

Easy synthesis of new series of pteridine analogs: di- and tetrahydropyrimido[4,5-*d*]pyrimidines via 5-pyrimidinecarbaldehydes

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In Memoriam to Professor Alan R. Katritzky, for his dedication and contribution to the world of chemistry

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Abstract

Pyrimidine-5-carbaldehyde derivatives, of easy access, were selected as precursors for the synthesis of mimic pteridine derivatives 5,6-dihydropyrimido[4,5-*d*]pyrimidines and 5,6,7,8-tetrahydropyrimido[4,5-*d*]pyrimidines via 4-amino-(5-aminomethyl)pyrimidine intermediates. Straightforward procedures allow a quick access to these compounds. The dihydro derivatives were prepared means of a final cyclocondensation carried out with orthoesters, catalysed by acid and assisted by microwaves irradiation under solvent free conditions. The final cyclocondensation with carbonyl compounds forming the tetrahydro derivatives was done under mild conditions, in which stereochemical induction was carried out on the building of this skeleton, and stereochemistry assignments corroborated by theoretical calculations.

Keywords: 4-Amino-5-(aminomethyl)pyrimidines; microwave irradiation; theoretical calculations; acid catalysis; cyclocondensation; stereoselectivity

Introduction

Pyrimidine-fused compounds are of interest in medicinal chemistry and chemical biology due to their wide range of biological activities.¹⁻⁴ See Figure 1 for compounds with the pyrimido[4,5-*d*]pyrimidine scaffold that have been found to show pharmacological activity, such as antiplatelet,⁵ tyrosine kinase inhibitor,⁶⁻⁸ antifolate (**I-II**),⁹ antibacterial (**III-IV**),^{10,11} anti-inflammatory,¹² hepatoprotective (**V**)¹³ and antiviral¹⁴ properties.

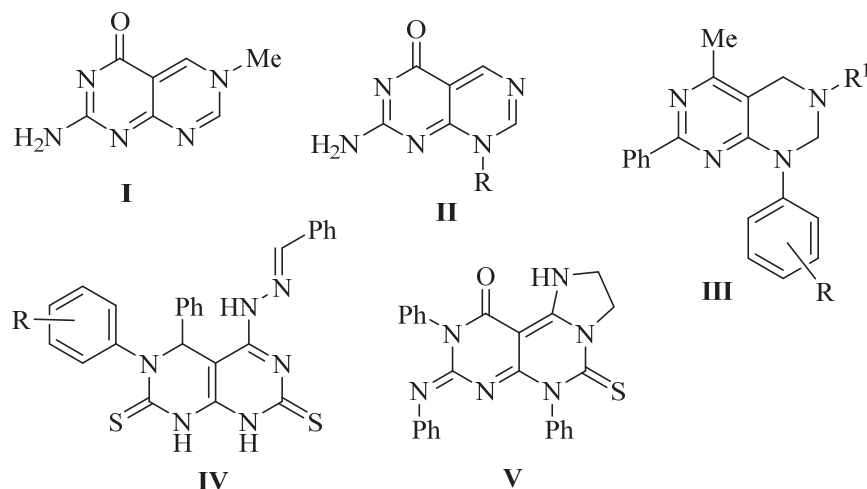


Figure 1. Some pyrimido[4,5-*d*]pyrimidine derivatives with diverse biological properties.

On account of the pharmaceutical interest in these compounds, the development of high-throughput methodologies for the synthesis of novel pyrimidine-fused heterocyclic scaffolds is in continuous expansion.¹⁵⁻¹⁹ It has become widely accepted that many classical reactions perform better under microwave irradiation than by conventional heating methods.²⁰⁻²⁴ Microwave irradiation has been used to carry out a wide range of reactions in short times, with high yields and regioselectivity, and frequently without the need of solvents.²⁵

With the aim to tune up simple reaction methodologies, we have focused our strategy in the use of 4-aminopyrimidine-5-carbaldehydes as key precursors for the preparation of cited fused heterocyclic system (see Figure 2) because they have the appropriate spatial arrangement of atoms. We have previously reported some 4-aminopyrimidine-5-carbaldehydes and employed them for the preparation of pyridopyrimidine derivatives.²⁶

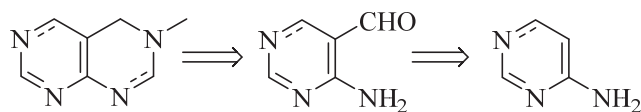


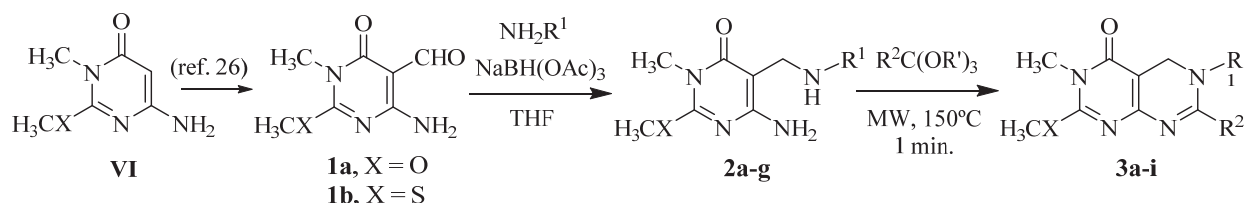
Figure 2

We here present the synthesis of a new series of 5,6-dihydro and 5,6,7,8-tetrahydro derivatives by a three step process starting from the corresponding 4-aminopyrimidines, *via* formation of 4-amino-(5-aminomethyl)pyrimidines as intermediates for further cyclocondensation with orthoesters and carbonyl compounds respectively.

In the tetrahydropyrimido[4,5-*d*]pyrimidines a stereogenic center is generated and the experimental conditions to induce stereoselectivity are discussed below.

Results and Discussion

The key precursor 6-aminopyrimidin-4(3*H*)-one-5-carbaldehydes, **1a,b**, were prepared by Vilsmeier-Haack formylation of 6-aminopyrimidin-4(3*H*)-ones **VI**,²⁶ and then reaction with various primary amines, such as aniline, benzylamine and cyclohexylamine, in order to get the required diamino intermediate **2** for further cyclocondensation *via* reaction with the carbaldehyde group. The standard reductive reagents and conditions were tested in order to afford compounds **2**, but just the use of sodium triacetoxyborohydride in THF was effective to perform the one step amino reduction. The reaction workup is simple and straightforward, and compounds **2** were isolated by filtration in good to excellent yields (Scheme 1 and Table 1).



Scheme 1. Synthetic route to pyrimidopyrimidines **3** from aminopyrimidines.

Table 1. Data for the synthesis of **2a-g** shown in **Scheme 1**

Entry	X	R ¹	Compounds 2	
			Yield (%)	Mp °C
2a	O	C ₆ H ₅	98	250
2b	O	CH ₂ C ₆ H ₅	82	125
2c	O	C ₆ H ₁₁	80	210
2d	S	C ₆ H ₅	92	175
2e	S	CH ₂ C ₆ H ₅	78	118
2f	S	C ₆ H ₁₁	90	140
2g/g'	S	CH(CH ₃)C ₆ H ₅	86/81	-

Compounds **2** were fully characterized by spectroscopic and analytical data. From the spectroscopic data of compounds **2**, the disappearance of signals corresponding to the carbaldehyde in **1**, and the appearance of the derived methylene group and the signals which correspond to the amine residue, are clearly observed. In the case of **2a**, the signal for 5-CH₂ is observed at 3.95 ppm (as a doublet, coupled with NH) and at 37.0 ppm, in the ¹H- and ¹³C-NMR spectra respectively.

For the cyclocondensation of the diamine derivative **2**, we first tried several acid derivatives such as acyl chlorides and anhydrides, and different conditions, but with no positive results. Finally we chose orthoesters, which provided the corresponding 5,6-dihydropyrimido[4,5-*d*]-pyrimidin-4(3*H*)-ones **3**, in satisfactory yields.

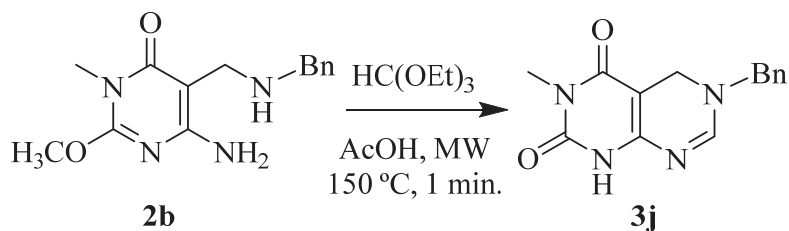
According to the literature, reactions with this kind of reagent need drastic conditions.^{27,28} An attempt to carry out these reactions by conventional heating gave decomposition of starting material because of the long reaction times required. To avoid conventional heating, we chose to assist the reaction with microwave irradiation, and we found that the reaction took place at 200 °C over 10–15 minutes with orthoester in excess (three- and four-fold molar) and in the absence of solvent to yield the desired pyrimidopyrimidines **3**, but not in good yields (< 45 %). This was probably due to the partial decomposition of the products under such conditions. From the different acid catalysts screened to optimize the reaction conditions, acetic acid was found to give the best results. After addition of acetic acid the reactions were greatly improved: the reaction time fell to 1 minute, the temperature for the reaction dropped to 150 °C, and yields were considerably increased (Table 2). Nevertheless no useful results were obtained when the 6-amino-5-(phenylaminomethyl)pyrimidin-4-one **2a** was taken as starting material.

