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2,4-Diaminopyrimidines as Inhibitors of Leishmanial and Trypanosomal Dihydrofolate Reductase

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Abstract—This paper describes the synthesis of 4′-substituted and 3′,4′-disubstituted 5-benzyl-2,4-diaminopyrimidines as selective inhibitors of leishmanial and trypanosomal dihydrofolate reductase. Compounds were then assayed against the recombinant parasite and human enzymes. Some of the compounds showed good activity. They were also tested against the intact parasites using in vitro assays. Good activity was found against *Trypanosoma cruzi*, moderate activity against *Trypanosoma brucei* and *Leishmania donovani*. Molecular modeling was undertaken to explain the results. The leishmanial enzyme was found to have a more extensive lipophilic binding region in the active site than the human enzyme. Compounds which bound within the pocket showed the highest selectivity.
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Introduction

Leishmaniasis, African trypanosomiasis and Chagas disease are major causes of mortality, mainly in the developing world. There is need for new treatments for these diseases as the current drugs are toxic, expensive and require long treatment. The situation has been compounded by increasing treatment failures by current drugs.[1] The causative organisms for these diseases are species and subspecies of *Leishmania* (in particular, *L. donovani*, *L. infantum*, *L. mexicana* and *L. amazonensis*), *Trypanosoma brucei* and *Trypanosoma cruzi*, respectively.

We have been interested in investigating dihydrofolate reductase as a drug target for these diseases.[2–7] Dihydrofolate reductase (DHFR) is responsible for the reduction of folates within the cell. A reaction of particular importance is the reduction of dihydrofolate to tetrahydrofolate. Tetrahydrofolate is then methylated to methylene tetrahydrofolate which is a vital cofactor for the methylation of deoxyuridine monophosphate to thymidine monophosphate. Thus inhibition of DHFR prevents biosynthesis of thymidine, leading to cell death. DHFR has been a successful drug target for antimalarials (pyrimethamine and cycloguanil), anti-bacterials (trimethoprim), and anti-cancer (methotrexate) amongst other diseases. However, these classical DHFR inhibitors show no selectivity for the leishmanial or trypanosome enzymes.[2] We have carried out modelling that suggests[3] that there are structural differences between the leishmanial and trypanosome DHFR and the corresponding human enzyme which could be exploited for selective inhibition.

Sirawaraporn et al.[8] reported some 5-benzyl-2,4-diaminopyrimidines as selective inhibitors of leishmanial DHFR. These compounds also showed some in vitro activity against *L. donovani* amastigotes. The most active and selective compound against the parasite enzyme was the 3′-octyloxy derivative **1** ($n=7$) (Fig. 1). We then conducted an extensive structure activity study with the 3′-series of compounds and extended the study to include *T. brucei* and *T. cruzi*.[4] Essentially, compounds with a chain length of 2–6 carbon atoms showed

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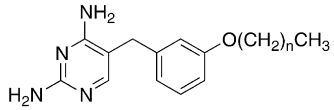


1

For n = 7, data obtained by Sirawaraporn *et al.*[8]

IC_{50} (*L. major* DHFR) 0.2μM

IC_{50} (human DHFR) 26μM

EC_{50} (*L. donovani* amastigotes) 4.6μM

Figure 1. The lead structure.

maximum enzyme activity and selectivity. We could rationalize the data using modeling; the alkyl chains extended towards a phenylalanine residue in the parasite enzyme active site. The closest (and presumably strongest) interaction occurred for a chain length of about 4–6 carbon atoms. With regards to selectivity, in the human enzyme, the phenylalanine residue has been replaced by an asparagine. The alkyl chain of compound **1** does not interact with the asparagine and is probably folded in a different conformation, explaining the selectivity of these compounds for the parasite enzymes. However, the most active compounds against the parasite enzymes were not the most active against the intact parasites. The most active compounds had a chain length of 8–10 carbon atoms and corresponded to the most lipophilic that were prepared. These longer chain compounds showed toxic effects on mammalian cells, presumably due, at least in part, to inhibition of the mammalian DHFR. The compounds ($n = 8$–10) showed good activity against *T. brucei* trypomastigotes, but minimal activity against *L. donovani* amastigotes. The lack of activity against *L. donovani* amastigotes may be due to problems of accessibility of the parasite to the compounds: *T. brucei* is an extracellular parasite, *T. cruzi* is an intracellular parasite in the cytoplasm of host cells, whilst *L. donovani* is also an intracellular parasite found in a vacuole within macrophages.

We decided to further investigate this class of compounds with the aim of maximizing activity and selectivity for the enzyme, whilst improving in vitro activity against the parasite. In particular it appeared that for compounds to have in vitro activity, they should be lipophilic. The proposed target compounds are shown in Figure 2. Firstly the substitution on the 4′-position (compound **2**) was investigated. Secondly substituents were added at both 3′- and 4′-positions to see if the presence of two alkyl substituents could maximize interaction with the phenylalanine residue and increase the lipophilicity. This was done in two ways: both substituents on the 3′- and 4′-positions were kept the same (compound **3**) or with both substituents being different (compound **4**).

In addition, further modeling studies were undertaken to try and understand interaction of the inhibitors with the active site and the basis of selectivity for the parasite enzymes.

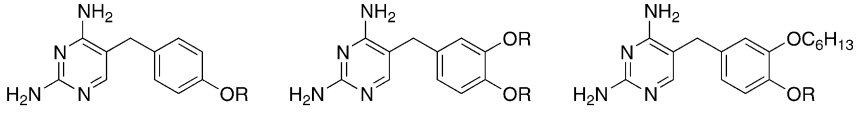
Chemistry

Compounds were prepared based on methodology reported previously.[4] A number of different strategies are described below.

Compounds of series 2

4-Hydroxybenzaldehyde (**6**) was alkylated (Scheme 1). The conditions for alkylation were optimized and it was found that the best conditions were the alkyl iodide in 2-butanone with potassium carbonate as base.[9] The ethers were then condensed with 3-ethoxypropionitrile using sodium ethoxide as base. Use of modified Dean–Stark conditions seemed to improve this step, where water was removed by molecular sieves. A final cyclisation with guanidine provided compounds **2c**, **2d**, **2f** and **2h** in good yields.

An alternative strategy to compounds of type **2** consisted in protection of 4-hydroxybenzaldehyde as the THP compound, condensation with 3-ethoxypropionitrile, cyclisation with guanidine and then removal of the protecting group (Scheme 2). The resulting 4-hydroxy phenol (**2a**) was alkylated, using potassium carbonate as



2

a, R = H
b, R = CH_3
c, R = C_5H_{11}
d, R = C_6H_{13}
e, R = C_7H_{15}
f, R = C_8H_{17}
g, R = C_9H_{19}
h, R = $C_{10}H_{21}$
i, R = CH_2Ph
j, R = THP

3

a, R = C_3H_7
b, R = C_6H_{13}
c, R = C_8H_{17}
d, R = $C_{10}H_{21}$

4

a, R = C_3H_7
b, R = C_8H_{17}

Figure 2. The target molecules.

base in ethanol to give other compounds in series **2** (**2e**, **2g**, **2i**).

Compounds of series 3

3,4-Dihydroxybenzaldehyde (**13**) was alkylated under a variety of conditions to give the di-substituted compounds **14** (Scheme 3). Condensation with 3-ethoxypropionitrile followed by condensation with guanidine gave the compounds of series **3**.

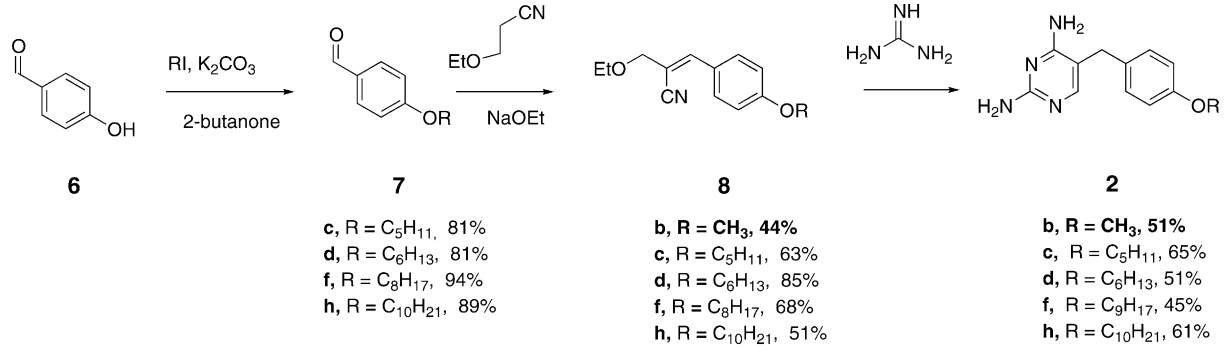
Compounds of series 4

These compounds are differentially substituted on the 3′- and 4′-positions, so a different strategy had to be applied (Scheme 4). 3,4-Dihydroxybenzaldehyde (**13**) was treated with one equivalent of benzyl bromide using acetone as solvent and potassium carbonate as base.[10] Only one regioisomer was found in 77% yield. This was deduced to be the 4′-substituted compound **16**. The reason for selective alkylation appears to be the greater acidity of the 4′-hydroxyl. The regioselectivity of alkylation was established by a long-range NOESY spectrum which showed a signal enhancement of the 5′-hydrogen, but not of the 2′-hydrogen on irradiation of the benzylic CH_2 (Fig. 3). Subsequently alkylation with hexyl iodide gave the differentially substituted compound **17**. Removal of the benzyl protecting group was initially accomplished with trimethylsilyl iodide. However due to the reactivity of the trimethylsilyl iodide, different conditions were investigated for deprotection of the benzyl group. Hydrogenolysis using 10% palladium on charcoal gave the required mono-alkylated compound **18**. Use of palladium hydroxide as catalyst lead to reduction of the aldehyde group to the methyl.[11,12] Finally alkylation of the free hydroxyl group was carried out with propyl iodide and octyl iodide, followed by condensation with 3-ethoxypropionitrile and then guanidine to give the required final compounds.

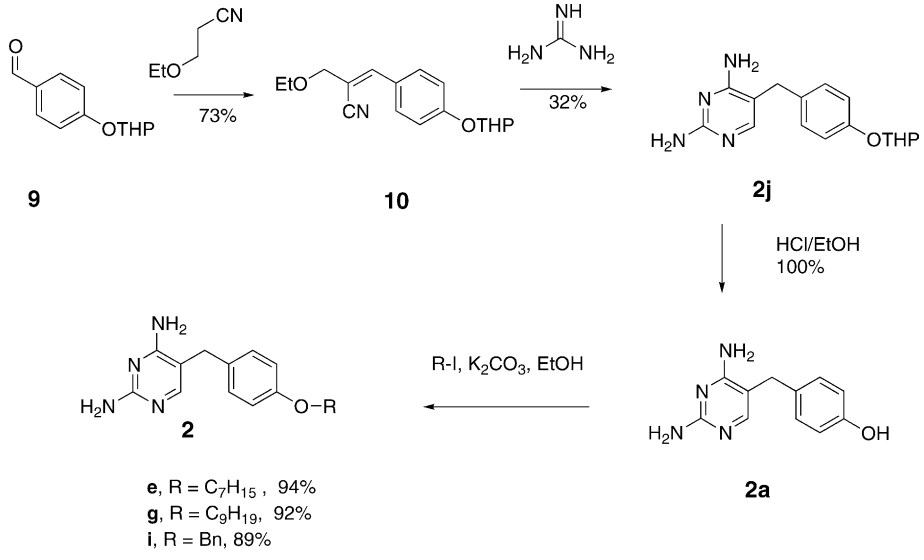
Biological Assays

Enzyme assays

Compounds were assayed against the recombinant *L. major*, *T. cruzi* and human enzymes. The data for this are shown in Table 1. The presence of a 4′-alkoxy derivative appeared to increase activity and selectivity for the parasite enzymes (series **2**). The most active and selective compounds were the hexyl derivative (**2d**) against the *L. major* enzyme and the methyl (**2b**) and heptyl (**2e**) derivatives against the *T. cruzi* enzyme. In addition both the benzyl and THP derivatives were



Scheme 1.



Scheme 2.

active and selective for both of the parasite enzymes. However no distinct trends could be observed. The disubstituted derivatives (series **3**) did not seem to make a significant difference in selectivity or activity compared to the corresponding mono-substituted derivatives. See the section of modeling for further discussion of this data.

In vitro assays

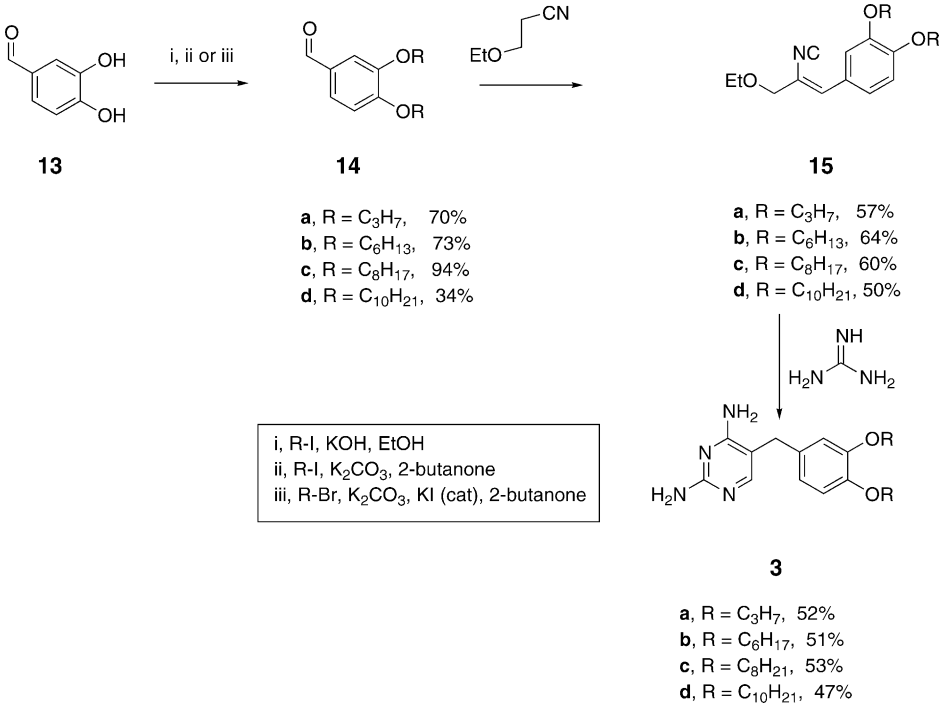
The compounds were also assayed against the clinically relevant stage of the intact parasites (Table 2). For *L. donovani* and *T. cruzi* this was the amastigote stage cultured in mammalian cells. The compounds were also assayed against the related parasite, *Trypanosoma brucei rhodesiense*, which causes African trypanosomiasis (sleeping sickness). For series **2** compounds, none of the compounds were notably active against *L. donovani*. Against *T. cruzi* and *T. b. rhodesiense*, the activity appeared to increase for the longer alkyl chain derivatives (**2c**–**2h**); however the selectivity for the parasite over the mammalian cells did not seem to be improved. Interestingly, the THP derivative **2j** appeared to have good activity and selectivity for both of these organisms. For bis-substituted compounds (series **3** and **4**), a number of compounds showed moderate activity, most notably the bis-hexyl derivative **3b**. However, the selectivity was not high.

In vivo assays

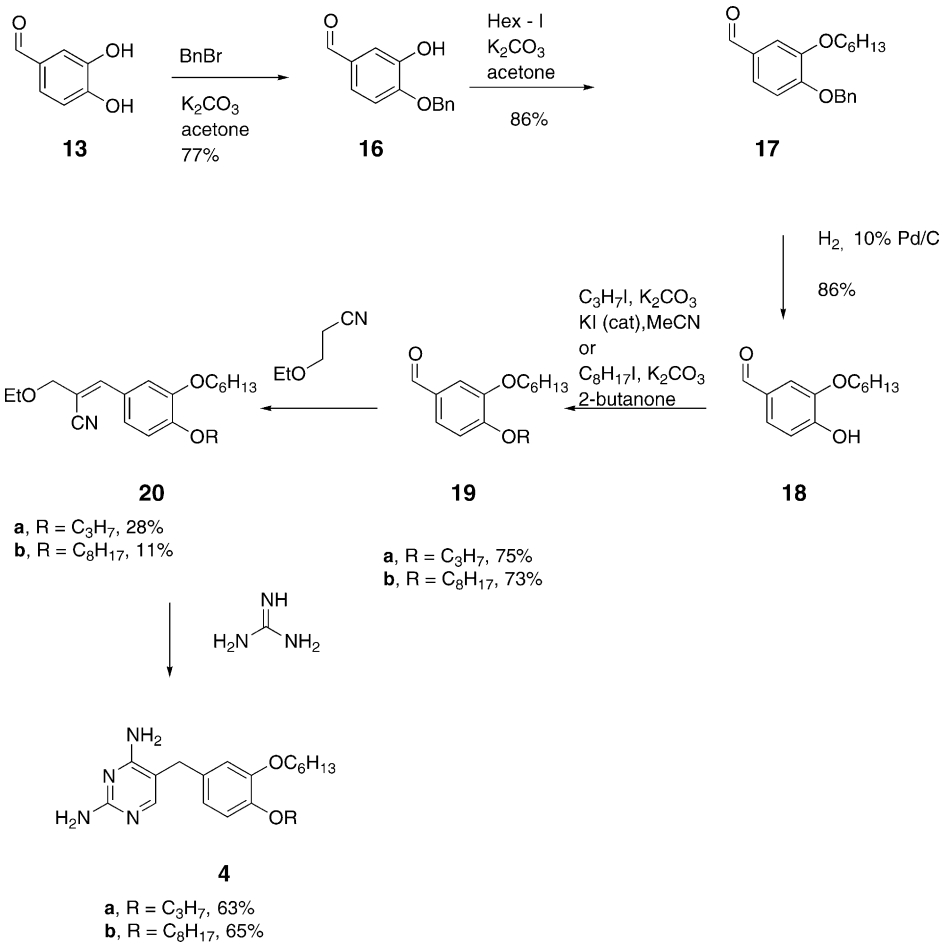
Compounds **2d**, **2g**, **2h** and **3b** were considered active enough for assays against a rodent model of Chagas' disease. At a dose of 50 mg/kg these compounds showed no effect on the progression of the disease.

