12.3$, 4.9 Hz, 3’-H), 3.55 (1 H, dd, $J = 12.3$, 4.5 Hz, 3’-H), 3.74 (1 H, m, 2’-H), 4.49 (1 H, d, $J = 6.2$ Hz, 1’-H), 5.94 (2 H, br, NH$_2$), 7.03 (2 H, br, NH$_2$), 7.29 (2 H, d, $J = 8.0$ Hz, Ar 2,6-H$_2$), 7.71 (2 H, d, $J = 8.0$ Hz, Ar 3,5-H$_2$); NMR (CD$_3$CN) $\delta$C 62.47, 68.98, 72.30, 108.83, 123.37, 129.70, 132.92, 132.98, 133.81, 156.05, 162.06, 164.91; MS m/z 379/377 (M + Na), 357.0396 (M + H) (C$_{13}$H$_{16}$Cl$_6$BrN$_4$O$_3$ requires 357.0385), 355.0412 (M + H) (C$_{13}$H$_{15}$Cl$_3$BrN$_4$O$_3$ requires 355.0405).

6.21. 5-(3,4-Dichlorophenyl)-6-((1S,2R)-1,2,3-trihydroxypropyl)pyrimidine-2,4-diamine (10d)

Compound 52d was treated with aq. CF$_3$CO$_2$H, as for the synthesis of 10c, to give 10d (87%) as a pale yellow solid: mp 120-121 ºC; $[\alpha]_{D}^{20} = -3.0^\circ$ (c 4.7, MeOH); IR $\nu_{\text{max}}$ 3549, 3476, 3415, 1618 cm$^{-1}$; NMR (CD$_3$CN) $\delta$H 3.41 (1 H, d, $J = 13.1$ Hz, 3’-H), 3.45 (1 H, d, $J = 13.1$ Hz, 3’-H), 3.63-3.68 (1 H, m, 2’-H), 4.35 (1 H, d, $J = 4.7$ Hz, 1’-H), 5.25 (2 H, br, NH$_2$), 5.67 (2 H, br, NH$_2$), 7.20 (1 H, dd, $J = 8.0$, 1.9 Hz, Ar 6-H), 7.46 (1 H, d, $J = 1.9$ Hz, Ar 2-H), 7.60 (1 H, d, $J = 8.0$ Hz, Ar 5-H); NMR ((CD$_3$)$_2$SO) $\delta$C 63.67, 69.38, 74.29, 106.16, 130.30, 131.08, 131.44, 131.63, 132.68, 134.09, 162.01, 162.81, 164.00; MS m/z 371/369/367 (M + Na), 349.0469 (M + H) (C$_{13}$H$_{15}$Cl$_2$N$_4$O$_3$ requires 349.0462), 347.0501 (M + H) (C$_{13}$H$_{15}$Cl$_3$N$_4$O$_3$ requires 347.0491), 345.0521 (M + H) (C$_{13}$H$_{15}$Cl$_3$N$_4$O$_3$ requires 345.0521).
6.22. 1-Cyano-1,4-diphenyl-2-methoxybut-1-ene (56) and 5-phenyl-6-(2-phenylethyl)-pyrimidine-2,4-diamine (11)

Compound 54/55 was treated with CH₂N₂, as for the synthesis of 24a, to give 56 (95%) as a pale yellow oil: IR νmax 2204 cm⁻¹; MS m/z 264.1390 (M + H) (C₁₈H₁₈NO requires 264.1388), 236 (M – HCN), 91 (Bn). Compound 56 was treated with guanidine, as for the synthesis of 25a (chromatographic eluant CH₂Cl₂ / MeOH (8:1)), to give 11 (32%) as a pale yellow solid: mp 116-118°C; NMR δH 2.54 (2 H, t, J = 8.0 Hz, CH₂), 2.83 (2 H, t, J = 8.0 Hz, CH₂), 4.62 (2 H, br, NH₂), 4.99 (2 H, br, NH₂), 6.94 (2 H, d, J = 6.8 Hz, Ph 2,6-H₂), 7.05 (2 H, d, J = 6.5 Hz, Ph’, 2,6-H₂), 7.14 (1H, t, J = 6.8 Hz, Ph 4-H), 7.17 (2 H, t, J = 6.8 Hz, Ph 3,5-H₂) 7.35 (2 H, t, J = 6.5 Hz, Ph’ 4-H), 7.39 (2 H, t, J = 6.5 Hz, Ph’ 3,5-H₂); MS m/z 291.1616 (M + H) (C₁₈H₁₉N₄ requires 291.1609), 199 (M – Bn).

6.23. 1-Cyano-2-methoxy-1-phenylprop-1-ene (60) and 6-methyl-5-phenylpyrimidine-2,4-diamine (12)

Compound 58/59 was treated with CH₂N₂, as for the synthesis of 24a, to give 60 (87%) as a pale yellow oil: IR νmax 2204, 1606 cm⁻¹; NMR δH 2.45 (3 H, s, CMe), 3.85 (3 H, s, OMe), 7.26 (1 H, t, J = 7.0 Hz, Ph 4-H), 7.30 (2 H, t, J = 7.0 Hz, Ph 3,5-H₂), 7.61 (2 H, t, J = 7.0 Hz, Ph 2,6-H₂); MS m/z 174.0921 (M + H) (C₁₁H₁₂NO requires 174.0918). Compound 60 was treated with guanidine, as for the synthesis of 25a (chromatographic eluant CH₂Cl₂ / MeOH (4:1)), to give 12 (38%) as a pale yellow solid: mp 250-251°C (lit. mp 249-251°C); IR νmax 3395, 3323 cm⁻¹; NMR ((CD₃)₂SO) δH 1.85 (3 H, s, Me), 5.62 (2 H, br, NH₂), 6.00 (2 H, br, NH₂), 7.20 (2 H, d, J = 7.3 Hz, Ph 2,6-H₂), 7.33 (1 H, t, J = 7.3 Hz, Ph 4-H), 7.43 (2 H, t, J = 7.3 Hz, Ph 3,5-H₂); MS m/z 201.1145 (M + H) (C₁₁H₁₃N₄ requires 201.1140), 123 (M – C₆H₅), 109 (M – C₇H₇).

6.24. 2,3-O-Isopropylidene-L-erythrose (18)

L-Arabinose 17 (10.0 g, 67 mmol), TsOH.H₂O (150 mg, 0.79 mmol) and 2,2-dimethoxy-propane (23.0 g, 221 mol) were stirred in dry DMF (130 mL) under N₂ for 90 min. The mixture was neutralised with Na₂CO₃. The evaporation residue was added to water (120 mL) and hexane (60 mL). NaIO₄ (35.5 g, 0.17 mol) was added to the aq. layer and the mixture was stirred for 2 h. Na₂CO₃ was added and the slurry was stirred for 1 h. The mixture was extracted with EtOAc. Evaporation and chromatography (Et₂O / hexane 2:1) gave 18 (5.8 g,
54%) as a colourless oil (lit.45 oil): NMR δH 1.31 (3 H, s, Me), 1.46 (3 H, s, Me), 3.89 (1 H, d, J = 2.5 Hz, OH), 4.01 (1 H, d, J = 10.5 Hz, 4-H), 4.05 (1 H, dd, J = 10.5, 3.5 Hz, 4-H), 4.55 (1 H, d, J = 6.0 Hz, 2-H), 4.82 (1 H, dd, J = 6.0, 3.5 Hz, 3-H), 5.39 (1 H, d, J = 2.5 Hz, 1-H); MS m/z 181 (M + Na), 159.0650 (M - H) (C7H11O4 requires 159.0657).

6.25. Ethyl (Z,4S,5R)-4-hydroxymethyl-2,2-dimethyl-1,3-dioxolane-5-propenoate (19Z) and ethyl (E,4S,5R)-4-hydroxymethyl-2,2-dimethyl-1,3-dioxolane-5-propenoate (19E)

Ethyl triphenylphosphoranylidineacetate (9.3 g, 27 mmol) was stirred with 18 (2.9 g, 18 mmol) in CH2Cl2 (130 mL) for 16 h. The evaporation residue was extracted with Et2O. Evaporation and chromatography (Et2O / hexane 1:1) gave 19Z (2.2 g, 54%) as a colourless oil (lit.46 oil): NMR δH 1.29 (3 H, t, J = 7.0 Hz, CH2C6H5), 1.40 (3 H, s, 2-Me), 1.53 (3 H, s, 2-Me), 2.44 (1 H, dd, J = 7.4, 5.5 Hz, OH), 3.45 (1 H, m, CHHOH), 3.59 (1 H, m, CHHOH), 4.16 (2 H, q, J = 7.4 Hz, CH2CH3), 4.53-4.57 (1 H, m, 4-H), 5.58 (1 H, dt, J = 7.1, 1.7 Hz, 5-H), 5.91 (1 H, dd, J = 11.7, 1.7 Hz, CHCO2), 6.36 (1 H, dd, J = 11.7, 7.1 Hz, CH=C=CO2). Further elution gave 19E (600 mg, 15%) as a colourless oil (lit.46 oil): NMR δH 1.29 (3 H, t, J = 7.2 Hz, CH2CH3), 1.40 (3 H, s, 2-Me), 1.52 (3 H, s, 2-Me), 2.41 (1 H, t, J = 5.9 Hz, OH), 3.55 (2 H, t, J = 5.9 Hz, CH2OH), 4.18 (2 H, q, J = 7.2 Hz, CH2CH3), 4.35 (1 H, m, 4-H), 4.79 (1H, dt, J = 5.5, 1.6 Hz, 5-H), 6.12 (1 H, dd, J = 15.6, 1.6 Hz, CHCO2), 6.88 (1 H, dd, J = 15.6, 5.5 Hz, CH=C=CO2); MS m/z 231.1240 (M + H) (C11H19O5 requires 231.1232), 215 (M - CH3).

6.26. Ethyl (4S,5R)-4-hydroxymethyl-2,3-dimethyl-1,3-dioxolane-5-propanoate (20)

A mixture of 19Z and 19E (2.3 g, 10 mmol) was stirred in EtOH (100 mL) with Pd/C (5%, 150 mg) under H2 for 3 h. Filtration (Celite®) and evaporation gave 20 (2.3 g, 99%) as a pale yellow oil (lit.46 oil): NMR δH 1.26 (3 H, t, J = 7.0 Hz, CH2CH3), 1.33 (3 H, s, 2-Me), 1.42 (3 H, s, 2-Me), 1.82 (2 H, m, CH2CH2CO2), 2.39 (1 H, br, OH), 2.40 (1 H, dt, J = 16.4, 7.8 Hz, CHCO2), 2.53 (1 H, dt, J = 16.4, 7.4 Hz, CHCO2), 3.65 (2 H, d, J = 5.1 Hz, CH2OH), 4.09-4.20 (4 H, m, 4-H + 5-H + CH2CH3); MS m/z 233.1396 (M + H) (C11H19O5 requires 233.1388), 217 (M - CH3), 173 (M – C3H6O), 143 (M – C4H3O2).

6.27. Ethyl (4S,5R)-4-benzyloxymethyl-2,2-dimethyl-1,3-dioxolane-4-propanoate (21)

LiN(SiMe3)2 (1.0 M in THF, 10 mL, 10 mmol) was stirred with 20 (2.3 g, 10 mmol) and BnBr (3.4 g, 20 mmol) in dry DMF (5 mL). After 2 h, water was added. The mixture was ex-
tracted (Et$_2$O). The extract was washed with water and brine and was dried. Evaporation and chromatography (Et$_2$O / hexane 1:4) afforded 21 (1.6 g, 48%) as a pale yellow oil: [α]$_{20}^D = +24.8^\circ$ (c 4.4, CHCl$_3$); NMR δ$_H$ 1.24 (3 H, t, $J = 7.0$ Hz, CH$_2$CH$_3$), 1.33 (3 H, s, 2-Me), 1.42 (3 H, s, 2-Me), 1.72-1.86 (2 H, m, CH$_2$CH$_2$CO$_2$), 2.50 (1 H, m, CHCO$_2$), 2.48-2.54 (1 H, m, CHCO$_2$), 3.50 (2 H, m, CH$_2$OBn), 4.08-4.15 (3 H, m, 5-H + CH$_2$CH$_3$), 4.28 (1 H, dd, $J = 11.9$, 6.1 Hz, 4-H), 4.50 (1 H, d, $J = 12.1$ Hz, CHPh), 4.57 (1 H, d, $J = 12.1$ Hz, CHPh), 7.24-7.33 (5H, m, Ph-H$_5$); MS m/z 323.1864 (M + H) (C$_{18}$H$_{27}$O$_5$ requires 323.1858), 265 (M − C$_3$H$_2$O), 91 (Bn).

6.28. (4R,5S)-5-Benzyloxymethyl-4-(4-cyano-3-oxo-4-phenylbutyl)-2,2-dimethyl-1,3-dioxolane (22a) / (4R,5S)-5-benzyloxymethyl-4-(4-cyano-3-hydroxy-4-phenylbut-3-enyl)-2,2-dimethyl-1,3-dioxolane (23a)

LiN(SiMe$_3$)$_2$ (1.0 M in THF, 9.1 mL, 9.1 mmol) was added to phenylacetonitrile (1.1 g, 9.4 mmol) in dry Et$_2$O (10 mL) under N$_2$ at -78°C. After 10 min, 21 (2.9 g, 9.0 mmol) was added. The mixture was allowed to warm to 20°C and was stirred for 72 h. Water was added. The solution was washed twice (Et$_2$O) before being acidified to pH 6 with aq. citric acid (1 M) in the presence of EtOAc. The EtOAc phase was separated and washed with water. Drying, evaporation and chromatography (EtOAc / hexane, 2:1) gave 22a/23a (1.5 g, 21%) as a yellow oil: IR $\nu_{max}$ 2207, 1728 cm$^{-1}$; NMR δ$_H$ 1.26 (3 H, s, 2-Me), 1.34 (3 H, s, 2-Me), 1.66-1.80 (2 H, m, CH$_2$CH$_2$CO), 2.63 (1 H, m, CHCO), 2.75-2.80 (1 H, m, CHCO), 3.45 (2 H, d, $J = 6.0$ Hz, CH$_2$OBn), 3.98 (1 H, m, 4-H), 4.20 (1 H, q, $J = 6.0$ Hz, 5-H), 4.45 (1 H, d, $J = 11.5$ Hz, CHPh), 4.52 (1 H, d, $J = 11.5$ Hz, CHPh), 7.21-7.41 (10 H, m, 2 × Ph-H$_5$), 8.98 (1 H, s, OH); MS m/z 394.2016 (M + H) (C$_{24}$H$_{28}$NO$_4$ requires 394.2018), 91 (Bn).

