Synthesis and Biological Testing of Purine Derivatives as Potential ATP-Competitive Kinase Inhibitors

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On the basis of ATP adenine, a series of adenine and purine derivatives was prepared and tested for their ability to inhibit a spectrum of disease-related kinases. There has been scant research investigating the potential of cosubstrate derived kinase inhibitors for other kinases than CDKs. Our inhibitor design combined the purine system from the original cosubstrate ATP and phenyl moieties in order to explore possible interactions with the different regions of the ATP binding site in several disease-related protein kinases. There have been a number of hits for the assayed substances, which led us to conclude that the spectrum of compounds may prove to be a valuable tool kit for the evaluation of bonding and selectivity patterns for a wide variety of kinases.

Introduction

The protein kinase family offers both a challenge and a huge opportunity for drug discovery. About 22% of the “druggable” human genome encodes target protein kinases.[1] Approximately 30% of all marketed drugs target G-protein-coupled receptors, about 7% address ion channels, and roughly 4% bind to nuclear hormone receptors. There are, however, only two drugs on the market that address kinase targets [imatinib (Gleevec) and gefitinib (Iressa)].[2] These facts dictate a great need for fundamental research in this field and for the development and design of new lead structures.

Kinases regulate many different cell proliferation, differentiation, and signaling processes by adding phosphate groups to target protein substrates. Reversible protein phosphorylation is the main strategy for the control of eukaryotic cell activities. Signal transduction pathways maintain the balanced steady-state functioning of a cell. Disease arises when signal transduction in a cell breaks down, thereby removing the tight control that typically exists over cellular functions. Devastating diseases such as cancer, autoimmune diseases, inflammation, psoriasis, allergic reactions, neurological disorders, and hormone-related diseases can result from abnormal signal transduction.

At present, 518 protein kinases[3] are identified, in which all of them the cofactor ATP (adenosine triphosphate) binds in a very similar way. The conservation of structural features within the ATP binding cleft initially indicated that specificity for ATP-site-directed inhibitors would be difficult to achieve. Structure elucidation of ATP complexes or the nonhydrolyzable ATP analogue adenylyl-β,γ-imidodiphosphate (AMP−PNP)[4] bound to protein kinases has revealed that there are regions within or close to the binding cleft that ATP does not fully occupy. These regions, unoccupied by ATP, show structural diversity between members of the kinase family. This provides opportunities for the discovery or design of selective and small molecule ATP-competitive inhibitors.

Architecture of the ATP Binding Site. Figure 1 shows a general scheme of the important interactions of a kinase with its cofactor ATP. The ATP binding site is located in the center of the enzyme, which formed a molecular hinge. This allows the opening or closure of the ATP binding cleft in case of kinase activation.[5,6]

Exploring Hydrophobic Region I. Many kinase inhibitors target hydrophobic region I with a phenyl moiety (Figure 2) as in the case of p38 mitogen activated protein kinase (p38 MAP kinase) inhibitors.[10] Hydrophobic region I is formed as a selectivity pocket, just big enough to incorporate a 4-fluorophenyl moiety. The pyridine nitrogen forms an H-bond with the amidic NH of Met 109, which mimics the H-bond interaction typically formed by the N-1 of ATP adenine.

The Purine System as a Central Scaffold in Kinase Inhibitors. Purine derivatives have already been reported as kinase inhibitors for some kinases, mainly cyclin-dependent kinases (CDKs).[11] Structures such as olomoucine, a 2,6,9-substituted purine (Figure 3), were initially identified as CDK-inhibitors (IC_{50} = 7 μM for CDK2) with only minor inhibitory effects on other kinases.[12] Olomoucine was used as a lead structure and was optimized in combinatorial chemistry approaches[13] to generate the more efficient analogues related to purvalanol A (IC_{50} = 0.004 μM for CDK2) (Figure 3). The 2,6,9-substitution pattern has been retained nearly unchanged, and most variations have been carried out at C-2 of the purine with variations of mainly aliphatic substituents. Substituents at N-9 have been kept very small (methyl, ethyl, isopropyl) in order to enable the imidazole ring of the purine to enter the pocket formed by hydrophobic region I (see Figure 1).[14]

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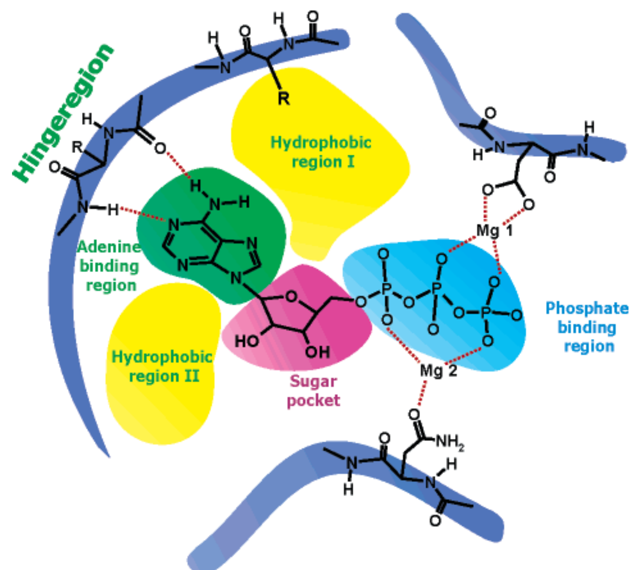