Modelling

In order to find a correlation between the compounds structure and their activity, molecular modelling was undertaken. The first step was to dock our compounds into the active site of the human, *L. major*[13] and *T. cruzi*[3] enzymes. Two programmes were considered, Genetic Optimisation for Ligand Docking (GOLD)[14] and FlexX[15] part of the Sybyl suite of software.[16] These have scoring functions, which may give rise to some correlation between enzyme inhibition and score. To validate the methodology, two known structures were used: the crystal structure of the human enzyme with folate bound in the active site (pdb 1DFR), and the crystal structure of the *L. major* enzyme[13,17] with methotrexate bound in the active site. Firstly, folate was removed from the active site of the human enzyme and then docked into the same active site using the programmes FlexX and GOLD. The same operation was carried out with the active site of the *L. major* enzyme by removal and subsequent redocking of the methotrexate. With GOLD, the docking of folate and methotrexate failed as the two ligands did not fit properly into their own active sites. It is reported that this programme failed with very flexible compounds or with a strong lipophilic character, which is the case with folate and methotrexate.[14] With the progamme FlexX, the folate was docked in a very similar orientation to that found in the crystal structure (RMSD = 1.43 Å) (Fig. 4a). Simi-



Scheme 3.



Scheme 4.

Table 1. Inhibition constants (K_i) of compounds against *L. major*, *T. cruzi* and human dihydrofolate reductase

No.	R	*L. major* K_i (μM)	*T. cruzi* K_i (μM)	Human K_i (μM)
2a	H	2.8 (1.2)	2.3 (1.5)	3.4
2b	Me	2.8 (2.0)	0.034 (163)	5.5
2c	Pentyl	0.45 (3.6)	0.99 (1.6)	1.6
2d	Hexyl	0.007 (331)	0.42 (5.8)	2.4
2e	Heptyl	0.86 (7.1)	0.04 (152)	6.1
2f	Octyl	16.7 (1.2)	0.48 (40.4)	19.4
2g	Nonyl	0.81 (3.1)	1.1 (2.2)	2.5
2h	Decyl	5.6 (1.5)	2.8 (3.0)	8.3
2i	Benzyl	0.22 (11)	0.03 (84)	2.5
2j	THP	0.32 (19)	0.037 (163)	6.0
3a	Propyl	2.1 (2.9)	0.55 (10.8)	5.9
3b	Hexyl	3.2 (0.51)	0.35 (4.6)	1.6
3c	Octyl	17.4 (1.5)	4.4 (5.8)	25.4
3d	Decyl	0.22 (7.4)	2.7 (0.61)	1.7
4a	Propyl	0.68 (4.5)	0.049 (62.5)	3.0
4b	Octyl	3.2	N.D.	N.D.
1[a]	Octyl	0.097 (25)	1.1 (2.1)	2.4

Selectivity values [calculated as K_i (human)/K_i (parasite)] are shown in parentheses.
[a]Data obtained in our laboratories[4] for compound **1**, $n = 7$.

larly, methotrexate was docked into the active site of the *L. major* enzyme and gave a very similar orientation to that of methotrexate in the active site in the crystal structure (RMSD = 1.65 Å) (Fig. 4b).

FlexX was then used to dock inhibitors **2–4** into the active sites of the human and *L. major* enzymes, and also into the active site of a homology model of the *T. cruzi* enzyme.[3]

The compounds with their highest Consensus Score[18–20] appeared to dock in a reasonable conformation.

Consensus Score or Cscore takes into account a number of different scoring functions:

- G_Score: computes the hydrogen bonding energies, complex–ligand energies and internal ligand–ligand energies.[15]
- PMF_Score: analyses the free energies of interactions for protein–ligand atom-contact pairs.[21]
- D_Score: computes the charge and Van der Waals interactions between the protein and ligand.[22]
- ChemScore: includes hydrogen bonding, metal–ligand interaction, lipophilic contact and rotational energy.[20]

There was no clear correlation between any of these scoring functions and enzyme inhibition for each compound series.

Table 2. In vitro activities of compounds against *L. donovani* amastigotes, *T. cruzi* amastigotes and *T. b. rhodesiense* bloodstream form trypomastigotes

No.	R	*L. donovani*	*T. cruzi*	*T. brucei*	Cytotoxicity
2a	H	>139	16.6 (20)	14.3 (24)	340
2b	Me	>130	12.2 (14)	3.7 (47)	174
2c	Pentyl	19.9 (2.1)	3.8 (11)	1.8 (23)	41.6
2d	Hexyl	ne	2.0 (7.8)	1.8 (8.7)	15.6
2e	Heptyl	>95	1.6 (21.4)	1.74 (20)	34.3
2f	Octyl	ne	1.5 (9.6)	0.73 (19)	14.0
2g	Nonyl	ne	1.8 (17.5)	1.6 (20)	31.8
2h	Decyl	ne	2.0 (7.0)	1.0 (13.5)	14.0
2i	Benzyl	>33	26.1 (2.3)	4.6 (12.9)	59.7
2j	THP	90 (1.4)	6.0 (21)	4.0 (32)	127
3a	Propyl	61 (1.1)	8.8 (7.5)	7.0 (9.5)	66.4
3b	Hexyl	3.7 (1.5)	1.2 (4.5)	0.77 (7.1)	5.5
3c	Octyl	6.6 (1.5)	3.3 (3.1)	4.2 (2.4)	10.0
3d	Decyl	12.1 (6.2)	92 (0.8)	33 (2.3)	75
4a	Propyl	ne	2.2 (7.7)	2.4 (7.0)	17
4b	Octyl	>70	84 (2.5)	42 (5.0)	>210

Values quoted are IC_{50} in μM. Selectivity values [calculated as IC_{50} (mammalian)/IC_{50} (parasite)] are shown in parentheses. Standards as follows: *L. donovani*, no standard was available; *T. cruzi*, benznidazole, $IC_{50} = 2.3$ μM; *T. brucei rhodesiense*, melarsoprol, $IC_{50} = 0.009$ μM. ne, data not determined.

Having docked the compounds into the active site, we analysed the active site using the programme GRID.[23] We wanted to use this programme in a qualitative manner to determine the differences in the active sites of the human and leishmanial enzymes which could explain the enzyme inhibition results that we had obtained and which could be useful for the design of more potent and selective inhibitors. GRID is a computational procedure to determine energetically favorable interaction sites of known three-dimensional structure of proteins. This is performed by calculating the energies of interaction between a defined chemical group (the probe) and the chemical functions of the protein in the region of interest. The interactions energy includes contributions from Van der Waals, hydrogen bond interactions and electrostatic interactions.

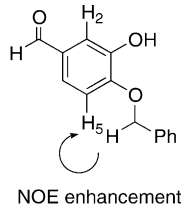


Figure 3. NOE enhancement for compound **16**.

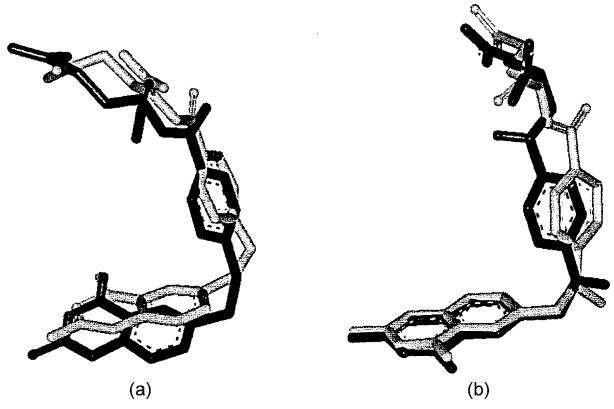


Figure 4. (a) Overlay of folate from the active site of the human DHFR (black) and the predicted orientation from FlexX (grey); (b) overlay of methotrexate from the active site of the *L. major* DHFR (black) and the predicted orientation of methotrexate in the active site by FlexX (grey).

The ligand was removed from the active sites of the human and leishmanial enzymes and then the active sites were examined with various probes. The following probes were used: lipophilic, carboxylate ion, hydroxyl and ammonium ion. The results could be displayed as a series of contoured surfaces, which represent a defined binding energy of the probe. Essentially all the probes gave identical surfaces for both the human and parasite enzymes, except the lipophilic probe. The lipophilic probe for both the human (Fig. 5a) and *L. major* enzymes at an energy of −0.1 kcal/mol gave a surface which extended into the active site along the binding cavity where both the glutamate and benzamide moieties of the folate bind. This can be seen in Figure 5b and d where the ligands folate and methotrexate, respectively, have been superimposed. At this energy, the lipophilic binding probe gave a larger surface in the case of the *L. major* enzyme than in the case of the human enzyme in the regions of the enzyme active site. The contours were displayed using a small negative value, as this should show any location where a lipophilic moiety of the molecule would show a 'favourable-binding' interaction with the protein. This result suggests that the active site of the *L. major* enzyme can accommodate larger lipophilic substituents than the human enzyme. This lipophilic binding site extended from where the pteridine binding pocket finished and encompassed the residues Ile45, Met53, Val56, Lys57, Val87, Pro88, Phe91, Leu94 and Arg97 of the *L. major* enzyme. Interestingly the active site of the *L. major* enzyme has been shown to be slightly larger than the human enzyme in this region.[3]

In the next stage of the study, we wished to see if this model could explain (a) the selectivity of compounds for

the *L. major* enzyme compared to the human and (b) the reasons for different activities of compounds against one of the enzymes. In order to accomplish this, the inhibitors described above were docked into the active site of the *L. major* and human enzymes using FlexX (as described above). The surface showing a binding energy of −0.1 kcal/mol was then superimposed. It was now possible to see which parts of the inhibitors were interacting with the enzyme within this contour and which parts of the inhibitors are interacting outside of this contour (or lipophilic binding region). The results from this are shown in Figures 6 and 7. To simplify interpretation of the data, the residues of the enzyme have not been displayed.

When the orientation of the inhibitors, as found by docking with FlexX were overlayed with this lipophilic binding pocket, the following observations were made:

- In compound pentyl (**2c**) (Fig. 6), the alkyl chain fitted well into both the lipophilic pocket of the *L. major* enzyme and protruded slightly out of the lipophilic pocket of the human enzyme. With the longer hexyl (**2d**) derivative, this difference was exaggerated further; the alkyl chain was still completely in the lipophilic pocket of the *L. major* enzyme, but extended significantly out of the human enzyme. Presumably when the alkyl chain protrudes out of the lipophilic binding region of the enzyme, it will not have favorable interactions with residues in the enzyme active site. This may explain the increased activity and selectivity for the *L. major* enzyme. However, as the chain length was now extended further to the heptyl (**2e**) and octyl (**2f**) derivatives, the alkyl chain extends beyond the lipophilic pocket for both the *L. major* and human active sites, perhaps

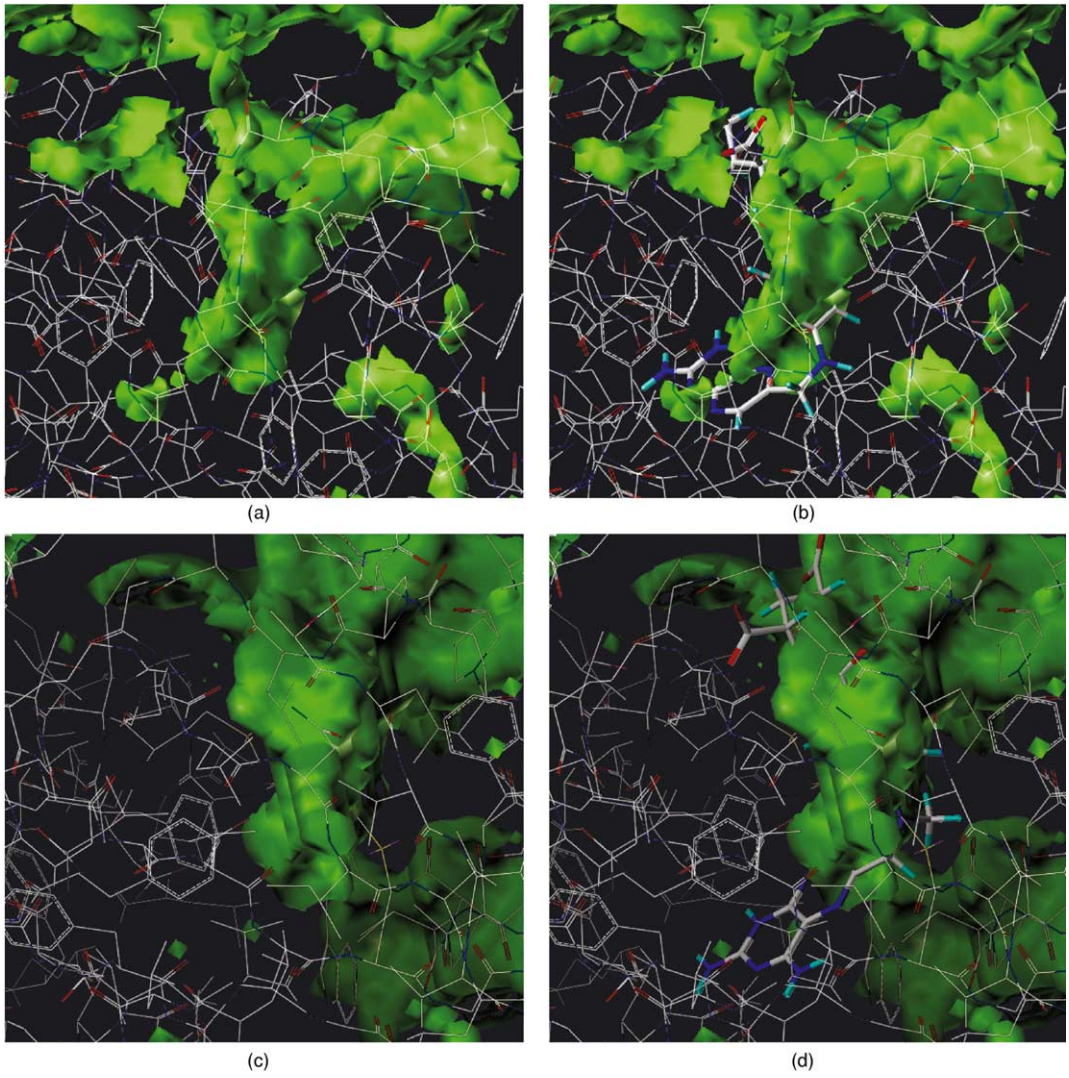


Figure 5. (a) The binding site for human DHFR with the ligand (folate) removed. The green surface represents where a lipophilic probe has a binding energy of −0.1 kcal/mol. (b) The same diagram as for (a), but now with the ligand folate superimposed onto the active site. The folate is shown in stick mode. (c) The binding site for *L. major* DHFR with the ligand (methotrexate) removed. The green surface represents where a lipophilic probe has a binding energy of −0.1 kcal/mol. (d) The same diagram as for (c), but now with the ligand methotrexate superimposed onto the active site. The methotrexate is shown in stick mode.

explaining the decreased activity and selectivity. This could explain the maximum activity against the *L. major* enzyme seen with the hexyl derivative **2d**. The greater activity of the nonyl (**2g**) compared to the octyl (**2f**) derivative cannot be readily explained by this model.

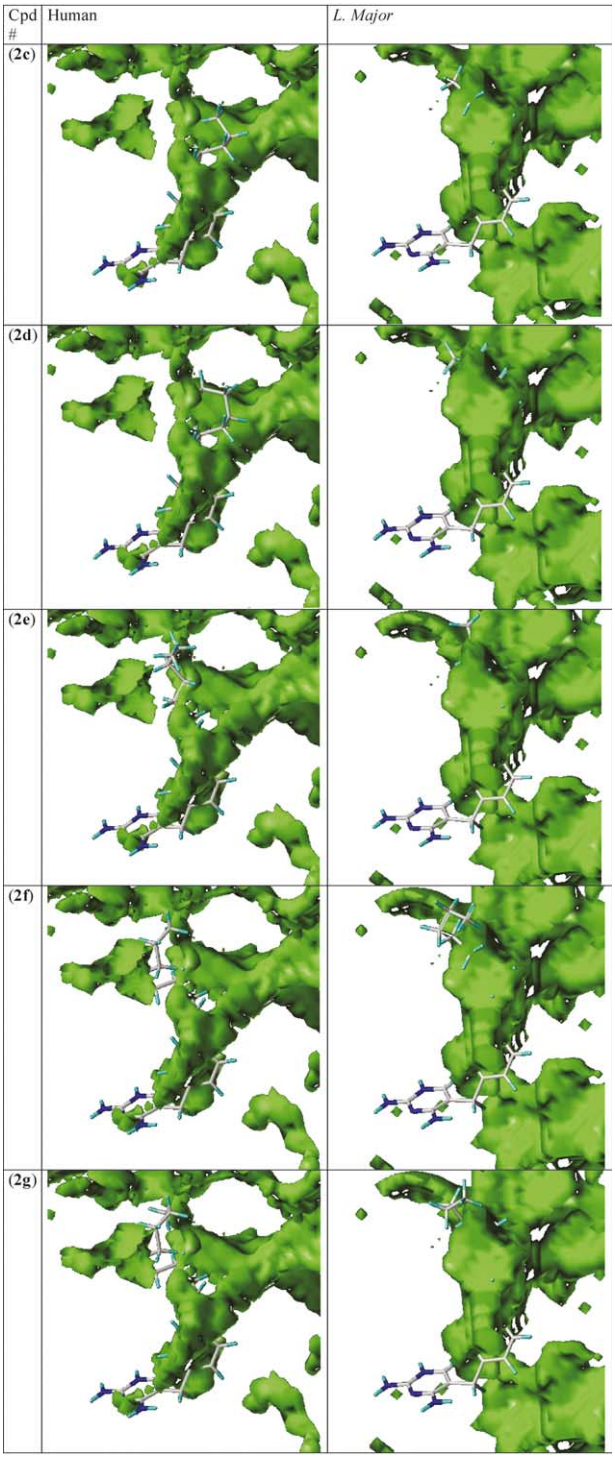


Figure 6. Overlay of the hydrophobic binding sites defined by GRID for the human and *L. major* DHFR active sites and key inhibitors (**2c–g**) in the conformations present in the active site as predicted by FlexX. The lipophilic binding site is larger in case of the *L. major* enzyme compared to the human enzyme. The residues of the protein are not shown for clarity.