6.29. (4R,5S)-5-Benzylxymethyl-4-(4-(4-chlorophenyl)-4-cyano-3-oxobutyl)-2,2-dimethyl-1,3-dioxolane (22b) / (4R,5S)-5-benzyloxymethyl-4-(4-cyano-3-hydroxy-4-(4-chlorophenyl)but-3-enyl)-2,2-dimethyl-1,3-dioxolane (23b)

4-Chlorophenylacetonitrile and 21 were condensed as for the synthesis of 22a/23a (chromatographic eluant EtOAc / hexane (1:1)), to give 22b/23b (26%) as a yellow oil: NMR δ$_H$ 1.25 (2.7 H, s, Me), 1.34 (2.7 H, s, Me), 1.40 (0.3 H, s, Me), 1.51 (0.3 H, s, Me), 1.67-1.88 (2 H, m, CH$_2$CH$_2$CO), 1.98-2.08 (0.2 H, m, CH$_2$CO), 2.60-2.80 (1.8 H, m, CH$_2$CO), 3.45 (1 H, dd, $J = 12.1$, 6.0 Hz, CHOBN), 3.47 (1 H, dd, $J = 12.1$, 6.0 Hz, CHOBN), 4.00 (0.9 H, ddd, $J = 8.6$, 6.0, 2.3 Hz, 4-H), 4.22 (0.9 H, q, $J = 6.0$ Hz, 5-H), 4.29 (0.1 H, ddd, $J = 10.14$, 6.0, 3.9 Hz, 4-H).
Hz, 4-H), 4.39 (0.1 H, q, J = 6.0 Hz, 5-H), 4.45 (0.1 H, d, J = 11.5 Hz, CHPh), 4.49 (0.1 H, d, J = 11.5 Hz, CHPh), 4.51 (0.9 H, d, J = 11.9 Hz, CHPh), 4.56 (0.9 H, d, J = 11.9 Hz, CHPh), 5.52 (0.1 H, s, CHCN), 7.16 (0.2 H, d, J = 8.6 Hz, Ar 2,6-H2), 7.25-7.32 (5 H, m, Ph-H5), 7.35 (1.8 H, d, J = 8.6 Hz, Ar 3,5-H2), 7.84 (1.8 H, d, J = 8.6 Hz, Ar 2,6-H2), 9.33 (0.9 H, br, OH); MS m/z 430.1604 (M + H) (C24H26\textsuperscript{37}ClNO4 requires 430.1599), 428.1618 (M + H) (C24H27\textsuperscript{35}ClNO4 requires 428.1628), 370 (M - C2H4NO), 91 (Bn).

6.30. (4R,5S)-5-Benzoxymethyl-4-(4-(4-bromophenyl)-4-cyano-3-oxobutyl)-2,2-dimethyl-1,3-dioxolane (22c) / (4R,5S)-5-benzyloxymethyl-4-(4-cyano-3-hydroxy-4-(4-bromophenyl)but-3-enyl)-2,2-dimethyl-1,3-dioxolane (23c)

4-Bromophenylacetonitrile and 21 were condensed, as for the synthesis of 22a/23a, to give 22c/23c (18%) as a pale yellow oil: NMR δH 1.27 (3 H, s, 2-Me), 1.35 (3 H, s, 2-Me), 1.70-1.82 (2H, m, CH\textsubscript{2}CHO), 2.62-2.78 (2 H, m, CH\textsubscript{2}C=O), 3.45-3.47 (2 H, m, CH\textsubscript{2}OBn), 4.00 (1 H, ddd, J = 10.1, 6.2, 3.9 Hz, 4-H), 4.22 (1 H, q, J = 6.2 Hz, 5-H), 4.45 (1 H, d, J = 11.9 Hz, CHPh), 4.49 (1 H, d, J = 11.9 Hz, CHPh), 5.48 (0.35 H, s, CHCN), 7.21-7.35 (5 H, m, Ph-H5), 7.45 (1.3 H, d, J = 8.8 Hz, Ar 2,6-H2), 7.55 (1.3 H, d, J = 8.8 Hz, Ar 3,5-H2), 7.59 (0.7 H, d, J = 8.6 Hz, Ar 3,5-H2), 7.76 (0.7 H, d, J = 8.6 Hz, Ar 2,6-H2), 9.35 (0.65 H, s, OH); MS m/z 474.1100 (M + H) (C24H27\textsuperscript{81}BrNO4 requires 474.1102), 472.1094 (M + H) (C24H26\textsuperscript{79}BrNO4 requires 472.1123), 415/413 (M - C2H4NO), 91 (Bn).

6.31. (4R,5S)-5-Benzoxymethyl-4-(4-(3,4-dichlorophenyl)-4-cyano-3-oxobutyl)-2,2-dimethyl-1,3-dioxolane (22d) / (4R,5S)-5-benzyloxymethyl-4-(4-cyano-3-hydroxy-4-(3,4-dichlorophenyl)but-3-enyl)-2,2-dimethyl-1,3-dioxolane (23d)

3,4-Dichlorophenylacetonitrile and 21 were condensed, as for the synthesis of 22a/23a, to give 22d/23d (16%) as a highly hygroscopic white solid: NMR δH 1.41 (3 H, s, 2-Me), 1.52 (3 H, s, 2-Me), 1.73-1.87 (2 H, m, CH\textsubscript{2}CHO), 2.71-2.84 (2 H, m, CH\textsubscript{2}C=O), 3.47 (1 H, dd, J = 11.7, 6.0 Hz, CHOBn), 3.49 (1 H, dd, J = 11.7, 6.0 Hz, CHOBn), 3.98 (1 H, m, 4-H), 4.22 (1 H, d, J = 6.0 Hz, 5-H), 4.47 (1 H, d, J = 12.3 Hz, CHPh), 4.56 (1 H, d, J = 12.3 Hz, CHPh), 7.26-7.37 (5 H, m, Ph-H5), 7.45 (1 H, d, J = 8.6 Hz, Ar 5-H), 7.50 (1 H, dd, J = 8.6, 2.0 Hz, Ar 6-H), 7.83 (1 H, d, J = 2.0 Hz, Ar 2-H), 9.61 (1 H, s, OH); MS m/z. 466.1178 (M + H) (C24H26\textsuperscript{37}Cl\textsubscript{2}NO4 requires 466.1179), 464.1193 (M + H) (C24H26\textsuperscript{37}Cl\textsuperscript{35}ClNO4 requires 464.1209), 462.1217 (M + H) (C24H26\textsuperscript{35}Cl\textsubscript{2}NO4 requires 462.1238), 407/405/403 (M - C2H4NO), 91 (Bn).
6.32. (4R,5S)-5-Benzylxoyethyl-4-(4-cyano-3-methoxy-4-phenylbut-3-enyl)-2,2-dimethyl-1,3-dioxolane (24a) and 6-(2-((4R,5S)-5-benzylxoyethyl-2,2-dimethyl-1,3-dioxolan-4-yl)ethyl)-5-phenylpyrimidine-2,4-diamine (25a)

Compound 22a/23a (1.5 g, 3.7 mmol) in THF (5 mL) was treated with CH$_2$N$_2$ (8.0 mmol) in Et$_2$O (20 mL) at 10°C for 16 h. Excess CH$_2$N$_2$ was destroyed by careful addition of AcOH (30% in THF). Evaporation gave 24a (1.2 g, 81%) as a yellow oil: NMR $\delta$H 1.37 (3 H, s, 2-Me), 1.46 (3 H, s, 2-Me), 1.77-1.88 (2 H, m, CH$_2$CHO), 2.78-2.86 (1 H, m, CH=C), 2.90-2.98 (1 H, m, CHC=C), 3.53 (2 H, d, $J$ = 5.9 Hz, CH$_2$OBn), 3.78 (3 H, s, OMe), 4.22 (1 H, m, 4-H), 4.33 (1 H, m, 5-H), 4.44 (1 H, d, $J$ = 12.1 Hz, CHPh), 4.51 (1 H, d, $J$ = 12.1 Hz, CHPh), 7.21-7.59 (10 H, m, 2 × Ph-H 5); MS m/z 408.2166 (M + H) (C$_{25}$H$_{30}$NO$_4$ requires 408.2174), 350 (M – C$_2$H$_3$NO), 91 (Bn). NaOMe (140 g, 2.6 mmol) was stirred with guanidine.HCl (300 mg, 2.6 mmol) in MeO(CH$_2$)$_2$OH (10 mL) stirred for 5 min at 30°C. The filtered solution was boiled under reflux with 24a (700 mg, 1.8 mmol) for 16 h. Evaporation and chromatography (CHCl$_3$ / MeOH 19:1) gave 25a (400 mg, 46%) as a highly hygroscopic pale yellow solid: IR $\nu$$_{max}$ 3415, 1685 cm$^{-1}$; NMR $\delta$H 1.23 (3 H, s, Me), 1.24 (3 H, s, Me), 1.61-1.67 (2 H, m, CH$_2$CHO), 2.24 (1 H, m, Pyr-CH), 2.51 (1 H, m, Pyr-CH), 3.4 (2 H, d, $J$ = 6.0 Hz, CH$_2$OBn), 3.95-4.00 (1 H, m, dioxolane 4-H), 4.15 (1 H, q, $J$ = 6.0 Hz, dioxolane 5-H), 4.44 (1H, d, $J$ = 12.3 Hz, CHPh), 4.53 (1 H, d, $J$ = 12.3 Hz, CHPh), 4.68 (2 H, br, NH$_2$), 5.01 (2 H, br, NH$_2$), 7.09-7.39 (10 H, m, 2 × Ph-H$_3$); MS m/z 435.2423 (M + H) (C$_{25}$H$_{31}$N$_4$O$_3$ requires 435.2396), 200 (M – C$_{14}$H$_{18}$O$_3$), 91 (Bn).

6.33. (4R,5S)-5-Benzylxoyethyl-4-(-4-(4-chlorophenyl)-4-cyano-3-methoxybut-3-enyl)-2,2-dimethyl-1,3-dioxolane (24b) and 6-(2-((4R,5S)-5-benzylxoyethyl-2,2-dimethyl-1,3-dioxolan-4-yl)ethyl)-5-(4-chlorophenyl)pyrimidine-2,4-diamine (25b)

Compound 22b/23b was treated with CH$_2$N$_2$, as for the synthesis of 24a, to give 24b (97%) as a pale yellow oil: NMR $\delta$H 1.36 (3 H, s, Me), 1.45 (3 H, s, Me), 1.74 (1 H, m, CHCHO), 1.85 (1 H, m, CHCHO), 2.80 (1 H, m, CHC=C), 2.94 (1 H, m, CHC=C), 3.51 (1 H, dd, $J$ = 11.7, 6.0 Hz, CHOBn), 3.53 (1 H, dd, $J$ = 11.7, 6.0 Hz, CHOBn), 3.80 (3 H, s, OMe), 4.22 (1 H, ddd, $J$ = 9.4, 6.0, 3.1 Hz, 4-H), 4.33 (1 H, q, $J$ = 6.0 Hz, 5-H), 4.50 (1 H, d, $J$ = 12.1 Hz, CHPh), 4.59 (1 H, d, $J$ = 12.1 Hz, CHPh), 7.2 (2 H, d, $J$ = 8.6 Hz, Ar 2,6-H$_2$), 7.30-7.36 (5 H, m, Ph-H$_3$), 7.52 (2 H, d, $J$ = 8.6 Hz, Ar 3,5-H$_2$); MS m/z 444.1746 (M + H) (C$_{25}$H$_{29}$ClNO$_4$ requires 444.1755), 442.1764 (M + H) (C$_{25}$H$_{29}$ClNO$_4$ requires 442.1785), 428/426 (M - CH$_3$), 386/384 (M – C$_2$H$_3$NO), 91 (Bn). Compound 24b was treated with
guanidine, as for the synthesis of 25a (chromatographic eluant CHCl₃ / MeOH (9:1)) to give 25b (25%) as a pale buff solid: mp 55-56°C; NMR δH 1.28 (6 H, s, Me₂), 1.62-1.71 (2 H, m, CH₂CHO), 2.25 (1 H, ddd, J = 13.5, 10.1, 5.9 Hz, Pyr-CH), 2.51 (1 H, ddd, J = 13.5, 10.1, 5.9 Hz, Pyr-CH), 3.43 (2 H, d, J = 5.9 Hz, CH₂OBn), 4.01 (1 H, ddd, J = 10.1, 5.9, 4.3 Hz, dioxolane 4-H), 4.21 (1 H, q, J = 5.9 Hz, dioxolane 5-H), 4.47 (1 H, d, J = 12.1 Hz, CPh), 4.56 (1 H, d, J = 12.1 Hz, CPh), 4.82 (2 H, br, NH₂), 5.23 (2 H, br, NH₂), 7.14 (1 H, d, J = 8.2 Hz, Ar 2-H), 7.15 (1 H, d, J = 8.2 Hz, Ar 6-H), 7.27-7.37 (5H, m, Ph-H₅), 7.39 (2 H, d, J = 8.2 Hz, Ar 3,5-H₂); MS m/z 471.1992 (M + H) (C₂₅H₃₀ClN₄O₃ requires 471.1976), 469.2005 (M + H) (C₂₅H₂₈ClN₄O₄ requires 469.2006), 236/234 (M - C₁₄H₁₈O₃), 91 (Bn).