Table 2. Data for the reaction of **2** with orthoesters to products **3a-i** with AcOH as catalyst (see Scheme 1)

Entry	X	R ¹	R ²	Compounds 3	
				Yield (%)	Mp °C
3a	O	CH ₂ C ₆ H ₅	H	65	178
3b	O	CH ₂ C ₆ H ₅	CH ₃	49	142
3c	O	C ₆ H ₁₁	H	80	227
3d	S	C ₆ H ₅	H	75	174
3e	S	C ₆ H ₅	CH ₃	60	146
3f	S	CH ₂ C ₆ H ₅	H	70	126
3g	S	CH ₂ C ₆ H ₅	CH ₃	67	115
3h	S	C ₆ H ₁₁	H	69	189
3i	S	C ₆ H ₁₁	CH ₃	53	237

Compounds **3** were fully characterized by spectroscopic and analytical data. From spectroscopic data of compounds **3**, the formation of the new pyrimidine ring is clearly observed: in the ¹H-NMR spectra the signals for NH are absent and instead the proton or methyl attached to C(7) appears at 7.40-8.12 ppm or around 2.0 ppm respectively; in the ¹³C-NMR the C(7) appears at 152-164 ppm.

In the hope of improving the reaction efficiency, equimolar amounts of 6-amino-5-(benzylaminomethyl)pyrimidine **2b**, ethyl orthoformate and acetic acid were tried; but in this case in addition to the cyclization reaction we found loss of the methoxy group at C(2), clearly indicated in the $^1\text{H-NMR}$ in which the NH of the lactam moiety was found (broad singlet at 10.90 ppm) for **3j** (Scheme 2). Something similar has been reported in other acid reactions in the presence of a nucleophile such as ethanol, which is a byproduct of the cyclocondensation step.^{26,29}



Scheme 2. Acetic acid in equimolar amount leads to demethylation and cyclization in the reaction of diaminopyrimidine **2b** with ethyl orthoformate.

All the pyrimidopyrimidines **3** were fully characterized, the most characteristic NMR feature being the afore-mentioned disappearance of the NH signals of **2** and the appearance of a new signal for C(7)-H in the case of **3a,c,d,f,h** or $-\text{CH}_3$ for **3b,e,g,i** depending on whether ethyl orthoformate or methyl orthoacetate were used.

X-Ray diffraction analysis was carried out on the pyrimido[4,5-*d*]pyrimidine **3a**, and its structure was unambiguously confirmed. The most relevant feature observed in its supra-molecular structure is the $\pi - \pi$ stacking interactions between phenyls which is reinforced by weak $\pi - \pi$ interactions between pyrimidine rings.

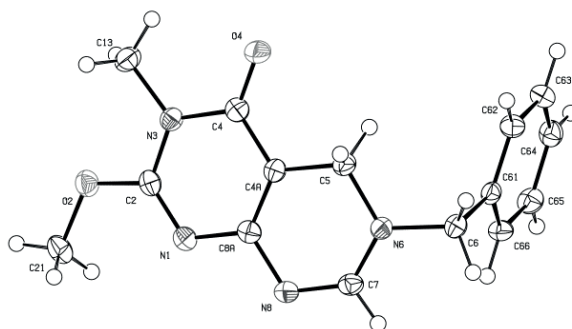
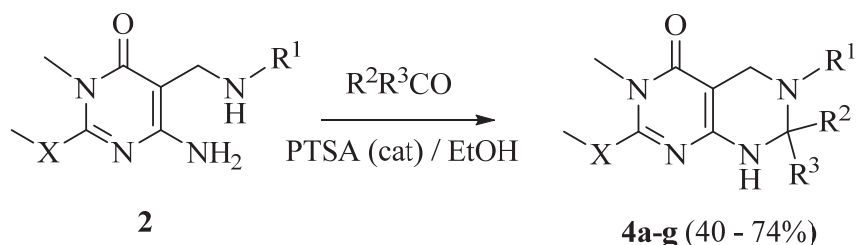


Figure 3. ORTEP drawing of the structure **3a** with 50% probability ellipsoids.

In order to extend the variety of hydroxypyrimido[4,5-*d*]pyrimidines available, the tetrahydro derivatives were prepared from the intermediates **2** and carbonyl compounds such as acetone and

benzaldehyde in a similar fashion to those reported for the preparation of tetrahydroquinazolines.³⁰ Mild reaction conditions were used: EtOH as solvent, a temperature of 50 °C, and *p*-toluenesulfonic acid as catalyst, which is needed because of the low nucleophilicity of the 6-amino group in **2** (Scheme 3 and Table 3).³¹



Scheme 3. Synthesis of 5,6,7,8-tetrahydropyrimido[4,5-*d*]pyrimidin-4(3*H*)-ones **4**.

Table 3. Data for products **4a-g**

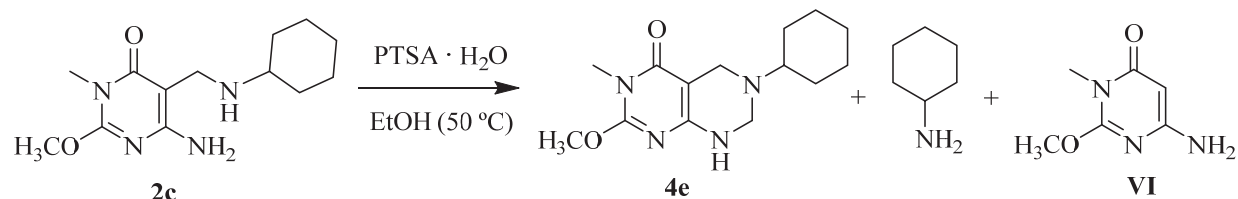
Entry	X	R ¹	R ²	R ³	Compounds 3	
					Yield (%)	Mp °C
4a	O	CH ₂ C ₆ H ₅	CH ₃	CH ₃	70	222
4b	S	CH ₂ C ₆ H ₅	CH ₃	CH ₃	74	220
4c	O	CH ₂ C ₆ H ₅	H	C ₆ H ₅	72	169
4d	S	CH ₂ C ₆ H ₅	H	C ₆ H ₅	55	156
4e^a	O	C ₆ H ₁₁	H	H	40	261
4f	S	C ₆ H ₁₁	H	C ₆ H ₅	67	126
4g	S	CH(CH ₃)C ₆ H ₅	H	C ₆ H ₅	38-72 ^b	115

^a Compound **4e** was isolated from the reaction of **2c** with acetone. ^b See Table 4.

The tetrahydropyrimidopyrimidines **4** were obtained in moderate to good yields and also fully characterized by spectroscopic and analytical data. From the spectroscopic data of compounds **4**, the formation of the new tetrahydropyrimidine ring is clearly observed, for example in ¹H-NMR spectra; as in the case of compounds **3**, the signals for NH largely disappear, there remaining just the N(8)-H, and substituents attached to C(7) are clearly observed, with the new C(7) appearing in the ¹³C-NMR at 59-71 ppm.

The reaction between **2c** and acetone afforded an unexpected product, which was characterized as **4e**. Its ¹H-NMR spectrum shows a signal at 4.78 ppm corresponding to the methylene for C(7), which is coupled with the N(8)-H, but not those corresponding to the methyl groups expected from acetone. So the acetone is not involved in the reaction in this case, as was also confirmed by mass spectrometry. To prove this possibility **2c** was reacted in ethanol with

PTSA in the absence of acetone. Under these conditions compound **4e**, together with the initial pyrimidine precursor **VI** and cyclohexylamine were obtained. Therefore, compound **2c** is acting in this reaction both as a diamine and a formaldehyde-releasing agent, to render **4e**, as shown in Scheme 4. This behavior can be explained by the higher basicity of the dialkylamino residue in molecule **2c**, compared to the other diamine derivatives **2**, that in acid media leads to its partial hydrolysis that releases formaldehyde (like in acetals) which is then trapped by another molecule of **2c**, forming compound **4e**.



Scheme 4. Compound **2c**: decomposition in acid medium.

From the mass spectrometry analysis for the dihydro (**3**) and tetrahydro (**4**) pyrimido[4,5-*d*]pyrimidine derivatives, similar fragmentation patterns were observed, characterized by progressive loss of pendant substituents and ring opening.

A single crystal X-ray diffraction on the tetrahydropyrimido[4,5-*d*]pyrimidine derivative **4c** was carried out (Figure 4), showing the predicted structure. Some considerations about the structure observed can be made: the puckering analysis shows a conformation of the saturated 6-membered ring close to the twist-boat, with substituents at N(6) and C(7) in a axial disposition to minimize the steric interaction between them.

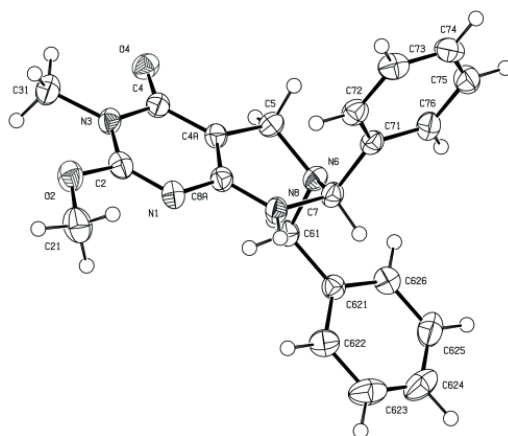


Figure 4. ORTEP drawing of the structure **4c** with 50% probability ellipsoids.