- For the THP derivative (**2j**) (Fig. 7), the substituent fitted well into the lipophilic pocket of the *L. major* enzyme, but a large part of the substituent protruded out of the lipophilic pocket of the human enzyme, perhaps explaining the activity and selectivity.
- For the disubstituted compound, **3c** (di-octyl) the 3′-substituent was no longer binding in the lipophilic binding region, probably leading to high-energy interactions in the active site, again being a possible explanation for the low selectivity of this series of compounds.

Discussion

For the 4-substituted series **2**, the best activity and selectivity against the *L. major* enzyme was seen with the pentyl (**2c**) to heptyl (**2e**) derivatives, presumably due to the strong interaction of the alkyl chains with the lipophilic binding region of the enzyme. In the case of the *T. cruzi* enzyme (for which there is no crystal structure) similar trends were observed, except that best activity and selectivity was seen over a larger range of compounds, methyl (**2b**) to octyl (**2f**). This is also presumably due to a larger, more lipophilic region of the *T. cruzi* enzyme compared to the human enzyme around the lipophilic binding pocket. The benzyl (**2i**) and THP (**2j**) groups fit well within the lipophilic binding region of the *L. major* enzyme and presumably also of the *T. cruzi* enzyme, whilst protruding in the case of the human enzyme, explaining their good activity and selectivity for the enzyme.

There is little activity of compounds of series **2** against *L. donovani* amastigotes. This may be due to lack of accessibility to the parasites that are found within a vacuole within a cell. However, there was activity

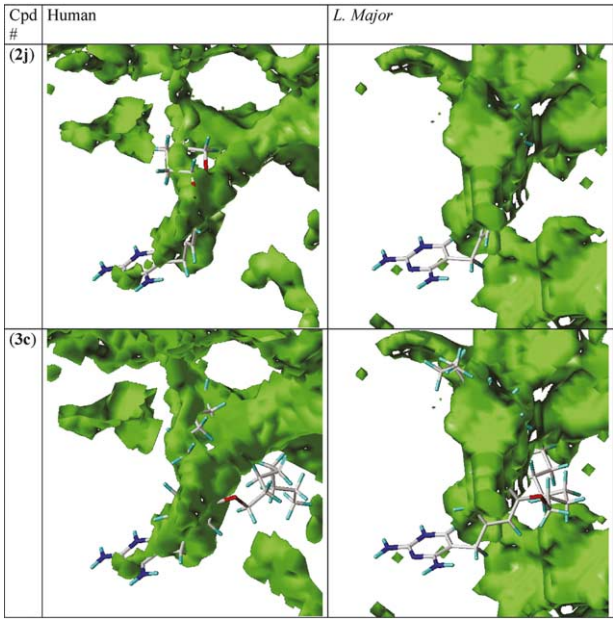


Figure 7. As for Figure 6, except with inhibitors **2j** and **3c**.

against *T. cruzi* and *T. brucei*. For the straight chain alkyl derivatives **2a–h**, there was some correlation between activity against the *T. cruzi* enzyme and against *T. cruzi* amastigotes, suggesting that inhibition of DHFR is important in their mode of action. There are some important exceptions, such as compound **2b**. Interestingly the benzyl and THP derivatives (**2i** and **2j**) are less active in vitro than the alkyl derivatives **2b–h**, which contrasts to the situation with inhibition against the enzyme, where **2i** and **2j** were relatively active. Therefore there is not total correlation between enzyme activity and anti-parasitic activity and other factors must be important.

The disubstituted compounds (**3** and **4**) show moderate activity against the *L. major* enzyme, but generally poor selectivity presumably due to protrusion of the alkyl chains from the lipophilic binding regions in both the *L. major* and human enzymes, which presumably gives little selective advantage. Interestingly these compounds show greater in vitro activity against *L. donovani* amastigotes, compared to some of the other derivatives (**2**), which may be due to increased lipophilicity allowing better penetration through membranes. This effect was not observed in the case of *T. cruzi* or *T. brucei* where there are fewer membranes for the compounds to cross before reaching the target. This may suggest that accessibility of the parasites to these DHFR inhibitors may be a problem in the case of *L. donovani*, but probably of much less significance in other organisms.

Conclusion

The chemical synthesis of a number of selective inhibitors of *L. major* and *T. cruzi* DHFR has been successfully carried out. The most active and selective compounds are the 4′-hexyloxy derivative (**2e**) against the *L. major* enzyme and the methoxy (**2b**), heptyloxy (**2f**) and octyloxy (**2g**) against the *T. cruzi* enzyme. In addition both the THP and benzyl substituted compounds (**2i**, **2j**) showed good activity and selectivity against both enzymes. Having two substituents on the aromatic ring (**3a–d**, **4a**,**b**) generally did not give selective compounds. The compounds were successfully docked into the active site using FlexX. This gave a qualitative explanation for the activity and selectivity of compounds.

The compounds showed relatively little activity against *L. donovani* and where there was a measurable effect, there appeared to be little selectivity for the parasite over the mammalian host cell. Against *T. cruzi*, the most active compounds appeared to be the longer chain derivatives (**2c–f**). Interestingly, for the disubstituted compounds, those with medium length chains showed good activity (**3b**, **3c**, **4a**). The THP-substituted compound, whilst not as active as others, showed good selectivity. The compounds were also assessed for activity against *T. b. rhodesiense*. They followed a similar trend to the case of *T. cruzi*. The lack of activity against *L. donovani* could be due to a number of factors. Firstly, there may be poor accessibility of the compound to the parasite, which is found in a vacuole within macrophages. Secondly, the parasites may be able to salvage the required folates from the host and finally there may be some kind of resistance, such as use of ptr1 to bypass DHFR. In the case of *T. cruzi*, the lack of complete correlation between activity against the enzyme and the intact organism may be due to penetration of the compound into the parasite. Another factor may be that the compound is acting on other enzymes in the folate pathway in addition to DHFR.

None of the compounds assayed showed any in vivo activity in a rodent model of Chagas' disease. This may be due to lack of efficacy or specificity of the compounds, compound absorption and distribution within the animal, metabolism or excretion of the compound. Whilst these compounds make interesting and selective compounds against trypanosomes and leishmania, there is insufficient in vivo activity to make them drug candidates. More work needs to be done to modify the structure to overcome these delivery and possibly pharmacokinetic problems.

Experimental

Biological assays

Enzyme assays. As DHFR catalyses the NADPH dependant reduction of dihydrofolate to tetrahydrofolate, the relative velocity of the reaction in the absence (n0) and presence (ni) of an inhibitor was assayed spectrophotometrically by measuring the difference in the rate of decrease in NADPH absorbance at 340 nm at 28 °C. The dissociation constant of a competitive inhibitor (K_i) of DHFR was calculated from eq 5 by using the values of ni and n0 and also by using the Dixon plot. The standard DHFR assay was performed with the enzyme, substrate, inhibitor and DHFR from *L. major*, *T. cruzi* and human.

Standard reactant concentrations were: For *L. major* DHFR enzyme 2.72 nM, dihydrofolate (DHF) 30 μM, NADPH 100 μM, bovine serum albumin (BSA) (1 mg/mL), variable inhibitor concentration (0.4–2.4 μM). For *T. cruzi* DHFR enzyme: 1.89 nM, DHF 30 μM, NADPH 100 μM, BSA (1 mg/mL), variable inhibitor concentration (0.4–2.4 μM). For human DHFR enzyme: 8.5 nM, DHF 30 μM, NADPH 100 μM, BSA (1 mg/mL), variable inhibitor concentration (5–60 μM).

In each case, a control was carried out which contained all reagents except the inhibitor. The DHFR assays were performed by adding the above-mentioned reagents to a quartz cuvette with 500 μL of 2×TES buffer pH 7.0 (100 mM TES, 150 mM β-mercaptoethanol, 2 mM EDTA). Reaction mixtures containing enzyme plus all standard assay components except the initiating substrate were first monitored to determine any background activity. Reactions were then initiated by adding the measured amount of substrate and were assayed by measuring the rate in decrease of NADPH absorbance at 340 nm. Absorbance was recorded in a

Hewlett-Packard model 8543 spectrophotometer. Dihydrofolate was prepared from folic acid by reduction with sodium dithionite according to the method of Futterman.[24] The following K_m values were used: *L. major* DHFR, 1.3 μM; T. *cruzi* DHFR, 1.2 μM; human DHFR, 0.64 μM. NADPH and BSA were purchased from SIGMA. The dihydrofolate was stored at −80 °C. At the start of each assay the concentration of dihydrofolate was checked by the spectrophotometer at 282 nm and calculated by using the Beer–Lambert law.

Analogous compounds showed competitive inhibition.[4]

In vitro assays. *L. donovani*. Mouse peritoneal macrophages were seeded in RPMI 1640 medium with 10% heat-inactivated FBS into Lab-tek 16-chamber slides. After 24 h *L. donovani* amastigote were added at a ratio of 3:1 (amastigotes to macrophages). The medium containing free amastigotes was replaced by fresh medium 4 h later. Next day, the medium was replaced by fresh medium containing different drug concentrations. The slides were incubated at 37 °C under a 5% CO_2 atmosphere for 96 h. Then the medium was removed, the slides fixed with methanol and stained with Giemsa. The ratio of infected to non-infected macrophages was determined microscopically, expressed as percentage of the control and the IC_{50} value calculated by linear regression.

***T. cruzi*.** Rat skeletal myoblasts (L-6 cells) were seeded in 96-well microtiter plates at 2000 cells/well/100 μL in RPMI 1640 medium with 10% FBS and 2 mM L-glutamine. After 24 h, 5000 trypomastigotes of *T. cruzi* [Tulahuen strain C2C4 containing the β-galactosidase (Lac Z) gene] were added in 100 μL per well with 2× of a serial drug dilution. The plates were incubated at 37 °C in 5% CO_2 for 4 days. Then the substrate CPRG/Nonidet was added to the wells. The colour reaction, which developed during the following 2–4 h, was read photometrically at 540 nm. From the sigmoidal inhibition curve IC_{50} values were calculated. Cytotoxicity was assessed in the same assay using non-infected L-6 cells and the same serial drug dilution.

***T.b. rhodesiense*.** Minimum Essential Medium (50 μL) supplemented according to Baltz et al.[25] with 2-mercaptoethanol and 15% heat-inactivated horse serum was added to each well of a 96-well microtiter plate. Serial drug dilutions were added to the wells. Then 50 μL of trypanosome suspension (*T.b. rhodesiense* STIB 900) was added to each well and the plate incubated at 37 °C under a 5% CO_2 atmosphere for 72 h. Alamar Blue (10 μL) was then added to each well and incubation continued for a further 2–4 h. The plates are read in a microplate fluorometer system (Spectramax Gemini by Molecular Devices) using an excitation wavelength of 536 nm and an emission wavelength of 588 nm.

Cytotoxicity. Rat skeletal myoblasts (L-6 cells) were seeded in 96-well microtiter plates in RPMI 1640 medium with 10% FBS and 2 mM L-glutamine at a density of 4×10^4 cells/mL. After 24 h, the medium was removed and replaced by fresh medium containing a serial drug dilution, and the plate incubated at 37 °C under a 5% CO_2 atmosphere for 72 h. Alamar Blue (10 μL) was then added to each well and incubation continued for a further 2–4 h. The plates are read in a microplate fluorometer system (Spectramax Gemini by Molecular Devices) using an excitation wavelength of 536 nm and an emission wavelength of 588 nm.

In vivo assays. BALB/c specific pathogen free mice were infected intraperitoneally on day 0 with 0.2 mL of inoculum (equivalent to 5×10^4 trypanosomes) from an infected donor mouse. Mice were monitored three times/day for a period of up to 60 days post-infection (if experimental compounds are active); Parasitemia is monitored by tail blood examination on days 7, 14, 21, and so on; post-infection, counting the number of parasites/microscope field at ×40. The mean survival time (MST) of each group of mice was compared. Benznidazole treated mice survived until the end of the experiment. Drugs were prepared in 0.25% carboxymethylcellulose vehicle. Mice were dosed intraperitoneally once/day on days 7–11 with 50 mg/kg. Negative control mice were dosed with 0.2 mL drug vehicle only. Positive control mice were dosed with 45 mg/kg benznidazole by an oral route.

Modelling. Modeling was performed on Silicon Graphics O2 workstations using Sybyl 6.7 and GRID19. For docking, water molecules were removed from the protein. For FlexX, the active site was defined as all residues within a radius of 6.5 Å from the folate ligand (in the case of the human enzyme) or 6.5 Å from the methotrexate ligand (in case of the *L. major* enzyme). The compounds for docking were drawn in Sybyl. Before docking, the compounds were subject to energy minimization using the Powell method using Sybyl with a gradient cut-off of 0.01 kcal/mol or a limit of 1000 iterations. For GRID v19, water molecules were removed from the proteins. The proteins were prepared using the default settings of the programme. The energy thresholds used for probes were as follows: COO^- −8 kcal/mol; hydrophobic −0.1 kcal/mol; OH −6 kcal/mol; $NH3^+$ −6 kcal/mol. The hydrophobic probe finds places at which hydrophobic atoms on the surface of one molecule will make favourable interactions with hydrophobic atoms of another molecule. The parameters for each probe are predefined in the programme and can be found in the literature.[24]

Chemistry. General experimental detail. Where applicable, all glassware was oven dried overnight and all reactions were carried out under nitrogen. All dry solvents, ethanol (EtOH), methanol (MeOH), dichloromethane (CH_2Cl_2), acetonitrile (CH_3CN) were purchased from Aldrich or Fluka in sure seal bottles. All reactions were monitored by Thin Layer Chromatography (TLC) using silica gel 60 F_{254} plates (Merck). Column chromatography was perfomed on Uetikon silica gel C-560 Act (0.035–0.070 mm). The infrared spectroscopy (IR) of the compound was recorded on a

Perkin-Elmer 1600 series FTIR spectrometer as films on sodium chloride discs (thin film) or as solids via a diffuse reflectance accessory using a potassium bromide matrix. The ^{1}H and ^{13}C NMR spectra were recorded on a Brücker Avance DPX 300 MHz spectrometer at 300 and 75 MHz, respectively. Assignment of ^{13}C NMR shifts was done with the aid of ACD labs software.[26] Elemental analyses were determined on a Perkin-Elmer 240C elemental analyser. Low resolution mass spectra (MS) were recorded on a Fison VG Platform II spectrometer using electrospray ionisation (ES) technique either in positive or negative ion modes. Mobile phases were acetonitrile/water 1/1 or methanol (ES). High resolution mass spectra (HRMS) in chemical ionisation (CI) and fast atom bombardment (FAB) were determined by the EPSRC Mass Spectroscopy Centre, Swansea, UK.

General procedure for monoalkylation of hydroxybenzaldehyde (7c, 7d, 7f, 7h, 16, 19a, 19b)

A mixture of 4-hydroxybenzaldehyde (1 equiv), 1-iodoalkane (2 equiv) (1 equiv in the case of compound **16**) and anhydrous potassium carbonate (3 equiv) in 2-butanone (1 M) was heated under reflux for 24 h under nitrogen. Most of the solvent was removed on a rotary evaporator. Water (100 mL) and ether (100 mL) were then added. The organic layer was separated, washed with water (3×150 mL) and dried over $MgSO_4$. The solvent was removed under reduced pressure and the crude compound was purified by column chromatography on silica gel using a gradient of elution of 10–20% EtOAc/hexane.

General procedure for condensation with ethoxypropionitrile using azeotropic distillation (8b, 15a, 15b, 15d)

Ethoxypropionitrile (2 equiv) was added to a solution of alkyloxybenzaldehyde (1 equiv) in dry ethanol (0.75 M) under nitrogen. Sodium ethoxide (2 equiv) was added to the mixture, and the reaction was heated for 12 h to remove water by azeotropic distillation with ethanol. Then, the mixture was refluxed for 12 h. Most of the solvent was removed on a rotary evaporator. Water (100 mL) and ether (100 mL) were added. The organic layer was separated, washed with water (3×150 mL) and dried over $MgSO_4$. The solvent was removed under reduced pressure and the crude compound was purified by column chromatography on silica gel using an elution of 10% EtOAc/hexane.