6.34. (4R,5S)-5-Benzylxymethyl-4-(4-(4-bromophenyl)-4-cyano-3-methoxybut-3-enyl)-2,2-dimethyl-1,3-dioxolane (24c) and 6-(2-((4R,5S)-5-benzyloxymethyl-2,2-dimethyl-1,3-dioxolan-4-yl)ethyl)-5-(4-bromophenyl)pyrimidine-2,4-diamine (25c)

Compound 22c/23c was treated with CH₂N₂, as for the synthesis of 24a, to give 24c (87%) as a pale yellow oil: NMR δH 1.36 (3 H, s, 2-Me), 1.45 (3 H, s, 2-Me), 1.75-1.88 (2 H, m, CH₂CHO), 2.80 (1 H, m, CHC=), 2.93 (1 H, m, CHC=C), 3.51 (1 H, ddd, J = 11.7, 5.9 Hz, CH₂OBn), 3.53 (1 H, ddd, J = 11.7, 5.9 Hz, CH₂OBn), 3.80 (3 H, s, OMe), 4.21 (1 H, ddd, J = 9.4, 5.9, 3.1 Hz, 4-H), 4.32 (1 H, q, J = 5.9 Hz, 5-H), 4.50 (1 H, d, J = 11.9 Hz, CPh), 4.59 (1 H, d, J = 11.9 Hz, CPh), 7.27-7.32 (5H, m, Ph-H₅), 7.43 (2 H, d, J = 8.6 Hz, Ar 2,6-H₂), 7.47 (2 H, d, J = 9.0 Hz, Ar 3,5-H₂); MS m/z 488.1255 (M + H) (C₂₅H₂₉BrNO₄ requires 488.1259), 486.1263 (M + H) (C₂₅H₂₇BrNO₄ requires 486.1279), 430/428 (M - CH₃), 91 (Bn). Compound 24c was treated with guanidine, as for the synthesis of 25a, to give 25c (57%) as a pale buff solid: mp 62-64°C; IR νmax 3462, 1635 cm⁻¹; NMR δH 1.26 (6 H, s, Me₂), 1.58-1.63 (2 H, m, CH₂CHO), 2.22 (1 H, ddd, J = 13.3, 10.1, 5.9 Hz, Pyr-CH), 2.49 (1 H, ddd, J = 13.3, 10.1, 5.9 Hz, Pyr-CH), 3.41 (2 H, d, J = 5.9 Hz, CH₂OBn), 4.00 (1 H, ddd, J = 9.8, 5.9, 3.5 Hz, dioxolane 4-H), 4.20 (1 H, q, J = 5.9 Hz, dioxolane 5-H), 4.45 (1 H, d, J = 12.1 Hz, CPh), 4.54 (1 H, d, J = 12.1 Hz, CPh), 4.65 (2 H, br, NH₂), 5.06 (2 H, br, NH₂), 7.04 (1 H, d, J = 7.8 Hz, Ar 2-H), 7.06 (1 H, d, J = 8.2 Hz, Ar 6-H), 7.23-7.33 (5 H, m, Ph-H₅), 7.50 (2 H, d, J = 8.2 Hz, Ar 3,5-H₂); MS m/z 515.1483 (M + H) (C₂₅H₂₉BrNO₄ requires 515.1480), 513.1497 (M + H) (C₂₅H₂₇BrNO₄ requires 513.1501), 499/497 (M - CH₃), 91 (Bn).
6.35. (4R,5S)-5-Benzylloxymethyl-4-(4-(3,4-dichlorophenyl)-4-cyano-3-methoxybut-3-en-yl)-2,2-dimethyl-1,3-dioxolane (24d) and 6-(2-((4R,5S)-5-benzyloxymethyl-2,2-dimethyl-1,3-dioxolan-4-yl)ethyl)-5-(3,4-dichlorophenyl)pyrimidine-2,4-diamine (25d)

Compound 22d/23d was treated with CH₂N₂, as for the synthesis of 24a, to give 24d (97%) as a pale yellow oil: NMR δH 1.36 (3 H, s, 2-Me), 1.45 (3 H, s, 2-Me), 1.77 (1 H, m, CHCHO), 1.85 (1 H, m, CHCHO), 2.81 (1 H, m, CHC=CH), 2.94 (1 H, m, CHC=CH), 3.51 (1 H, dd, J = 11.3, 6.0 Hz, CHOBn), 3.53 (1 H, dd, J = 11.3, 6.0 Hz, CHOBn), 3.84 (3 H, s, OMe), 4.21 (1 H, ddd, J = 9.4, 6.0, 3.1 Hz, 4-H), 4.32 (1 H, q, J = 6.0 Hz, 5-H), 4.49 (1 H, d, J = 12.1 Hz, CHPh), 4.58 (1 H, d, J = 12.1 Hz, CHPh), 7.26-7.32 (5H, m, Ph-H 5), 7.37 (1 H, d, J = 8.6 Hz, Ar 5-H), 7.42 (1 H, dd, J = 8.6, 2.1 Hz, Ar 6-H), 7.73 (1 H, d, J = 2.1 Hz, Ar 2-H); MS m/z 480.1319 (M + H) (C₂₅H₂₈₃₇Cl₂NO₄ requires 480.1336), 478.1344 (M + H) (C₂₅H₂₈₃₇Cl 35ClNO₄ requires 478.1365), 476.1367 (M + H) (C₂₅H₂₈₃₅Cl₂NO₄ requires 476.1395), 422/420/418 (M - C₂H₃NO), 91 (Bn). Compound 24d was treated with guanidine, as for the synthesis of 25a, to give 25d (47%) as a pale buff solid: mp 67-69°C; IR νmax 3411, 1637 cm⁻¹; NMR δH 1.27 (6 H, s, Me₂), 1.60 (1 H, m, CHCHO), 1.73 (1 H, m, CHCHO), 2.25 (1 H, m, Pyr-CH), 2.47 (1 H, m, Pyr-CH), 3.44 (2 H, d, J = 6.0 Hz, CH₂OBn), 3.99 (1 H, d, J = 9.8, 6.0, 3.5 Hz, dioxolane 4-H), 4.20 (1 H, q, J = 6.0 Hz, dioxolane 5-H), 4.47 (1 H, d, J = 12.3 Hz, CHPh), 4.56 (1 H, d, J = 12.3 Hz, CHPh), 4.85 (2 H, br, NH₂), 5.21 (2 H, br, NH₂), 7.03 (1 H, dd, J = 8.0, 2.0 Hz, Ar 6-H), 7.26-7.32 (5 H, m, Ph-H₃), 7.35 (1 H, d, J = 2.0 Hz, Ar 2-H), 7.46 (1 H, d, J = 8.0 Hz, Ar 5-H); MS m/z 507.1563 (M + H) (C₂₅H₂₉₃₇Cl₂N₄O₃ requires 507.1557), 505.1586 (M + H) (C₂₅H₂₉₃₇Cl 35ClN₄O₃ requires 505.1587), 503.1617 (M + H) (C₂₅H₂₉₃₅Cl₂N₄O₃ requires 503.1616), 91 (Bn).

6.36. 6-((3R,4S)-5-Benzylxyloxy-3,4-dihydroxypentyl)-5-phenylpyrimidine-2,4-diamine (26a)

Compound 25a (700 mg, 1.6 mmol) was stirred for 16 h with aq. CF₃CO₂H (30%, 70 mL). Evaporation and chromatography (CHCl₃ / MeOH 7:3) to give 26a (600 mg, 95%) as a highly hygroscopic pale yellow solid: NMR (CD₃OD) δH 1.69 (1 H, m, 2'-H), 1.88 (1 H, m, 2'-H), 2.43 (1 H, m, 1'-H), 2.60 (1 H, ddd, J = 15.3, 10.6, 5.5 Hz, 1'-H), 3.43-3.47 (2 H, m, 3’,4’-H₂), 3.49-3.56 (2 H, m, 5’-H₂), 4.52 (1 H, d, J = 12.6 Hz, CHPh), 4.56 (1 H, d, J = 12.6 Hz, CHPh), 7.27-7.41 (6 H, m, 2 × Ph 3,4,5-H₃), 7.47-7.58 (4 H, m, 2 × Ph 2,6-H₂); MS m/z 417 (M + Na), 395.2097 (M + H) (C₂₂H₂₇N₄O₃ requires 395.2083), 243 (M - C₉H₁₄O₂), 213 (M – C₁₀H₁₃O₃), 91 (Bn).
6.37. 6-((3R,4S)-5-Benzylxoy-3,4-dihydroxypentyl)-5-(3-chlorophenyl)pyrimidine-2,4-diamine (26b)

Compound 25b was treated with aq. CF₃CO₂H, as for the synthesis of 25a, to give 26b (87%) as a white solid: mp 131-133°C; NMR (CD₃OD) δ_H 1.64 (1 H, m, 2'-H), 1.82 (1 H, m, 2'-H), 2.37 (1 H, m, 1'-H), 2.51 (1 H, m, 1'-H), 3.49-3.55 (2 H, m, 3',4'-H₂), 3.57-3.64 (2 H, m, 5'-H₂), 4.52 (1 H, d, J = 12.5 Hz, CHPh), 4.56 (1 H, d, J = 12.5 Hz, CHPh), 7.24 (2 H, d, J = 8.6 Hz, Ar 2,6-H₂), 7.33-7.37 (5 H, m, Ph-H₅), 7.47 (2 H, d, J = 8.6 Hz, Ar 3,5-H₂); NMR (CD₃OD) δ_C 29.02, 30.84, 71.03, 71.61, 72.09, 72.99, 107.42, 121.01, 127.36, 127.58, 128.01, 129.32, 132.16, 134.14, 138.10, 161.81, 162.16, 163.66; MS m/z 453/451 (M + Na), 431.1657 (M + H) (C₂₂H₂₆₃₇ClN₄O₃ requires 431.1663), 429.1680 (M + H) (C₂₂H₂₆₃₅ClN₄O₄ requires 429.1693), 279/277 (M - C₉H₁₁O₂), 249/247 (M - C₁₀H₁₃O₃), 91 (Bn).

6.38. 6-((3R,4S)-5-Benzylxoy-3,4-dihydroxypentyl)-5-(3-bromophenyl)pyrimidine-2,4-diamine (26c)

Compound 25c was treated with aq. CF₃CO₂H, as for the synthesis of 25a, to give 26c (91%) as a highly hygroscopic pale buff solid: NMR δ_H 1.65-1.84 (2 H, m, 2'-H₂), 2.35-2.55 (2 H, m, 1'-H₂), 3.52-3.71 (4 H, m, 3',4',5'-H₄), 4.49 (1 H, d, J = 12.1 Hz, CHPh), 4.54 (1 H, d, J = 12.1 Hz, CHPh), 4.71 (2 H, br, NH₂), 5.09 (2 H, br, NH₂), 7.05 (2 H, d, J = 8.4 Hz, Ar 2,6-H₂), 7.24-7.34 (5 H, m, Ph-H₅), 7.54 (2 H, d, J = 8.4 Hz, Ar 3,5-H₂); MS m/z 497/495 (M + Na), 475.1184 (M + H) (C₂₂H₂₆₈₁BrN₄O₃ requires 475.1167), 473.1179 (M + H) (C₂₂H₂₆₇₉BrN₄O₄ requires 473.1188), 323/321 (M - C₉H₁₁O₂), 293/291 (M - C₁₀H₁₃O₃), 91 (Bn).

6.39. 6-((3R,4S)-5-Benzylxoy-3,4-dihydroxypentyl)-5-(3,4-dichlorophenyl)pyrimidine-2,4-diamine (26d)

Compound 25d was treated with aq. CF₃CO₂H, as for the synthesis of 25a, to give 26d (74%) as a pale yellow solid: mp 123-125°C; NMR (CD₃OD) δ_H 1.60 (1 H, m, 2'-H), 1.82 (1 H, m, 2'-H), 2.31 (1 H, ddd, J = 14.2, 9.0, 5.9 Hz, 1'-H), 2.47 (1 H, ddd, J = 14.2, 9.0, 5.9 Hz, 1'-H), 3.44-3.51 (2 H, m, 3',4'-H₂), 3.54-3.59 (2 H, m, 5'-H₂), 4.47 (1 H, d, J = 14.1 Hz, CHPh), 4.52 (1 H, d, J = 14.1 Hz, CHPh), 7.15 (1 H, dd, J = 8.2, 1.9 Hz, Ar 6-H), 7.22-7.33 (5 H, m, Ph-H₅), 7.41 (1 H, d, J = 1.9 Hz, Ar 2-H), 7.57 (1 H, d, J = 8.2 Hz, Ar 5-H); NMR (CD₃OD) δ_C 23.30, 30.97, 71.23, 71.58, 72.99, 106.22, 181.16, 127.31, 127.55, 127.99, 130.64, 131.15,
131.95, 132.61, 134.54, 138.17, 162.09, 162.83, 163.16; MS m/z 489/487/485 (M + Na), 467.1258 (M + H) (C_{22}H_{25}\text{Cl}_{2}N_{4}O_{3} \text{ requires} 467.1244), 465.1272 (M + H) (C_{22}H_{25}\text{Cl}_{3}N_{4}O_{3} \text{ requires} 465.1274), 463.1293 (M + H) (C_{22}H_{25}\text{Cl}_{2}N_{4}O_{3} \text{ requires} 463.1303), 315/313/311 (M - C_{9}H_{11}O_{2}), 91 (Bn).

6.40. \((4R,5S)-4-(4-Cyano-3-oxo-4-phenylbutyl)-5-hydroxymethyl-2,2-dimethyl-1,3-dioxolane (27a) / (4R,5S)-4-(4-cyano-3-hydroxy-4-phenylbut-3-enyl)-5-hydroxymethyl-2,2-dimethyl-1,3-dioxolane (28a)\)

LiN(SiMe\text{3})\text{2} (1.0 M in THF, 30 mL, 30 mmol) was added to phenylacetonitrile (1.75 g, 15 mmol) in dry Et\text{2}O (15 mL) under N\text{2} at -78°C. After 10 min, 20 (3.5 g, 15 mmol) was added. The mixture was warmed to 20°C and was stirred for 72 h. Water was added. The solution was washed twice (Et\text{2}O) before being acidified to pH 6 with aq. citric acid (1 M) in the presence of EtOAc. The EtOAc phase was separated and washed with water. Drying, evaporation and chromatography (EtOAc / hexane, 3:1) gave 27a/28a (450 mg, 10%) as a pale yellow oil: NMR δH 1.30 (3 H, s, Me), 1.40 (3 H, s, Me), 1.74-1.81 (2 H, m, CH\text{2}CHO), 2.71 (1 H, m, CHC=O), 2.83 (1 H, m, CHC=O), 3.62 (2 H, d, J = 5.5 Hz, CH\text{2}OH), 4.07 (1 H, m, 4-H), 4.15 (1 H, m, 5-H), 4.79 (1 H, s, CHCN), 7.36-7.50 (5 H, m, Ph-H); MS m/z 315.1464 (M + H) (C\text{17}H\text{21}NO\text{4} requires 315.1470), 287 (M - CH\text{3}), 271 (M - CH\text{2}O), 245 (M - C\text{2}H\text{3}NO).