When benzaldehyde is used as reagent, a stereogenic center is generated at C(7) (see Figure 5). In the first instance we observed that in order to induce stereoselectivity in this reaction, the presence of a chiral residue in the starting material was needed, and therefore the enantiomeric

compounds **2g/2g'** were prepared by reductive amination of **1b** with (*R*)- and (*S*)-methylbenzylamine. The stereoselectivity of the reaction of **2g/2g'** with benzaldehyde was studied under several conditions. The conclusions of the chirality study are: slight importance of solvent (better results in acetonitrile than in ethanol), the necessity for a chiral auxiliary introduced in the molecule (R^1) (**2g/2g'**) and also a chiral acid catalyst (camphorsulfonic acid). Without chiral acid catalyst the d.e. of the reaction was 4% in EtOH and 33% in acetonitrile, while it reaches 88% upon the addition of camphorsulfonic acid (Table 4). When the substituent is the isomer *S* and the acid is *S* too, the preferred configuration generated is *S*. Likewise, when starting material and catalyst are *R*, *C*(7) is also *R*, and in both cases the diastereomeric excess obtained is around 88% (see Table 4).

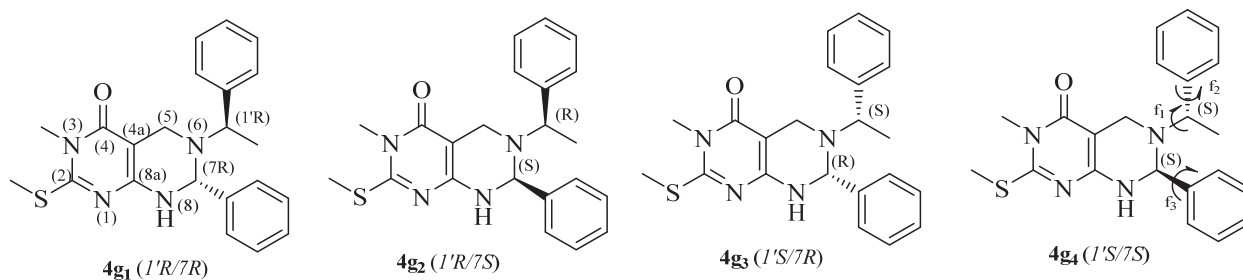


Figure 5. Set of diastereomeric structures formed by reaction of **2g/2g'** with benzaldehyde.

Table 4. Results of the stereochemical analysis of the chiral diaminopyrimidines **2g/2g'**

Major isomer	Starting chiral diamine (2)	Conditions	d.e [α (D)]	Yield (%)
4g₁	2g [<i>1'R</i> (+)]	EtOH, refl. / PTSA·H ₂ O (10%)	4% [+16.6°]	38
4g₁	2g [<i>1'R</i> (+)]	CH ₃ CN, 50 °C / PTSA·H ₂ O (10%)	33 %	44
4g₁	2g [<i>1'R</i> (+)]	EtOH, 50 °C / CSA (+) (10%)	86 %	72
4g₁	2g [<i>1'R</i> (+)]	CH ₃ CN, 50 °C / CSA (+) (10%)	88 % [+84.4°]	66
4g₁	2g [<i>1'R</i> (+)]	CH ₃ CN, 50 °C / CSA (-) (10%)	78 %	72
4g₄	2g' [<i>1'S</i> (-)]	EtOH, 50 °C / PTSA·H ₂ O (10%)	58 %	72
4g₄	2g' [<i>1'S</i> (-)]	CH ₃ CN, 50 °C / CSA (+) (10%)	50 %	56
4g₄	2g' [<i>1'S</i> (-)]	CH ₃ CN, 50 °C / CSA (-) (10%)	82 % [-77.2°]	68

Analysis by NMR was essential to determine the stereochemistry in the **4g** diastereomers and to calculate the diastereomeric excess for the reactions. NOESY experiments permitted

assignment of **4g₁** as the major diastereoisomer (see figures 1S and 2S in supporting information). The NOESY (figure 1S) shows the correlation between C(7)-H with 1'-H for **4g₁**, but in the case of **4g₂** are observed between C(7)-H with 1'-CH₃ and N(8)-H with 1'-H, which agrees with their optimized conformations.

Theoretical calculations

To support the experimental results, theoretical calculations have been done on compound **4g₁₋₄**. An exhaustive conformational study has been carried out using the Multidimensional Conformational Analysis (MDCA).³² A combination of semiempirical (PM6) and DFT (B3LYP/6-31G(d)) computations were employed in order to obtain the energetically preferred conformations. A detailed explanation about the conformational search is given in the experimental section. From the low-energy conformations obtained for both diastereomers *R/R* and *R/S* we calculated the shielding tensors using the GIAO approach^{33,34} from the program Gaussian 03.³⁵ Our study also includes the calculation of ¹³C-NMR chemical shift for the diastereoisomers (*R/R*; *R/S*; *S/R* and *S/S*) by using the gauge independent atomic orbital (GIAO) approximation.

Observing the curves obtained in figure 6, it is evident that the correlation obtained for the *R/R* enantiomer is excellent indicating that such assignment for **4a-g** is correct. Figures 6a and 6b give the correlations obtained for the experimentally obtained chemical shifts versus calculated chemical shifts obtained for *R/R* and *R/S*, respectively.

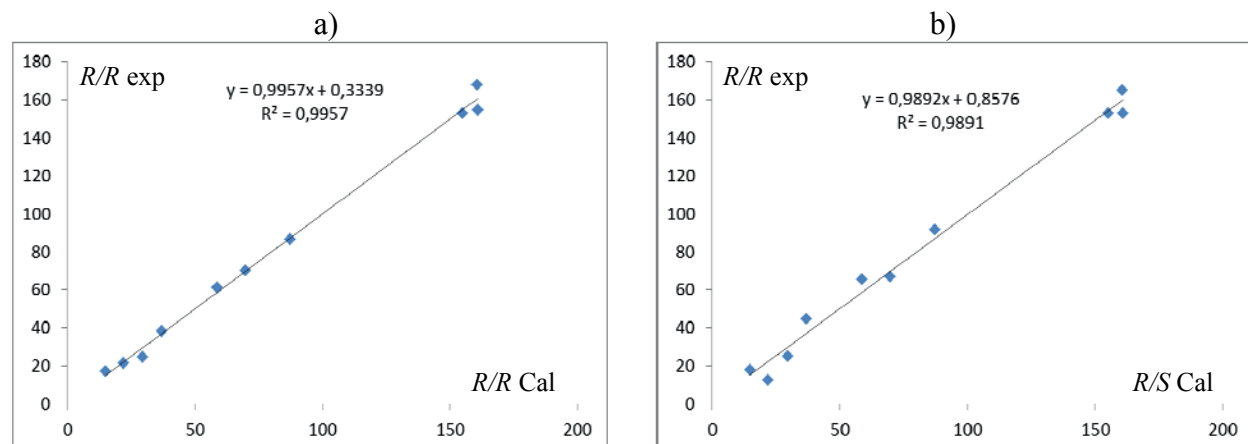


Figure 6. Chemical shifts correlations: (a) assigned to *R/R* vs calculated for *R/R*, and (b) assigned to *R/R* vs calculated for *R/S*

It is pertinent to remark that the theoretical calculations reported here are in agreement with the experimental results obtained from X-ray measurements. Figure 3S (supporting information) gives in a comparative fashion the conformations obtained from X-ray and from theoretical

calculations. The very similar spatial ordering obtained for both forms can be appreciated very well in this figure.

Antimicrobial activity

It should be noted that the synthesized compounds have a certain structural similarity with compounds **IV** and **V** shown in Figure 1 which have been reported as antibacterial agents. Thus, we decide to test these compounds against different pathogenic fungi and bacteria. Minimum Inhibitory Concentration (MIC) of each compound was determined by using broth microdilution techniques according to the guidelines of the National Committee for Clinical Laboratory Standards for yeasts (M27-A3) and for filamentous fungi (M 38-A2).³⁶ More details about these bioassays might be obtained from previous works.³⁷⁻³⁸

Most of the compounds described herein were evaluated against *Candida albicans*, *C. neoformans*, *Staphylococcus aureus* methicillin-sensitive ATCC 29213, *Staphylococcus aureus* methicillin-resistant ATCC 43300, *Escherichia coli* ATCC 25922, LM₁-*Escherichia coli* LM₂, *Pseudomonas aeruginosa* ATCC 27853, *PI-Yersinia enterocolitica*, *MI-Salmonella enteritidis* and *Salmonella sp* (LM). Unfortunately, none of the test compounds displayed antifungal nor antibacterial activity at the highest concentration tested (250 µg/mL). Other bioassays are in progress and will be the subject of another report in the near future.

Conclusions

We have developed the synthesis of a new series of 5,6-dihydro- and 5,6,7,8-tetrahydropyrimido[4,5-*d*]pyrimidin-4(3*H*)-ones starting from the corresponding 4-aminopyrimidines, via formation of 4-amino-(5-aminomethyl)pyrimidines as intermediates. The cyclocondensation was achieved using orthoesters under MW irradiation and solvent-free conditions. This latter reaction was optimized by acid catalyst to get the desired products in just 1 minute in good yields. This methodology is straightforward and the isolation of desired products is simple. These compounds have an attractive skeleton from a biological point of view, and they are being tested with the aim to find potential pharmacological applications.