General procedure for condensation with ethoxypropionitrile using Dean Stark trap (15c, 8c, 8d, 8f, 8h, 10, 20a, 20b). Ethoxypropionitrile (2 equiv) was added to a solution of 3,4-dioctyloxybenzaldehyde **14c** (1 equiv) in dry ethanol (0.2 M) under nitrogen. Sodium ethoxide (2 equiv) was added to the mixture. The reaction was heated with removal of the methanol via a Dean–Stark trap for 24 h. Most of the solvent was removed on a rotary evaporator. Water (100 mL) and ether (100 mL) were added. The organic layer was separated, washed with water (3×150 mL) and dried over $MgSO_4$. The solvent was removed under reduced pressure and the crude compound was purified by column chromatography on silica gel using a gradient of elution of 2–10% EtOAc/hexane.

General procedure for cyclisation with guanidine (3a–3d, 2b, 2c, 2d, 2f, 2h, 2j, 4a, 4b)

A solution of guanidine hydrochloride (6 equiv) in warm anhydrous ethanol (1 M) and a solution of sodium ethoxide (6 equiv) in warm anhydrous ethanol (1 M) were prepared separately and allowed to cool to room temperature. The guanidine solution was added to the stirred solution of sodium ethoxide under nitrogen. The mixture was stirred for 5 min, filtered and added to cinnamonitrile derivative (1 equiv). The reaction mixture was heated to reflux for 24 h. Then, the mixture was concentrated in vacuo. The crude compound was purified by column chromatography on silica gel using a gradient of elution of 5–40% MeOH/EtOAc.

General procedure for alkylation of 5-[4-hydroxylbenzyl]-2,4-diaminopyrimidine (2e, 2g, 2h, 2i)

A mixture of 5-[4-hydroxybenzyl]-2,4-diaminopyrimidine **2a** (1 equiv), 1-iodoalkane (2 equiv), anhydrous potassium carbonate (2 equiv) ethanol (0.1 M) was heated under reflux and stirred for 18 h under nitrogen. Most of the solvent was removed on a rotary evaporator to get a white powder. The latter was washed with water (100 mL) and dried.

General procedure for dialkylation of 3,4-dihydroxybenzaldehyde (14a, 14b)

A mixture of 3,4-dihydroxybenzaldehyde (1 equiv), 1-bromoalkane (3 equiv), anhydrous potassium carbonate (5 eqiv) and potassium iodide (0.1 equiv) in 2-butanone (1 M) was heated under reflux and stirred for 18 h under nitrogen. Most of the solvent was removed on a rotary evaporator. Water (100 mL) and ether (100 mL) were then added. The organic layer was separated, washed with water (3×150 mL) and dried over $MgSO_4$. The solvent was removed under reduced pressure and the crude compound was purified by column chromatography on silica gel using a gradient of elution of 5–10% EtOAc/hexane.

4-Pentyloxybenzaldehyde (7c). Following the general procedure of monoalkylation of hydroxylbenzaldehyde and starting from 4-hydroxybenzaldehyde (1.952 g, 16.00 mmol), 1-iodopentane (6.336 g, 32.00 mmol) and anhydrous potassium carbonate (6.624 g, 48.00 mmol) in 2-butanone (100 mL), compound **7c** was isolated as a yellow oil (2.498 g, 12.99 mmol, 81%). TLC R_f 0.50 (20% EtOAc/hexane). IR (neat): $\nu = 2942$, 2870, 1691, 1257 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 9.94 (s, 1H, C<u>H</u>O), 7.89 (dt, 2H, $J = 8.8$, 2.3 Hz, H2 + H6), 7.05 (dt, 2H, $J = 8.8$, 2.3 Hz, H3 + H5), 4.10 (t, 2H, $J = 6.6$ Hz, H1′), 1.87 (quint, 2H, $J = 6.6$ Hz, H2′), 1.38–1.60 (m, 4H, H3′ + H4′), 1.00 (t, 3H, $J = 6.6$ Hz, H3′). ^{13}C NMR ($CDCl_3$) δ 191.3 (<u>C</u>HO), 164.7 (4), 132.4 (2 + 6), 130.2 (1), 115.1 (3 + 5), 68.8 (1′), 29.2 (3′), 28.5 (2′), 22.8 (4′), 14.5 (5′). Found: C, 74.92; H, 8.46. calcd for $C_{12}H_{16}O_2$: C, 74.97; H, 8.39%.

4-Hexyloxybenzaldehyde (7d). Following the general procedure of monoalkylation of hydroxylbenzaldehyde and starting from 4-hydroxybenzaldehyde (1.952 g, 16.00 mmol), 1-iodohexane (6.784 g, 32.00 mmol) and anhydrous potassium carbonate (6.624 g, 48.00 mmol) in 2-butanone (100 mL), compound **7d** was isolated as a pale yellow oil (2.674 g, 12.96 mmol, 81%). TLC R_f 0.50 (20% EtOAc/hexane). IR (neat): ν = 2929, 2857, 1690, 1251 cm^{-1}. ^1H NMR ($CDCl_3$) δ 9.94 (s, 1H, C<u>H</u>O), 7.89 (dt, 2H, J = 8.8, 2.3 Hz, H2 + H6), 7.05 (dt, 2H, J = 8.8, 2.3 Hz, H3 + H5), 4.10 (t, 2H, J = 6.6 Hz, H1′), 1.87 (quint, 2H, J = 6.6 Hz, H2′), 1.35–1.60 (m, 6H, H3′-H5′), 1.00 (t, 3H, J = 6.6 Hz, H3′). ^{13}C NMR ($CDCl_3$) δ 191.3 (<u>C</u>HO), 164.7 (4), 132.4 (2 + 6), 130.2 (1), 115.1 (3 + 5), 68.8 (1′), 31.9 (4′), 29.4 (2′), 26.0 (3′), 23.1 (5′), 14.5 (6′). Found: C, 75.36; H, 8.88. calcd for $C_{13}H_{18}O_2$: C, 75.69; H, 8.79%.

4-Octyloxybenzaldehyde (7f). Following the general procedure of monoalkylation of hydroxylbenzaldehyde and starting from 4-hydroxybenzaldehyde (1.986 g, 16.28 mmol), 1-iodooctane (7.814 g, 32.56 mmol) and anhydrous potassium carbonate (6.740 g, 48.84 mmol) in 2-butanone (100 mL), compound **7f** was isolated as a pale yellow oil (3.563 g, 15.23 mmol, 94%). TLC R_f 0.50 (20% EtOAc/hexane). IR (neat): ν = 2924, 2855, 1690, 1255 cm^{-1}. ^1H NMR ($CDCl_3$) δ 9.94 (s, 1H, C<u>H</u>O), 7.89 (dt, 2H, J = 8.8, 2.3 Hz, H2 + H6), 7.05 (dt, 2H, J = 8.8, 2.3 Hz, H3 + H5), 4.09 (t, 2H, J = 6.6 Hz, H1′), 1.85 (quint, 2H, J = 6.6 Hz, H2′), 1.30–1.60 (m, 10H, H3′-H7′), 1.00 (t, 3H, J = 6.6 Hz, H8′). ^{13}C NMR ($CDCl_3$) δ 191.3 (<u>C</u>HO), 164.7 (4), 132.4 (2 + 6), 130.1 (1), 115.2 (3 + 5), 68.9 (1′), 32.2 (6′), 29.7 (4′), 29.6 (5′), 29.5 (2′), 26.4 (3′), 23.1 (7′), 14.5 (8′). Found: C, 76.88, H, 9.60. calcd for $C_{15}H_{22}O_2$: C, 76.88; H, 9.46%.

4-Decyloxybenzaldehyde (7h). Following the general procedure of monoalkylation of hydroxylbenzaldehyde and starting from 4-hydroxybenzaldehyde (1.937 g, 15.88 mmol), 1-iododecane (8.511 g, 31.76 mmol) and anhydrous potassium carbonate (6.574 g, 47.64 mmol) in 2-butanone (100 mL), compound **7h** was isolated as a pale yellow oil (3.559 g, 13.58 mmol, 86%). TLC R_f 0.50 (20% EtOAc/hexane). IR (neat): ν = 2925, 2852, 1686, 1256 cm^{-1}. ^1H NMR ($CDCl_3$) δ 9.94 (s, 1H, C<u>H</u>O), 7.88 (dt, 2H, J = 8.8, 2.3 Hz, H2 + H6), 7.03 (dt, 2H, J = 8.8, 2.3 Hz, H3 + H5), 4.09 (t, 2H, J = 6.6 Hz, H1′), 1.86 (quint, 2H, J = 6.6 Hz, H2′), 1.30–1.60 (m, 14H, H3′-H9′), 0.95 (t, 3H, J = 6.6 Hz, H10′). ^{13}C NMR ($CDCl_3$) δ 191.3 (<u>C</u>HO), 164.7 (4), 132.4 (2 + 6), 130.1 (1), 115.2 (3 + 5), 68.9 (1′), 32.3 (8′), 30.0 (6′), 29.8 (2′), 29.7 (5′), 29.6 (7′), 29.5 (4′), 26.4 (3′), 23.1 (9′), 14.6 (10′). Found: C, 77.97; H, 10.17. calcd for $C_{17}H_{26}O_2$: C, 77.82; H, 9.99%.

(*E*)3-(4′-Methoxyphenyl)-2-(ethoxymethyl)-2-propenenitrile (8b). Following the general procedure of condensation with ethoxypropionitrile using azeotropic distillation with ethoxypropionitrile (1.089 g, 11.00 mmol), 4-methoxybenzaldehyde (1.496 g, 11.00 mmol) and sodium ethoxide (928 mg, 13.64 mmol) in dry ethanol (25 mL), compound **8b** was isolated as a yellow oil (1.047 g, 4.82 mmol, 44%).

(*E*)3-(4-Pentyloxyphenyl)-2-(ethoxymethyl)-2-propenenitrile (8c). Following the general procedure of condensation with ethoxypropionitrile using Dean–Stark trap and starting from ethoxypropionitrile (1.426 g, 14.40 mmol), 4-pentyloxybenzaldehyde **7c** (1.383 g, 7.20 mmol) and sodium ethoxide (0.979 g, 14.40 mmol) in dry ethanol (150 mL), compound **8c** was isolated as a yellow oil (1.257 g, 4.60 mmol, 63%). TLC R_f 0.60 (40% EtOAc/hexane). IR (neat): ν = 2933, 2871, 2210, 1258, 1180 cm^{-1}. ^1H NMR ($CDCl_3$) δ 7.81 (dt, 2H, J = 8.8, 1.9 Hz, H2′ + H6′), 7.13 (br s, 1H, H3), 6.98 (dt, 2H, J = 8.8, 1.9 Hz, H3′ + H5′), 4.26 (s, 2H, C<u>H</u>$_2$OEt), 4.06 (t, 2H, J = 6.7 Hz, H1″), 3.64 (q, 2H, J = 7.0 Hz, $CH_3C\underline{H}_2O$), 1.85 (m, 2H, H2″), 1.40–1.65 (m, 4H, H3″-4″), 1.33 (t, 3H, J = 7.0 Hz, C<u>H</u>$_3$CH$_2$O), 1.00 (t, 6H, J = 6.6 Hz, H5″). ^{13}C NMR ($CDCl_3$) δ 160.5 (4′), 145.0 (3), 131.3 (2′ + 6′), 126.0 (1′), 118.9 (1), 115.2 (3′ + 5′), 105.4 (2), 72.5 (1″), 72.5 ($CH_3\underline{C}H_2O$), 66.6 (EtO<u>C</u>H$_2$), 29.3 (2″), 28.6 (3″), 22.9 (4″), 15.5 (<u>C</u>H$_3$CH$_2$O), 14.5 (5″). LRMS (ES+ mode): m/z = 296.3 [$(M + Na)^+$, 20%], 569.0 [$(2M + Na)^+$, 80%], 551.1 [$(2M + 3Na)^+$, 100%]. HRMS (ES+ mode) : calculated mass 291.2073 $(M + NH_4)^+$, measured mass 291.2073 $(M + NH_4)^+$.

(*E*)3-(4-Hexyloxyphenyl)-2-(ethoxymethyl)-2-propenenitrile (8d). Following the general procedure of condensation with ethoxypropionitrile using Dean–Stark trap and starting from ethoxypropionitrile (1.494 g, 15.10 mmol), 4-hexyloxybenzaldehyde **7d** (1.556 g, 7.55 mmol) and sodium ethoxide (1.026 g, 15.10 mmol) in dry ethanol (150 mL), compound **8d** was isolated as a yellow oil (1.861 g, 15.10 mmol, 85%). TLC R_f 0.60 (40% EtOAc/hexane). IR (neat): ν = 2930, 2855, 2210, 1256, 1179 cm^{-1}. ^1H NMR ($CDCl_3$) δ 7.81 (dt, 2H, J = 8.8, 1.9 Hz, H2′ + H6′), 7.13 (br s, 1H, H3), 6.98 (dt, 2H, J = 8.8, 1.9 Hz, H3′ + H5′), 4.26 (s, 2H, C<u>H</u>$_2$OEt), 4.05 (t, 2H, J = 6.7 Hz, H1″), 3.64 (q, 2H, J = 7.0 Hz, $CH_3C\underline{H}_2O$), 1.85 (quint, 2H, J = 6.6 Hz, H2″), 1.35–1.65 (m, 6H, H3″-5″), 1.33 (t, 3H, J = 7.0 Hz, C<u>H</u>$_3$CH$_2$O), 0.95 (t, 3H, J = 6.6 Hz, H5″). ^{13}C NMR ($CDCl_3$) δ 161.5 (4′), 145.0 (3), 131.3 (2′ + 6′), 126.0 (1′), 118.9 (1), 115.2 (3′ + 5′), 105.4 (2), 72.5 (1″), 68.6 ($CH_3\underline{C}H_2O$), 66.6 (EtO<u>C</u>H$_2$), 40.0 (4″), 29.7 (2″), 26.1 (3″), 23.0 (5″), 15.5 (<u>C</u>H$_3$CH$_2$O), 14.6 (6″). LRMS (ES+ mode): m/z = 309.8 [$(M + Na)^+$, 30%], 596.8 [$(2M + Na)^+$, 100%], 642.5 [$(2M + 3Na)^+$, 90%]. HRMS (ES+ mode): calculated mass 305.2229 $(M + NH_4)^+$, measured mass 305.2231$(M + NH_4)^+$.

(*E*)3-(4-Octyloxyphenyl)-2-(ethoxymethyl)-2-propenenitrile (8f). Following the general procedure of condensation with ethoxypropionitrile using Dean–Stark trap and starting from ethoxypropionitrile (1.736 g, 17.54 mmol), 4-octyloxybenzaldehyde **7f** (2.052 g, 8.77 mmol) and sodium ethoxide (1.192 g, 17.54 mmol) in dry ethanol (150 mL), compound **8f** was isolated as a yellow oil (1.869 g, 5.93 mmol, 68%). TLC R_f 0.60 (40% EtOAc/hexane). IR (neat): ν = 2928, 2856, 2210, 1259, 1180 cm^{-1}. ^1H NMR ($CDCl_3$) δ 7.81 (dt, 2H, J = 8.8, 1.9 Hz, H2′ + H6′), 7.13 (br s, 1H, H3), 6.98 (dt, 2H, J = 8.8, 1.9 Hz, H3′ + H5′), 4.26 (s, 2H, C<u>H</u>$_2$OEt), 4.05 (t, 2H, J = 6.7 Hz, H1″), 3.64 (q, 2H, J = 7.0 Hz, $CH_3C\underline{H}_2O$), 1.85 (quint, 2H, J = 6.6 Hz, H2″), 1.35–1.65 (m, 10H,

H3″-7″), 1.33 (t, 3H, $J=7.0$ Hz, C$\underline{H}_3$CH$_2$O), 0.93 (t, 3H, $J=6.6$ Hz, H8″). ^{13}C NMR ($CDCl_3$) δ 161.5 (4′), 145.0 (3), 131.3 (2′+6′), 126.0 (1′), 118.9 (1), 115.2 (3′+5′), 105.4 (2), 72.5 (1″), 68.6 (CH$_3$C$\underline{H}_2$O), 66.6 (EtO$\underline{C}$H$_2$), 32.2 (6″), 29.8 (2″), 29.7 (4″), 29.6 (5″), 26.4 (3″), 23.1 (7″), 15.5 ($\underline{C}$H$_3$CH$_2$O), 14.6 (8″). LRMS (ES+ mode): $m/z=337.5$ [(M+Na)$^+$, 20%], 653.3 [(2 M+Na)$^+$, 80%], 699.4 [(2 M+3Na)$^+$, 50%]. HRMS (ES+ mode): calculated mass 333.2542 (M+NH$_4$)$^+$, measured mass 333.2540 (M+NH$_4$)$^+$.