6.41. \((4R,5S)-4-(4-(4-Chlorophenyl)-4-cyano-3-oxobutyl)-5-hydroxymethyl-2,2-dimethyl-1,3-dioxolane (27b) / (4R,5S)-4-(4-(4-chlorophenyl)-4-cyano-3-hydroxybut-3-enyl)-5-hydroxymethyl-2,2-dimethyl-1,3-dioxolane (28b)\)

4-Chlorophenylacetonitrile was condensed with 20, as for the synthesis of 27a/28a, to give 27b/28b (7%) as a pale yellow solid; mp 133-135°C; NMR δH 1.42 (3 H, s, Me), 1.52 (3 H, s, Me), 2.35-2.52 (2 H, m, CH\text{2}CHO), 2.66-2.84 (2 H, m, CH\text{2}C=O), 3.63 (1 H, dd, J = 12.7, 4.9 Hz, CH\text{2}OH), 3.73 (1 H, dd, J = 12.7, 4.9 Hz, CH\text{2}OH), 4.21-4.28 (2 H, m, 4,5-H 2), 7.44 (2 H, d, J = 9.0 Hz, Ar 2,6-H 2), 7.44 (2 H, d, J = 9.0 Hz, Ar 3,5-H 2); MS m/z 340 (M + H) (C\text{17}H\text{20}\text{Cl} \text{ requires} 340.1464), 338 (M + H) C\text{17}H\text{20}\text{Cl} \text{ requires} 338.1464), 322/320 (M – OH), 308/306 (M - CH\text{3}O).

6.42. \((4R,5S)-4-(4-(4-Bromophenyl)-4-cyano-3-oxobutyl)-5-hydroxymethyl-2,2-dimethyl-1,3-dioxolane (27c) / (4R,5S)-4-(4-(4-bromophenyl)-4-cyano-3-hydroxybut-3-enyl)-5-hydroxymethyl-2,2-dimethyl-1,3-dioxolane (28c)\)

4-Bromophenylacetonitrile was condensed with 20, as for the synthesis of 27a/28a, to give
27c/28c (6%) as a pale yellow solid: mp 128-130°C; NMR δH 1.41 (3 H, s, Me), 1.51 (3 H, s, Me), 2.39-2.48 (2 H, m, CH₂CHO), 2.69-2.79 (2 H, m, CH₂C=O), 3.62 (1 H, dd, J = 11.6, 5.3 Hz, CHO₇), 3.73 (1 H, dd, J = 11.6, 5.3 Hz, CHO₂), 4.19-4.27 (2 H, m, 4,5-H₂), 7.30 (2 H, d, J = 8.5 Hz, Ar 2,6-H₂), 7.47 (2 H, d, J = 8.5 Hz, Ar 3,5-H₂); MS m/z 382.0480 (M + H) (C₁₇H₂₀₈¹BrNO₄ requires 382.0476), 380.0475 (M + H) (C₁₇H₂₀₇⁹BrNO₄ requires 380.0497), 368/366 (M - CH₃), 342/340 (M - C₂H₃N), 326/324 (M – C₂H₃NO).

6.43. (4R,5S)-4-(4-Cyano-4-(3,4-dichlorophenyl)-3-oxobutyl)-5-hydroxymethyl-2,2-dimethyl-1,3-dioxolane (27d) / (4R,5S)-4-(4-cyano-4-(3,4-dichlorophenyl)-3-hydroxybut-3-enyl)-5-hydroxymethyl-2,2-dimethyl-1,3-dioxolane (28d)

3,4-Dichlorophenylacetonitrile was condensed with 20, as for the synthesis of 27a/28a, to give 27d/28d (7%) as a pale yellow solid: mp 117-118 °C; IR νmax 3424, 2209, 1718 cm⁻¹; NMR δH 1.42 (3 H, s, Me), 1.52 (3 H, s, Me), 2.38-2.47 (2 H, m, CH₂CHO), 2.72-2.84 (2 H, m, CH₂C=O), 3.64 (1 H, dd, J = 11.5, 5.5 Hz, CHO₀), 3.75 (1 H, dd, J = 11.5, 5.5 Hz, CHO₂), 4.18-4.29 (2 H, m, 4,5-H₂), 7.27 (1 H, dd, J = 8.5, 2.2 Hz, Ar 6-H), 7.43 (1 H, d, J = 8.5 Hz, Ar 5-H), 7.54 (1 H, d, J = 2.2 Hz, Ar 2-H). MS m/z 375/373/371 (M + H), 334/332/330 (M - C₂H₂N), 316/314/312 (M – C₂H₅NO).

6.44. (4R,5S)-4-(4-Cyano-3-methoxy-4-phenylbut-3-enyl)-2,2-dimethyl-5-hydroxymethyl-1,3-dioxolane (29a) and 6-(2-((4R,5S)-2,2-dimethyl-5-hydroxymethyl-1,3-dioxolan-4-yl)ethyl)-5-phenylpyrimidine-2,4-diamine (30a)

Compound 27a/28a was treated with CH₂N₂, as for the synthesis of 24a, to give 29a (330 mg, 95%) as a pale yellow oil: NMR δH 1.40 (3 H, s, 2-Me), 1.51 (3 H, s, 2-Me), 1.87-2.01 (2 H, m, CH₂CHO), 2.84-3.02 (2 H, m, CH₂C=C), 3.69 (2 H, d, J = 5.9 Hz, CH₂OH), 3.86 (3 H, s, OMe), 4.22-4.27 (2 H, m, 4,5-H₂), 7.24-7.42 (5 H, m, Ph-H 5); MS m/z 318.1707 (M + H) (C₁₈H₂₄NO₄ requires 318.1705), 302 (M – Me), 277 (M - C₂H₂N), 258 (M – C₂H₃NO). Compound 29a was treated with guanidine, as for the synthesis of 25a (reaction time 4 h, chromatographic eluant CHCl₃ / MeOH (9:1)), to give 30a (42%) as a pale buff solid: mp 72-75°C; NMR (D₂O) δH 1.27 (3 H, s, Me), 1.31 (3 H, s, Me), 1.75-1.88 (2 H, m, CH₂CHO), 2.32 (1 H, ddd, J = 15.9, 9.7, 6.0 Hz, Pyr-CH), 2.49 (1 H, ddd, J = 15.9, 8.7, 7.4 Hz, Pyr-CH), 3.53 (1 H, ddd, J = 11.6, 5.9 Hz, CHO₀), 3.65 (1 H, dd, J = 11.6, 5.9 Hz, CHO₂), 3.65 (1 H, d, J = 11.6, 5.9 Hz, CHO₂), 4.05 (1 H, dd, J = 12.2, 5.9 Hz, dioxolane 4-H), 4.12 (1 H, q, J = 5.9 Hz, dioxolane 5-H), 4.63 (2 H, br, NH₂), 4.95 (2 H, br, NH₂), 7.21 (1 H, d, J = 7.9 Hz, Ar 2-H), 7.22 (1 H, d, J = 7.9 Hz, Ar 2-H), 7.36
(1 H, t, J = 7.9 Hz, Ar 4-H), 7.21 (2H, t, J = 7.9 Hz, Ar 3,5-H2); NMR (D2O) δ C 23.01, 25.58, 25.70, 28.17, 58.60, 74.07, 75.63, 105.27, 106.26, 125.50, 126.82, 126.85, 127.94, 158.71, 159.82, 162.92; MS m/z 345.1935 (M + H) (C19H23N4O3 requires 345.1926), 329 (M - Me), 200 (M – C7H12O3).

6.45. (4R,5S)-4-(4-(4-Chlorophenyl)-4-cyano-3-methoxybut-3-enyl)-2,2-dimethyl-5-hydroxymethyl-1,3-dioxolane (29b) and 5-(4-chlorophenyl)-6-(2-((4R,5S)-2,2-dimethyl-5-hydroxymethyl-1,3-dioxolan-4-yl)ethyl)pyrimidine-2,4-diamine (30b)

Compound 27b/28b was treated with CH2N2, as for the synthesis of 24a, to give 29b (90%) as a pale yellow oil. Compound 29b was treated with guanidine, as for the synthesis of 25a (reaction time 10 h, chromatographic eluant CHCl3 / MeOH (9:1)), to give 30b (12%) as a white solid: mp 94-95°C; NMR δ H 1.27 (3 H, s, Me), 1.30 (3 H, s, Me) 1.73 (1 H, m, CHCHO), 1.84 (1 H, m, CHCHO), 2.29 (1 H, ddd, J = 13.4, 10.8, 5.1 Hz, Pyr-CH), 2.46 (1 H, ddd, J = 13.4, 10.4, 5.9 Hz, Pyr-CH), 3.56 (1 H, dd, J = 11.7, 6.1 Hz, CHO), 3.66 (1 H, dd, J = 11.7, 6.1 Hz, CHO), 4.02 (1 H, dt, J = 8.1, 5.8 Hz, dioxolane 4-H), 4.13 (1 H, q, J = 5.8 Hz, dioxolane 5-H), 5.04 (2 H, br, NH2), 5.76 (2 H, br, NH2), 7.16 (2 H, d, J = 7.0 Hz, Ar 2,6-H2), 7.42 (2 H, d, J = 7.0 Hz, Ar 3,5-H2); NMR δ C 25.57, 28.04, 28.42, 30.46, 60.90, 76.77, 77.09, 107.39, 107.94, 129.71, 132.64, 134.29, 160.82, 162.48, 164.40; MS m/z 381.1506 (M + H) (C18H2437ClN4O3 requires 381.1507), 379.1525 (M + H) (C18H2435ClN4O4 requires 379.1536), 236/234 (M - C7H12O3), 188/186 (M – C8H13ClO3).

6.46. (4R,5S)-4-(4-(4-Bromophenyl)-4-cyano-3-methoxybut-3-enyl)-2,2-dimethyl-5-hydroxymethyl-1,3-dioxolane (29c) and 5-(4-bromophenyl)-6-(2-((4R,5S)-2,2-dimethyl-5-hydroxymethyl-1,3-dioxolan-4-yl)ethyl)pyrimidine-2,4-diamine (30c)

Compound 27c/28c was treated with CH2N2, as for the synthesis of 24a, to give 29c (90%) as a pale yellow oil: IR νmax 3435, 2243, 1592 cm⁻¹; NMR δ H 1.42 (3 H, s, Me), 1.52 (3 H, s, Me), 2.38-2.49 (2 H, m, CH2CHO), 2.74-2.80 (2 H, m, CH2C=C), 3.33 (3 H, s, OMe), 3.64 (1 H, dd, J = 11.1, 5.0 Hz, CHO), 3.74 (1 H, dd, J = 11.1, 5.0 Hz, CHO), 4.21-4.27 (2 H, m, dioxolane 4,5-H2), 7.29 (2 H, d, J = 8.5 Hz, Ar 2,6-H2), 7.50 (2 H, d, J = 8.5 Hz, Ar 3,5-H2); NMR δ C 23.01, 25.58, 25.70, 28.17, 58.60, 74.07, 75.63, 105.27, 106.26, 125.50, 126.82, 126.85, 127.94, 158.71, 159.82, 162.92; MS m/z 345.1935 (M + H) (C19H23N4O3 requires 345.1926), 329 (M - Me), 200 (M – C7H12O3).
11.1, 5.9 Hz, CHOH), 3.54 (1 H, dd, J = 11.1, 5.9 Hz, CHOH), 4.01 (1 H, ddd, J = 9.9, 5.9, 3.9 Hz, dioxolane 4-H), 4.08 (1 H, q, J = 5.9 Hz, dioxolane 5-H), 7.21 (2 H, d, J = 7.7 Hz, Ar 2,6-H2), 7.66 (2 H, d, J = 7.7 Hz, Ar 3,5-H2); NMR (CD3OD) δC 24.43, 27.11, 28.49, 30.66, 60.32, 76.63, 77.89, 107.20, 107.82, 121.84, 132.21, 132.57, 133.50, 160.82, 162.98, 163.71; MS m/z 425.1007 (M + H) (C18H2481BrN4O3 requires 425.1011), 423.1019 (M + H) (C18H2479BrN4O4 requires 423.1013), 280/278 (M - C7H12O3), 186 (M – C8H13BrO3).

6.47. (4R,5S)-4-(4-(3,4-Dichlorophenyl)-4-cyano-3-methoxybut-3-enyl)-2,2-dimethyl-5-hydroxymethyl-1,3-dioxolane (29d) and 5-(3,4-dichlorophenyl)-6-(2-((4R,5S)-5-hydroxy-2,2-dimethyl-1,3-dioxolan-4-yl)ethyl)pyrimidine-2,4-diamine (30d)

Compound 27c/28c was treated with CH2N2, as for the synthesis of 24a, to give 29d (91%) as a pale yellow oil: IR νmax 3467, 2210, 1597 cm⁻¹; NMR δH 1.40 (3 H, s, 2-Me), 1.50 (3 H, s, 2-Me), 2.30-2.40 (2 H, m, CH2CHO), 2.72-2.80 (2 H, m, CH2C=C), 3.37 (3 H, s, OMe), 3.58-3.74 (2 H, m, CH2OH), 4.18-4.26 (2 H, m, 4,5-H2), 7.51 (1 H, d, J = 8.3 Hz, Ar 5-H), 7.84 (1 H, dd, J = 8.3, 2.0 Hz, Ar 6-H), 8.10 (1 H, d, J = 2.0 Hz, Ar 2-H). Compound 29d was treated with guanidine, as for the synthesis of 25a (reaction time 6 h, chromatographic eluant CHCl3 / MeOH (9:1)), to give 30d (9%) as a white solid: mp 114-115°C; NMR δH 1.29 (3 H, s, Me), 1.33 (3 H, s, Me), 1.78 (1 H, m, CHCHO), 1.88 (1 H, m, CHCHO), 2.34 (1 H, m, Pyr-CH), 2.48 (1 H, m, Pyr-CH), 3.58 (1 H, dd, J = 11.4, 5.9 Hz, CHOH), 3.67 (1 H, dd, J = 11.4, 5.9 Hz, CHOH), 4.05 (1 H, m, dioxolane 4-H), 4.15 (1 H, q, J = 5.6 Hz, dioxolane 5-H), 4.95 (2 H, br, NH2), 5.53 (2 H, br, NH2), 7.08 (1 H, dd, J = 8.4, 1.7 Hz, Ar 6-H), 7.38 (1 H, d, J = 1.7 Hz, Ar 2-H), 7.52 (1 H, d, J = 8.4 Hz, Ar 5-H); NMR δC 25.53, 28.04, 28.12, 30.35, 61.05, 65.83, 70.51, 106.62, 108.04, 130.15, 131.41, 131.45, 133.51, 134.4, 160.93, 162.22, 168.33; MS m/z 417.1091 (M + H) (C18H2337Cl2N4O3 requires 417.1088), 415.1103 (M + H) (C18H2335ClN4O3 requires 415.1117), 413.1129 (M + H) (C18H2335Cl2N4O3 requires 413.1147), 272/270/268 (M - C7H12O3), 186 (M – C8H13Cl2O3).