Experimental Section

General. Melting points were determined on a Barstead Electrothermal 9100 melting point apparatus. IR spectra were recorded in KBr disks on a Bruker TENSOR 27 spectrophotometer at “Centro de Instrumentación Científico-Técnica (CICT) at Universidad de Jaén”. ¹H and ¹³C NMR spectra were recorded on a Bruker Avance 400 spectrophotometer (CICT) operating at 400 MHz and 100 MHz respectively, using CDCl₃ and DMSO-d₆ as solvents and tetramethylsilane as internal standard; the carbon type described p (primary), s (secondary), t (tertiary) and q

(quaternary) at ^{13}C -NMR was done using DEPT-135 and 2D-NMR experiments. Mass spectra were run on a Shimadzu-GCMS 2010-DI-2010 spectrometer (equipped with a direct inlet probe) operating at 70 eV. High resolution mass spectra were run on a Waters Micromass AutoSpec-Ultima spectrometer equipped with a direct inlet probe operating at 70 eV. Silica gel aluminum plates (Merck 60 F₂₅₄) were used for analytical TLC. The microwave-assisted reactions were carried out in a 10 ml glass sealed vial for focused mono-mode microwaves oven (Discover, by CEM Corp.) working in standard mode by controlling the target temperature. The starting amines and orthoesters were purchased from Aldrich, Fluka and Acros (analytical reagent grades) and were used without further purification.

Reductive amination of 6-aminopyrimidine-5-carbaldehydes (1): 6-amino-5-[(substituted-amino)methyl]pyrimidin-4(3H)-ones (2). General procedure. The 6-aminopyrimidine-5-carbaldehyde (**1**) (1 mmol) was suspended in anhydrous THF under argon. The requisite primary amine (2 mmol) and then sodium triacetoxyborohydride (2 mmol) were added and stirred was continued overnight at room temperature. The solvent was removed under reduced pressure and water (20 mL) was added. The solution was neutralized with aqueous sodium hydroxide (2 M). The desired colorless product (**2**) was precipitated, filtered off, and washed with water.

6-Amino-2-methoxy-3-methyl-5-[(phenylamino)methyl]pyrimidin-4(3H)-one (2a) from **1a** and aniline, yield 98 %. Mp 250-3 °C. IR (KBr): 3358 (b,m); 3182 (m); 2953 (w); 1644 (b,s); 1541 (b,s); 1485 (m); 1452 (m); 1411 (m); 1216 (m). ^1H -NMR (DMSO-*d*₆) δ (ppm): 3.19 (s, 3H); 3.87 (s, 3H); 3.94 (d, 5.4 Hz, 2H); 5.47 (t, 5.4 Hz, 1H); 6.35 (s, 2H); 6.46 (t, 7.2 Hz, 1H); 6.61 (d, 7.2 Hz, 2H); 7.00 (t, 7.2 Hz, 2H). ^{13}C -NMR (DMSO-*d*₆) δ (ppm): 27.0 p; 37.1 s; 55.0 p; 87.9 q; 112.3 t; 115.3 t; 128.6 t; 148.7 q; 155.1 q; 159.4 q; 162.0 q. MS (70 eV) *m/z* (%): 260 (M^+ , 10); 257 (23); 168 (100); 111 (10); 93 (3); 77 (5). HRMS (EI, 70 eV): calc for C₁₃H₁₄N₄O₂ 260.1273; found 260.1270.

6-Amino-5-[(benzylamino)methyl]-2-methoxy-3-methylpyrimidin-4(3H)-one (2b) from **1a** and benzylamine, yield 82 %. Mp 125-7 °C. IR (KBr): 3360 (m); 3311 (m); 3200 (b,m); 2952 (w); 2853 (w); 1623 (b,s); 1540 (b,s); 1487 (m); 1221 (m). ^1H -NMR (DMSO-*d*₆) δ (ppm): 3.16 (s, 3H), 3.52 (s, 2H), 3.60 (s, 2H); 3.88 (s, 3H); 6.47 (s, 2H); 7.17 – 7.24 (m, 2H); 7.25 – 7.30 (m, 4H). ^{13}C -NMR (DMSO-*d*₆) δ (ppm): 26.9 p; 42.9 s; 52.0 s; 55.0 p; 88.1 q; 127.4 t; 127.9 t; 128.0 t; 141.0 q; 155.0 q; 159.7 q; 161.9 q. MS (70 eV) *m/z* (%): 272 (M^+ , 4); 183 (100); 169 (83); 91 (30). HRMS (IE, 70 eV): calc for C₁₄H₁₆N₄O₂ 272.1273; found 272.1271.

6-Amino-5-[(cyclohexylamino)methyl]-2-methoxy-3-methylpyrimidin-4(3H)-one (2c) from **1a** and cyclohexylamine, yield 80 %. Mp 210-2 °C. IR (KBr): 3371 (s); 3296 (m); 3182 (b,m); 2920 (m); 2853 (m); 1635 (b,s); 1598 (m); 1546 (b,s); 1452 (s). ^1H -NMR (DMSO-*d*₆) δ (ppm): 0.94 – 1.02 (m, 2H); 1.08 – 1.18 (m, 3H); 1.48 – 1.52 (m, 1H); 1.61 – 1.63 (m, 2H); 1.79 – 1.82 (m, 2H); 2.26 – 2.31 (m, 1H); 3.15 (s, 3H); 3.50 (s, 2H); 3.87 (s, 3H); 6.49 (s, 2H). ^{13}C -NMR (DMSO-*d*₆) δ (ppm): 24.4 s; 25.9 s; 26.9 p; 32.8 s; 40.3 s; 54.8 t; 54.9 p; 88.5 q; 154.9 q;

159.7 q; 161.7 q. MS (70 eV) m/z (%): 183 (100); 168 (61); 111 (13); 98 (20); 56 (22). HRMS (IE, 70 eV): calc for $C_{13}H_{20}N_4O_2$ 264.1586; found 264.1588.

6-Amino-3-methyl-2-(methylthio)-5-[(phenylamino)methyl]pyrimidin-4(3H)-one (2d) from **1b** and aniline, yield 92 %. Mp 175-7 °C. IR (KBr): 3486 (m); 3386 (s); 3298 (m); 2922 (w); 2844 (w); 1600 (b,s); 1524 (b,s); 1317 (m); 1093 (m). 1H -NMR (DMSO- d_6) δ (ppm): 2.48 (s, 3H); 3.31 (s, 3H); 3.96 (d, 5.6 Hz, 2H); 5.51 (t, 5.6 Hz, 1H); 6.40 (sa, 2H); 6.47 (pt, 6.4 Hz, 1H); 6.61 (pd, 6.4 Hz, 2H); 7.00 (pt, 7.2 Hz, 2H). ^{13}C -NMR (DMSO- d_6) δ (ppm): 14.1 p; 29.5 p; 37.0 s; 89.8 q; 112.4 t; 115.4 t; 128.7 t; 148.6 q; 158.7 q; 159.8; 161.5 q. MS (70 eV) m/z (%): 276 (M^+ , 10); 184 (100); 138 (5); 93 (15). HRMS: calc for $C_{13}H_{16}N_4OS$ 276.1045; found 276.1047.

6-Amino-5-[(benzylamino)methyl]-3-methyl-2-(methylthio)pyrimidin-4(3H)-one (2e) from **1b** and benzylamine (0.22 ml) (78 %). M. p. 118-21 °C. IR (KBr): 3381 (m); 3284 (m); 2922 (w); 2859 (w); 1605 (b,s); 1513 (s); 1459 (m); 1411 (m); 1089 (m). 1H -NMR (DMSO- d_6) δ (ppm): 2.45 (s, 3H); 3.24 (s, 3H); 3.50 (s, 2H); 3.56 (s, 2H); 6.49 (sa, 2H); 7.10 – 7.20 (m, 1H); 7.20 – 7.30 (m, 4H). ^{13}C -NMR (DMSO- d_6) δ (ppm): 14.1 p; 29.4 p; 42.8 s; 52.1 s; 90.1 q; 126.5 t; 127.9 t; 128.1 t; 140.9 q; 159.2 q; 159.6 q; 161.5 q. MS (70 eV) m/z (%): 288 (8); 199 (100); 185 (68); 138 (15); 91 (17). HRMS: calc for $C_{14}H_{18}N_4OS$ 288.1045; found 288.1039.

6-Amino-5-[(cyclohexylamino)methyl]-3-methyl-2-(methylthio)pyrimidin-4(3H)-one (2f) from **1b** and cyclohexylamine (0.2 ml) (90 %). IR (KBr): 3380 (s); 3184 (b,m); 2918 (m); 2850 (m); 1607 (b,s); 1576 (m); 1521 (s); 1410 (m); 1095 (m). Mp 140-2 °C. 1H -NMR (DMSO- d_6) δ (ppm): 0.96 – 1.03 (m, 2H); 1.09 – 1.22 (m, 3H); 1.50 – 1.54 (m, 3H); 1.80 – 1.83 (m, 2H); 2.26 – 2.31 (m, 1H); 2.50 (s, 3H); 3.28 (s, 3H); 3.53 (s, 2H); 6.53 (sa, 2H). ^{13}C -NMR (DMSO- d_6) δ (ppm): 14.0 p; 24.4 s; 25.9 s; 29.3 p; 32.9 s; 40.2 s; 54.8 t; 90.6 q; 159.1 q; 159.2 q; 161.2 q. MS (70 eV) m/z (%): 199 (100); 184 (64); 138 (11); 98 (28); 56 (21). HRMS: calc for $C_{13}H_{22}N_4OS$ 282.1514; found 282.1507.