(*E*)3-(4-Decyloxyphenyl)-2-(ethoxymethyl)-2-propenenitrile (8h). Following the general procedure of condensation with ethoxypropionitrile using Dean–Stark trap and starting from ethoxypropionitrile (0.971 mg, 9.80 mmol), 4-decyloxybenzaldehyde **7h** (1.285 g, 4.90 mmol) and sodium ethoxide (666 mg, 9.80 mmol) in dry ethanol (100 mL), compound **8h** was isolated as a yellow oil (856 mg, 2.50 mmol, 51%). TLC R_f 0.59 (40% EtOAc/hexane). IR (neat): ν=2922, 2854, 2211, 1259, 1180 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 7.81 (dt, 2H, $J=8.8$, 1.9 Hz, H2′+H6′), 7.13 (br s, 1H, H3), 6.98 (dt, 2H, $J=8.8$, 1.9 Hz, H3′+H5′), 4.26 (s, 2H, C$\underline{H}_2$OEt), 4.05 (t, 2H, $J=6.7$ Hz, H1″), 3.65 (q, 2H, $J=7.0$ Hz, CH$_3$C$\underline{H}_2$O), 1.85 (quint, 2H, $J=6.6$ Hz, H2″), 1.35-1.60 (m, 14H, H3″-9″), 1.33 (t, 3H, $J=7.0$ Hz, C$\underline{H}_3$CH$_2$O), 0.93 (t, 3H, $J=6.6$ Hz, H10″). ^{13}C NMR ($CDCl_3$) δ 161.5 (4′), 145.0 (3), 131.3 (2′+6′), 126.0 (1′), 118.9 (1), 115.2 (3′+5′), 105.4 (2), 72.5 (1″), 68.6 (CH$_3$C$\underline{H}_2$O), 66.6 (EtO$\underline{C}$H$_2$), 32.3 (8″), 30.0 (6″), 29.8 (2″), 29.7 (5″), 29.6 (7″), 29.5 (4″), 26.4 (3″), 23.1 (9″), 15.5 ($\underline{C}$H$_3$CH$_2$O), 14.6 (10″). LRMS (ES+ mode): $m/z=365.9$ [(M+Na)$^+$, 30%], 709.3 [(2 M+Na)$^+$, 100%]. HRMS (ES+ mode): calculated mass 361.2855 (M+NH$_4$)$^+$, measured mass 361.2850 (M+NH$_4$)$^+$.

5-(4′-Methyloxybenzyl)-2,4-diaminopyrimidine (2c). Following general procedure **D** with a solution of guanidine hydrochloride (2.750 g, 28.95 mmol) in dry ethanol (25 mL), a solution of sodium ethoxide (1.978 g, 28.95 mmol) in dry ethanol (25 mL) and (*E*)-3-(4′-methyloxyphenyl-2-(ethoxymethyl)-2-propememitrile **8b** (1.047 g, 4.825 mmol), compound **2c** was isolated as a yellow solid (566 mg, 2.46 mmol, 51%). ^{1}H NMR ($CDCl_3$) δ 7.42 (s, 1H, H6), 7.10 (m, 2H, H2′+H6′), 6.85 (m, 2H, H3′+H5′), 3.75 (s, 3H, C$\underline{H}_3$OAr), 3.60 (s, 2H,C$\underline{H}_2$Ar).

5-[4-Pentyloxybenzyl]-2,4-diaminopyrimidine (2c). Following the general procedure of cyclisation with guanidine and starting from guanidine hydrochloride (2.331 g, 24.54 mmol) in dry ethanol (50 mL), (*E*)-3-(4-pentyloxyphenyl)-2-(ethoxymethyl)-2-propenenitrile **8c** (1.117 g, 4.09 mmol) and sodium ethoxide (1.669 g, 24.54 mmol) in dry ethanol (50 mL), compound **2c** was isolated as a yellow solid (760 mg, 2.66 mmol, 65%). TLC R_f 0.43 (40% MeOH/EtOAc). IR (KBr): ν=3324, 3150, 2934, 1567, 1250 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 7.71 (s, 1H, H6), 7.03 (dt, 2H, $J=8.7$, 1.9 Hz, H2′+H6′), 6.78 (dt, 2H, $J=8.8$, 1.9 Hz, H3′+H5′), 4.75 (s, 2H, NH$_2$), 4.55 (s, 2H, NH$_2$), 3.87 (t, 2H, $J=6.5$ Hz, H1″), 3.60 (s, 2H, C$\underline{H}_2$Ph), 1.73 (quint, 2H, H2″), 1.25–1.45 (m, 4H, H3″+4″), 0.88 (t, 3H, $J=6.6$ Hz, H5″). ^{13}C NMR ($CDCl_3$) δ 163.0 (4), 162.4 (2), 158.5 (4′), 156.8 (6), 130.1 (1′), 129.5 (2′+6′), 115.3 (3′+5′), 107.3 (5), 68.5 (1″), 33.9 ($\underline{C}$H$_2$Ph), 29.4 (2″), 28.6 (3″), 22.9 (4″), 14.5 (5″). LRMS (ES+ mode): $m/z=287.2$ [(M+H)$^+$, 100%]. Found: C, 67.01; H, 7.48; N, 19.70. calcd for $C_{16}H_{22}N_4O$: C, 67.11; H, 7.74; N, 19.56%.

5-[4-Hexyloxybenzyl]-2,4-diaminopyrimidine (2d). Following the general procedure of cyclisation with guanidine and starting from guanidine hydrochloride (2.947 g, 31.02 mmol) in dry ethanol (50 mL), (*E*)-3-(4-hexyloxyphenyl)-2-(ethoxymethyl)-2-propenenitrile **8d** (1.483 g, 5.17 mmol) and sodium ethoxide (2.109 g, 31.02 mmol) in dry ethanol (50 mL), compound **2d** was isolated as a yellow solid (791 mg, 2.64 mmol, 51%). TLC R_f 0.43 (40% MeOH/EtOAc). IR (KBr): ν=3325, 3144, 2920, 1565, 1242 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 7.61 (s, 1H, H6), 7.03 (dt, 2H, $J=8.7$, 1.9 Hz, H2′+H6′), 6.78 (dt, 2H, $J=8.8$, 1.9 Hz, H3′+H5′), 5.51 (s, 2H, NH$_2$), 4.75 (s, 2H, NH$_2$), 3.87 (t, 2H, $J=6.5$ Hz, H1″), 3.60 (s, 2H, C$\underline{H}_2$Ph), 1.70 (quint, 2H, H2″), 1.25–1.45 (m, 6H, H3″-5″), 0.85 (t, 3H, $J=6.6$ Hz, H6″). ^{13}C NMR ($CDCl_3$) δ 163.1 (4), 162.1 (2), 158.5 (4′), 156.1 (6), 129.9 (1′), 129.5 (2′+6′), 115.3 (3′+5′), 107.6 (5), 68.5 (1″), 33.9 ($\underline{C}$H$_2$Ph), 32.0 (4″), 29.7 (2″), 26.2 (3″), 23.1 (5″), 14.5 (6″). LRMS (ES+ mode): $m/z=301.2$ [(M+H)$^+$, 100%]. Found: C, 67.57; H, 8.25; N, 18.91. calcd for $C_{17}H_{24}N_4O$: C, 67.97; H, 8.05; N, 18.65%.

5-[4-Octyloxybenzyl]-2,4-diaminopyrimidine (2f). Following the general procedure of cyclisation with guanidine and starting from guanidine hydrochloride (3.688 g, 38.82 mmol) in dry ethanol (50 mL), (*E*)-3-(4-octyloxyphenyl)-2-(ethoxymethyl)-2-propenenitrile **8f** (2.038 g, 6.47 mmol) and sodium ethoxide (2.640 g, 38.82 mmol) in dry ethanol (50 mL), compound **2f** was isolated as a yellow solid (955 mg, 291 mmol, 45%). TLC R_f 0.43 (40% MeOH/EtOAc). IR (KBr): ν=3333, 3162, 2926, 1567, 1255 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 7.72 (s, 1H, H6), 7.04 (dt, 2H, $J=8.7$, 1.9 Hz, H2′+H6′), 6.80 (dt, 2H, $J=8.8$, 1.9 Hz, H3′+H5′), 4.75 (s, 2H, NH$_2$), 4.55 (s, 2H, NH$_2$), 3.88 (t, 2H, $J=6.5$ Hz, H1″), 3.61 (s, 2H, C$\underline{H}_2$Ph), 1.71 (quint, 2H, H2″), 1.25–1.45 (m, 10H, H3″-7″), 0.86 (t, 3H, $J=6.6$ Hz, H8″). ^{13}C NMR ($CDCl_3$) δ 163.2 (4), 162.1 (2), 158.5 (4′), 156.1 (6), 129.8 (1′), 129.6 (2′+6′), 115.3 (3′+5′), 107.6 (5), 68.5 (1″), 33.8 ($\underline{C}$H$_2$Ph), 32.2 (6″), 29.8 (4″), 29.7 (5″), 29.7 (2″), 26.5 (3″), 23.1 (7″), 14.5 (8″). LRMS (ES+ mode): $m/z=329.2$ [(M+H)$^+$, 100%]. Found: C, 69.12; H, 8.77; N, 17.38. calcd for $C_{19}H_{28}N_4O$: C, 69.48; H, 8.59; N, 17.06%.

5-[4-Decyloxybenzyl]-2,4-diaminopyrimidine (2h). Following the general procedure of cyclisation with guanidine and starting from guanidine hydrochloride (1.288 g, 13.56 mmol) in dry ethanol (25 mL), (*E*)-3-(4-decyloxyphenyl)-2-(ethoxymethyl)-2-propenenitrile **8h** (774 mg, 2.26 mmol) and sodium ethoxide (922 mg, 13.56 mmol) in dry ethanol (25 mL), compound **2h** was isolated as a yellow solid (491 mg, 1.38 mmol, 61%). TLC R_f 0.43 (40% MeOH/EtOAc). IR (KBr): ν=3323, 3157, 2918, 1560, 1240 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 7.72 (s, 1H, H6), 7.04 (dt, 2H, $J=8.7$, 1.9 Hz, H2′+H6′), 6.80 (dt, 2H, $J=8.8$, 1.9 Hz, H3′+H5′), 4.75 (s, 2H, NH$_2$), 4.55

(s, 2H, NH_2), 3.88 (t, 2H, $J=6.5$ Hz, H1″), 3.61 (s, 2H, C$\underline{H}_2$Ph), 1.71 (quint, 2H, H2″), 1.25–1.50 (m, 14H, H3″-9″), 0.86 (t, 3H, $J=6.6$ Hz, H10″). ^{13}C NMR ($CDCl_3$) δ 163.0 (4), 162.5 (2), 158.5 (4′), 157.1 (6), 130.1 (1′), 129.5 (2′ + 6′), 115.3 (3′ + 5′), 107.6 (5), 68.5 (1″), 33.9 (C$\underline{H}_2$Ph), 32.3 (8″), 30.0 (6″), 29.9 (7″), 29.8 (5″), 29.7 (2″ + 4″), 26.5 (3″), 23.1 (9″), 14.5 (10″). LRMS (ES+ mode): $m/z=357.2$ [$(M+H)^+$, 100%]. Found: C, 70.73; H, 9.24; N, 16.01. calcd for $C_{21}H_{32}N_4O$: C, 70.75; H, 9.05; N, 15.72%.

4-(Tetrahydro-2-pyranoxy)benzaldehyde (9). A solution of 3,4-dihydro-2*H*-pyran (1.870 g, 22.00 mmol) in dichloromethane (12 mL) was added dropwise into a well stirred suspension of 4-hydroxybenzaldehyde (1.000 g, 7.20 mmol) and pyridinium *p*-toluene sulfonate (0.04 g, 0.16 mmol) in dichloromethane (34 mL). The mixture was stirred at room temperature for 1.5 h, then extracted with brine. The organic layer was separated, washed with brine (3×15 mL) and dried over $MgSO_4$. The solvent was removed under reduced pressure and the crude compound was purified by column chromatography on silica gel using an elution of 20% EtOAc/hexane to give **9** as a yellow oil (1.484 g, 7.20 mmol, 100%). TLC R_f 0.27 (20% EtOAc/hexane). IR (neat): ν = 2945 (Ar), 2861 (C=O), 1691 (C=O), 1247 (ArOR), 955 (OCH_2O) cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 9.95 (s, 1H, C$\underline{H}$O), 7.88 (dt, 2H, $J=8.8$, 1.9 Hz, H2 + H6), 7.23 (dt, 2H, $J=8.8$, 1.9 Hz, H3 + H5), 5.60 (br t, 1H, $J=2.9$ Hz, H2′), 3.90 (td, 1H, $J=11.4$, 3.0 Hz, H6′), 3.69 (dtd, 1H, $J=11.4$, 3.9, 1.5 Hz, H6′), 1.65–1.98 (m, 6H, H3′-5′). ^{13}C NMR ($CDCl_3$) δ 191.5 (C$\underline{H}$O), 162.6 (4), 132.7 (6), 132.3 (2), 130.9 (1), 116.9 (3), 116.4 (5), 96.5 (2′), 62.5 (6′), 30.5 (3′), 25.4 (5′), 18.8 (4′). LRMS (ES+ mode): $m/z=209.1$ [$(M+H)^+$, 30%], 435.1 [$(2M+Na)^+$, 100%].

(*E*)-2-(Ethoxymethyl)-2-[4-(tetrahydro-2-pyranyloxy)phenyl]-2-propenenitrile (10). Following the general procedure of condensation with ethoxypropionitrile using Dean–Stark trap and starting from ethoxypropionitrile (4.700 g, 47.48 mmol), 4-(tetrahydro-2-pyranoxy)-benzaldehyde **9** (4.891 g, 23.74 mmol) and sodium ethoxide (3.229 g, 47.48 mmol) in dry ethanol (300 mL), compound **10** was isolated as a yellow oil (4.560 g, 15.87 mmol, 71%). TLC R_f 0.58 (40% EtOAc/hexane). IR (neat): ν = 2937, 2868, 2210, 1271, 1143, 940 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 7.80 (dt, 2H, $J=8.8$, 1.9 Hz, H2′ + H6′), 7.13 (dt, 2H, $J=8.8$, 1.9 Hz, H3′ + H5′), 7.12 (br s, 1H, H3), 5.54 (t, 1H, $J=3.0$ Hz, H2″), 4.25 (s, 2H, EtOC$\underline{H}_2$), 3.93 (td, 1H, $J=11.4$, 3.0 Hz, H6″), 3.61 (q, 2H, $J=7.0$ Hz, $CH_3C\underline{H}_2O$), 2.95–3.20 (m, 1H, H6″), 1.60–1.98 (m, 6H, H3″-H5‴), 1.30 (t, 3H, $J=7.0$ Hz, C$\underline{H}_3CH_2O$). ^{13}C NMR ($CDCl_3$) δ 159.3 (4′), 144.9 (3), 131.2 (2′ + 6′), 126.9 (1′), 118.8 (1), 117.0 (3′ + 5′), 105.9 (2), 96.5 (2″), 67.2 ($CH_3\underline{C}H_2O$), 65.5 (6″), 62.5 (Et$O\underline{C}H_2$), 30.8 (3″), 25.5 (5″), 19.0 (4″), 15.5 ($\underline{C}H_3CH_2O$). LRMS (ES+ mode): $m/z=310.1$ [$(M+Na)^+$, 70%], 597.0 [$(2M+Na)^+$, 100%]. HRMS (ES+ mode): calculated mass 288.1599 $(M+H)^+$, measured mass 288.1605 $(M+H)^+$.

5-[4-(Tetrahydro-2-pyranoxy)benzyl]-2,4-diaminopyrimidine. (2j). Following the general procedure of cyclisation with guanidine and starting from guanidine hydrochloride (4.064 g, 42.78 mmol) in dry ethanol (50 mL), (*E*)-2-(ethoxymethyl)-2-[4-(tetrahydro-2-pyranyloxy)phenyl]-2-propenenitrile **10** (2.045 g, 7.13 mmol) and sodium ethoxide (2.909 g, 42.78 mmol) in dry ethanol (50 mL), compound **2j** was isolated as a yellow solid (688 mg, 2.29 mmol, 32%). TLC R_f 0.29 (10% MeOH/EtOAc). IR (neat): ν = 3418, 3140, 2948, 1509, 1235, 935 cm^{-1}. ^{1}H NMR (MeOD) δ 7.47 (s, 1H, H6), 7.11 (dt, 2H, $J=8.8$, 1.9 Hz, H2′ + H6′), 6.97 (dt, 2H, $J=8.8$, 1.9 Hz, H3′ + H5′), 5.37 (t, 1H, $J=2.9$ Hz, H2″), 3.88 (td, 1H, $J=11.4$, 3.0 Hz, H6″), 3.61 (s, 2H, C$\underline{H}_2$Ph), 3.52–3.62 (m, 1H, H6″), 1.55–1.99 (m, 6H, H3‴-H4‴). ^{13}C NMR (MeOD) δ 163.5 (4), 162.9 (2), 157.5 (6), 156.1 (4′), 133.5 (1′), 126.4 (2′ + 6′), 118.2 (3′ + 5′), 108.9 (5), 98.3 (2″), 63.6 (6″), 33.8 (C$\underline{H}_2$Ph), 31.9 (3″), 26.8 (5″), 18.8 (4″). LRMS (CI+ mode, NH_3): $m/z=310.2$ [$(M+H)^+$, 100%]. HRMS (ES+ mode): calculated mass 301.1664 $(M+H^+)$, measured mass 301.1661 $(M+H^+)$. Found: C, 63.69; H, 6.82; N, 18.67. calcd for $C_{16}H_{20}N_4O_2$: C, 63.98; H, 6.71; N, 18.65%.