6.48. Diethyl (R,R)-2,2-diethyl-1,3-dioxolane-4,5-dicarboxylate (32)

Diethyl (R,R)-2,3-dihydroxybutanedioate 31 (15.0 g, 70 mmol), 2,2-dimethoxypropane (8.0 g, 80 mmol) and 4-methylbenzenesulfonic acid (66 mg, 0.34 mmol) in dichloromethane (200 mL) were heated under reflux through activated 4 Å molecular sieves (33 g) in a Soxhlet apparatus for 3 h. Na2CO3 (83 mg, 1.0 mmol) was added. Filtration, drying and evaporation gave
32 (16.0 g, 89%) as a pale buff oil (lit.\textsuperscript{47} oil): NMR $\delta\text{H}$ 1.32 (6 H, t, $J = 7.2$ Hz, 2 × CH$_2$CH$_3$), 1.50 (6 H, s, CMe$_2$), 4.28 (4 H, q, $J = 7.2$ Hz, 2 × CH$_2$), 4.77 (2 H, s, 4,5-H$_2$).

6.49. (S,S)-4,5-Di(hydroxymethyl)-2,2-dimethyl-1,3-dioxolane (33)

LiAlH$_4$ (6.0 g, 150 mmol) was heated in dry THF (60 mL) for 30 min. Compound 32 (18.0 g, 70 mmol) in dry THF (80 mL) was added during 1.5 h. The mixture was heated under reflux for 5 h, then cooled to 0ºC. Water (10 mL), aq. NaOH (4 M, 10 mL) and water (30 mL) were added. Filtration and evaporation gave 33. The solid was extracted with hot 1,4-dioxane; evaporation gave further 33 (total 7.0 g, 60%) as a pale yellow oil (lit.\textsuperscript{48} oil): NMR $\delta\text{H}$ 1.41 (6 H, s, Me$_2$), 2.65 (2 H, br, 2 × OH), 3.68-3.78 (4 H, m, 2 × CH$_2$), 3.97 (2 H, m, 2 × CH$_2$).

6.50. (S,S)-4,5-Di(benzyloxymethyl)-2,2-dimethyl-1,3-dioxolane (34) and (S,S)-4-benzyl-oxymethyl-5-hydroxymethyl-2,2-dimethyl-1,3-dioxolane (35)

NaH (60% oil, 1.4 g, 34 mmol) was stirred in dry DMF (20 mL) under N$_2$ for 30 min. Compound 33 (5.0 g, 31 mmol) in DMF (20 mL) was added dropwise and the mixture was stirred for 30 min before BnCl (4.0 g, 32 mmol) was added. The mixture was stirred for 1.5 h, then poured into ice-water (250 mL) and extracted thrice with Et$_2$O. The combined extracts were washed with water and brine. Drying, evaporation and chromatography (hexane / Et$_2$O 1:1) gave 34 (2.2 g, 28%) as a pale yellow oil (lit.\textsuperscript{49} oil): NMR $\delta\text{H}$ 1.42 (6 H, s, Me$_2$), 3.54-3.66 (4 H, m, 2 × CH$_2$OBn), 4.02 (2 H, m, 4,5-H$_2$), 4.54 (2 H, d, $J = 12.3$ Hz, 2 × CHPh), 4.58 (2 H, d, $J = 12.3$ Hz, 2 × CHPh), 7.35 (10 H, m, 2 × Ph-H$_5$). Further elution gave 35 (3.2 g, 64%) as a pale yellow oil. [\(\alpha\)]$_{20}^D = +8.0^o$ (c 3.2, CHCl$_3$) (lit.\textsuperscript{50} [\(\alpha\)]$_{23}^D = +8.2^o$ (c 1.0, CHCl$_3$)); NMR $\delta\text{H}$ 1.41 (3 H, s, Me), 1.42 (3 H, s, Me), 2.33 (1 H, dd, $J = 8.6, 4.3$ Hz, OH), 3.55 (1 H, dd, $J = 9.8, 4.3$ Hz, CHOBN), 3.64-3.70 (2 H, m, CHO + CHOBN), 3.75 (1 H, dt, $J = 11.7, 4.3$ Hz, CHO), 3.94 (1H, dt, $J = 8.3, 4.3$ Hz, 5-H), 4.05 (1 H, dt, $J = 8.3, 4.3$ Hz, 4-H), 4.58 (2 H, s, CH$_2$Ph), 7.29-7.35 (5H, m, Ph-H$_5$).

6.51. (4S,5R)-4-Benzylxoxymethyl-2,2-dimethyl-1,3-dioxolane-5-carboxaldehyde (4-O-benzyl-2,3-O-isopropylidene-L-threose) (36)

Compound 35 (3.6 g, 14 mmol) was stirred with pyridinium chlorochromate (3.6 g, 35 mmol), NaOAc (300 mg, 3.5 mmol) and powdered 4 Å molecular sieves (3.0 g) in CH$_2$Cl$_2$ (215 mL) under N$_2$ for 3 h. The mixture was passed through a bed of silica. The silica was extracted with Et$_2$O. Evaporation of the solvent from the combined filtrate and extract gave 36
(3.3 g, 93%) as a pale yellow oil: $[\alpha]^{20}_D = +14^\circ$ (c 3, CHCl$_3$) (lit.$^{51}$ $[\alpha]^{20}_D = +16.2^\circ$ (c 1, CHCl$_3$)); NMR $\delta_{H}$ 1.43 (3 H, s, Me), 1.50 (3 H, s, Me), 3.67 (2 H, d, $J = 4.0$ Hz, CH$_2$OBn), 4.19-4.29 (2 H, m, 4,5-H$_2$), 4.58 (1 H, d, $J = 10.5$ Hz, CHPh), 4.61 (1 H, d, $J = 10.5$ Hz, CHPh), 7.25-7.36 (5 H, m, Ph-H$_5$), 9.76 (1 H, d, $J = 1.5$ Hz, CHO).

6.52. Ethyl ($Z$,4$S$,5$S$)-4-benzyloxymethyl-2,2-dimethyl-1,3-dioxolane-5-propenoate (37$Z$) and ethyl ($E$,4$S$,5$S$)-4-benzyloxymethyl-2,2-dimethyl-1,3-dioxolane-5-propenoate (37$E$)

Compound 36 (2.0 g, 8.0 mmol), ethyl triphenylphosphoranylideneacetate (4.2 g, 16 mmol) and benzoic acid (50 mg, 0.4 mmol) were heated at reflux in PhMe (200 mL) under N$_2$ for 4 h. The evaporation residue was extracted thrice with Et$_2$O. Evaporation and chromatography (hexane / Et$_2$O 5:1) gave 37$Z$ (800 g, 31%) as a colourless oil (lit.$^{52}$ oil): NMR $\delta_{H}$ 1.25 (3 H, t, $J = 7.1$ Hz, CH$_2$CH$_3$), 1.45 (6 H, s, CMe$_2$), 3.68 (2 H, d, $J = 3.1$ Hz, CH$_2$OBn), 3.97 (1 H, m, 4-H), 4.12 (2 H, q, $J = 7.1$ Hz, , CH$_2$Me), 4.56 (1 H, d, $J = 12.1$ Hz, CHPh), 4.62 (1 H, d, $J = 12.1$ Hz, CHPh), 5.38 (1 H, td, $J = 8.3$, 1.2 Hz, 5-H), 5.92 (1 H, dd, $J = 11.7$, 1.2 Hz, CHCO$_2$), 6.18 (1 H, dd, $J = 11.7$, 8.3 Hz, CH=CCO$_2$), 7.32-7.37 (5H, m, Ph-H$_5$). Further elution gave 37$E$ (800 mg, 31%) as a colourless oil (lit.$^{53}$ oil): NMR $\delta_{H}$ 1.29 (3 H, t, $J = 7.0$ Hz, CH$_2$CH$_3$), 1.43 (3 H, s, 2-Me), 1.45 (3 H, s, 2-Me), 3.62 (2 H, d, $J = 4.7$ Hz, CH$_2$OBn), 3.95 (1 H, dt, $J = 8.6$, 4.7 Hz, 4-H), 4.19 (2 H, q, $J = 7.0$ Hz, CH$_2$Me), 4.42 (1 H, ddd, $J = 8.6$, 5.5, 1.4 Hz, 5-H), 4.56 (1 H, J = 12.1 Hz, CHPh), 4.61 (1 H, d, $J = 12.1$ Hz, CHPh), 6.09 (1 H, dd, $J = 15.6$, 1.4 Hz, CHCO$_2$), 6.88 (1 H, dd, $J = 15.6$, 5.5 Hz, CH=CCO$_2$), 7.27-7.36 (5 H, m, Ph-H$_5$).

6.53. Ethyl (4$S$,5$S$)-4-benzyloxymethyl-2,2-dimethyl-1,3-dioxolane-5-propanoate (38)

A mixture of 37$Z$ and 37$E$ (620 mg, 1.9 mmol) was stirred in EtOH (25 mL) with Pd/C (5%, 30 mg) under H$_2$ for 1 h. Filtration (Celite®), evaporation and chromatography (hexane / Et$_2$O 4:1) gave 38 (400 mg, 63%) as a pale yellow oil: $[\alpha]^{20}_D = -15^\circ$ (c 4.0, CHCl$_3$); NMR $\delta_{H}$ 1.23 (3 H, t, $J = 7.0$ Hz, CH$_2$CH$_3$), 1.38 (3 H, s, 2-Me), 1.39 (3 H, s, 2-Me), 1.84 (1 H, m, CHCH$_2$CO$_2$), 1.96 (1 H, m, CHCH$_2$CO$_2$), 2.37-2.54 (2 H, m, CH$_2$CO$_2$), 3.53-3.60 (2 H, m, CH$_2$OBn), 3.80-3.87 (2 H, m, 4,5-H$_2$), 4.12 (2 H, q, $J = 7.0$ Hz, , CH$_2$Me), 4.56 (1 H, d, $J = 12.3$ Hz, CHPh), 4.59 (1 H, d, $J = 12.3$ Hz, CHPh), 7.32-7.34 (5 H, m, Ph-H$_5$); MS m/z 323.1856 (M + H) (C$_{19}$H$_{26}$O$_5$ requires 323.1858), 265 (M – C$_3$H$_6$O), 91 (Bn).
6.54. **(4S,5S)-5-Benzoxymethyl-4-(4-cyano-3-oxo-4-phenylbutyl)-2,2-dimethyl-1,3-dioxolane** (39a) / **(4S,5S)-5-benzoxymethyl-4-(4-cyano-3-hydroxy-4-phenylbut-3-enyl)-2,2-dimethyl-1,3-dioxolane** (40a)

Phenylacetonitrile was condensed with 38, as for the synthesis of 22a/23a, to give 39a/40a (14%) as a pale yellow solid: mp 75-77ºC; IR \( \nu_{\text{max}} \) 2206, 1731 cm\(^{-1}\); NMR \( \delta_{\text{H}} \) 1.39 (3 H, s, Me), 1.41 (3 H, s, Me), 1.86 (1 H, m, CHCHO), 2.00 (1 H, m, CHCHO), 2.48-2.65 (2 H, m, CH\(_2\)=O), 3.54-3.63 (2 H, m, 4,5-H\(_2\)), 3.87-3.89 (2 H, m, CH\(_2\)OBn), 4.57 (1 H, d, \( J = 12.5 \) Hz, CHPh), 4.61 (1 H, d, \( J = 12.5 \) Hz, CHPh), 5.59 (1 H, s, CHCN), 7.33-7.64 (8 H, m, Ph' 3,4,5-H\(_3\) + Ph-H\(_5\)), 8.11 (2 H, d, \( J = 7.0 \) Hz, Ph 2,6-H\(_2\)); MS \( m/z \) 392.1859 (M - H) (C\(_{24}\)H\(_{26}\)NO\(_4\) requires 392.1861), 335 (M - C\(_2\)H\(_4\)NO), 317 (M - C\(_7\)H\(_6\)), 91 (Bn).

6.55. **(4S,5S)-5-Benzoxymethyl-4-(4-(4-chlorophenyl)-4-cyano-3-oxobutyl)-2,2-dimethyl-1,3-dioxolane** (39b) / **(4S,5S)-5-benzoxymethyl-4-(4-(4-chlorophenyl)-4-cyano-3-hydroxybut-3-enyl)-2,2-dimethyl-1,3-dioxolane** (40b)

4-Chlorophenylacetonitrile was condensed with 38, as for the synthesis of 22a/23a (chromatographic eluant EtOAc / hexane (1:1)), to give 39b/40b (41%) as a yellow oil: IR \( \nu_{\text{max}} \) 2209, 1731 cm\(^{-1}\); NMR \( \delta_{\text{H}} \) 1.39 (3 H, s, Me), 1.40 (3 H, s, Me), 1.85 (1 H, m, CHCHO), 2.00 (1 H, m, CHCHO), 2.46-2.63 (2 H, m, CH\(_2\)=O), 3.53-3.62 (2 H, m, 4,5-H\(_2\)), 3.83-3.87 (2 H, m, CH\(_2\)OBn), 4.57 (1 H, d, \( J = 12.1 \) Hz, CHPh), 4.60 (1 H, d, \( J = 12.1 \) Hz, CHPh), 7.26-7.40 (5H, m, Ph-H\(_5\)), 7.44 (2 H, d, \( J = 8.6 \) Hz, Ar 3,5-H\(_2\)), 8.02 (2 H, d, \( J = 8.6 \) Hz, Ar 2,6-H\(_2\)); MS \( m/z \) 430.1608 (M + H) (C\(_{24}\)H\(_{27}\)\(_37\)ClNO\(_4\) requires 430.1599), 428.1623 (M + H) (C\(_{24}\)H\(_{27}\)\(_{35}\)ClNO\(_4\) requires 428.1628), 372/370 (M – C\(_2\)H\(_3\)NO), 91 (Bn).