(R)-6-Amino-3-methyl-2-methylthio-5-[(1-phenylethyl)amino]methyl]pyrimidin-4(3H)-one (2g) from **1b** and D(+)- α -methylbenzylamine. The product was extracted with DCM (3 \times 20 mL). The organic extracts were dried over anhydrous Na_2SO_4 , and solvents removed under reduced pressure to afford an oily residue. The product was purified by flash chromatography (eluent DCM/MeOH 9:1; column diam. 2cm) to give a colorless oil (86%). $[\alpha]_D + 75.8$ (DCM; 25 °C; 0.01g/L). IR (KBr): 3442 (m, b); 2959 (w); 2924 (m); 2852 (w); 1630 (s, b); 1516 (s, b); 1452 (m); 1412 (m); 1095 (m, b). 1H -NMR (DMSO- d_6) δ (ppm): 1.41 (d, 6.6 Hz, 3H); 2.49 (s, 3H); 3.41 (s, 3H); 3.47 (d, 13.2 Hz, 1H), 3.74 (d, 13.2 Hz, 1H); 3.84 (q, 6.6 Hz, 1H); 5.45 (sa, 2H); 7.24 – 7.27 (m, 1H); 7.31 – 7.37 (m, 4H). ^{13}C -NMR (DMSO- d_6) δ (ppm): 14.6 p; 23.5 p; 30.0 p; 42.1 s; 58.2 t; 91.8 q; 126.7 t; 127.3 t; 128.6 t; 144.6 q; 159.6 q; 160.8 q; 162.5 q. MS (70 eV) m/z (%): 211 (47); 199 (100); 184 (48); 138 (8); 120 (74); 105 (25); 88 (15). HRMS: calc for $C_{15}H_{20}N_4OS$: 316.1358; found 316.1356.

(S)-6-Amino-3-methyl-2-methylthio-5-[(1-phenylethyl)amino]methyl]pyrimidin-4(3H)-one (2g') from **1b** (1 mmol) and L(-)- α -methylbenzylamine. The workup as for **2g** gave a colorless oil (81%). $[\alpha]_D -74.4$ (DCM; 25 °C; 0.01g/L). NMR and IR are the same as for **2g**. MS (70 eV)

m/z (%): 211 (4547); 199 (100); 184 (5348); 138 (8); 120 (7374); 105 (25); 88 (1315).. HRMS: calc for $C_{15}H_{20}N_4OS$: 316.1358; found 316.1355.

Synthesis of pyrimido[4,5-*d*]pyrimidines (3) by cyclocondensation of 2 with orthoesters: general procedure. A mixture of 6-amino-5-aminomethylpyrimidine (2) (1 mmol), the orthoester (0.5 ml) and acetic acid (one drop) was placed into a sealed MW glass tube and irradiated in the microwave oven for 1 minute (150 °C, max. power 200W). After cooling, hexane was added and the solid that separated was filtered off, washed with cold diethyl ether, and recrystallized from ethanol.

6-Benzyl-2-methoxy-3-methyl-5,6-dihydropyrimido[4,5-*d*]pyrimidin-4(3*H*)-one (3a) from **2b** and triethyl orthoformate: colorless solid (65 %). Mp 175-82 °C. IR (KBr): 2955 (b,w); 1705 (m); 1654 (b,s); 1556 (b,m); 1496 (m); 1455 (m); 1436 (m); 1212 (m); 1156 (m). 1H -NMR ($CDCl_3$) δ (ppm): 3.33 (s, 3H); 4.03 (s, 3H); 4.23 (s, 2H); 4.33 (s, 2H); 7.28 (pd, 7.0 Hz, 2H); 7.33 – 7.41 (m, 3H); 7.40 (s, 1H). ^{13}C -NMR ($CDCl_3$) δ (ppm): 27.6 p; 43.3 s; 55.8 p; 57.4 s; 91.5 q; 127.9 t; 128.5 t; 129.0 t; 133.5 q; 155.9 t; 156.5 q; 157.9 q; 161.6 q. MS (70 eV) m/z (%): 284 (M^+ , 5); 193 (20); 182 (13); 168 (20); 133 (17); 106 (35); 91 (100); 75 (31); 65 (37). HRMS: calc for $C_{15}H_{16}N_4O_2$ 284.1273; found 284.1271.

Crystal data for **3a** were deposited at CCDC with reference 809643: Chemical formula $C_{15}H_{16}N_4O_2$, M_r 284.32, Orthorhombic, *Pbca*, 120 K, cell dimensions *a*, *b*, *c* (Å) 17.948 (5), 7.8393 (15), 18.8278 (13); α , β , γ (°) 90, 90, 90, V (Å³) 2649.1 (9), Z = 8, $F(000)$ = 1200, D_x (Mg m⁻³) = 1.426, $Mo K\alpha$, μ (mm⁻¹) = 0.10, Crystal size (mm) = 0.3 × 0.24 × 0.22. Data collection: Diffractometer KappaCCD diffractometer, Monochromator graphite, CCD rotation images, thick slices ϕ & θ scans, absorption correction *SADABS* 2.10, T_{min} , T_{max} 0.971, 0.979. No. of measured, independent and observed [$I > 2\sigma(I)$] reflections 23485, 3043, 2071, R_{int} = 0.064, θ values (°): θ_{max} = 27.5, θ_{min} = 3.1; Range $h = -23 \rightarrow 23$, $k = -10 \rightarrow 8$, $l = -22 \rightarrow 24$, Refinement on F^2 : $R[F^2 > 2\sigma(F^2)]$ = 0.048, $wR(F^2)$ = 0.128, S = 1.09. No. of reflections 3043, No. of parameters 192, No. of restraints 0. Weighting scheme: $w = 1/[\sigma^2(F_o^2) + (0.0505P)^2 + 1.7324P]$ where $P = (F_o^2 + 2F_c^2)/3$. (Δ/σ)_{max} = 0.001, $\Delta\sigma_{max}$, $\Delta\sigma_{min}$ (e Å⁻³) 0.31, -0.34.

6-Benzyl-2-methoxy-3,7-dimethyl-5,6-dihydropyrimido[4,5-*d*]pyrimidin-4(3*H*)-one (3b) from **2b** and trimethyl orthoacetate: yellow solid (49 %). Mp 142-3 °C. IR (KBr): 3006 (w); 2955 (b,w); 2861 (b,w); 2811 (w); 1736 (w); 1666 (s); 1599 (m); 1558 (s); 1533 (s); 1471 (b,s); 1430 (m); 1412 (m). 1H -NMR ($CDCl_3$) δ (ppm): 2.29 (s, 3H); 3.34 (s, 3H); 4.05 (s, 3H); 4.34 (s, 2H); 4.51 (s, 2H); 7.24-7.39 (m, 5H). ^{13}C -NMR ($CDCl_3$) δ (ppm): 22.5 p; 27.6 p; 46.2 s; 54.8 s; 55.8 p; 91.5 q; 126.6 t; 128.1 t; 129.1 t; 134.1 q; 156.6 q; 158.0 q; 161.4 q; 164.2 q. MS (70 eV) m/z (%): 298 (M^+ , 2); 207 (27); 91 (100). HRMS: calc for $C_{16}H_{18}N_4O_2$ 298.1430; found 298.1429.

6-Cyclohexyl-2-methoxy-3-methyl-5,6-dihydropyrimido[4,5-*d*]pyrimidin-4(3*H*)-one (3c) from **2c** and triethyl orthoformate: colorless solid (80 %). Mp 226-8 °C. IR (KBr): 3177 (m); 2932 (m); 2856 (w); 1635 (b,s); 1548 (b,s); 1487 (m); 1455 (m); 1410 (m); 1377 (m). 1H -NMR ($CDCl_3$) δ (ppm): 1.15-1.24 (m, 1H); 1.32-1.41 (m, 2H); 1.57-1.66 (m, 2H); 1.72 (pd, 13.8 Hz, 1H); 1.93 (pd, 10.7 Hz, 4H); 3.31-3.38 (m, 1H); 3.38 (s, 3H); 4.05 (s, 3H); 4.44 (s, 2H); 8.12 (s, 1H). ^{13}C -NMR ($CDCl_3$) δ (ppm): 24.8 s; 25.1 s; 27.9 p; 30.1 s; 41.4 s; 56.4 p; 64.4 t; 90.3 q;

153.0 q; 154.1 t; 157.2 q; 161.1 q. MS (70 eV) m/z (%): 276 (M^+ , 55); 275 (71); 261 (22); 193 (100); 179 (24); 136 (17); 83 (4). HRMS: calc for $C_{14}H_{20}N_4O_2$ 276.1586; found 276.1589.

3-Methyl-2-(methylthio)-6-phenyl-5,6-dihydropyrimido[4,5-*d*]pyrimidin-4(3*H*)-one (3d) from **2d** and triethyl orthoformate. Colorless solid (75 %). Mp 173-5 °C. IR (KBr): 3063 (w); 2924 (w); 2854 (w); 1655 (b,s); 1594 (m); 1547 (m); 1516 (s); 1437 (m); 1405 (b,m); 1225 (m). 1H -NMR ($CDCl_3$) δ (ppm): 2.64 (s, 3H); 3.51 (s, 3H); 4.85 (s, 2H); 7.24-7.31 (m, 3H); 7.45 (pt, 7.4 Hz, 2H); 7.79 (s, 1H). ^{13}C -NMR ($CDCl_3$) δ (ppm): 15.0 p; 30.1 p; 44.2 s; 94.4 q; 119.2 t; 126.4 t; 129.8 t; 142.0 q; 153.2 t; 156.5 q; 160.9 q; 162.6 q. MS (70 eV) m/z (%): 286/288 ($M/M+2$, 66/13); 271 (47); 198 (24); 77 (100). HRMS: calc for $C_{14}H_{14}N_4OS$ 286.0888; found 286.0891.