5-[4-Hydroxybenzyl]-2,4-diaminopyrimidine (2a). A solution of 36% hydrochloric acid (2 mL) was added dropwise into a solution of 5-[4-(tetrahydro-2-pyranoxy)-benzyl]-2,4-pyrimidinediamine **2j** (0.349 g, 1.16 mmol) in methanol (50 mL). The mixture was stirred at room temperature for 2 h, then a white solid started to appear. The solid was filtered and washed with methanol to give **2a** as a white solid (250 mg, 1.16 mmol, 100%). TLC R_f 0.29 (40% MeOH/EtOAc). IR (KBr): ν = 3354, 3128, 2985,1503, 1210 cm^{-1}. ^{1}H NMR (MeOD) δ 7.15 (br s, 1H, H6), 7.03 (dt, 2H, $J=8.5$, 2.8 Hz, H2′ + H6′), 6.78 (dt, 2H, $J=8.5$, 2.8 Hz, H3′ + H5′), 3.62 (s, 2H, C$\underline{H}_2$Ph). ^{13}C NMR (MeOD) δ 166.8 (4), 158.0 (4′), 156.5 (2), 140.5 (1′), 131.5 (2′ + 6′), 128.6 (6), 117.2 (3′ + 5′), 112.1 (5), 33.3 (C$\underline{H}_2$Ph). LRMS (ES+ mode): $m/z=216.8$ [$(M+H)^+$, 100%]. HRMS (ES+ mode): calculated mass 217.1089 $(M+H)^+$, measured mass 217.1091 $(M+H)^+$. Found: C, 61.15; H, 5.43; N, 25.95. calcd for $C_{11}H_{12}N_4O$: C, 61.10; H, 5.59; N, 25.91%.

5-[4-heptyloxybenzyl]-2,4-diaminopyrimidine (2e). Following the general procedure of alkylation of 5-[4-hydroxylbenzyl]-2,4-diaminopyrimidine and starting from 5-[4-hydroxybenzyl]-2,4-diaminopyrimidine **2a** (111 mg, 0.51 mmol), 1-iodoheptane (230 mg, 1.02 mmol), anhydrous potassium carbonate (140 mg, 1.02 mmol) ethanol (20 mL), compound **2e** was isolated as a white solid (150 mg, 0.47 mmol, 94%). IR (KBr): ν = 3308, 3164, 2925, 1565, 1242 cm^{-1}. ^{1}H NMR (MeOD) δ 7.15 (br s, 1H, H6), 7.03 (dt, 2H, $J=8.5$, 2.8 Hz, H2′ + H6′), 6.78 (dt, 2H, $J=8.5$, 2.8 Hz, H3′ + H5′), 3.62 (s, 2H, C$\underline{H}_2$Ph), 1.75 (quint, 2H, H2″), 1.25–1.45 (m, 8H, H3″-6″), 0.85 (t, 3H, $J=6.5$ Hz, H7″). ^{13}C NMR (MeOD) δ 163.0 (4), 162.9 (2), 157.7 (6), 150.1 (3′), 149.8 (4′), 140.5 (1′), 123.5 (6′), 115.6 (5′), 115.1 (2′), 111.3 (5), 68.2 (1″), 34.6 (C$\underline{H}_2$Ph), 31.8 (5″), 29.2 (2″), 29.1 (4″), 26.0 (3″), 22.6 (6″), 14.1 (7″). LRMS (ES+ mode): $m/z=315.1$ [$(M+H)^+$, 30%]. HRMS (ES+ mode): calculated mass 217.1089 $(M+H)^+$, measured mass 217.1091 $(M+H)^+$. Found: C, 68.57; H, 8.46; N,

17.89. calcd for $C_{18}H_{26}N_4O$: C, 68.76; H, 8.33; N, 17.82%.

5-[4-Nonyloxybenzyl]-2,4-diaminopyrimidine (2g). Following the general procedure of alkylation of 5-[4-hydroxylbenzyl]-2,4-diaminopyrimidine and starting from 5-[4-hydroxybenzyl]-2,4-diaminopyrimidine **2a** (122 mg, 0.56 mmol), 1-iodononane (285 mg, 1.12 mmol), anhydrous potassium carbonate (155 mg, 1.12 mmol) ethanol (20 mL), compound **2g** was isolated as a white solid (174 mg, 0.51 mmol, 91%). IR (KBr): $\nu = 3312$, 3154, 2918, 1563, 1242 cm^{-1}. ^1H NMR ($CDCl_3$) δ 7.72 (s, 1H, H6), 7.04 (dt, 2H, $J = 8.7$, 1.9 Hz, H2′ + H6′), 6.80 (dt, 2H, $J = 8.8$, 1.9 Hz, H3′ + H5′), 4.75 (s, 2H, NH_2), 4.55 (s, 2H, NH_2), 3.88 (t, 2H, $J = 6.5$ Hz, H1″), 3.61 (s, 2H, C$\underline{H}_2$Ph), 1.71 (quint, 2H, H2″), 1.25–1.50 (m, 12H, H3″-8″), 0.89 (t, 3H, $J = 6.6$ Hz, H9″). ^{13}C NMR ($CDCl_3$) δ 163.2 (4), 162.1 (2), 158.5 (4″), 156.1 (6), 129.8 (1″), 129.6 (2″ + 6″), 115.3 (3″ + 5″), 107.6 (5), 68.2 (1‴), 34.5 (C$\underline{H}_2$Ph), 32.2 (7‴), 29.6 (6‴), 29.2 (4‴), 28.9 (2‴), 27.4 (5‴), 25.9 (3‴), 22.9 (8‴), 14.5 (9‴). LRMS (ES+ mode): $m/z = 343.2$ [$(M+H)^+$, 40%]. Found: C, 70.31; H, 8.92; N, 16.47. calcd for $C_{20}H_{30}N_4O$: C, 70.14; H, 8.83; N, 16.36%.

5-[4-Benzyloxybenzyl]-2,4-diaminopyrimidine (2i). Following the general procedure of alkylation of 5-[4-hydroxylbenzyl]-2,4-diaminopyrimidine and starting from 5-[4-hydroxybenzyl]-2,4-diaminopyrimidine **2a** (117 mg, 0.54 mmol), benzyl bromide (185 mg, 1.08 mmol), anhydrous potassium carbonate (149 mg, 1.08 mmol) ethanol (20 mL), compound **2i** was isolated as a white solid (138 mg, 0.43 mmol, 79%). IR (KBr): $\nu = 3312$, 3164, 3075, 1568, 1252 cm^{-1}. ^1H NMR (MeOD) δ 7.21 (br s, 1H, H6), 7.12 (dt, 2H, $J = 8.1$, 2.8 Hz, H2′ + H6′), 6.98–6.69 (m, 8H, H3′ + H5′ + H1″-H6″), 5.11 (s, 2H, OC$\underline{H}_2$Ph), 3.62 (s, 2H, C$\underline{H}_2$Ph). ^{13}C NMR (MeOD) δ 163.1 (4), 162.9 (2), 157.7 (6), 150.2 (3′), 149.8 (4′), 140.5 (1″), 136.8 (1″), 128.2 (2″ + 6″), 127.6 (4″), 127.0 (3″ + 5″), 123.5 (6′), 115.6 (5′), 115.3 (2′), 111.3 (5), 69.8 (O$\underline{C}H_2$Ph), 34.5 ($\underline{C}H_2$Ph). LRMS (ES+ mode): $m/z = 307.0$ [$(M+H)^+$, 10%]. Found: C, 70.45; H, 5.98; N, 18.40. calcd for $C_{18}H_{18}N_4O$: C, 70.57; H, 5.92; N, 18.29%.

3,4-Dipropoxybenzaldehyde (14a)

Following the general procedure of dialkylation of 3,4-dihydroxybenzaldehyde starting from 3,4-dihydroxybenzaldehyde **13** (2.200 g, 16.00 mmol), 1-bromopropane (5.904 g, 48.00 mmol), anhydrous potassium carbonate (11.040 g, 80.00 mmol) and potassium iodide (0.266 g, 1.60 mmol) in 2-butanone (100 mL), compound **14a** was isolated as a pale yellow oil (2.460 g, 11.07 mmol, 70%). TLC R_f 0.21 (10% EtOAc/hexane). IR (neat): $\nu = 2916$, 2754, 1691, 1276 cm^{-1}. ^1H NMR ($CDCl_3$) δ 9.89 (s, 1H, C$\underline{H}$O), 7.47 (dd, 1H, $J = 8.0$, 1.9 Hz, H6), 7.45 (d, 1H, $J = 1.9$ Hz, H2), 7.00 (d, 1H, $J = 8.0$ Hz, H5), 4.10 (t, 2H, $J = 6.7$ Hz, H1″), 4.07 (t, 2H, $J = 6.7$ Hz, H1′), 1.85–2.00 (m, 4H, H2′ + H2″), 1.11 (td, 6H, $J = 6.6$, 3.2 Hz, H3′ + H3″). ^{13}C NMR ($CDCl_3$) δ 191.5 (C$\underline{H}$O), 155.1 (4), 149.8 (3), 130.3 (1), 127.1 (6), 112.2 (5), 111.4 (2), 70.9 (1′ + 1″), 22.8 (2′ + 2″), 10.8 (3′ + 3″). Found: C, 70.36; H, 8.32. calcd for $C_{13}H_{18}O_3$: C, 70.25; H, 8.16%.

3,4-Dihexyloxybenzaldehyde (14b). Following the general procedure of dialkylation of 3,4-dihydroxybenzaldehyde starting from 3,4-dihydroxybenzaldehyde **13** (2.200 g, 16.00 mmol), 1-bromohexane (7.920 g, 48.00 mmol), anhydrous potassium carbonate (11.04 g, 80.00 mmol) and potassium iodide (0.266 g, 1.60 mmol) in 2-butanone (100 mL), compound **14b** was isolated as a white solid (3.580 g, 11.68 mmol, 73%). TLC R_f 0.33 (10% EtOAc/hexane). Mp = 41–42 °C. IR (KBr): $\nu = 2925$, 2855, 1688, 1277 cm^{-1}. ^1H NMR ($CDCl_3$) δ 9.89 (s, 1H, C$\underline{H}$O), 7.47 (dd, 1H, $J = 8.0$, 1.7 Hz, H6), 7.45 (d, 1H, $J = 1.7$ Hz, H2), 7.00 (d, 1H, $J = 8.0$ Hz, H5), 4.14 (t, 2H, $J = 6.7$ Hz, H1″), 4.10 (t, 2H, $J = 6.7$ Hz, H1′), 1.85–2.00 (m, 4H, H2′ + H2″), 1.45–1.60 (m, 12H, H3′-5′ + H3″-5″), 0.90 (t, 6H, $J = 6.6$ Hz, H6′ + H6″). ^{13}C NMR ($CDCl_3$) δ 191.5 (C$\underline{H}$O), 155.1 (4), 149.8 (3), 130.3 (1), 127.1 (6), 112.1 (5), 111.2 (2), 69.5 (1′ + 1″), 31.9 (4′ + 4″), 29.4 (2′ + 2″), 26.0 (3′ + 3″), 23.0 (5′ + 5″), 14.4 (6′ + 6″). Found: C, 74.39; H, 10.00. calcd for $C_{19}H_{30}O_3$: C, 74.47; H, 9.87%.

3,4-Dioctyloxybenzaldehyde (14c). A mixture of 3,4-dihydroxybenzaldehyde **13** (2.200 g, 16.00 mmol), 1-iodooctane (11.520 g, 48.00 mmol) and anhydrous potassium carbonate (11.040 g, 80.00 mmol) in 2-butanone (100 mL) was heated under reflux and stirred for 18 h under nitrogen. Most of the solvent was removed on a rotary evaporator. Water (100 mL) and ether (100 mL) were then added. The organic layer was separated, washed with water (3×150 mL) and dried over $MgSO_4$. The solvent was removed under reduced pressure and the crude compound was purified by column chromatography on silica gel using a gradient of elution of 5–10% EtOAc/hexane to give **14c** as a white solid (5.456 g, 15.05 mmol, 94%). TLC R_f 0.35 (10% EtOAc/hexane). Mp = 53–54 °C. IR (KBr): $\nu = 2924$, 2851, 1687, 1279 cm^{-1}. ^1H NMR ($CDCl_3$) δ 9.89 (s, 1H, C$\underline{H}$O), 7.47 (dd, 1H, $J = 8.0$, 1.8 Hz, H6), 7.45 (d, 1H, $J = 1.8$ Hz, H2), 7.00 (d, 1H, $J = 8.0$ Hz, H5), 4.09 (t, 2H, $J = 6.6$ Hz, H1″), 4.07 (t, 2H, $J = 6.6$ Hz, H1′), 1.85–2.00 (m, 4H, H2′ + H2″), 1.25–1.55 (m, 20H, H3′-7′ + H3″-7″), 0.90 (t, 6H, $J = 6.6$ Hz, H8′ + H8″). ^{13}C NMR ($CDCl_3$) δ 191.5 (C$\underline{H}$O), 155.1 (4), 149.8 (3), 130.2 (1), 127.1 (6), 112.1 (5), 111.3 (2), 69.5 (1′ + 1″), 32.0 (6′ + 6″), 29.7 (5′ + 5″), 29.5 (4′ + 4″), 29.4 (2′ + 2″), 26.4 (3′ + 3″), 23.1 (7′ + 7″), 14.5 (8′ + 8″). Found: C, 76.18; H, 10.68. calcd for $C_{23}H_{38}O_3$: C, 76.20; H, 10.56%.

3,4-Didecyloxybenzaldehyde (14d). To a solution of 3,4-dihydroxybenzaldehyde **13** (2.200 g, 16.00 mmol) in dry ethanol (100 mL) was added potassium hydroxide (2.150 g, 38.40 mmol). 1-iododecane (10.291 g, 38.40 mmol) was added dropwise to the reaction mixture after 10 min. The mixture was heated to reflux for 24 h. Water (100 mL) was added and the extraction was carried out with ether (3×100 mL). The organic layer was dried over $MgSO_4$ and concentrated in vacuo. The crude compound was concentrated and purified by column chromatography on silica gel using an elution of 10% EtOAc/hexane to give **8** as a white solid (2.251 g,

5.38 mmol, 34%). TLC R_f 0.25 (10% EtOAc/hexane). Mp = 64–65 °C. IR (KBr): ν = 2921, 2850, 1688, 1278 cm^{-1}. ^1H NMR ($CDCl_3$) δ 9.89 (s, 1H, C<u>H</u>O), 7.47 (dd, 1H, J = 8.0, 1.8 Hz, H6), 7.45 (d, 1H, J = 1.8 Hz, H2), 7.00 (d, 1H, J = 8.0 Hz, H5), 4.13 (t, 2H, J = 6.7 Hz, H1″), 4.10 (t, 2H, J = 6.7 Hz, H1′), 1.85–2.00 (m, 4H, H2′ + H2″), 1.30–1.60 (m, 28H, H3′-9′ + H3″-9″), 0.90 (t, 6H, J = 6.6 Hz, H10′ + H10″). ^{13}C NMR ($CDCl_3$) δ 191.5 (<u>C</u>HO), 151.8 (4), 149.4 (3), 130.3 (1), 127.2 (6), 112.1 (5), 111.2 (2), 69.5 (1′ + 1″), 32.4 (8′ + 8″), 30.1 (6′ + 6″), 30.0 (7′ + 7″), 29.8 (5′ + 5″), 29.6 (2′ + 2″), 29.5 (4′ + 4″), 26.5 (3′ + 3″), 23.0 (9′ + 9″), 14.5 (10′ + 10″). Found: C, 77.16; H, 11.20. calcd for $C_{27}H_{46}O_3$: C, 77.46; H, 11.07%.

(*E*)-3-(3,4-Dipropoxyphenyl)-2-(ethoxymethyl)-2-propenenitrile (15a). Following the general procedure of condensation with ethoxypropionitrile using azeotric distillation starting from ethoxypropionitrile (1.794 g, 18.12 mmol), 3,4-dipropoxybenzaldehyde **14a** (2.012 g, 9.06 mmol), sodium ethoxide (1.232 g, 18.12 mmol) in dry ethanol (60 mL), compound **15a** was isolated as a yellow oil (1.543 g, 5.09 mmol, 57%). TLC R_f 0.55 (40% EtOAc/hexane). IR (neat): ν = 2933, 2871, 2210, 1271, 1143 cm^{-1}. ^1H NMR ($CDCl_3$) δ 7.62 (d, 1H, J = 2.1 Hz, H2′), 7.27 (dd, 1H, J = 8.4, 2.1 Hz, H6′), 7.10 (br s, 1H, H3), 6.92 (d, 1H, J = 8.4 Hz, H5′), 4.25 (s, 2H, C<u>H</u>$_2$OEt), 4.13 (t, 2H, J = 6.7 Hz, H1‴), 4.10 (t, 2H, J = 6.7 Hz, H1″), 3.64 (q, 2H, J = 7.0 Hz, $CH_3C\underline{H}_2O$), 1.85–2.00 (m, 4H, H2″ + H2‴), 1.33 (t, 3H, J = 7.0 Hz, $C\underline{H}_3CH_2O$), 1.10 (t, 6H, J = 6.6 Hz, H3″ + H3‴). ^{13}C NMR ($CDCl_3$) δ 151.8 (4′), 149.3 (3′), 145.4 (3), 126.3 (1′), 124.2 (6′), 119.0 (1), 113.3 (5′), 113.0 (2′), 105.4 (2), 72.5 ($CH_3\underline{C}H_2O$), 70.9 (1″ + 1‴), 66.6 (EtO<u>C</u>H$_2$), 23.0 (2″ + 2‴), 15.5 (<u>C</u>H$_3$CH$_2$O), 10.9 (3″ + 3‴). LRMS (ES+ mode) : m/z = 325.9 [(M + Na)$^+$, 20%], 628.9 [(2 M + Na)$^+$, 100%]. HRMS (ES+ mode): calculated mass 321.2178 (M + NH$_4$)$^+$, measured mass 321.2178 (M + NH$_4$)$^+$.