Figure 1. Mapping important regions within the ATP binding site of kinases (modified from Traxler[7]). *The adenine binding region:* N-1 of adenine acts as a hydrogen-bond acceptor from the amidic NH of the protein backbone. A proton from the amino group at C-6 forms a hydrogen bond with the carbonyl oxygen of the backbone. This bidentate hydrogen-bond-donor/-acceptor system in the hinge region anchors the adenine deep inside the binding cleft.[7] *The sugar pocket:* The ribose binding pocket is hydrophilic.[7] Surprisingly, the ribose can be replaced by the phenyl ring of an aniline moiety[8] without causing substantial loss to its binding ability. *The phosphate binding region:* The region is surrounded by several residues that stabilize the phosphates by chelation together with one or two metal ions (in most cases Mg^{2+}) and thus position the γ-phosphate for a facilitated phosphoryl transfer.[9] *Hydrophobic region I:* This region is formed as a lipophilic pocket[8] located deep inside the ATP binding cleft in the direction of N-7 and C-6 of the adenine moiety of ATP. Its expanse is controlled by the size of the amino acid side chains surrounding this area which is not occupied by ATP. The extent of this cavity, which varies between different kinases, provides opportunities for the design of selective inhibitors. *Hydrophobic region II:* The region is also unoccupied by ATP. It is formed as a cleft that opens to solvent with the lipophilic surface above and below the level of the adenine heterocycle. The upper and lower part of this surface provide two additional interaction possibilities, thus gaining further affinity and selectivity for potential inhibitors.[7]

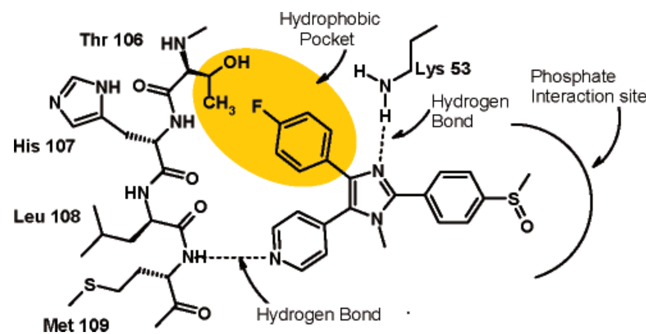


Figure 2. Schematic drawing of important interactions between inhibitor SB203580 (a p38 MAP kinase inhibitor) and the ATP binding site of p38 highlighting the selectivity pocket.[10]

The hydroxyethylamino moiety of C-2 binds in the sugar pocket.[14] If the crystal structure data provided by Schulze-Gahmen[14] are carried forward to the map of the ATP binding site introduced to the literature by Traxler,[7] the benzylamino moiety of C-6 should, however, bind in hydrophobic region II. Therefore, it is obvious that the purine moiety of adenine-derived ATP-competitive inhibitors of CDKs does not necessarily take the same orientation as seen for adenine of ATP.[13,15]

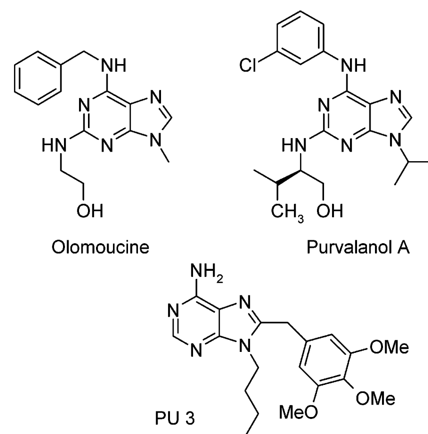


Figure 3. Purine-based CDK inhibitors and the HSP90 inhibitor PU 3.

Another purine-based compound library of 8,9-disubstituted purine derivatives was derived from the compound PU 3 (Figure 3) to provide inhibitors of the ATPase HSP90.[16,17] On binding, these inhibitors induce a conformational change of the ATP binding region of the HSP90 protein.[17]

Our review of the literature revealed that there has been scant research investigating the potential of cosubstrate derived (adenine- or purine-based) kinase inhibitors for kinases other than CDKs. Moreover, an approach to the design of adenine-derived kinase inhibitors has been urgently missed in the literature.[18]

As discussed above, the selectivity pocket (hydrophobic region I) of CDKs is too small to incorporate the benzylamino moiety of olomoucine,[14] whereas other kinases, such as p38[10,19,20] or JNK,[21] are able to bind phenyl moieties in their selectivity pocket. Therefore, the goal of our work was to establish the optimal design of a potential kinase inhibitor wherein the purine system from the original cosubstrate is combined with phenyl moieties at various positions (Figure 4). We started our investigations with the synthesis of compounds bearing only one aryl moiety at different positions of the purine system. Furthermore, these first aryls were supplemented with second aryl moieties. The introduction of phenyl moieties with various linkers is necessary to exploit all possible orientations of the purine system according to the binding properties of the phenyl moieties in the hydrophobic regions. Thus, the probability of finding the best inhibitor, which can mimic the interaction observed for the adenine of ATP, increases. In the ATP−adenine model, N-9 is linked to the sugar moiety, thus effectively blocking any interaction possible (i.e. H-bond) through this position. In circumspect, any subsequent kinase inhibitor designed to mimic the adenine of ATP should probably also possess this inherent characteristic. This can be achieved by blocking the N-9 position using small and simple substituents (i.e. methyl). A series of about 50 derivatives was synthesized and tested for inhibitory potency on a panel of 15 disease-related kinases, including p38 MAP kinase.[22] This research project demonstrates that