6.56. **(4S,5S)-5-Benzoxymethyl-4-(4-(4-bromophenyl)-4-cyano-3-oxobutyl)-2,2-dimethyl-1,3-dioxolane** (39c) / **(4S,5S)-5-benzoxymethyl-4-(4-(4-bromophenyl)-4-cyano-3-hydroxybut-3-enyl)-2,2-dimethyl-1,3-dioxolane** (39c)

4-Bromophenylacetonitrile was condensed with 38, as for the synthesis of 22a/23a, to give 39c/40c (24%) as a yellow oil: IR \( \nu_{\text{max}} \) 2208, 1718 cm\(^{-1}\); NMR \( \delta_{\text{H}} \) 1.33 (3 H, s, Me), 1.34 (3 H, s, Me), 1.75 (1 H, m, CHCHO), 1.91 (1 H, m, CHCHO), 2.69 (1 H, m, CHC=O), 2.79 (1 H, m, CHC=O), 3.55-3.65 (2 H, m, CH\(_2\)OBn), 3.93 (1 H, m, 5-H), 4.03 (1 H, dt, \( J = 8.0, 3.7 \) Hz, 4-H), 4.53 (1 H, d, \( J = 12.0 \) Hz, CHPh), 4.61 (1 H, d, \( J = 12.0 \) Hz, CHPh), 7.22 (2 H, d, \( J = 8.1 \) Hz, Ar 2,6-H\(_2\)), 7.26-7.36 (5H, m, Ph-H\(_5\)), 7.53 (2 H, d, \( J = 8.8 \) Hz, Ar 3,5-H\(_2\)); MS \( m/z \)
474.1103 (M + H) (C<sub>24</sub>H<sub>27</sub>BrNO<sub>4</sub> requires 474.1102), 472.1103 (M + H) (C<sub>24</sub>H<sub>27</sub>BrNO<sub>4</sub> requires 472.1123), 415/413 (M – C<sub>2</sub>H<sub>4</sub>NO), 91 (Bn).

6.57. (4S,5S)-5-Benzyloxymethyl-4-(4-cyano-3-methoxy-4-phenylbut-3-enyl)-2,2-dimethyl-1,3-dioxolane (41a) and 6-(2-((4S,5S)-5-benzyloxymethyl-2,2-dimethyl-1,3-dioxolan-4-yl)ethyl)-5-phenylpyrimidine-2,4-diamine (42a)

Compound 39a/40a was treated with CH<sub>2</sub>N<sub>2</sub>, as for the synthesis of 24a, to give 41a (79%) as a pale yellow oil: IR ν<sub>max</sub> 2208, 1605 cm<sup>-1</sup>; NMR δ<sub>H</sub> 1.45 (3 H, s, Me), 1.49 (3 H, s, Me), 1.83 (1 H, m, CHCHO), 1.98 (1 H, m, CHCHO), 2.40-2.59 (2 H, m, CH<sub>2</sub>C=C), 3.55-3.60 (2 H, m, 4,5-H<sub>2</sub>), 3.75 (3 H, s, OMe), 3.81-3.85 (2 H, m, CH<sub>2</sub>OBn), 4.55 (1 H, d, J = 12.7 Hz, CHPh), 4.58 (1 H, d, J = 12.7 Hz, CHPh), 7.14-7.45 (10 H, m, 2 × Ph-H<sub>5</sub>); MS m/z 408.2184 (M + H) (C<sub>25</sub>H<sub>30</sub>NO<sub>4</sub> requires 408.2174), 391 (M - CH<sub>4</sub>), 380 (M – HCN), 91 (Bn). Compound 41a was condensed with guanidine, as for the synthesis of 25a (reaction time 4 h, chromatographic eluant CHCl<sub>3</sub> / MeOH (9:1)), to give 42a (67%) as a highly hygroscopic pale yellow solid: IR ν<sub>max</sub> 3454, 1664 cm<sup>-1</sup>; NMR δ<sub>H</sub> 1.26 (3 H, s, Me), 1.29 (3 H, s, Me), 1.71-1.91 (2 H, m, CH<sub>2</sub>CHO), 2.33 (1 H, ddd, J = 13.5, 10.3, 5.8 Hz, Pyr-CH), 2.45 (1 H, ddd, J = 13.5, 10.5, 5.7 Hz, PyR-CH), 3.44 (2 H, d, J = 4.6 Hz, CH<sub>2</sub>OBn), 3.67 (1 H, dt, J = 7.9, 4.6 Hz, dioxolane 4-H), 3.75 (1 H, q, J = 4.6 Hz, dioxolane 5-H), 4.28 (1 H, d, J = 12.1 Hz, CHPh), 4.54 (1 H, d, J = 12.1 Hz, CHPh), 4.64 (2 H, br, NH<sub>2</sub>), 5.14 (2 H, br, NH<sub>2</sub>), 7.19-7.43 (10 H, m, 2 × Ph-H<sub>3</sub>); MS m/z 435.2398 (M + H) (C<sub>25</sub>H<sub>31</sub>N<sub>4</sub>O<sub>3</sub> requires 435.2396), 327 (M – C<sub>3</sub>H<sub>2</sub>O), 91 (Bn).

6.58. (4S,5S)-5-Benzyloxymethyl-4-(4-(4-chlorophenyl)-4-cyano-3-methoxybut-3-enyl)-2,2-dimethyl-1,3-dioxolane (41b) and 6-(2-((4S,5S)-5-benzyloxymethyl-2,2-dimethyl-1,3-dioxolan-4-yl)ethyl)-5-(4-chlorophenyl)pyrimidine-2,4-diamine (42b)

Compound 39b/40b was treated with CH<sub>2</sub>N<sub>2</sub>, as for the synthesis of 24a, to give 41b (91%) as a pale yellow oil: IR ν<sub>max</sub> 2208, 1605 cm<sup>-1</sup>; NMR δ<sub>H</sub> 1.39 (3 H, s, Me), 1.41 (3 H, s, Me), 1.62-1.75 (2 H, m, CH<sub>2</sub>CHO), 2.86-2.96 (2 H, m, CH<sub>2</sub>C=C), 3.52-3.65 (2 H, m, 4,5-H<sub>2</sub>), 3.81 (3 H, s, OMe), 3.84-3.93 (2 H, m, CH<sub>2</sub>OBn), 4.54 (1 H, d, J = 11.1 Hz, CHPh), 4.58 (1 H, d, J = 11.1 Hz, CHPh), 7.24-7.38 (9H, m, Ph-H<sub>5</sub> + Ar-H<sub>4</sub>). Compound 41b was condensed with guanidine, as for the synthesis of 25a (reaction time 4 h, chromatographic eluant CHCl<sub>3</sub> / MeOH (4:1)), to give 42b (48%) as a highly hygroscopic pale yellow solid: IR ν<sub>max</sub> 3475, 3414, 1618 cm<sup>-1</sup>; NMR δ<sub>H</sub> 1.31 (3 H, s, Me), 1.73 (1 H, m, CHCHO), 1.83 (1 H, m, CHCHO), 2.30 (1 H, ddd, J =
13.5, 10.5, 5.7 Hz, Pyr-CH), 2.45 (1 H, ddd, J = 13.5, 10.5, 5.7 Hz, Pyr-CH), 3.41-3.49 (2 H, m, CH₂OBn), 3.66 (1 H, dt, J = 8.2, 3.5 Hz, dioxolane 4-H), 3.51 (1 H, m, dioxolane 5-H), 4.50 (1 H, d, J = 12.1 Hz, CHPh), 4.54 (1 H, d, J = 12.1 Hz, CHPh), 4.69 (2 H, br, NH₂), 5.11 (2 H, br, NH₂), 7.10-7.35 (9 H, m, Ph-H₅ + Ar-H₄); MS m/z 471.1979 (M + H) (C₂₅H₃₀³⁷ClN₄O₃ requires 471.1976), 469.1999 (M + H) (C₂₅H₃₀³⁵ClN₄O₃ requires 469.2006), 363/361 (M – C₇H₇O), 91 (Bn).

6.59. (4S,5S)-5-Benzylxomethyl-4-(-4-(4-bromophenyl)-4-cyano-3-methoxybut-3-enyl)-2,2-dimethyl-1,3-dioxolane (41c) and 6-(2-((4S,5S)-5-benzyloxymethyl-2,2-dimethyl-1,3-dioxolan-4-yl)-ethyl)-5-(4-bromophenyl)pyrimidine-2,4-diamine (42c)

Compound 39c/40c was treated with CH₂N₂, as for the synthesis of 24a, to give 41c (93%) as a pale yellow oil: NMR δH 1.39 (3 H, s, Me), 1.41 (3 H, s, Me), 1.78-1.86 (2 H, m, CH₂CHO), 2.85-2.95 (2 H, m, CH₂C=C), 3.52-3.68 (2 H, m, 4,5-H₂), 3.81 (3 H, s, OMe), 3.84-3.94 (2 H, m, CH₂OBn), 4.54 (1 H, d, J = 12.1 Hz, CHPh), 4.59 (1 H, d, J = 12.1 Hz, CHPh), 7.22-7.42 (9 H, m, Ph-H₅ + Ar-H₄); MS m/z 488.1255 (M + H) (C₂₅H₂₉⁸¹BrNO₄ requires 488.1259), 486.1258 (M + H) (C₂₅H₂₉⁷⁹BrNO₄ requires 486.1279), 91 (Bn). Compound 41c was condensed with guanidine, as for the synthesis of 42a, to give 42c (53%) as a highly hygroscopic buff solid: NMR δH 1.31 (3 H, s, Me), 1.34 (3 H, s, Me), 1.75 (1 H, m, CHCHO), 2.33 (1 H, ddd, J = 13.7, 10.5, 5.9 Hz, Pyr-CH), 2.48 (1 H, ddd, J = 13.7, 10.5, 5.9 Hz, Pyr-CH), 3.45-3.53 (4 H, m, 3’,4’,5’-H₄), 4.48 (1 H, d, J = 11.7 Hz, CHPh), 4.52 (1 H, d, J = 11.7 Hz, CHPh), 7.09 (2 H, d, J = 8.6 Hz, Ar 2,6-H₂), 7.28-7.38 (5 H, m, Ph-H₅), 7.54 (2 H, d, J = 8.6 Hz, Ar 3,5-H₂); MS m/z 515.1488 (M + H) (C₂₅H₃₀⁸¹BrN₄O₃ requires 515.1480), 513.1500 (M + H) (C₂₅H₃₀⁷⁹BrN₄O₄ requires 513.1501), 487/485 (M – C₂H₅), 407/405 (M - C₇H₇O), 91 (Bn).

6.60. 6-((3S,4S)-5-Benzylxoyo-3,4-dihydroxypentyl)-5-phenylpyrimidine-2,4-diamine (43a)

Compound 42a was treated with aq. CF₃CO₂H, as for the synthesis of 26a, to give 43a (210 mg, 76%) as a pale buff solid: mp 101-102ºC; NMR (CD₃OD) δH 1.70 (1 H, q, J = 7.6 Hz, 2’-H₂), 2.36 (1 H, dt, J = 14.2, 7.6 Hz, 1’-H), 2.48 (1 H, dt, J = 14.2, 7.6 Hz, 1’-H), 3.42-3.55 (4 H, m, 3’,4’,5’-H₄), 4.48 (1 H, d, J = 11.7 Hz, CHPh), 4.52 (1 H, d, J = 11.7 Hz, CHPh), 7.22-
7.49 (10 H, m, 2 × Ph-H); MS m/z 395.2082 (M + H) (C_{22}H_{27}N_4O_3 requires 359.2083), 91 (Bn).

6.61. 6-((3S,4S)-5-Benzxyloxy-3,4-dihydroxypentyl)-5-(4-chlorophenyl)pyrimidine-2,4-diamine (43b)

Compound 42b was treated with aq. CF₃CO₂H, as for the synthesis of 26a, to give 43b (75%) as a pale yellow solid: mp 141-143°C; IR ν_{max} 3562, 3492, 3430, 3343, 1618 cm⁻¹; NMR (CD₃OD) δ_{H} 1.65-1.73 (2 H, m, 2'-H₂), 2.28 (1 H, ddd, J = 13.7, 9.4, 6.6 Hz, 1'-H), 2.42 (1 H, ddd, J = 13.7, 9.4, 6.6 Hz, 1'-H), 3.42-3.54 (4 H, m, 3',4',5'-H₄), 4.48 (1 H, d, J = 11.7 Hz, CHPh), 4.52 (1 H, d, J = 11.7 Hz, CHPh), 7.20 (2 H, d, J = 8.6 Hz, Ar 2,6-H₂), 7.30-7.36 (5 H, m, Ph-H₅), 7.44 (2 H, d, J = 8.6 Hz, Ar 3,5-H₂); MS m/z 431.1680 (M + H) (C_{22}H_{26}^{37}ClN_4O_3 requires 431.1663), 429.1702 (M + H) (C_{22}H_{26}^{35}ClN_4O_3 requires 429.1693), 91 (Bn).

6.62. 6-((3S,4S)-5-Benzxyloxy-3,4-dihydroxypentyl)-5-(3-bromophenyl)pyrimidine-2,4-diamine (43c)

Compound 42c was treated with aq. CF₃CO₂H, as for the synthesis of 26a, to give 43c (95%) as a highly hygroscopic pale yellow solid: IR ν_{max} 3582, 3350, 1613 cm⁻¹; NMR (CD₃OD) δ_{H} 1.71-1.78 (2 H, m, 2'-H₂), 2.36 (1 H, dt, J = 14.6, 7.8 Hz, 1'-H), 2.46 (1H, dt, J = 14.6, 7.8 Hz, 1'-H), 3.43-3.61 (4 H, m, 3',4',5'-H₄), 4.51 (1 H, d, J = 11.9 Hz, CHPh), 4.56 (1 H, d, J = 11.9 Hz, CHPh), 7.18 (2 H, d, J = 8.6 Hz, Ar 2,6-H₂), 7.34-7.36 (5 H, m, Ph-H₅), 7.63 (2 H, d, J = 8.6 Hz, Ar 3,5-H₂); NMR (CD₃OD) δ_{C} 29.26, 31.59, 70.80, 72.32, 71.11, 72.99, 107.30, 120.98, 122.10, 127.39, 127.58, 128.04, 130.83, 132.31, 138.05, 161.45, 161.80, 162.50; MS m/z 475.1184 (M + H) (C_{22}H_{26}^{81}BrN_4O_3 requires 475.1167), 473.1186 (M + H) (C_{22}H_{26}^{79}BrN_4O_3 requires 473.1188), 91 (Bn).