(*E*)-3-(3,4-Dihexyloxyphenyl)-2-(ethoxymethyl)-2-propenenitrile (15b). Following the general procedure of condensation with ethoxypropionitrile using azeotric distillation starting from ethoxypropionitrile (1.766 g, 17.84 mmol), 3,4-dihexyloxybenzaldehyde (2.730 g, 8.92 mmol) and sodium ethoxide (1.213 g, 17.84 mmol) in dry ethanol (50 mL), compound **15b** was isolated as a yellow oil (2.210 g, 5.70 mmol, 64%). TLC R_f 0.56 (40% EtOAc/hexane). IR (neat): ν = 2930, 2866, 2210, 1269, 1141 cm^{-1}. ^1H NMR ($CDCl_3$) δ 7.62 (d, 1H, J = 2.1 Hz, H2′), 7.29 (dd, 1H, J = 8.4, 2.1 Hz, H6′), 7.13 (br s, 1H, H3), 6.92 (d, 1H, J = 8.4 Hz, H5′), 4.27 (s, 2H, C<u>H</u>$_2$OEt), 4.13 (t, 2H, J = 6.7 Hz, H1‴), 4.10 (t, 2H, J = 6.7 Hz, H1″), 3.66 (q, 2H, J = 7.0 Hz, $CH_3C\underline{H}_2O$), 1.85–2.00 (m, 4H, H2″ + H2‴), 1.45–1.60 (m, 12H, H3″-5″ + H3‴-5‴), 1.33 (t, 3H, J = 7.0 Hz, $C\underline{H}_3CH_2O$), 0.90 (t, 6H, J = 6.6 Hz, H6″ + H6‴). ^{13}C NMR ($CDCl_3$) δ 151.8 (4′), 149.4 (3′), 145.4 (3), 126.3 (1′), 124.2 (6′), 119.0 (1), 113.3 (5′), 113.0 (2′), 105.4 (2), 72.5 ($CH_3\underline{C}H_2O$), 69.5 (1″ + 1‴), 66.6 (EtO<u>C</u>H$_2$), 32.0 (4″ + 4‴), 29.5 (2″ + 2‴), 26.5 (3″ + 3‴), 23.0 (5″ + 5‴), 15.5 (<u>C</u>H$_3$CH$_2$O), 14.5 (6″ + 6‴). LRMS (ES+ mode): m/z = 410.0 [(M + Na)$^+$, 20%], 796.9 [(2 M + Na)$^+$, 100%]. HRMS (ES+ mode): calculated mass 405.3117 (M + NH$_4$)$^+$, measured mass 405.3109 (M + NH$_4$)$^+$.

(*E*)-3-(3,4-Dioctyloxyphenyl)-2-(ethoxymethyl)-2-propenenitrile (15c). Following the general procedure of condensation with ethoxypropionitrile using Dean–Stark trap and starting from ethoxypropionitrile (851.4 mg, 8.60 mmol), 3,4-dioctyloxybenzaldehyde **14c** (1.557 g, 4.30 mmol), sodium ethoxide (585 mg, 8.60 mmol), in dry ethanol (150 mL), compound **15c** was isolated as a yellow oil (1.152 g, 2.60 mmol, 60%). TLC R_f 0.63 (40% EtOAc/hexane). IR (neat): ν = 2927, 2855, 2211, 1270, 1143 cm^{-1}. ^1H NMR ($CDCl_3$) δ 7.60 (d, 1H, J = 2.1 Hz, H2′), 7.27 (dd, 1H, J = 8.4, 2.1 Hz, H6′), 7.10 (br s, 1H, H3), 6.91 (d, 1H, J = 8.4 Hz, H5′), 4.26 (s, 2H, C<u>H</u>$_2$OEt), 4.11 (t, 2H, J = 6.7 Hz, H1‴), 4.09 (t, 2H, J = 6.7 Hz, H1″), 3.66 (q, 2H, J = 7.0 Hz, $CH_3C\underline{H}_2O$), 1.85–2.00 (m, 4H, H2″ + H2‴), 1.45–1.60 (m, 20H, H3″-7″ + H3‴-7‴), 1.33 (t, 3H, J = 7.0 Hz, $C\underline{H}_3CH_2O$), 0.95 (t, 6H, J = 6.6 Hz, H8″ + H8‴). ^{13}C NMR ($CDCl_3$) δ 151.8 (4′), 149.4 (3′), 145.4 (3), 126.3 (1′), 124.2 (6′), 119.0 (1), 113.3 (5′), 113.0 (2′), 105.4 (2), 72.5 ($CH_3\underline{C}H_2O$), 69.5 (1″ + 1‴), 66.6 (EtO<u>C</u>H$_2$), 32.4 (6″ + 6‴), 29.8 (5″ + 5‴), 29.6 (4″ + 4‴), 29.4 (2″ + 2‴), 26.4 (3″ + 3‴), 23.1 (7″ + 7‴), 15.6 (<u>C</u>H$_3$CH$_2$O), 14.5 (8″ + 8‴). LRMS (ES+ mode): m/z = 466.0 [(M + Na)$^+$, 60%], 909.3 [(2 M + Na)$^+$, 100%]. HRMS (ES+ mode): calculated mass 444.3477 (M + H)$^+$, measured mass 444.3472 (M + H)$^+$.

(*E*)-3-(3,4-Didecyloxyphenyl)-2-(ethoxymethyl)-2-propenenitrile (15d). Following the general procedure of condensation with ethoxypropionitrile using azeotric distillation starting from ethoxypropionitrile (651 mg, 6.58 mmol), 3,4-didecyloxybenzaldehyde **8** (2.218 g, 5.31 mmol) and sodium ethoxide (447 mg, 6.58 mmol) in dry ethanol (50 mL), compound **15d** was isolated as a yellow oil (1.331 g, 2.66 mmol, 50%). TLC R_f 0.62 (40% EtOAc/hexane). IR (neat): ν = 2928, 2847, 2210, 1270, 1145 cm^{-1}. ^1H NMR ($CDCl_3$) δ 7.60 (d, 1H, J = 2.1 Hz, H2′), 7.27 (dd, 1H, J = 8.4, 2.1 Hz, H6′), 7.10 (br s, 1H, H3), 6.92 (d, 1H, J = 8.4 Hz, H5′), 4.25 (s, 2H, C<u>H</u>$_2$OEt), 4.11 (t, 2H, J = 6.7 Hz, H1‴), 4.09 (t, 2H, J = 6.7 Hz, H1″), 3.66 (q, 2H, J = 7.0 Hz, $CH_3C\underline{H}_2O$), 1.85–2.00 (m, 4H, H2″ + H2‴), 1.45–1.60 (m, 28H, H3″-9″ + H3‴-9‴), 1.33 (t, 3H, J = 7.0 Hz, $C\underline{H}_3CH_2O$), 0.90 (t, 6H, J = 6.6 Hz, H10″ + H10‴). ^{13}C NMR ($CDCl_3$) δ 151.8 (4′), 149.4 (3′), 145.4 (3), 126.3 (1′), 124.2 (6′), 119.0 (1), 113.3 (5′), 113.0 (2′), 105.4 (2), 72.5 ($CH_3\underline{C}H_2O$), 69.5 (1″ + 1‴), 66.6 (EtO<u>C</u>H$_2$), 32.4 (8″ + 8‴), 30.1 (6″ + 6‴), 30.0 (7″ + 7‴), 29.8 (5″ + 5‴), 29.6 (2″ + 2‴), 29.5 (4″ + 4‴), 26.5 (3″ + 3‴), 23.0 (9″ + 9‴), 15.5 (<u>C</u>H$_3$CH$_2$O), 14.5 (10″ + 10‴). LRMS (ES+ mode): m/z = 522.0 [(M + Na)$^+$, 40%], 1021.9 [(2 M + Na)$^+$, 100%]. HRMS (ES+ mode): calculated mass 517.4369 (M + NH$_4$)$^+$, measured mass 517.4372 (M + NH$_4$)$^+$.

5-(3,4-Dipropoxybenzyl)-2,4-diaminopyrimidine (3a). Following the general procedure of cyclisation with guanidine and starting from guanidine hydrochloride (2.901 g, 30.54 mmol) in dry ethanol (25 mL), (*E*)-3-(3,4-dipropoxyphenyl)-2-(ethoxymethyl)-2-propenenitrile

15a (1.543 g, 5.09 mmol) and a solution of sodium ethoxide (2.067 g, 30.54 mmol) in dry ethanol (25 mL), compound **3a** was isolated as a yellow solid (840 mg, 2.66 mmol, 52%). TLC R_f 0.61 (40% MeOH/EtOAc). IR (KBr): ν = 3368, 3146, 2985,1565, 1259 cm^{-1}. ^1H NMR (MeOD) δ 7.55 (s, 1H, H6), 6.96 (d, 1H, J = 8.1 Hz, H5′), 6.87 (d, 1H, J = 1.8 Hz, H2′), 6.80 (dd, 1H, J = 8.1, 1.8 Hz, H6′), 4.00 (t, 2H, J = 6.5 Hz, H1‴), 3.98 (t, 2H, J = 6.5 Hz, H1″), 3.40 (s, 2H, C$\underline{H}_2$Ph), 1.77–1.94 (m, 4H, H2″ + H2‴), 1.13 (td, 6H, J = 6.6, 2.8 Hz, H3″ + H3‴). ^{13}C NMR (MeOD) δ 164.8 (4), 163.6 (2), 156.2 (6), 151.0 (3′), 149.5 (4′), 133.5 (1′), 122.5 (6′), 116.2 (5′), 116.1 (2′), 108.8 (5), 72.5 (1″ + 1‴), 34.2 (C$\underline{H}_2$Ph), 24.2 (2″ + 2‴), 11.3 (3″ + 3‴). LRMS (ES+ mode): m/z = 317.2 [(M + H)$^+$, 100%]. Found: C, 60.31; H, 7.24; N, 16.67. calcd for $C_{17}H_{24}N_4O_2 \cdot 1.2H_2O$: C, 60.44; H, 7.16; N, 16.59%.

5-(3,4-Dihexyloxybenzyl)-2,4-diaminopyrimidine (3b). Following the general procedure of cyclisation with guanidine and starting from guanidine hydrochloride (2.188 g, 23.04 mmol) in dry ethanol (25 mL), (*E*)-3-(3,4-dihexyloxyphenyl)-2-(ethoxymethyl)-2-propenenitrile **15b** (1.486 g, 3.84 mmol) and sodium ethoxide (1.566 g, 23.04 mmol) in dry ethanol (25 mL), compound **3b** was isolated as a yellow solid (790 mg, 1.98 mmol, 51%). TLC R_f 0.50 (40% MeOH/EtOAc). IR (KBr): ν = 3354, 3146, 2984, 1565, 1259 cm^{-1}. ^1H NMR (MeOD) δ 7.45 (s, 1H, H6), 6.85 (d, 1H, J = 8.1 Hz, H5′), 6.77 (d, 1H, J = 1.8 Hz, H2′), 6.70 (dd, 1H, J = 8.1, 1.8 Hz, H6′), 3.94 (t, 2H, J = 6.5 Hz, H1‴), 3.91 (t, 2H, J = 6.5 Hz, H1″), 3.59 (s, 2H, C$\underline{H}_2$Ph), 1.67–1.80 (m, 4H, H2″ + H2‴), 1.30–1.55 (m, 12H, H3″-5″ + H3‴-5‴), 0.90 (t, 6H, J = 6.6 Hz, H6″ + H6‴). ^{13}C NMR (MeOD) δ 163.0 (4), 162.9 (2), 157.7 (6), 150.1 (3′), 149.8 (4′), 140.6 (1′), 123.5 (6′), 115.6 (5′), 115.0 (2′), 111.3 (5), 68.5 (1″ + 1‴), 34.6 (C$\underline{H}_2$Ph), 31.5 (4″ + 4‴), 29.1 (2″ + 2‴), 25.7 (3″ + 3‴), 22.6 (5″ + 5‴), 14.0 (6″ + 6‴). LRMS (ES+ mode): m/z = 401.0 [(M + H)$^+$, 100%]. HRMS (ES+ mode): calculated mass 401.2916 (M + H)$^+$, measured mass 401.2911 (M + H)$^+$. Found: C, 68.61; H, 8.43; N, 13.96. calcd for $C_{23}H_{36}N_4O_2$: C, 68.97; H, 8.06; N, 13.99%.

5-(3,4-Dioctyloxybenzyl)-2,4-diaminopyrimidine (3c). Following the general procedure of cyclisation with guanidine and starting from guanidine hydrochloride (1.922 g, 16.02 mmol) in dry ethanol (25 mL), (*E*)-3-(3,4-dioctyloxyphenyl)-2-(ethoxymethyl)-2-propenenitrile **15c** (1.185 g, 2.67 mmol) and sodium ethoxide (1.089 g, 16.02 mmol) in dry ethanol (25 mL), compound **3c** was isolated as a yellow solid (1.051 g, 2.05 mmol, 53%). TLC R_f 0.50 (40% MeOH/EtOAc). IR (KBr): ν = 3360, 3150, 2922, 1554, 1252 cm^{-1}. ^1H NMR (MeOD) δ 7.34 (s, 1H, H6), 6.85 (d, 1H, J = 8.1 Hz, H5′), 6.85 (d, 1H, J = 1.8 Hz, H2′), 6.75 (dd, 1H, J = 8.1, 1.8 Hz, H6′), 3.97 (t, 2H, J = 6.5 Hz, H1‴), 3.95 (t, 2H, J = 6.5 Hz, H1″), 3.55 (s, 2H, C$\underline{H}_2$Ph), 1.67–1.88 (m, 4H, H2″ + H2‴), 1.30–1.55 (m, 20H, H3″-7″ + H3‴-7‴), 0.90 (t, 6H, J = 6.6 Hz, H8″ + H8‴). ^{13}C NMR (MeOD) δ 163.0 (4), 162.9 (2), 157.7 (6), 150.1 (3′), 149.8 (4′), 140.5 (1′), 123.5 (6′), 115.6 (5′), 115.1 (2′), 111.3 (5), 68.2 (1″ + 1‴), 34.6 (C$\underline{H}_2$Ph), 31.8 (6″ + 6‴), 29.3 (5″ + 5‴), 29.2 (4″ + 4‴), 29.1 (2″ + 2‴), 26.0 (3″ + 3‴), 22.6 (7″ + 7‴), 14.1 (8″ + 8‴). Found: C, 66.73; H, 9.27; N, 11.64. calcd for $C_{27}H_{44}N_4O_2 \cdot 1.6H_2O$): C, 66.84; H, 9.14; N, 11.55%.

5-(3,4-Didecyloxybenzyl)-2,4-diaminopyrimidine (3d). Following the general procedure of cyclisation with guanidine and starting from guanidine hydrochloride (1.404 g, 14.78 mmol) in dry ethanol (25 mL), (*E*)-3-(3,4-didecyloxyphenyl)-2-(ethoxymethyl)-2-propenenitrile **15d** (1.229 g, 2.46 mmol) and sodium ethoxide (1.005 g, 14.78 mmol) in dry ethanol (25 mL), compound **3d** was isolated as a yellow solid (348 mg, 0.68 mmol, 47%). TLC R_f 0.50 (40% MeOH/EtOAc). IR (KBr): ν = 3334, 3116, 2918, 1564, 1263 cm^{-1}. ^1H NMR (MeOD) δ 7.16 (s, 1H, H6), 6.90 (d, 1H, J = 8.1 Hz, H5′), 6.82 (d, 1H, J = 1.8 Hz, H2′), 6.73 (dd, 1H, J = 8.1, 1.8 Hz, H6′), 3.96 (t, 4H, J = 6.5 Hz, H1″ + H1‴), 3.61 (s, 2H, C$\underline{H}_2$Ph), 1.67–1.82 (m, 4H, H2″ + H2‴), 1.30–1.55 (m, 28H, H3″-9″ + H3‴-9‴), 0.89 (t, 6H, J = 6.6 Hz, H10″ + H10‴). ^{13}C NMR (MeOD) δ 163.0 (4), 162.9 (2), 157.7 (6), 150.1 (3′), 149.8 (4′), 140.6 (1′), 123.5 (6′), 115.6 (5′), 115.0 (2′), 111.3 (5), 70.9 (1″ + 1‴), 48.7 (C$\underline{H}_2$Ph), 33.6 (8″ + 8‴), 31.2 (6″ + 6‴), 31.1 (5″ + 5‴), 30.9 (7″ + 7‴), 30.8 (4″ + 4‴), 27.7 (2″ + 2‴), 24.1 (9″ + 9‴), 18.6 (3″ + 3‴), 14.8 (10″ + 10‴). Found: C, 70.73; H, 10.04; N, 10.29. calcd for $C_{31}H_{52}N_4O_2 \cdot 0.7H_2O$: C, 70.91; H, 9.98; N, 10.67%.

4-Benzyloxy-3-hydroxybenzaldehyde (16). 3,4-Dihydroxybenzaldehyde (2.041 g, 14.79 mmol) was dissolved in acetone (150 mL). Benzylbromide (2.529 g, 14.79 mmol), anhydrous potassium carbonate (3.062 g, 22.19 mmol) and a catalytic amount of potassium iodide were then added. The mixture was heated to reflux overnight. The inorganic salts were filtered off and the filtrate was diluted with EtOAc and brine, extracted with EtOAc, dried over $MgSO_4$. The solvent was removed under reduced pressure and the crude compound was purified by column chromatography on silica gel using an elution of 50% EtOAc/hexane to give **16** as a white solid (2.583 g, 11.33 mmol, 77%). TLC R_f 0.56 (70% EtOAc/hexane). IR (KBr): ν = 3204, 2969, 1644, 1259 cm^{-1}. ^1H NMR ($CDCl_3$) δ 9.89 (s, 1H, C$\underline{H}$O), 7.37–7.60 (m, 7H, H2 + H6 + H2′-6′), 7.12 (d, 1H, J = 8.0 Hz, H5), 5.28 (s, 2H, OC$\underline{H}_2$Ph). ^{13}C NMR ($CDCl_3$) δ 191.5 (C$\underline{H}$O), 154.7 (4), 149.6 (3), 135.7 (1′), 130.7 (1), 129.3 (2′,6′), 128.4 (3′,5′),127.5 (4′), 124.8 (6) 114.8 (2), 111.9 (5), 71.7 (O$\underline{C}$H$_2$Ph). Found: C, 73.63; H, 5.34. calcd for $C_{14}H_{12}O_3$: C, 73.67; H, 5.30%.