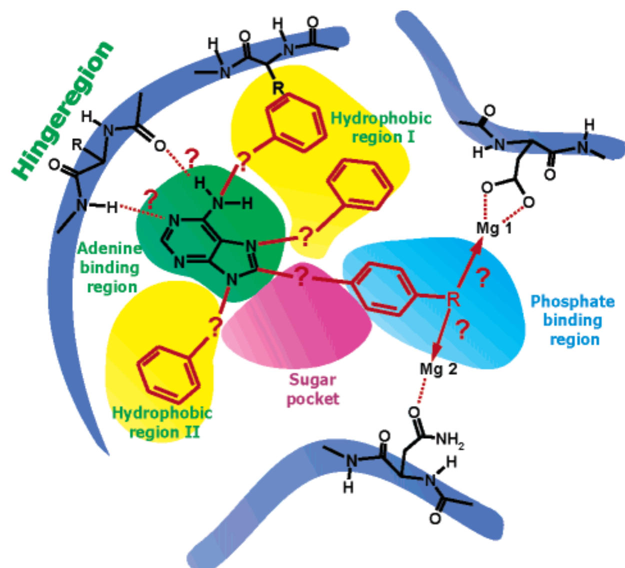
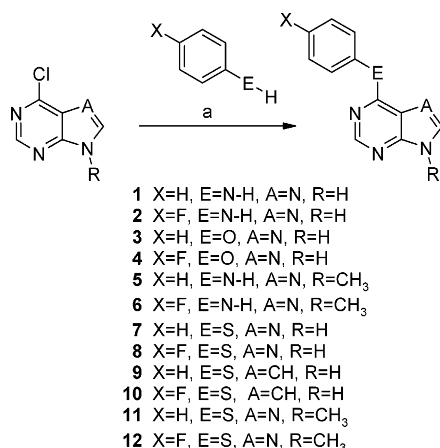


Figure 4. The ATP binding site showing the purine core having the same orientation as the adenine of ATP. (Possible phenyl substitutions, linkers, and interactions are highlighted in red.)

Scheme 1[a]



[a] Reagents: (a) *n*-butanol, triethylamine, appropriate anilines or thiophenols, reflux (melt in the case of phenols).

systematic variation of cosubstrate-based analogues shows promise as a way to develop selective kinase inhibitors.

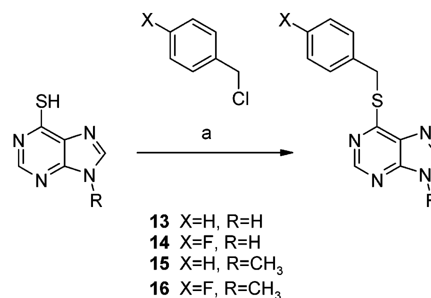
Chemistry

Purines and deazapurines substituted at C-6 (compounds **1**−**12**) were synthesized by nucleophilic substitution of the chlorine of 6-chloropurine,[23] 6-chloro-9-methyl-9*H*-purine,[24] or 4-chloro-7*H*-pyrrolo[2,3-*d*]pyrimidine[25] with the appropriate anilines, phenols, or thiophenols (Scheme 1).

The 6-benzylmercaptopurines **13**−**16** were synthesized by nucleophilic substitution of benzyl chloride or 4-fluorobenzyl chloride with 6-mercaptopurine or 6-mercapto-9-methylpurine[24] (Scheme 2).

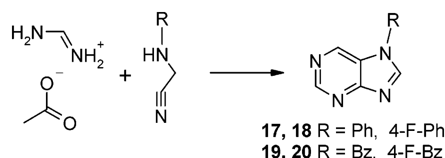
The 7-substituted purines **17**−**20** were synthesized in a one-step reaction starting from formamidine acetate and the appropriately substituted aminoacetonitriles[26] following the Bredereck method for preparing compound **17**[27] (Scheme 3).

Scheme 2[a]



[a] Reagents: (a) 2 N NaOH, appropriate benzyl chlorides, rt.

Scheme 3[a]



[a] Solvent: (a) *n*-butanol at reflux or ethylene glycol at 140 °C.

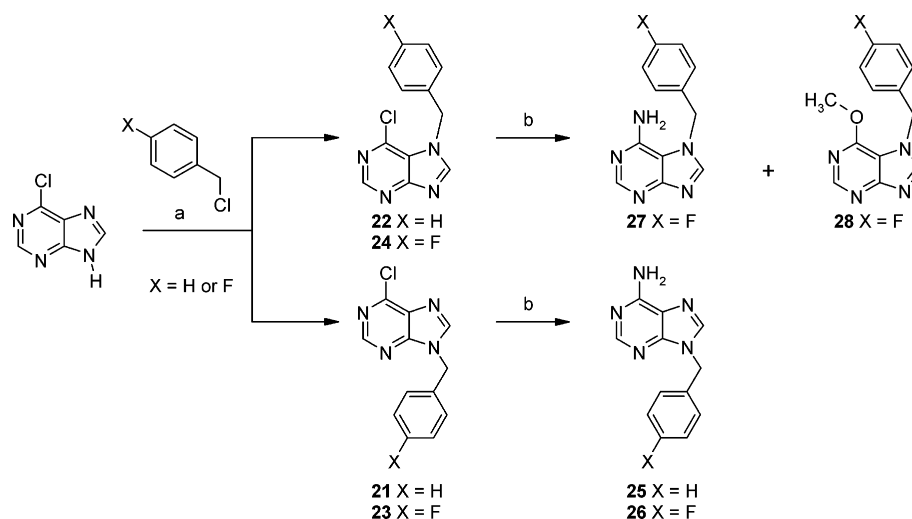
6-Chloropurine was N-alkylated with benzyl chloride or 4-fluorobenzyl chloride to obtain the 7- and 9-regioisomeric compounds **21**−**24**. The reaction provided the N-7 benzylated isomers as the minor product.[28] Treatment of compounds **21**, **23**, and **24** with methanolic ammonia[24] in a sealed bottle yielded the adenine derivatives **25**−**27** and 6-methoxy-substituted **28** as a side product (Scheme 4).