6.63. 1-Cyano-7-hydroxy-1-phenylheptan-2-one (45a) / 1-cyano-1-phenylhept-1-en-1,7-diol (46a)

Phenylacetonitrile and tetrahydrooxepin-2-one 44 were treated with LiN(SiMe₃)₂, as for the synthesis of 22a/23a, to give 45a/46a (21%) as a pale yellow solid: mp 98-99°C; IR ν_{max} 3402, 2205, 1718 cm⁻¹; NMR ((CD₃)$_2$SO) δ_{H} 1.35-1.42 (2 H, m, 5-H₂), 1.44-1.51 (2 H, m, 6-H₂), 1.65 (2 H, quin, J = 7.4 Hz, 4-H₂), 2.60 (2 H, t, J = 7.4 Hz, 3-H₂), 3.40 (2 H, t, J = 6.2 Hz, 7-H₂), 4.36 (1 H, br, OH), 7.20 (1 H, t, J = 7.6 Hz, Ph 4-H), 7.30 (2 H, t, J = 7.6 Hz, Ph 3,5-
H₂), 7.61 (2 H, d, J = 7.6 Hz, Ph 2,6-H₂); MS m/z 232.1329 (M + H) (C₁₄H₁₈NO₂ requires 232.1337), 214 (M - OH), 185 (M – C₂H₆O), 115 (M – C₆H₁₂O₂).

6.64. 1-(4-Chlorophenyl)-1-cyano-7-hydroxyheptan-2-one (45b) / 1-(4-chlorophenyl)-1-cyanohept-1-en-1,7-diol (46b)

4-Chlorophenylacetonitrile and tetrahydrooxepin-2-one 44 were treated with LiN(SiMe₃)₂, as for the synthesis of 22a/23a, to give 45b/46b (11%) as a white solid: mp 92-94°C; NMR δH 1.21-1.30 (2 H, m, 5-H₂), 1.50 (2 H, qn, J = 6.8 Hz, 6-H₂), 1.58 (2 H, qn, J = 7.4 Hz, 4-H₂), 2.58 (1 H, dt, J = 18.2, 7.4 Hz, 3-H), 2.66 (1 H, dt, J = 18.2, 7.4 Hz, 3-H), 3.60 (2 H, t, J = 6.8 Hz, 7-H₂), 4.65 (1 H, br, OH), 7.32 (2 H, d, J = 8.4 Hz, Ar 2,6-H₂), 7.41 (2 H, d, J = 8.4 Hz, Ar 3,5-H₂); MS m/z 268.0912 (M + H) (C₁₄H₁₇³⁷ClNO₂ requires 268.0918), 266.0942 (M + H) (C₁₄H₁₇³⁵ClNO₂ requires 266.0947), 250/248 (M – OH), 207/205 (M - C₃H₈O).

6.65. 1-(4-Bromophenyl)-1-cyano-7-hydroxyheptan-2-one (45c) / 1-(4-bromophenyl)-1-cyanohept-1-en-1,7-diol (46c)

4-Bromophenylacetonitrile and tetrahydrooxepin-2-one 44 were treated with LiN(SiMe₃)₂, as for the synthesis of 22a/23a (chromatographic eluant EtOAc / hexane (3:1)), to give 45c/46c (8%) as a pale yellow solid: mp 76-78°C; NMR δH 1.28 (2 H, qn, J = 7.3 Hz, 5-H₂), 1.50 (2 H, qn, J = 7.3 Hz, 6-H₂), 1.58 (2 H, qn, J = 7.3 Hz, 4-H₂), 2.62 (1 H, dt, J = 18.0, 7.3 Hz, 3-H), 2.65 (1 H, dt, J = 18.0, 7.3 Hz, 3-H), 3.60 (2 H, t, J = 6.4 Hz, 7-H₂), 4.64 (1 H, br, OH), 7.26 (2 H, d, J = 8.2 Hz, Ar 2,6-H₂), 7.41 (2 H, d, J = 8.2 Hz, Ar 3,5-H₂); MS m/z 312.0430 (M + H) (C₁₄H₁₇³⁷BrNO₂ requires 312.0422), 310.0449 (M + H) (C₁₄H₁₇³⁵BrNO₂ requires 310.0442), 294/292 (M – OH).

6.66. 1-Cyano-1-(3,4-dichlorophenyl)-7-hydroxyheptan-2-one (45d) / 1-cyano-1-(3,4-dichlorophenyl)hept-1-en-1,7-diol (46d)

3,4-Dichlorophenylacetonitrile and tetrahydrooxepin-2-one 44 were treated with LiN(SiMe₃)₂, as for the synthesis of 45c/46c, to give 45d/46d (25%) as a pale yellow solid: mp 95-97°C; NMR δH 1.26 (2 H, qn, J = 7.3 Hz, 5-H₂), 1.53 (2 H, qn, J = 7.3 Hz, 6-H₂), 1.62 (2 H, qn, J = 7.3 Hz, 4-H₂), 2.66 (2 H, dt, J = 15.4, 7.3 Hz, 3-H₂), 3.60 (2 H, t, J = 6.4 Hz, 7-H₂), 4.69 (1 H, br, OH), 7.39 (1 H, d, J = 8.4 Hz, Ar 6-H), 7.51 (1 H, d, J = 8.4 Hz, Ar 5-H), 7.83 (1 H, s, Ar 2-H); MS m/z 304.0520 (M + H) (C₁₄H₁₆³⁷Cl₂NO₂ requires 304.0499),
302.0537 (M + H) \(\text{C}_{14}\text{H}_{16}\text{Cl}_{35}\text{ClNO}_2\) requires 302.0528), 300.0559 (M + H) \(\text{C}_{14}\text{H}_{16}\text{Cl}_{35}\text{ClNO}_2\) requires 300.0558), 286/284/282 (M – OH).

6.67. \((4R,5R)-4-(2-Cyano-1-oxo-2-phenylethyl)-5-hydroxymethyl-2,2-dimethyl-1,3-dioxolane (49a) / (4R,5R)-4-(2-cyano-1-hydroxy-2-phenylethenyl)-5-hydroxymethyl-2,2-dimethyl-1,3-dioxolane (50a)

Phenylacetonitrile and 2,3-O-isopropylidene–D-erythronolactone 48 were treated with LiN(SiMe\(_3\))\(_2\), as for the synthesis of 22a/23a, to give 49a/50a (27%) as a pale yellow oil: IR \(\nu_{\text{max}}\) 3408, 2246, 1694 cm\(^{-1}\); NMR \(\delta_H\) 1.41 (3 H, s, Me), 1.49 (3 H, s, Me), 4.41 (1 H, dd, \(J = 11.0, 3.5\) Hz, \(\text{CH}_2\text{OH}\)), 4.48 (1 H, d, \(J = 11.0\) Hz, \(\text{CH}_2\text{OH}\)), 4.75 (1 H, d, \(J = 5.5\) Hz, 4-H), 4.88 (1 H, m, 5-H), 7.47 (2 H, t, \(J = 7.4\) Hz, Ph 3,5-H), 7.61 (1 H, t, \(J = 7.4\) Hz, Ph 4-H), 8.10 (2 H, d, \(J = 8.6\) Hz, Ph 2,6-H); MS \(m/z\) 276.1225 (M + H) \((\text{C}_{15}\text{H}_{18}\text{NO}_4\) requires 276.1235), 258 (M – OH).

6.68. \((4R,5R)-4-(2-(4-Chlorophenyl)-2-cyano-1-oxoethyl)-5-hydroxymethyl-2,2-dimethyl-1,3-dioxolane (49b) / (4R,5R)-4-(2-(4-chlorophenyl)-2-cyano-1-hydroxyethenyl)-5-hydroxymethyl-2,2-dimethyl-1,3-dioxolane (50b)

4-Chlorophenylacetonitrile and 2,3-O-isopropylidene–D-erythronolactone 48 were treated with LiN(SiMe\(_3\))\(_2\), as for the synthesis of 22a/23a, to give 49b/50b (21%) as a pale yellow oil: NMR \(\delta_H\) 1.30 (3 H, s, Me), 1.59 (3 H, s, Me), 4.05 (1 H, dd, \(J = 10.1, 3.1\) Hz, \(\text{CH}_2\text{OH}\)), 4.09 (1 H, d, \(J = 10.1\) Hz, \(\text{CH}_2\text{OH}\)), 4.86 (1 H, m, 5-H), 4.73 (1 H, d, \(J = 5.8\) Hz, 4-H), 7.27 (2 H, d, \(J = 8.4\) Hz, Ar 2,6-H), 7.48 (2 H, d, \(J = 8.4\) Hz, Ar 3,5-H); MS \(m/z\) 312.0849 (M + H) \((\text{C}_{15}\text{H}_{17}\text{ClNO}_4\) requires 312.0816), 310.0855 (M + H) \((\text{C}_{15}\text{H}_{16}\text{ClNO}_4\) requires 310.0846), 294/292 (M – OH).

6.69. \((4R,5R)-4-(2-(4-Bromophenyl)-2-cyano-1-oxoethyl)-5-hydroxymethyl-2,2-dimethyl-1,3-dioxolane (49c) / (4R,5R)-4-(2-(4-bromophenyl)-2-cyano-1-hydroxyethenyl)-5-hydroxymethyl-2,2-dimethyl-1,3-dioxolane (50c)

4-Bromophenylacetonitrile and 2,3-O-isopropylidene–D-erythronolactone 48 were treated with LiN(SiMe\(_3\))\(_2\), as for the synthesis of 22a/23a (chromatographic eluant EtOAc / hexane (3:1)), to give 49c/50c (38%) as a pale yellow oil: IR \(\nu_{\text{max}}\) 3422, 2208, 1777 cm\(^{-1}\); NMR \(\delta_H\) 1.39 (3 H, s, Me), 1.47 (3 H, s, Me), 4.40 (1 H, dd, \(J = 10.9, 3.7\) Hz, \(\text{CH}_2\text{OH}\)), 4.45 (1 H, d, \(J = 10.9\) Hz, \(\text{CH}_2\text{OH}\)), 4.74 (1 H, d, \(J = 5.5\) Hz, 4-H), 4.87 (1 H, m, 5-H), 5.57 (1 H, s, CHCN),
7.27 (2 H, d, J = 8.6 Hz, Ar 2,6-H₂), 7.51 (2 H, d, J = 8.6 Hz, Ar 3,5-H₂); MS m/z 356.0326 (M + H) (C₁₅H₁₇^8¹BrNO₄ requires 356.0320), 354.0327 (M + H) (C₁₅H₁₇^7⁹BrNO₄ requires 354.0340), 338/336 (M – OH).

6.70. (4'R,5'R)-4-(2-Cyano-2-(3,4-dichlorophenyl)-1-oxoethyl)-5-hydroxymethyl-2,2-dimethyl-1,3-dioxolane (49d) / (4'R,5'R)-4-(2-cyano-2-(3,4-dichlorophenyl)-1-hydroxyethenyl)-5-hydroxymethyl-2,2-dimethyl-1,3-dioxolane (50d)

3,4-Dichlorophenylacetonitrile and 2,3-O-isopropylidene-D-erythronolactone 48 were treated with LiN(SiMe₃)₂, as for the synthesis of 22a/23a (chromatographic eluant EtOAc / hexane (1:1)), to give 49d/50d (22%) as a pale yellow oil: IR νmax 3404, 2250, 1782 cm⁻¹; NMR δH 1.30 (3 H, s, Me), 1.38 (3 H, s, Me), 3.93 (1 H, dd, J = 10.3, 3.7 Hz, CHO), 4.00 (1 H, d, J = 10.3 Hz, CHO), 4.70 (1 H, d, J = 5.9 Hz, 4-H), 4.91 (1 H, dd, J = 5.9, 3.7 Hz, 5-H), 7.33 (1 H, dd, J = 8.2, 2.0 Hz, Ar 6-H), 7.36 (1 H, d, J = 8.2 Hz, Ar 5-H), 7.58 (1 H, d, J = 2.0 Hz, Ar 2-H); MS m/z 348.0411 (M + H) (C₁₅H₁₆^3₁Cl₂NO₄ requires 348.0397), 346.0901 (M + H) (C₁₅H₁₆^3₁Cl₃₅ClNO₄ requires 346.0906), 344.0448 (M + H) (C₁₅H₁₆^3₁Cl₂NO₄ requires 344.0456), 330/328/326 (M – OH).

6.71. (4'R,5'R)-4-(2-Cyano-1-methoxy-2-phenylethenyl)-5-hydroxymethyl-2,2-dimethyl-1,3-dioxolane (51a) and 6-((4'S,5'R)-2,2-dimethyl-5-hydroxymethyl-1,3-dioxolan-4-yl)-5-phenylpyrimidine-2,4-diamine (52a)

Compound 49a/50a was treated with CH₂N₂, as for the synthesis of 24a, to give 51a (85%) as a pale yellow oil: IR νmax 3492, 2209 cm⁻¹; NMR δH 1.44 (3 H, s, 2-Me), 1.58 (3 H, s, 2-Me), 3.53 (3 H, s, OMe), 4.39 (1 H, dd, J = 11.0, 3.7 Hz, CHO), 4.45 (1 H, d, J = 11.0 Hz, CHO), 4.60 (1 H, m, 5-H), 5.37 (1 H, d, J = 7.4 Hz, 4-H), 7.30-7.41 (5 H, m, Ph-H 5); MS m/z 290.1388 (M + H) (C₁₅H₂₀NO₄ requires 290.1392), 274 (M – Me), 258 (M - OMe).