4-Benzyloxy-3-hexyloxybenzaldehyde (17). Following the general procedure of monoalkylation of hydroxylbenzaldehyde and starting from 4-benzyloxy-3-hydroxybenzaldehyde **16** (2.118 g, 9.29 mmol), 1-bromohexane (4.514 g, 27.36 mmol), anhydrous potassium carbonate (5.664 g, 41.04 mmol) and a catalytic amount of potassium iodide in 2-butanone (100 mL), compound **17** was isolated as a yellow oil (2.493 g, 7.99 mmol, 86%). TLC R_f 0.38 (33% EtOAc/hexane). IR (neat): ν = 3064, 2927, 1686, 1267 cm^{-1}. ^1H NMR ($CDCl_3$) δ 9.89 (s, 1H, C$\underline{H}$O), 7.35–7.55 (m, 7H, H2 + H6 + H2′-H6′), 7.05 (d, 1H, J = 7.9 Hz, H5), 5.30 (s, 2H, OC$\underline{H}_2$Ph), 4.12 (t, 2H, J = 6.7 Hz, H1″), 1.95 (sext, 2H, J = 7.3 Hz, H2″),

1.35–1.65 (m, 6H, H3″-H5″), 0.95 (t, 3H, $J=7.4$ Hz, H6″). ^{13}C NMR ($CDCl_3$) δ 191.5 (CHO), 154.4 (4), 150.1 (3), 136.8 (1′), 130.8 (1), 129.1 (2′,6′), 128.5 (4′), 127.5 (3′,5′), 126.8 (6) 113.3 (5), 111.2 (2), 71.2 (OCH_2Ph), 69.5 (1″), 32.0 (4″), 29.4 (2″), 26.1 (3″), 23.0 (5″), 14.6 (6″). Found: C, 76.97; H, 7.79. calcd for $C_{20}H_{24}O_3$: C, 76.89; H, 7.74%.

4-Hydroxy-3-hexyloxybenzaldehyde (18). 4-Benzyloxy-3-hexyloxybenzaldehyde **17** (2.400 g, 7.69 mmol) was dissolved in ethanol (40 mL), then palladium on actived carbon 10% (400 mg, 0.38 mmol) was added. The reaction was stirred under an atmosphere of hydrogen at room temperature for 2 h. The mixture was filtered and the solvent was removed to give as yellow oil **18** (1.472 g, 6.63 mmol, 86%). TLC R_f 0.18 (10% EtOAc/hexane). IR (neat): ν = 3373 (OH), 2944 (Ar), 2729 (C=O), 1682 (C=O), 1269 (ArOR) cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 9.90 (s, 1H, CHO), 7.32 (dd, 1H, $J=8.5$ Hz, 1.8 Hz, H6), 7.30 (d, 1H, $J=1.8$ Hz, H2), 6.92 (d, 1H, $J=8.5$ Hz, H5), 6.30 (br s, 1H, OH), 4.07 (t, 2H, $J=6.7$ Hz, H1′), 1.80 (sext, 2H, $J=6.6$ Hz, H2′), 1.35–1.60 (m, 6H, H3′-H5′), 0.90 (t, 3H, $J=6.6$ Hz, H6′). ^{13}C NMR ($CDCl_3$) δ 191.4 (CHO), 152.2 (4), 147.0 (3), 130.3 (1), 127.8 (6), 114.7 (5), 109.9 (2), 69.7 (1′), 31.9 (4′) 29.4 (2′), 26.1 (3′), 23.0 (5′), 14.5 (6′). LRMS (ES− mode): $m/z=220.92$ [$(M-H)^+$, 40%], 442.7 [$(2M-H)^+$, 40%]. HRMS (ES+ mode): calculated mass 240.1600 $(M+NH_4)^+$, measured mass 240.1600 $(M+NH_4)^+$.

3-Hexyloxy-4-propyloxybenzaldehyde (19a). Following the general procedure of monoalkylation of hydroxylbenzaldehyde and starting from 4-hydroxy-3-hexyloxy benzaldehyde **18** (609 mg, 2.74 mmol), 1-bromopropane (674 mg, 5.48 mmol), anhydrous potassium carbonate (1.134 g, 8.22 mmol) and a catalytic amount of potassium iodide in acetonitrile (150 mL), compound **19a** was isolated as a white oil (539 mg, 2.04 mmol, 75%). TLC R_f 0.27 (10% EtOAc/hexane). IR (neat): ν = 2947, 2826, 1682, 1270 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 9.90 (s, 1H, CHO), 7.39 (dd, 1H, $J=8.1$ Hz, 1.9 Hz, H6), 7.34 (d, 1H, $J=1.9$ Hz, H2), 6.92 (d, 1H, $J=8.1$ Hz, H5), 4.02 (t, 2H, $J=6.7$ Hz, H1′), 4.01 (t, 2H, $J=6.7$ Hz, H1″), 1.75–1.90 (m, 4H, H2′ + H2″), 1.35–1.60 (m, 6H, H3′-H5′), 1.05 (t, 3H, $J=6.6$ Hz, H3″), 0.90 (t, 3H, $J=6.6$ Hz, H6′). ^{13}C NMR ($CDCl_3$) δ 191.5 (CHO), 160.4 (4), 155.1 (3), 130.3 (1), 127.1 (6), 112.1 (5), 111.3 (2), 70.9 (1′), 69.7 (1″), 32.0 (4′) 29.4 (2′), 26.1 (3′), 23.0 (2″), 22.8 (5′), 14.5 (6′), 10.8 (3″). LRMS (ES+ mode): $m/z=265.2$ [$(M+H)^+$, 40%], 287.0 [$(M+Na)^+$, 80%], 551.1 [$(2M+Na)^+$, 100%]. HRMS (ES+ mode): calculated mass 265.1803 $(M+H)^+$, measured mass 265.1807 $(M+H)^+$.

3-Hexyloxy-4-octyloxybenzaldehyde (19b). Following the general procedure of monoalkylation of hydroxylbenzaldehyde and starting from 4-hydroxy-3-hexyloxy benzaldehyde **18** (838 mg, 3.77 mmol), 1-iodooctane (1.809 g, 7.54 mmol), anhydrous potassium carbonate (1.563 g, 11.32 mmol) in 2-butanone (100 mL), compound **19b** was isolated as a white solid (925 mg, 2.77 mmol, 73%). TLC R_f 0.32 (10% EtOAc/hexane). IR (neat): ν = 2924, 2853, 1686, 1275 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 9.90 (s, 1H, CHO), 7.39 (dd, 1H, $J=8.1$ Hz, 1.9 Hz, H6), 7.34 (d, 1H, $J=1.9$ Hz, H2), 6.92 (d, 1H, $J=8.1$ Hz, H5), 4.02 (t, 2H, $J=6.7$ Hz, H1′), 4.01 (t, 2H, $J=6.7$ Hz, H1″), 1.75–1.90 (m, 4H, H2′ + H2″), 1.35–1.60 (m, 6H, H3′-H5′), 0.93 (t, 3H, $J=6.6$ Hz, H3″), 0.90 (t, 3H, $J=6.6$ Hz, H6′). ^{13}C NMR ($CDCl_3$) δ 191.5 (CHO), 155.1 (4), 149.8 (3), 130.3 (1), 127.1 (6), 112.1 (5), 111.2 (2), 69.5 (1′ + 1″), 32.2 (6″), 32.0 (4′), 29.8 (5″), 29.7 (4″), 29.5 (2″), 29.4 (2′), 26.4 (3″), 26.1 (3′), 23.1 (7″), 23.0 (5′), 14.6 (8″), 14.5 (6′). Found: C, 75.40; H, 10.28. calcd for $C_{21}H_{39}O_3$: C, 75.41; H, 10.24%.

(*E*)-3-(3-Hexyloxy-4-propyloxyphenyl)-2-(ethoxymethyl)-2-propenenitrile (20a). Following the general procedure of condensation with ethoxypropionitrile using Dean–Stark trap and starting from ethoxypropionitrile (750 mg, 7.58 mmol), 3-hexyloxy-4-propyloxybenzaldehyde **19a** (523 mg, 3.79 mmol) and sodium ethoxide (515 mg, 7.58 mmol) in dry ethanol (100 mL), compound **20a** was isolated as a yellow oil (372 mg, 1.08 mmol, 28%). TLC R_f 0.55 (40% EtOAc/hexane). IR (neat): ν = 2931, 2871, 2210, 1269, 1140 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 7.54 (d, 1H, $J=1.9$ Hz, H2′), 7.27 (dd, 1H, $J=8.1$ Hz, 1.9 Hz, H6′), 7.11 (br s, 1H, H3), 6.98 (d, 1H, $J=8.8$, H5′), 4.26 (s, 2H, CH_2OEt), 4.10 (t, 2H, $J=6.7$ Hz, H1″), 4.05 (t, 2H, $J=6.7$ Hz, H1‴), 3.64 (q, 2H, $J=7.0$ Hz, CH_3CH_2O), 1.75–1.90 (m, 4H, H2″ + H2‴), 1.35–1.65 (m, 6H, H3″-5″), 1.33 (t, 3H, $J=7.0$ Hz, CH_3CH_2O), 1.05 (t, 3H, $J=6.6$ Hz, H3‴), 0.93 (t, 3H, $J=6.6$ Hz, H6″). ^{13}C NMR ($CDCl_3$) δ 151.7 (4′), 149.3 (3), 145.3 (3), 126.3 (1′), 124.2 (6′), 119.0 (1), 113.2 (5′), 113.0 (2′), 105.4 (2), 72.5 (CH_3CH_2O), 70.8 (1″), 69.6 (1‴), 66.6 ($EtOCH_2$), 31.4 (4″), 29.5 (2″), 26.1 (3″), 29.7 (5″), 23.0 (2‴), 22.9 (5″), 15.5 (CH_3CH_2O), 14.5 (6″), 10.9 (3‴). LRMS (ES+ mode): $m/z=368.2$ [$(M+Na)^+$, 70%], 713.1 [$(2M+Na)^+$, 100%]. HRMS (ES+ mode): calculated mass 346.2382 $(M+H)^+$, measured mass 346.2383 $(M+H)^+$.

(*E*)-3-(3-Hexyloxy-4-octyloxyphenyl)-2-(ethoxymethyl)-2-propenenitrile (20b). Following the general procedure of condensation with ethoxypropionitrile using Dean–Stark trap and starting from ethoxypropionitrile (1.255 g, 12.68 mmol), 3-Hexyloxy-4-octyloxybenzaldehyde **19b** (628 mg, 6.34 mmol) and sodium ethoxide (863 mg, 12.68 mmol) in dry ethanol (100 mL), compound **20b** was isolated as a yellow oil (279 mg, 0.67 mmol, 11%). TLC R_f 0.45 (20% EtOAc/hexane). IR (film): ν = 2925, 2857, 2210, 1270, 1140 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 7.53 (d, 1H, $J=1.9$ Hz, H2′), 7.20 (dd, 1H, $J=8.1$ Hz, 1.9 Hz, H6′), 7.04 (br s, 1H, H3), 6.85 (d, 1H, $J=8.8$, H5′), 4.20 (s, 2H, CH_2OEt), 4.04 (t, 2H, $J=6.7$ Hz, H1″), 4.01 (t, 2H, $J=6.7$ Hz, H1‴), 3.57 (q, 2H, $J=7.0$ Hz, CH_3CH_2O), 1.75–1.90 (m, 4H, H2″ + H2‴), 1.35–1.65 (m, 16H, H3″-5″ + H3‴-7‴), 1.33 (t, 3H, $J=7.0$ Hz, CH_3CH_2O), 0.90 (t, 3H, $J=6.6$ Hz, H6″), 0.87 (t, 3H, $J=6.6$ Hz, H8‴). ^{13}C NMR ($CDCl_3$) δ 151.8 (4′), 149.3 (3′), 145.3 (3), 126.3 (1′), 124.2 (6′), 119.0 (1), 113.2 (5′), 113.3 (2′), 105.4 (2), 72.5 (CH_3CH_2O), 68.5 (1″), 68.2 (1‴), 66.6 ($EtOCH_2$), 31.8 (6‴), 31.6 (4″), 29.3 (5‴), 29.2 (2‴ + 4‴), 29.1 (2″), 25.9 (3″), 22.6 (7‴), 22.5 (5″), 15.5 (CH_3CH_2O), 14.0 (8‴), 13.9 (6″). LRMS (ES+ mode):

$m/z = 438.1$ [$(M+Na)^+$, 30%], 853.1 [$(2M+Na)^+$, 50%], 898.9 [(2 M + 3Na), 70%]. HRMS (ES+ mode): calculated mass 416.3164 $(M+H)^+$, measured mass 416.3163 $(M+H)^+$.

5-(3-Hexyloxy-4-propyloxybenzyl)-2,4-diaminopyrimidine (4a). Following the general procedure of cyclisation with guanidine and starting from guanidine hydrochloride (661 mg, 6.96 mmol) in dry ethanol (25 mL), (*E*)-3-(3-hexyloxy-4-propyloxyphenyl)-2-(ethoxymethyl)-2-propenenitrile **20a** (399 mg, 1.16 mmol) and sodium ethoxide (473 mg, 6.96 mmol) in dry ethanol (25 mL), compound **4a** was isolated as a yellow solid (262 mg, 0.73 mmol, 63%). TLC R_f 0.43 (40% MeOH/EtOAc). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.42 (s, 1H, H6), 7.00 (d, 1H, $J = 8.1$ Hz, H5′), 6.81 (d, 1H, $J = 1.8$ Hz, H2′), 6.79 (dd, 1H, $J = 8.1$, 1.8 Hz, H6′), 5.49 (s, 2H, NH_2), 4.95 (s, 2H, NH_2), 3.94 (m, 4H,H1″ + H1‴), 3.65 (s, 2H, C$\underline{H}_2$Ph), 1.67–1.80 (m, 4H, H2″ + H2‴), 1.30–1.55 (m, 6H, H3″-5″), 1.13 (t, 3H, $J = 6.6$ Hz, H3‴), 0.90 (t, 3H, $J = 6.6$ Hz, H6″). ^{13}C NMR (MeOD) δ 163.0 (4), 162.9 (2), 157.7 (6), 150.1 (3′), 149.8 (4′), 140.6 (1′), 123.5 (6′), 115.6 (5′), 115.0 (2′), 111.3 (5), 70.8 (1″), 69.6 (1‴), 34.6 (C$\underline{H}_2$Ph), 31.4 (4″), 29.5 (2″), 26.1 (3″), 29.7 (5″), 23.0 (2‴), 22.9 (5″), 14.5 (6″), 10.9 (3‴). LRMS (ES+ mode) : $m/z = 359.1$ [$(M+H)^+$, 100%]. Found: C, 63.58; H, 7.92; N, 14.85. calcd for $C_{20}H_{30}N_4O_2.1.0H_2O$: C, 63.84; H, 8.04; N, 14.89%.

5-(3-Hexyloxy-4-octyloxybenzyl)-2,4-diaminopyrimidine (4b). Following the general procedure of cyclisation with guanidine and starting from guanidine hydrochloride (383 mg, 4.03 mmol) in dry ethanol (20 mL), (*E*)-3-(3-hexyloxy-4-octyloxyphenyl)-2-(ethoxymethyl)-2-propenenitrile **20b** (278 mg, 0.67 mmol) and sodium ethoxide (274 mg, 4.03 mmol) in dry ethanol (20 mL), compound **4b** was isolated as a yellow solid (186 mg, 0.44 mmol, 65%). TLC R_f 0.43 (40% MeOH/EtOAc). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.42 (s, 1H, H6), 6.99 (d, 1H, $J = 8.1$ Hz, H5′), 6.83 (d, 1H, $J = 1.8$ Hz, H2′), 6.78 (dd, 1H, $J = 8.1$, 1.8 Hz, H6′), 5.49 (s, 2H, NH_2), 4.95 (s, 2H, NH_2), 3.94 (m, 4H,H1″ + H1‴), 3.58 (s, 2H, C$\underline{H}_2$Ph), 1.67–1.80 (m, 4H, H2″ + H2‴), 1.30–1.55 (m, 16H, H3″-5″ + H3‴-7‴), 0.92 (t, 6H, $J = 6.6$ Hz, H6″ + H8‴). ^{13}C NMR (MeOD) δ 163.1 (4), 162.9 (2), 157.6 (6), 150.1 (3′), 149.8 (4′), 140.6 (1′), 123.5 (6′), 115.6 (5′), 115.0 (2′), 111.3 (5), 68.5 (1″), 68.2 (1‴), 34.5 (C$\underline{H}_2$Ph), 31.8 (6‴), 31.6 (4″), 29.3 (5‴), 29.2 (2‴ + 4‴), 29.1 (2″), 25.9 (3″), 22.6 (7‴), 22.5 (5″), 14.0 (8‴), 13.9 (6″). LRMS (ES+ mode): $m/z = 429.2$ [$(M+H)^+$, 40%]. Found: C, 63.81; H, 8.44; N, 11.89. calcd for $C_{25}H_{340}N_4O_2.2.3H_2O$: C, 63.92; H, 8.58; N, 11.93%.

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