6-Substituted purines **29**−**31** having a one- or two-carbon linker were synthesized by Wittig reaction or a modified Wittig reaction starting from 9-tetrahydropyranyl-protected 6-chloropurine[29] following the procedures described by Taylor[30] (Scheme 5). On the other hand, the 9-methyl-substituted styrylpurine **32** could be obtained via Suzuki coupling of stryrylboronic acid with 6-chloro-9-methyl-9*H*-purine as recently described for 9-benzylpurines.[31] 9-Unsubstituted 6-chloropurine is unreactive toward Suzuki coupling conditions.

The 6-phenyl-substituted purines **39** and **40** and 6,8-diphenyl-substituted **41** were synthesized as shown in Scheme 6. Suzuki coupling of benzeneboronic acid or 4-fluorobenzeneboronic acid with 4,6-dichloro-5-nitropyrimidine[32] provided the arylpyrimidines **33** or **34**, which were treated with methylamine to deliver the 4-amino-5-nitro compounds **35** or **36**. Subsequent catalytic reduction of the 5-nitro group afforded the diamines **37** or **38**, which permitted acid-catalyzed ring closure with the suitable ortho esters

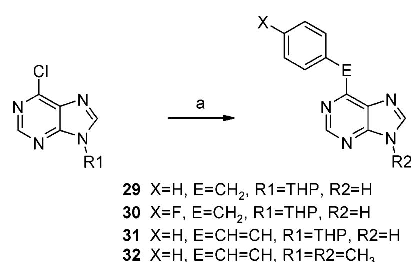
The starting compound for the synthesis of the 6,8,9-trisubstituted purines **44**, **45**, and **49**−**54** was 6-chloro-9-methyl-9*H*-purine, which was brominated to the 8-bromo derivative by the method described by Nolsoe.[33] The two different halogens at positions C-6 and C-8 of the purine moiety allowed us to selectively introduce substituents at both positions. Substitution at position C-8 was achieved by Suzuki coupling of benzeneboronic acid or 4-methylthiobenzeneboronic acid with the 8-bromo derivative. The sulfur of the methylthio moiety could be selectively oxidized to a methylsulfinyl group by hydrogen peroxide or to a methylsulfonyl group by *m*-chloroperbenzoic acid. The 8-substituted compounds were subjected to nucleophilic substitution at the 6-position with the appropriate thiophenols (Scheme 7).

Scheme 4[a]



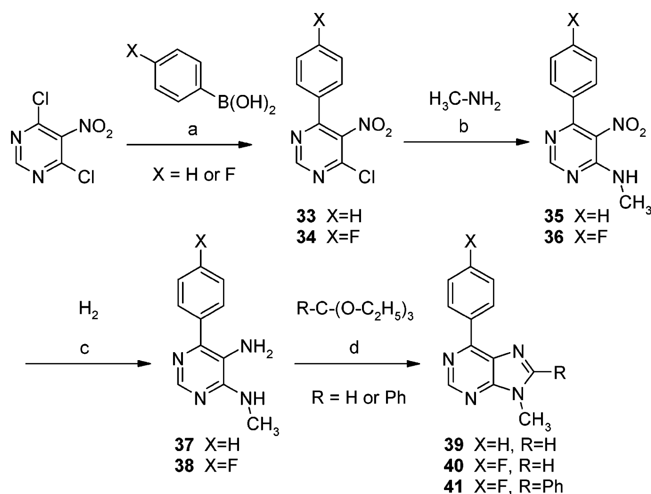
[a] Reagents: (a) DMF, K_2CO_3, appropriate benzyl chlorides; (b) MeOH, aqueous ammonia.

Scheme 5[a]



[a] Reagents: (a) for **29** and **30**: (1) benzyl(tri-*n*-butyl)phosphonium bromide or 4-fluoro-benzyl(tri-*n*-butyl)phosphonium bromide, DME, *n*-butyllithium, −35 °C; (2) Na_2CO_3, H_2O, rt; (3) 1 N HCl, EtOH, rt. For **31**: (1) methyltriphenylphosphonium bromide, DME, *n*-butyllithium, −35 °C; (2) benzaldehyde, rt; (3) 1 N HCl, EtOH, rt. For **32**: *trans*-2-phenylvinylboronic acid, K_2CO_3, DME, $Pd(PPh_3)_4$, 90 °C.

Scheme 6[a]



[a] Reagents: (a) appropriate boronic acid, Na_2CO_3, toluene, $Pd(PPh_3)_4$, 90 °C; (b) $H_2N{-}CH_3$ 40% in H_2O, reflux; (c) PtS/C, H_2, 4 bar, rt; (d) appropriate ortho ester, EtOH, *p*-toluenesulfonic acid, reflux.

This reaction sequence was not applicable for the synthesis of compound **56** because of the reactivity of the alkynyl moiety at the 8-position. Thus, 6-chloro-9-methyl-9*H*-purine was first subjected to nucleophilic substitution with thiophenol to provide compound **11**, which was brominated at the 8-position by the procedure described above for **42** to deliver compound **55**. Sonogashira coupling[34] with but-3-yn-1-ol afforded compound **56** (Scheme 8).