Compound 51a was treated with guanidine, as for the synthesis of 25a (chromatographic eluant CH₂Cl₂ / MeOH (4:1)) to give 52a (48%) as a pale yellow solid: mp 214-216°C; IR νmax 3492, 3465, 3422, 3318, 3178, 1624 cm⁻¹; [α]D²⁰ = +3.3° (c 4, CHCl₃); NMR δH 1.21 (3 H, s, Me), 1.62 (3 H, s, Me), 3.48 (1 H, dd, J = 12.7, 2.1 Hz, CHO), 3.57 (1 H, dd, J = 12.7, 3.3 Hz, CHO), 3.97 (1 H, m, dioxolane 5-H), 4.79 (1 H, d, J = 6.6 Hz, dioxolane 4-H), 4.90 (2 H, br, NH₂), 5.16 (2 H, br, NH₂), 7.10 (1 H, d, J = 7.4 Hz, Ph 2-H), 7.31 (1 H, d, J = 7.4 Hz, Ph 6-H), 7.41 (1 H, t, J = 7.4 Hz, Ph 4-H), 7.47 (2 H, t, J = 7.4 Hz, Ph 3,5-H₂); MS m/z 317.1622 (M + H) (C₁₆H₂₀N₄O₃ requires 317.1613).
6.72. (4R,5R)-4-(2-(4-Chlorophenyl)-2-cyano-1-methoxyethenyl)-5-hydroxymethyl-2,2-dimethyl-1,3-dioxolane (51b) and 5-(4-chlorophenyl)-6-((4S,5R)-2,2-dimethyl-5-hydroxy- methyl-2,2-dimethyl-1,3-dioxolan-4-yl)pyrimidine-2,4-diamine (52b)

Compound 49b/50b was treated with CH₂N₂, as for the synthesis of 24a, to give 51b (69%) as a pale yellow oil: MS m/z 326.0989 (M + H) (C₁₆H₁₉₃⁷ClNO₄ requires 326.0973), 324.1014 (M + H) (C₁₆H₁₉₃⁵ClNO₄ requires 324.1002), 307/305 (M - H₂O). Compound 51b was treated with guanidine, as for the synthesis of 52a, to give 52b (50%) as a pale buff solid: mp 172-174°C; IR νmax 3497, 3459, 3433, 3396, 3217, 1613 cm⁻¹; NMR δH 1.24 (3 H, s, Me), 1.63 (3 H, s, Me), 1.66 (1 H, br, OH), 3.47 (1 H, dd, J = 12.8, 2.3 Hz, CHOH), 3.58 (1 H, dd, J = 12.8, 3.3 Hz, CHOH), 3.98 (1 H, m, dioxolane 5-H), 4.66 (2 H, br, NH₂), 4.77 (1 H, d, J = 6.2 Hz, dioxolane 4-H), 4.93 (2 H, br, NH₂), 7.05 (1 H, dd, J = 8.8, 2.0 Hz, Ar 2-H), 7.27 (1 H, dd, J = 9.4, 2.0 Hz, Ar 6-H), 7.43 (1 H, dd, J = 8.8, 2.0 Hz, Ar 3-H), 7.47 (1 H, dd, J = 9.4, 2.0 Hz, Ar 5-H); MS m/z 353.1218 (M + H) (C₁₆H₂₀₇⁷ClN₄O₃ requires 353.1194), 351.1236 (M + H) (C₁₆H₂₀₇⁵ClN₄O₃ requires 351.1223), 295/293 (M – C₃H₅O).

6.73. (4R,5R)-4-(2-(4-Bromophenyl)-2-cyano-1-methoxyethenyl)-2,2-dimethyl-5-hydroxymethyl-1,3-dioxolane (51c) and 5-(4-bromophenyl)-6-((4S,5R)-2,2-dimethyl-5-hydroxy- methyl-2,2-dimethyl-1,3-dioxolan-4-yl)pyrimidine-2,4-diamine (52c)

Compound 49c/50c was treated with CH₂N₂, as for the synthesis of 24a, to give 51c (91%) as a pale yellow oil: NMR δH 1.44 (3 H, s, 2-Me), 1.48 (3 H, s, 2-Me), 3.57 (3 H, s, OMe), 4.40 (1 H, dd, J = 10.9, 3.5 Hz, CHOH), 4.46 (1 H, d, J = 10.9 Hz, CHOH), 4.61 (1 H, m, 5-H), 5.34 (1 H, d, J = 7.0 Hz, 4-H), 7.36 (2 H, d, J = 8.6 Hz, Ar 2,6-H₂), 7.50 (2 H, d, J = 8.6 Hz, Ar 3,5-H₂); MS m/z 370.0481 (M + H) (C₁₆H₁₉₈₁BrNO₄ requires 370.0476), 368.0501 (M + H) (C₁₆H₂₀₇⁹BrN₄O₃ requires 368.0497). Compound 51c was treated with guanidine, as for the synthesis of 52a, to give 52c (29%) as a pale yellow solid: mp 181-183°C; NMR ((CD₃)₂SO) δH 1.14 (3 H, s, Me), 1.48 (3 H, s, Me), 3.45 (2 H, d, J = 3.5 Hz, CH₂O), 4.03 (1 H, dt, J = 6.4, 3.5 Hz, dioxolane 5-H), 4.73 (1 H, d, J = 6.4 Hz, dioxolane 4-H), 5.70 (2 H, br, NH₂), 5.85 (2 H, br, NH₂), 7.20 (1 H, dd, J = 8.1, 2.0 Hz, Ar 2-H), 7.25 (1 H, dd, J = 7.7, 2.0 Hz, Ar 6-H), 7.61 (1 H, dd, J = 7.7, 2.0 Hz, Ar 5-H), 7.63 (1 H, dd, J = 8.1, 2.0 Hz, Ar 3-H); NMR ((CD₃)₂SO) δC 25.44, 26.37, 62.49, 76.46, 79.81, 108.35, 108.72, 121.70, 132.30, 132.35, 132.59, 133.38, 134.30, 159.21, 162.29, 163.21; MS m/z 397.0694 (M + H) (C₁₆H₂₀₈₁BrN₄O₃ requires 397.0698), 395.0712 (M + H) (C₁₆H₂₀₇⁹BrN₄O₃ requires 395.0718).
6.74. (4R,5R)-2-Cyano-4-(2-(3,4-dichlorophenyl)-1-methoxyethenyl)-2,2-dimethyl-5-hydroxymethyl-1,3-dioxolane (51d) and 5-(3,4-dichlorophenyl)-6-((4S,5R)-2,2-dimethyl-5-hydroxymethyl-1,3-dioxolan-4-yl)pyrimidine-2,4-diamine (52d)

Compound 49d/50d was treated with CH\textsubscript{2}N\textsubscript{2}, as for the synthesis of 24a, to give 51d (78%) as a pale yellow oil: IR \(\nu\) max 3534, 2247, 1595 cm\(^{-1}\); NMR \(\delta\)\(\text{H}\) 1.44 (3 H, s, 2-Me), 1.47 (3 H, s, 2-Me), 3.72 (3 H, s, OMe), 4.54 (1 H, dd, \(J = 10.7, 4.1\) Hz, \text{CHOH}), 4.73 (1 H, d, \(J = 10.7\) Hz, \text{CHOH}), 4.96 (1 H, m, 5-H), 5.52 (1 H, d, \(J = 5.9\) Hz, 4-H), 7.15 (1 H, d, \(J = 8.5\) Hz, Ar 5-H), 7.37 (1 H, dd, \(J = 8.5, 1.4\) Hz, Ar 6-H), 7.40 (1 H, d, \(J = 1.4\) Hz, Ar 2-H); MS \(m/z\) 360.0378 (M - H) (C\textsubscript{16}H\textsubscript{16}\textsuperscript{37}Cl\textsubscript{2}NO\textsubscript{4} requires 360.0397), 358.0433 (M - H) (C\textsubscript{16}H\textsubscript{16}\textsuperscript{37}Cl\textsubscript{3}ClNO\textsubscript{4} requires 358.0426), 356.0452 (M - H) (C\textsubscript{16}H\textsubscript{16}\textsuperscript{35}Cl\textsubscript{2}NO\textsubscript{4} requires 356.0456), 346/344/342 (M – Me).

Compound 51d was treated with guanidine, as for the synthesis of 52a, to give 52d (47%) as a pale yellow solid: mp 181-183ºC; NMR (CD\textsubscript{3}CN) \(\delta\)\(\text{H}\) 1.20 (3 H, s, Me), 1.49 (3 H, s, Me), 3.40-3.42 (2 H, m, \text{CH\textsubscript{2}OH}), 4.03 (1 H, m, 5-H), 4.72 (1 H, d, \(J = 7.0\) Hz, 4-H), 5.21 (2 H, br, \text{NH\textsubscript{2}}), 5.31 (2 H, br, \text{NH\textsubscript{2}}), 7.11 (0.5 H, dd, \(J = 8.2, 2.0\) Hz, Ph 6-H), 7.21 (0.5 H, dd, \(J = 8.2, 2.0\) Hz, Ph 6-H), 7.38 (0.5 H, d, \(J = 2.0\) Hz, Ph 2-H), 7.48 (0.5 H, d, \(J = 2.0\) Hz, Ph 2-H), 7.60 (0.5 H, d, \(J = 8.2\) Hz, Ph 5-H), 7.62 (0.5 H, d, \(J = 8.2\) Hz, Ph 5-H); NMR (CD\textsubscript{3})\textsubscript{2}CO) \(\delta\)\(\text{C}\) 24.90 (Me), 25.88 (Me), 61.93 (CH\textsubscript{2}OH), 76.13 (CH), 79.16 (CH), 107.43 (CMe\textsubscript{2}), 108.75 (Pyr 5-C), 130.36 (Ph C), 131.07 (Ph CH), 131.18 (Ph C), 131.51 (Ph CH), 132.89 (Ph CH), 135.14 (Ph C), 159.37 (Pyr 2-C), 161.03 (Pyr 4-C), 162.93 (Pyr 6-C); MS \(m/z\) 389.0777 (M + H) (C\textsubscript{16}H\textsubscript{16}\textsuperscript{37}Cl\textsubscript{2}N\textsubscript{2}O\textsubscript{3} requires 389.0775), 387.0810 (M + H) (C\textsubscript{16}H\textsubscript{16}\textsuperscript{37}Cl\textsubscript{3}ClNO\textsubscript{4} requires 387.0833), 385.0833 (M + H) (C\textsubscript{16}H\textsubscript{16}\textsuperscript{35}Cl\textsubscript{2}N\textsubscript{2}O\textsubscript{3} requires 385.0834), 331/329/327 (M – C\textsubscript{3}H\textsubscript{5}O).

6.75. 1-Cyano-1,4-diphenylbutan-2-one (54) / 1-cyano-1,4-diphenylbut-1-en-2-ol (55)

Phenylacetonitrile was condensed with ethyl 3-phenylpropanoate 53, as for the synthesis of 22a/23a, to give 54/55 (34%) as a pale buff solid: mp 53-54ºC (lit.\textsuperscript{54} mp 76-78ºC) ; IR \(\nu\) max 2200 cm\(^{-1}\); NMR ((CD\textsubscript{3})\textsubscript{2}SO) \(\delta\)\(\text{H}\) 2.88 (2 H, t, \(J = 6.4\) Hz, CH\textsubscript{2}), 2.94 (2 H, t, \(J = 6.4\) Hz, CH\textsubscript{2}), 7.20-7.61 (10 H, m, 2 \times Ph-H\textsubscript{2}), 11.70 (1 H, br s, OH) MS \(m/z\) 250.1240 (M + H) (C\textsubscript{17}H\textsubscript{16}NO requires 250.1231), 222 (M – HCN), 91 (Bn).

6.76. 1-Cyano-1-phenylpropan-2-one (58) / 1-cyano-1-phenylprop-1-en-2-ol (59)

Phenylacetonitrile was condensed with ethyl acetate 58, as for the synthesis of 22a/23a except...
that chromatography was omitted and the product was recrystallised (aq. EtOH), to give 58/59 (31%) as a pale buff solid: mp 87-88°C (lit. \(^{55}\) mp 87-89°C); NMR \(\delta_H\) 2.25 (3 H, s, Me), 4.66 (1 H, s, CHCN), 7.38-7.47 (5 H, m, Ph-H\(_3\)); MS \(m/z\) 160.0740 (M + H) (C\(_{10}\)H\(_{10}\)NO requires 160.0762), 144 (M - CH\(_3\)), 118 (M – C\(_2\)H\(_3\)N).

6.77. 1-(4-Chlorophenyl)-1-cyanobutan-2-one (68) / 1-(4-chlorophenyl)-1-cyano-but-1-en-2-ol (64)

4-Chlorophenylacetonitrile was condensed with ethyl propanoate 62, as for the synthesis of 22a/23a, to give 62/63 (31%) as a pale yellow solid: mp 50-51°C (lit. \(^{16}\) mp 50-52°C); NMR ((CD\(_3\))\(_2\)SO) \(\delta_H\) 1.24 (3 H, t, \(J = 7.4\) Hz, Me), 2.62 (2 H, q, \(J = 7.4\) Hz, CH\(_2\)), 7.42 (2 H, d, \(J = 8.8\) Hz, 2,6-H\(_2\)), 7.66 (2 H, d, \(J = 8.8\) Hz, 3,5-H\(_2\)).

6.78. Biological assay

The radial spoke assay was performed essentially as described by Gerum et al.\(^{38}\) and Sibley et al.\(^{56}\) The three yeasts were grown in media comprising 10% yeast extract, 10% peptone and 10% dextrose. Sulfanilamide (1.0 mM, 100 µL), an inhibitor of dihydropteroate synthase,\(^{57}\) was spread onto fresh agar plates and allowed to absorb into the medium overnight. Three template plates were streaked with the yeast cultures in two orthogonal lines and incubated at 30°C for 3 d. These plates were used to generate replica test plates. Test compounds 7a-d, 8a-c, 9a-d, 10a-d and control compounds 3, 6, 11, 12 were made up as 10 mM solutions in DMSO; a spot (10 µL) of each of these solutions was placed at the centre of each test plate. The assay plates were then incubated for 3 days at 30°C before the inhibition zone was measured. Each compound/yeast combination was assayed in triplicate.

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References


Table 1. Diameters of zones of inhibition of growth of *S. cerevisiae* carrying the DHFR gene from *M. tuberculosis*, *S. cerevisiae* carrying the human DHFR gene and wild-type *S. cerevisiae* by test pyrimidine-2,4-diamines 7-10 and by control pyrimidine-2,4-diamines 11, 12, 3 (pyrimethamine) and 6 (trimethoprim).

![Chemical structure](image)

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<th>R⁶</th>
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<th>Diameter of zone of inhibition (mm)</th>
<th>S. cerevisiae (human-DHFR)</th>
<th>Diameter of zone of inhibition (mm)</th>
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* Diameters of the zone of inhibition were measured for each of the orthogonal streaks on each of at least three test plates for each determination; data are expressed ± 1 mm.

* TB5 yeast engineered to contain DHFR from *M. tuberculosis* only.

* TB5 yeast engineered to contain human DHFR only.

* TB5 yeast engineered to contain yeast DHFR only.