3,7-Dimethyl-2-(methylthio)-6-phenyl-5,6-dihydropyrimido[4,5-*d*]pyrimidin-4(3*H*)-one (3e) from **2d** and trimethyl orthoacetate: yellow solid (60 %). Mp 145-8 °C. IR (KBr): 3039 (w); 2928 (w); 1724 (m); 1645 (b,s); 1513 (b,s); 1477 (m); 1448 (b,m); 1410 (m); 1342 (m). 1H -NMR ($DMSO-d_6$) δ (ppm): 1.81 (s, 3H); 2.50 (s, 3H); 3.34 (s, 3H); 4.54 (s, 2H); 7.35-7.41 (m, 1H); 7.44-7.51 (m, 4H). ^{13}C -NMR ($DMSO-d_6$) δ (ppm): 14.4 p; 23.0 p; 29.6 p; 48.8 s; 93.2 q; 126.3 t; 128.0 t; 129.9 t; 143.3 q; 157.1 q; 159.5 q; 161.0 q; 162.8 q. MS (70 eV) m/z (%): 300 (M^+ , 62); 299 (100); 285 (42); 77 (27). HRMS: calc for $C_{15}H_{16}N_4OS$ 300.1045; found 300.1044.

6-Benzyl-3-methyl-2-(methylthio)-5,6-dihydropyrimido[4,5-*d*]pyrimidin-4(3*H*)-one (3f) from **2e** and triethyl orthoformate: colorless solid (70 %). Mp 126 °C. IR (KBr): 2996 (w); 2931 (w); 1701 (s); 1661 (b,s); 1560 (s); 1519 (s); 1461 (m); 1428 (s); 1367 (b,s); 1284 (b,m). 1H -NMR ($CDCl_3$) δ_{ppm} : 2.61 (s, 3H); 3.43 (s, 3H); 4.25 (s, 2H); 4.33 (s, 2H); 7.27-7.38 (m, 5H); 7.41 (s, 1H). ^{13}C -NMR ($CDCl_3$) δ (ppm): 15.0 p; 29.9 p; 43.1 s; 57.5 s; 93.5 q; 127.9 t; 128.6 t; 129.1 t; 133.4 q; 156.0 t; 157.4 q; 161.1 q; 162.1 q. MS (70 eV) m/z (%): 300 (M^+ , 4); 209 (33); 91 (100). HR MS: calc for $C_{15}H_{16}N_4OS$ 300.1045; found 300.1042.

6-Benzyl-3,7-dimethyl-2-(methylthio)-5,6-dihydropyrimido[4,5-*d*]pyrimidin-4(3*H*)-one (3g) from **2e** and trimethyl orthoacetate: yellow solid (67 %). Mp 114-16 °C. 1H -NMR ($CDCl_3$) δ (ppm): 2.30 (s, 3H); 2.63 (s, 3H); 3.44 (s, 3H); 4.36 (s, 2H); 4.51 (s, 2H); 7.24-7.39 (m, 5H). ^{13}C -NMR ($CDCl_3$) δ (ppm): 14.9 p; 22.6 p; 29.6 p; 46.0 s; 54.8 s; 93.5 c; 126.6 t; 128.1 t; 129.1 t; 134.0 q; 157.6 q; 160.8 q; 162.0 q; 164.3 q. IR (KBr): 2999 (w); 2927 (w); 2841 (w); 1720 (w); 1650 (b,s); 1519 (b,s); 1484 (m); 1453 (m); 1408 (m). MS (70 eV) m/z (%): 314/316 ($M/M+2$, 4/1); 223 (37); 91 (100). HR MS: calc for $C_{16}H_{18}N_4OS$ 314.1197; found 314.1201.

6-Cyclohexyl-3-methyl-2-(methylthio)-5,6-dihydropyrimido[4,5-*d*]pyrimidin-4(3*H*)-one (3h) from **2f** and triethyl orthoformate: colorless solid (69 %). Mp 187-91 °C. IR (KBr): 2934 (w); 2856 (m); 1709 (m); 1650 (b,s); 1591 (m); 1520 (b,s); 1460 (m); 1412 (m). 1H -NMR ($CDCl_3$) δ (ppm): 1.15 (tq, 3.7 y 13.0 Hz, 1H); 1.35 (qt, 3.7, 13.0 Hz, 2H); 1.60 (qd, 3.5 Hz y 12.0 Hz, 2H); 1.70 (pd, 13.7 Hz, 1H); 1.89 (pt, 13.2 Hz, 4H); 2.60 (s, 3H); 3.20 (tt, 3.3 Hz y 12.0 Hz, 1H); 3.47 (s, 3H); 4.43 (s, 2H); 7.65 (s, 1H). ^{13}C -NMR ($CDCl_3$) δ (ppm): 15.0 p; 24.9 s; 25.2 s; 30.0 p; 30.1 s; 63.4 t; 92.6 q; 154.8 t; 155.6 q; 160.8 q; 162.8 q. MS (70 eV) m/z (%): 292/294 ($M/M+2$, 72/5); 277 (29); 209 (100); 195 (22); 83 (4). HRMS: calc for $C_{14}H_{20}N_4OS$ 292.1358; found 292.1351.

6-Cyclohexyl-3,7-dimethyl-2-(methylthio)-5,6-dihydropyrimido[4,5-*d*]pyrimidin-4(3*H*)-one (3i) from **2f** and trimethyl orthoacetate: yellow solid (53 %). Mp 235-40 °C. IR (KBr): 3180 (b,w); 3124 (b,w); 2935 (b,w); 2886 (w); 1691 (m); 1641 (b,s); 1591 (s); 1523 (s); 1485 (m); 1405 (m). ¹H-NMR (CDCl₃) δ (ppm): 1.12 (tq, 3.7 Hz, 13.2 Hz, 1H); 1.27-1.40 (m, 2H); 1.69-1.72 (m, 4H); 1.90 (pd, 13.8 Hz, 2H); 2.24 (s, 3H); 2.61 (s, 3H); 3.47 (s, 3H); 3.59-3.68 (m, 1H); 4.35 (s, 2H). ¹³C-NMR (CDCl₃) δ (ppm): 15.0 p; 22.8 p; 25.1 s; 25.6 s; 29.1 s; 29.9 p; 39.4 s; 59.0 t; 92.9 q; 158.0 q; 160.9 q; 161.8 q; 164.0 q. MS (70 eV) *m/z* (%): 305/307 (M/M+2, 26/7); 223 (100); 209 (17); 177 (10); 83 (20). HRMS: calc for C₁₅H₂₂N₄OS 306.1514; found 306.1509.

6-Benzyl-3-methyl-5,6-dihydropyrimido[4,5-*d*]pyrimidin-2,4(1*H*,3*H*)-dione (3j) from **2b**, triethyl orthoformate and 0.06 ml of acetic acid for 5 minutes: yellow solid (25%). Mp 239-41 °C. IR (KBr): 3097 (b,w); 2883 (b,w); 2782 (b,w); 1709 (m); 1662 (s); 1625 (s); 1581 (m); 1453 (b,m); 1436 (m). ¹H-NMR (DMSO-*d*₆) δ (ppm): 3.03 (s, 3H); 3.94 (s, 3H); 4.48 (s, 2H); 7.35-7.43 (m, 5H); 7.67 (s, 1H); 10.90 (ws, 1H). ¹³C-NMR (DMSO-*d*₆) δ (ppm): 26.0 p; 41.5 s; 55.8 s; 82.0 q; 128.0 t; 128.1 t; 128.6 t; 134.3 q; 150.2 q; 150.7 q; 156.6 t; 161.5 q. MS (70 eV) *m/z* (%): 270 (M⁺, 4); 179 (29); 91 (100); 65 (25). HRMS: calc for C₁₄H₁₄N₄O₂ 270.1117; found 270.1116.

Synthesis of pyrimido[4,5-*d*]pyrimidines (4) by reaction with carbonyl compounds: general procedure. The 6-amino-5-(aminomethyl)pyrimidine (**2**) (1 mmol) in a solvent (3ml), the carbonyl compound (1 mmol) and catalytic acid (0.05 mmol) were stirred and heated to 50 °C. for the indicated time. The solution was then cooled to room temperature, The product was collected by filtration, washed with cold diethyl ether and recrystallized from EtOH.

6-Benzyl-3,7,7-trimethyl-2-methoxy-5,6,7,8-tetrahydropyrimido[4,5-*d*]pyrimidin-4(3*H*)-one (4a) from **2b** in EtOH, acetone and *p*-TsOH·H₂O. Reaction time: 20h. Colorless solid (70%). Mp 222 °C. IR (KBr): 3285 (s); 2962 (m); 2801 (w); 2769 (w); 1639 (s); 1616 (s); 1598 (s); 1531 (s); 1223 (s). ¹H-NMR (CDCl₃). δ (ppm): 1.45 (s, 6H); 3.32 (s, 3H); 3.60 (s, 2H); 3.61 (s, 2H); 3.93 (s, 3H); 4.83 (s, 1H); 7.20 (pt, 7.0 Hz, 1H); 7.25-7.35 (m, 4H). ¹³C-NMR (CDCl₃). δ (ppm): 27.00 p; 27.14 p; 43.38 s; 53.19 s; 55.10 p; 69.45 q; 85.70 q; 126.74 t; 128.26 t; 128.39 t; 140.06 q; 154.64 q; 155.86 q; 161.48 q. MS (70eV) *m/z* (%): 314 (M⁺, 9); 299 (46); 223 (16); 207 (35); 194 (18); 168 (95); 146 (45); 91 (100). HRMS: calc for C₁₇H₂₂N₄O₂ 314.1743; found 314.1730.