6,9-Substituted purines **57**−**60** were obtained by nucleopilic substitution of compounds **21** or **23** with thiophenol or 4-fluorothiophenol (Scheme 9).

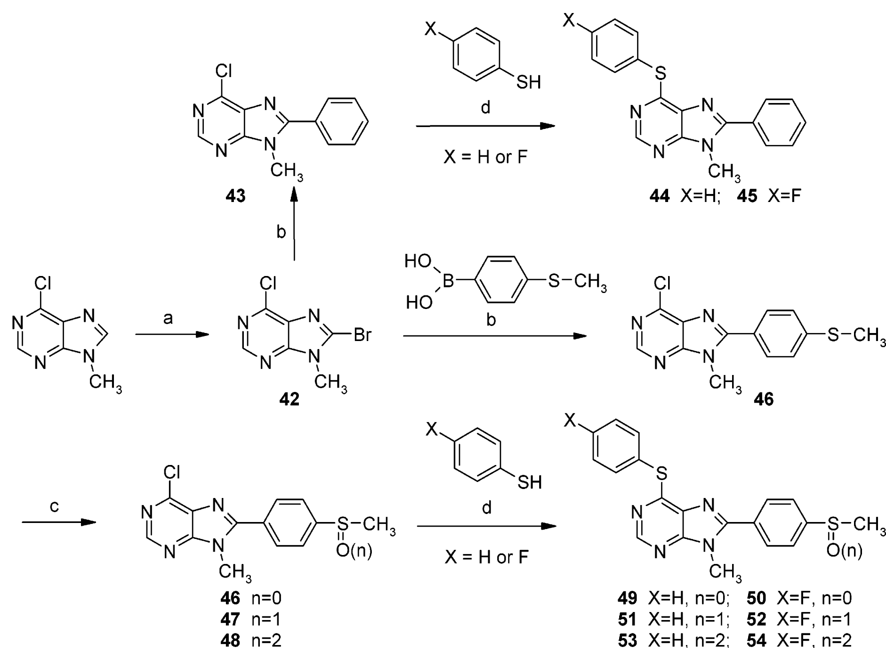
Biological Results and Discussion

The inhibitory potency of the compounds was evaluated with the Upstate Kinase Profiler.[22] The results are shown in Table 1 and will be discussed on the basis of protein−ligand interactions.

As reported for most kinase inhibitors,[35] our compounds were assumed to be ATP-competitive as well. In this line, inhibition values are usually dependent on the ATP concentrations in the test assay.[36] From an in vivo standpoint, the competitiveness of the potential inhibitor is even weaker because the intracellular concentrations of ATP are in the millimolar range.[37] On the basis of these important considerations, the ATP concentration was maintained at higher levels than the usual ATP concentrations. The concentrations of the potential inhibitors were kept at a level about 3-fold lower than that of ATP to create an especially challenging condition for the compounds.

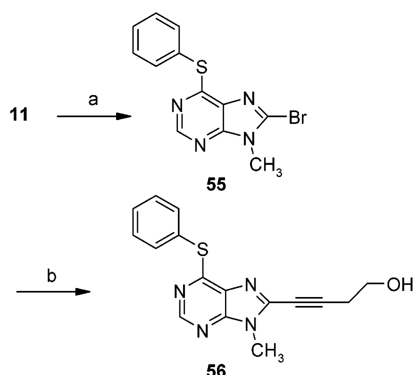
As mentioned earlier, the adenine of ATP exhibits key interactions when bound to kinases. Adenine in itself without the ATP substructure would then act more like an inhibitor, due to the fact that it would lose its ability to transfer phosphate groups together with the kinase. Since the central scaffold of all the potential inhibitors are purine or adenine, it would be pertinent to also include adenine in the assay as a comparison in judging the values obtained. To determine the effectiveness of an inhibitor, the cutoff value, which would be of interest, has to be set above this particular result. Although the compounds have not yet been optimized for a single kinase, the result should deliver selectivity criteria and structural clues for the substitutional pattern using diverse phenyl substituents. Purines substituted at the 6- and 7-positions were the primary candidates, because this allows the purine to have the same orientation as that of adenine when ATP is bound to the protein. Their

Scheme 7[a]



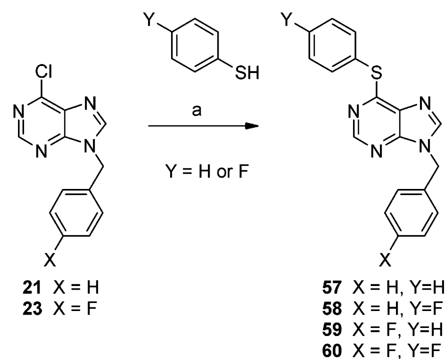
[a] Reagents: (a) (1) LDA, (2) $BrCCl_2CCl_2Br$, −78 °C; (b) appropriate boronic acid, K_2CO_3, toluene, $Pd(PPh_3)_4$, 90 °C; (c) for $n = 0$, no reaction; for $n = 1$, glacial acetic acid, H_2O_2, rt; for $n = 2$, CH_2Cl_2, *m*-chloroperbenzoic acid, reflux; (d) *n*-butanol, triethylamine, appropriate thiophenols, reflux.

Scheme 8[a]



[a] Reagents: (a) (1) LDA, (2) $BrCCl_2CCl_2Br$; (b) but-3-yn-1-ol, CH_2Cl_2, triethylamine, $PdCl_2(PPh_3)_2$, CuI, reflux.

Scheme 9[a]



[a] Reagents: (a) *n*-butanol, triethylamine, appropriate thiophenols, reflux.

aryl substituents should be directed to the lipophilic pocket formed by the amino acid residues of hydrophobic region I.

In ATP, the 6-position of adenine is substituted with an amino group. Similarly, **1**, **2**, **5**, and **6** contain the amino group linked to aryl moieties. This resulted in quite unselective inhibition. Eight kinases could be inhibited with stronger values as seen for adenine. For these four compounds, additional methyl substitution at the 9-position increased selectivity with four kinases targeted at better inhibition levels in the cases of c-RAF kinase and MAPK2 kinase. Replacement of the amino moiety at C-6 by carbon and other heteroatoms was done to investigate the relevance of possible alternative linkers. As exemplified by compounds **3** and **4**, the activity decreased with the oxygen linker. For that reason this approach was discontinued. Compounds **29** and **30** with a carbon linker at C-6 showed no activity. This perhaps is due to the absence of H-bond interactions or the reduced distance between the purine and the aryl moiety. In compound **31**, this distance was increased by addition of one carbon atom, keeping the linker rigid by introducing a double bond. In contrast to the single carbon counterpart, the compound showed activity and inhibition of the three kinases c-RAF kinase, Lck kinase, and MAPK1 kinase. The replacement of the amino moiety at C-6 of the purine with sulfur led to compounds with activity against two or three kinases (ROCK kinase, p38 MAP kinase, and c-RAF kinase) in the case of compounds **7** and **8**. However, the trend of selectivity was lost in their 7-deaza analogues **9** and **10**. On the basis of these findings, subsequent investigation involved retaining, in most compounds, the sulfur at C-6 and the nitrogen at the 7-position.

Compounds **17**−**20**, without substituents at C-6 and aryl substituted at N-7, did not show any activity. Their analogues **22** and **24**, with chlorine substituted at C-6, showed similar results. This, together with the facts discussed above, indicates the importance of heteroatom substitution with nitrogen or sulfur at this position for improving activity. The 6-amino-9-aryl combination of compounds **25** and **26**, where the aryls could in theory

Table 1. Inhibition Values of Kinase Activity[a,b]

no.	c-RAF[c]	CaMKII[d]	GSK3β[c]	JNK1α1[c]	JNK2α2[c]	JNK3[d]	Lck[c]	MAPK1[c]	MAPK2[c]	MEK1[c]	PKA[c]	PKBα[c]	PKCα[c]	ROCK-II[c]	SAPK2α[c]
1	19	0	13	0	0	11	*39*	22	*33*	19	*32*	14	6	*48*	*34*
2	24	0	13	0	0	10	*42*	33	*48*	*34*	*40*	18	17	*52*	*30*
3	3	0	0	1	1	0	11	0	6	1	11	0	8	16	12
4	1	0	0	0	0	0	0	0	0	0	0	0	3	9	18
5	*44*	0	*26*	0	0	5	18	16	*42*	4	0	0	2	0	0
6	*27*	8	14	5	6	19	9	36	*57*	8	0	0	11	7	13
7	6	0	1	0	0	0	5	3	13	0	8	0	3	*33*	*54*
8	*29*	0	0	0	1	8	13	2	12	2	10	0	9	*37*	*72*
9	22	6	11	0	0	0	*56*	11	4	*41*	0	0	23	*36*	*56*
10	*43*	3	4	2	6	5	*52*	10	2	*36*	0	0	15	*42*	*79*
11	10	8	3	3	6	7	6	12	12	3	0	0	13	6	17
12	12	5	0	10	10	9	2	9	12	6	1	0	7	6	*38*
13	*53*	0	*35*	1	0	7	22	15	12	7	13	4	11	*28*	*50*
14	*47*	3	0	0	0	12	16	9	5	0	12	6	4	18	*29*
15	12	0	10	1	7	4	6	6	0	6	0	4	13	1	22
16	16	5	14	10	6	9	5	11	8	7	0	11	12	6	21
17	0	0	4	0	0	3	2	0	0	0	0	0	4	0	0
18	0	0	0	1	0	4	9	0	0	0	0	0	2	2	0
19	10	7	13	0	4	13	7	11	7	4	0	9	18	7	6
20	7	5	10	0	0	12	13	5	4	0	0	8	15	4	5
21	4	7	0	0	0	16	0	9	0	3	2	0	17	3	11
22	12	0	9	0	1	11	0	10	1	0	0	0	12	6	7
23	4	0	8	0	0	20	0	13	0	1	0	0	14	0	10
24	20	8	0	6	9	21	0	16	1	4	0	0	13	7	17
25	*25*	0	7	1	0	17	19	17	11	7	1	0	2	5	7
26	16	9	5	0	0	9	22	22	0	7	0	0	16	0	8
27	*28*	8	0	4	7	*25*	0	*26*	5	8	7	0	14	6	8
28	9	2	5	0	0	*30*	0	19	3	3	7	0	7	1	8
29	2	0	0	0	0	0	0	0	0	8	0	0	0	3	1
30	0	8	2	0	0	0	0	0	0	0	0	3	0	2	1
31	*66*	3	20	0	0	2	*37*	*50*	20	7	4	7	13	22	17
32	8	4	0	0	0	8	1	2	0	0	0	0	7	0	12
39	12	8	11	9	11	14	21	23	9	9	7	15	14	8	16
40	9	12	3	5	3	14	5	17	0	1	0	0	8	10	16
41	24	5	*26*	2	8	12	17	20	1	5	0	*31*	17	0	23
44	12	1	0	0	0	12	5	10	0	4	3	*42*	13	1	*44*
45	14	8	22	2	0	16	20	11	1	7	3	*29*	17	9	*29*
49	5	0	3	0	1	0	0	1	0	4	0	5	0	0	11
50	1	7	5	3	5	3	1	1	0	4	0	0	0	0	15
51	0	8	4	0	0	6	*30*	0	0	3	0	4	8	1	*27*
52	4	12	4	0	1	10	*30*	0	0	1	4	10	15	0	*44*
53	10	8	0	1	8	7	16	9	3	4	11	14	18	5	*36*
54	6	0	0	0	2	5	10	5	0	0	3	4	7	0	19
55	0	0	19	0	0	0	23	0	0	0	0	1	5	0	23
56	0	10	0	0	0	0	11	0	0	0	0	*33*	18	0	3
57	0	3	1	0	0	0	0	0	0	6	0	1	0	1	*64*
58	0	0	0	0	0	0	16	0	0	4	0	1	0	5	*64*
59	0	0	0	0	0	0	12	17	0	1	0	13	0	2	*67*
60	0	0	8	0	0	0	16	6	0	3	0	0	0	0	*27*
61[e]	14	0	0	0	0	10	19	3	17	9	14	0	6	*28*	7