6-Benzyl-3,7,7-trimethyl-2-(methylthio)-5,6,7,8-tetrahydropyrimido[4,5-*d*]pyrimidin-4(3*H*)-one (4b) from **2e** in EtOH, acetone and *p*-TsOH·H₂O. Reaction time: 22h. Colorless solid (74%). Mp 219-21 °C. IR (KBr): 3293 (s); 2993 (w); 2927 (w); 2840 (w); 1628 (s); 1595 (s); 1539 (s); 1497 (s); 1164 (m). ¹H-NMR (CDCl₃). δ (ppm): 1.46 (s, 6H); 2.51 (s, 3H); 3.43 (s, 3H); 3.60 (s, 2H); 3.63 (s, 2H); 4.89 (s, 1H); 7.20 (pt, 7.0 Hz, 1H); 7.25-7.35 (m, 4H). ¹³C-NMR (CDCl₃). δ_{ppm}: 14.66 p; 27.07 p; 29.55 p; 43.25 s; 53.20 s; 69.39 q; 87.78 q; 126.79 t; 128.29 t; 128.38 t; 139.95 q; 154.52 q; 160.84 q. MS (70eV) *m/z* (%): 330 (M⁺, 7); 315 (49); 223 (33); 210 (17); 184 (55); 146 (65); 91 (100). HRMS: calc for C₁₇H₂₂N₄OS 330.1514; found 330.1511.

6-Benzyl-2-methoxy-3-methyl-7-phenyl-5,6,7,8-tetrahydropyrimido[4,5-*d*]pyrimidin-4(3*H*)-one (4c) from **2b** in EtOH, benzaldehyde and *p*-TsOH·H₂O. Reaction time: 1h 30min. 10 ml of water is added to the reaction mixture and neutralized with NaHCO₃, extracted with DCM (20ml

× 3) and dried with Na₂SO₄. The solvent is evaporated under reduced pressure. Colorless solid (72%). Mp 168-70 °C. IR (KBr): 3301 (m, b); 3026 (w); 2942 (w); 2903 (w); 2850 (w); 1658 (s, b); 1608 (m); 1548 (s); 1519 (s, b); 1428 (m). ¹H-NMR (CDCl₃). δ (ppm): 3.33 (s, 3H); 3.47 (d, 15.9Hz, 1H); 3.58 (d, 15.9Hz, 1H); 3.67 (d, 13.2Hz, 1H); 3.73 (d, 13.2Hz, 1H); 3.99 (s, 3H); 5.07 (bs, 1H); 5.16 (bs, 1H); 7.26-7.44 (m, 10H). ¹³C-NMR (CDCl₃). δ (ppm): 27.21 p; 43.00 s; 55.23 p; 56.97 s; 71.24 t; 86.18 q; 126.82 t; 127.22 t; 127.98 t; 128.38 t; 128.46 t; 128.97 t; 138.77 q; 141.04 q; 154.72 q; 155.94 q; 161.41 q. MS (70eV) *m/z* (%): 361 (M⁺-1, 50); 285 (59); 271 (59); 257 (45); 242 (41); 168 (83); 91(100). HR MS: calc for C₂₁H₂₂N₄O₂ 362.1743; found 362.1732.

Crystal data for **4c** were deposited at CCDC with reference CCDC 945842: Chemical formula C₂₁H₂₂N₄O₂, *M_r* 362.43, Monoclinic, *C2/c*, 120 K, cell dimensions *a*, *b*, *c* (Å) 21.527 (3), 11.1709 (8), 17.448 (4); α, β, γ (°) 90, 120, 144 (11), 90, *V* (Å³) 3628,5 (10), *Z* = 8, *F*(000)=1200, *D_x* (Mg m⁻³) = 1.426, Mo *Kα*, μ (mm⁻¹)=0.10, Crystal size (mm)= 0.32 × 0.25 × 0.22. Data collection: Diffractometer KappaCCD diffractometer, Monochromator graphite, CCD rotation images, thick slices φ & θ scans, absorption correction *SADABS* 2.10, *T_{min}*, *T_{max}* 0.9724, 0.9809. No. of measured, independent and observed [*I* > 2σ(*I*)] reflections 27121, 4163, 2179, *R_{int}*= 0.095, θ values (°): θ_{max} = 27.5, θ_{min} = 5.0; Range *h* = -23→23, *k* = -10→8, *l* = -22→24, Refinement on *F*²: *R*[*F*² > 2σ(*F*²)] = 0.058, *wR*(*F*²) = 0.167, *S* = 1.07. No. of reflections 4163, No. of parameters 246, No. of restraints 0. Weighting scheme: *w* = 1/[σ²(*F_o*²) + (0.0505*P*)² + 1.7324*P*] where *P* = (*F_o*² + 2*F_c*²)/3. (Δ/σ)_{max} < 0.001, Δσ_{max}, Δσ_{min} (e Å⁻³) 0.25, -0.31.

6-Benzyl-3-methyl-2-(methylthio)-7-phenyl-5,6,7,8-tetrahydropyrimido[4,5-*d*]pyrimidin-4(3*H*)-one (4d) from **2e** in EtOH, benzaldehyde and *p*-TsOH·H₂O. Reaction temperature: reflux. Reaction time: 40min. Colorless solid (55%). Mp 155-7 °C. IR (KBr): 3261 (m, b); 3030 (w); 2842 (w); 1654 (s); 1629 (s); 1595 (s); 1527 (s); 1508 (s); 1073 (m). ¹H-NMR (CDCl₃). δ (ppm): 2.55 (s, 3H); 3.44 (s, 3H); 3.49 (d, 16.4Hz, 1H); 3.60 (d, 16.4Hz, 1H); 3.67 (d, 13.5Hz, 1H); 3.73 (d, 13.5Hz, 1H); 5.08 (s, 1H); 5.26 (s, 1H); 7.26-7.44 (m, 10H). ¹³C-NMR (CDCl₃). δ (ppm): 14.72 p; 29.62 p; 42.81 s; 57.03 s; 71.08 t; 88.21 c; 126.79 t; 127.22 t; 128.00 t; 128.37 t; 128.46 t; 128.93 t; 138.66 q; 140.98 q; 154.54 q; 160.76 q; 160.88 q. MS (70eV) *m/z* (%): 378 (M⁺, 38); 301 (57); 287 (48); 273 (38); 256 (22); 184 (62); 91(100); 88 (34). HRMS: calc for C₂₁H₂₂N₄O₂S 378.1514; found 378.1508.

6-Cyclohexyl-3-methyl-2-methoxy-5,6,7,8-tetrahydropyrimido[4,5-*d*]pyrimidin-4(3*H*)-one (4e) from **2c** in EtOH and *p*-TsOH·H₂O. Reaction time: 15h. Colorless solid (40%). Mp 260-3 °C. IR (KBr): 3251 (m, b); 2927 (m); 2853 (m); 1640 (s, b); 1607 (m); 1557 (s); 1527 (s); 1462 (m); 1415 (m). ¹H-NMR (CDCl₃). δ (ppm): 1.10-1.29 (m, 5H); 1.60 (pd, 11.2 Hz, 1H); 1.74-1.81 (m, 2H); 1.97 (pd, 10.3 Hz, 2H); 2.44-2.52 (m, 1H); 3.33 (s, 3H); 3.76 (s, 2H); 3.91 (s, 3H); 4.23 (d, 2.9Hz, 2H); 4.78 (bs, 1H). ¹³C-NMR (CDCl₃). δ (ppm): 25.43 s; 25.94 s; 27.18 p; 30.63 s; 43.97 s; 55.12 p; 58.39 t; 59.60 s; 87.51 q; 155.68 q; 156.24 q; 161.27 q. MS (70eV) *m/z* (%): 278 (M⁺, 53); 277 (100); 195 (76); 180 (98); 168 (58); 72 (26); 55 (34). HRMS: calc for C₁₄H₂₂N₄O₂ 278.1743; found 278.1738.

6-Cyclohexyl-3-methyl-2-(methylthio)-7-phenyl-5,6,7,8-tetrahydropyrimido[4,5-*d*]pyrimidin-4(3*H*)-one (4f) From **2f** in EtOH, benzaldehyde and (±)camphor-10-sulfonic acid. Reaction time: 22h. Colorless solid (67%). Mp 189-90 °C. ¹H-NMR (CDCl₃). δ (ppm): 1.11-1.50 (m, 5H); 1.57 (bs, 1H); 1.69-1.83 (m, 2H); 1.89 (pd, 12.4 Hz, 1H); 1.98 (pd, 12.4 Hz, 1H); 2.49-2.58 (m, 4H); 3.32 (d, 16.7Hz, 1H); 3.43 (s, 3H); 3.80 (d, 16.7Hz, 1H); 5.22 (s, 1H); 5.41 (s, 1H); 7.22-7.40 (m, 5H). ¹³C-NMR (CDCl₃). δ (ppm): 14.7 p; 25.4 s; 25.5 s; 26.0 s; 29.6 p; 30.5 s; 31.9 s; 58.4 t; 69.8 t; 89.5 c; 126.8 t; 127.7 t; 128.4 t; 142.0 q; 155.2 q; 160.2 q; 160.6 q. IR (KBR): 3332 (m, b); 2930 (m); 2851 (m); 1644 (s, b); 1593 (s); 1581 (s); 1511 (s, b); 1073 (m). MS (70eV) *m/z* (%): 370 (M⁺, 64); 369 (42); 355 (32); 293 (83); 287 (82); 272 (100); 184 (56); 98 (30); 88 (60). HRMS: calc for C₂₀H₂₆N₄OS 370.1827; found 370.1823.