[a] Concentration of the compounds, 30 μM; concentration of ATP, 100 μM. Measured values are given as percent inhibition, and values ≥25% are italic. [b] Abbreviations: c-RAF, Raf kinase; CaMK, calcium/calmodulin-dependent protein kinase; GSK3β, glycogen synthase kinase 3β; JNK, c-Jun N-terminal kinase (also stress-activated protein kinase, SAPK); LCK, lymphocyte kinase; MAPK, mitogen-activated protein kinase; MEK, MAPK kinase (also called MKK); PKA, cAMP-dependent protein kinase; PKB, protein kinase B (also called Akt); PKC, protein kinase C; ROCK, Rho-dependent protein kinase; SAPK2α, stress-activated protein kinase 2a (also called p38 MAP kinase). [c] From human. [d] From rat. [e] Compound **61** is adenine.

occupy the hydrophobic region II, showed only weak inhibition. This was slightly improved in the 6-amino-7-aryl or 6-methoxy-7-aryl combinations of compounds **27** and **28**, where the aryls project toward the hydrophobic region I.

In general, most hits were found for p38 MAP kinase. This was not particularly astonishing, because the selectivity pocket formed by hydrophobic region I allows the exact incorporation of phenyl moieties. The best results for p38 MAP kinase were obtained with the simple purines **7** and **8** and the deazapurines **9** and **10**, which have a sulfanyl linker at C-6. The size of the sulfur linker provides greater flexibility for the bond angles. The unsubstituted positions at N-9 (purine) or N-7 (pyrrolopyrimidine) allow the molecule to form a bidentate H-bond in conjunction with N-3 (purine) and N-1 (pyrrolopyrimidine) (Figure 5). Thus, the N-9 methylated analogues **11** and **12** show weaker activity because one hydrogen bond site is unavailable. In analogues **1** and **2**, the activity should be higher than observed, since both N-9 (unsubstituted) and N-2 are available for H-bonding. This was not the case. This could be explained by an additional H-bonding site at the amino function at C-6, which could possibly cause unselective H-bonding patterns with the kinase. Compound **10** may not necessarily take the same orientation as the adenine in ATP because of its disposition to form this H-bond with the enzyme backbone. This forces the deazapurine in a different configuration needed for activity, even though the phenylsulfanyl moiety is still

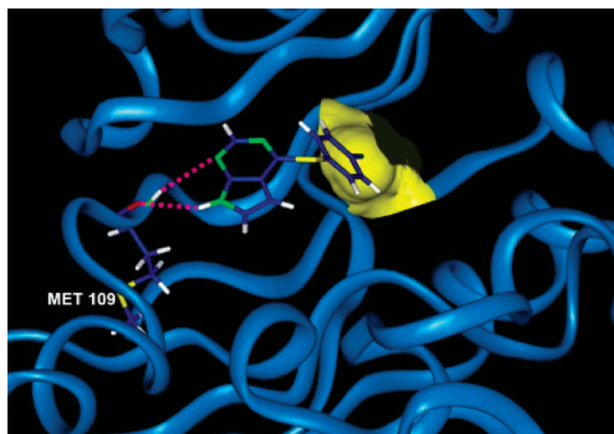


Figure 5. Active site of p38 MAP kinase with compound **10** occupying the lipophilic pocket (yellow) with a 4-fluorophenylsulfanyl moiety. The formation of H-bonds to the backbone Met109 similar to those of adenine in ATP seems to be possible (pink).