3-Methyl-2-(methylthio)-7(*R*)-phenyl-6-[(*R*)-1-phenylethyl]-5,6,7,8-tetrahydropyrimido[4,5-*d*]pyrimidin-4(3*H*)-one (4g₁) from **2g** in CH₃CN, benzaldehyde and (*R*)-camphor-10-sulfonic acid (CSA-*R*). Reaction time: 18h. Colorless solid (66%). Mp 160-2 °C. [α]_D +84.4 (DCM; 25°C; 0.01g/l). ¹H-NMR (CDCl₃). δ (ppm): 1.39 (d, 6.6Hz, 3H); 2.56 (s, 3H); 3.31 (d, 17.2Hz, 1H); 3.45 (s, 3H); 3.73 (q, 6.6Hz, 1H); 4.07 (dd, 1.9 y 17.2Hz, 1H); 4.98 (d, 3.5Hz, 1H); 5.14 (d, 3.5Hz, 1H); 7.23-7.29 (m, 6H); 7.34-7.36 (m, 2H); 7.50-7.55 (m, 2H). ¹³C-NMR (CDCl₃). δ (ppm): 14.7 p; 21.8 p; 36.9 s; 58.7 t; 69.8 t; 87.2 c; 126.5 t; 127.6 t; 128.3 t; 128.6 t; 129.0 t; 129.7 t; 134.4 t; 142.1 q; 145.4 q; 154.9 q; 160.6 q; 160.9 q. IR (KBR): 3266 (m, b); 3030 (w); 2975 (w); 2928 (w); 2865 (w); 1700 (m); 1638 (s); 1595 (s); 1539 (s); 1510 (s); 1554 (m); 1413 (m). MS (70eV) *m/z* (%): 392 (M⁺, 14); 315 (15); 287 (100); 273 (22); 258 (30); 184 (43); 105 (71); 88 (22). HRMS: calc for C₂₂H₂₄N₄OS 392.1671; found 392.1667.

3-Methyl-2-(methylthio)-7(*S*)-phenyl-6-[(*S*)-1-phenylethyl]-5,6,7,8-tetrahydropyrimido[4,5-*d*]pyrimidin-4(3*H*)-one (4g₄) from **2g'** in CH₃CN, benzaldehyde and (*S*)-camphor-10-sulfonic acid (CSA-*S*). Reaction time: 15h. Colorless solid (68%). [α]_D -77.2 (DCM; 25°C; 0,01g/l). ¹H-NMR (CDCl₃). δ (ppm): 1.39 (d, 6.6Hz, 3H); 2.56 (s, 3H); 3.31 (d, 17.2Hz, 1H); 3.45 (s, 3H); 3.73 (q, 6.6Hz, 1H); 4.07 (dd, 1.9 y 17.2Hz, 1H); 4.98 (d, 3.5Hz, 1H); 5.14 (d, 3.5Hz, 1H); 7.23-7.29 (m, 6H); 7.34-7.36 (m, 2H); 7.50-7.55 (m, 2H). ¹³C-NMR (CDCl₃). δ (ppm): 14.7 p; 21.8 p; 36.9 s; 58.7 t; 69.8 t; 87.2 c; 126.5 t; 127.6 t; 128.3 t; 128.6 t; 129.0 t; 129.7 t; 134.4 t; 142.1 q; 145.4 q; 154.9 q; 160.6 q; 160.9 q.

3-Methyl-2-(methylthio)-7(*S*)-phenyl-6-[(*R*)-1-phenylethyl]-5,6,7,8-tetrahydropyrimido[4,5-*d*]pyrimidin-4(3*H*)-one (4g₂); 3-methyl-2-(methylthio)-7(*R*)-phenyl-6-[(*S*)-1-phenylethyl]-5,6,7,8-tetrahydropyrimido[4,5-*d*]pyrimidin-4(3*H*)-one (4g₃) ¹H-NMR (CDCl₃). δ (ppm): 1.49 (d, 6.6 Hz, 3H); 2.55 (s, 3H); 3.18 (d, 17.2 Hz, 2H); 3.39 (s, 3H); 3.80 (q, 6.6 Hz, 1H); 5.30 (d, 3.5Hz, 1H); 5.52 (bs, 1H); 7.19-7.53 (m, 5H). ¹³C-NMR (CDCl₃). δ (ppm): 14.7 p; 21.9 p; 29.5 p; 39.5 s; 58.9 t; 68.4 t; 88.8 q; 126.7 t; 127.0 t; 127.3 t; 127.5 t; 127.7 t; 128.5 t; 141.6 c; 144.9 q; 155.0 q; 160.4 q; 160.7 q.

Methods of calculations

Multidimensional conformational analysis (MDCA). From the concepts of MDCA³² we performed a systematic conformational search for compounds **4g₁₋₄**. In such analysis the following variables were taken into account:

- (i) The three torsional angles ϕ_1 , ϕ_2 and ϕ_3 , which determine the different conformations. (MDCA predicts a multiplicity of 3 for the torsional angle ϕ_1 and a multiplicity of 2 for the torsional angles ϕ_2 and ϕ_3).
- (ii) The two possible spatial orderings for the N atom of ring B (up or down)(multiplicity 2)
- (iii) The two possible spatial orderings, axial or equatorial, for the alkyl-phenyl substituent (ring D) (multiplicity 2).
- (iv) The different configurational isomers due to both chiral centers (see figure 4) (multiplicity 2 for each chiral center).

Considering the above variables the conformational potential energy hypersurface (CPEH) might be raised as follow:

$$E=E(\phi_1,\phi_2,\phi_3,\text{up/down,ax/eq,R/S,R/S}) \quad (1)$$

Equation 1 might be also expressed in function of the multiplicities as follows:

$$E=E(3 \times 2 \times 2 \times 2 \times 2 \times 2)=192 \quad (2)$$

Equation 2 indicates that in theory there are 192 different forms for compounds **4g₁₋₄**. However, our preliminary calculations indicate that the torsional angles ϕ_2 and ϕ_3 adopt only two almost perpendicular conformations. Considering that rings C and D are symmetrical, they give non-identical but indistinguishable forms (energetically degenerate). Thus, Equation 2 might be reduced to Equation 3.

$$E=E(3 \times 2 \times 2 \times 2 \times 2)=48 \quad (3)$$

On the basis of Equation 3 MDCA predicts 48 different forms. All these different spatial orderings were analyzed in our study.

Computations. An extensive search for the low energy conformations on the PEHS was carried out by using a systematic search in connection with semiempirical PM6 calculations. Next to determine the minima on the PEHS, fully relaxed DFT optimizations were performed. Correlation effects were included using density functional theory (DFT) with the Beke-Lee-Yang Parr (B3LYP)³⁹⁻⁴¹ functional and the 6-31 G(d) basis set. Minima were characterized through harmonic frequency analysis employing B3LYP/6-31 G(d) calculations. GIAO³³⁻³⁴ (gauge independent atomic orbital) computations were carried out using B3LYP/6-31G(d) calculations. All the calculations reported here were performed using the Gaussian 03 Program.³⁵

From the 48 potentially available conformations of compound **4g**, only 41 conformers were obtained for these molecules; seven conformations were annihilated during the optimization process. Annihilation means that they do not converge or finally converge to one of the other 41 conformers. It should be noted that the synthesis of compound **4g₁₋₄** was carried out from a particular enantiomer in the first chiral center C(1'), so that the result of the synthesis can only give rise to two stereoisomers *R/R* and *R/S* or *S/S* and *S/R* (C(1')/C(7)). This assumption was supported by the theoretical calculations performed here.

Considering an energetic window of 6 Kcal/mol just eight conformers were obtained for both diastereomers (*R/R* and *R/S*) and their equivalents (*S/S* and *S/R*) (see Table 1S and Figure 4S in supporting information for angles analyzed in this study). All these conformations were optimized at DFT level using B3LYP/6/31G(d) calculations. These conformers and their relative energies are summarized in Table 1S. Figure 3S shows a spatial view of the lowest-energy conformation obtained for compound **4g₂**.

From the low-energy conformations obtained for both diastereomers *R/R* and *R/S* we calculated the shielding tensors using the GIAO approach^{33,34} from the program Gaussian 03.³⁵ From the tensors we obtain the value of calculated chemical shift (δ_{calc}) for each C atom of each isomer, using the following expression (Eq. 4):

$$\delta_{calc} = b + m.(I + A) \quad (4)$$

where *b* and *m* values were obtained from the linear correlation between δ_{exp} and the sum of *I* (isotropy) with *A* (asymmetry). Recently we demonstrated the importance to include asymmetry in such calculations for this type of molecule.⁴²⁻⁴³

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