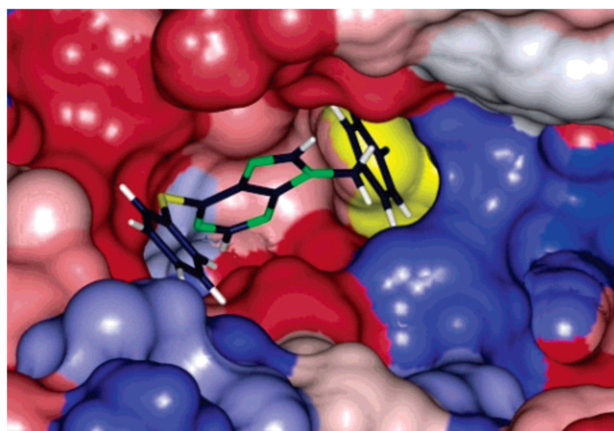


Figure 6. Active site of p38 MAP kinase with compound **59** occupying the lipophilic pocket (yellow) with a 4-fluorobenzyl moiety. The Conolly surface is colored by the lipophilicity of the amino acid side chains. Red, lipophilic; blue, hydrophilic.

oriented toward the lipophilic pocket, owing to the flexibility offered by the sulfur linker.

A possible arrangement may be envisioned for compound **59** in the active site of p38 MAP kinase (Figure 6). The 4-fluorobenzyl moiety is directed toward the lipophilic pocket and the phenylsulfanyl moiety in the area of the hydrophobic pocket II, providing additional lipophilic interactions. H-bonding between N-1 or N-7 and the protein backbone could still be feasible. For compounds **57**−**60**, activity was only observed for p38 MAP kinase. A possible reason may be the size of the phenyl substituents and their corresponding distance to each other, which is defined by the dimension of the purine moiety. The size of the hydrophobic regions and the distance between them is possibly different in the other kinases. Thus, the utilization of appropriate substituents should exploit the distance needed to attain additional selectivity for potential inhibitor molecules.

Surprisingly, there are only very few hits for JNK3 kinase, which is structurally closely related to p38 MAP kinase. The size of the lipophilic pocket of p38 is controlled by the side chain of the appropriate amino acid. For p38 MAP kinase, the expanse of the lipophilic pocket is controlled by Thr 106, and for JNK3, it is

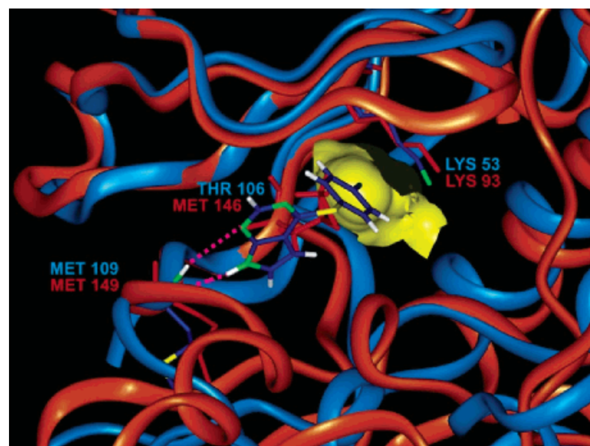


Figure 7. Overlay of p38 MAP kinase (blue) and JNK3 kinase (orange) with compound **10**. JNK3 does not form a wide lipophilic pocket: Met 146 clashes with the 4-fluorophenyl moiety. The highlighted amino acids were used for superposition of the protein backbones.

controlled by Met 146.[4] Since Met has a bulkier side chain than the Thr, this allows the p38 MAP kinase to accommodate the larger phenyl moiety (Figure 7).

Compounds **51** and **52** had hits for Lck and p38 MAP kinase. As opposed to compounds **11** and **12**, these compounds have an additional methylsulfinylphenyl ring at C-8, which could provide the possibility for either $\pi-\pi$-interactions with Tyr residues or interactions between the sulfoxide oxygen of the methanesulfinyl substituents and Arg residues at the phosphate binding region.[38] The 7-(4-fluorobenzyl)-substituted adenine **27** showed hits at the cutoff for three kinases at values that have been seen for unsubstituted adenine **61** in other kinases. The fluorobenzyl moiety possibly reaches the area of the lipophilic pocket, whereas adenine takes a similar orientation as in ATP but a full incorporation does not seem likely for JNK3 kinase for the reasons discussed above. Since the methoxy analogue **28** seems to be more selective than adenine analogue **27**, it may serve as a template to design more potent analogues as selective JNK3 inhibitors.

Conclusion

Although the compounds do not exhibit an outstanding selectivity or activity profile, we were able to show that simple substitution of purine or adenine templates with one or two aryl moieties bound by diverse linkers can provide the first selectivity pattern and point out general substitution requirements for selective kinase inhibitors. Overall, if the panel of the compounds is viewed as a whole, it may prove to be a valuable tool kit for the evaluation of bonding and selectivity patterns for a wide variety of kinases. This is especially true for kinases, for which no potent inhibitors are presently known. Due to its excellent H-bonding pattern, the purine moiety should be a useful scaffold in the design of new kinase inhibitors, if the inhibitor contains the following attributes to gain selectivity and potency: (i) a portion that closely mimics the ATP molecule, (ii) the H-bonding characteristics of adenine, and (iii) portions that protrude away from the ATP binding region and reach into a lipophilic pocket and/or into additional hydrophobic areas. However, other mechanisms of

achieving specificity may be possible as well. For instance, this could be achieved through hydrophilic interactions in the areas of the phosphate binding region and the sugar binding region. The substitution pattern of the purine moiety with other residues than phenyl substituents and/or mainly lipophilic substituents will be subject to further investigation. Some of the compounds may serve as lead structures for new and more potent kinase inhibitors on the basis of a new core structure closely related to the adenine moiety of ATP.

Experimental Section

Molecular Models. Each structure was built with Insight II, minimized under CVFF force field conditions, and subsequently docked manually into the ATP binding site of p38 MAP kinase. The crystal structure of p38 MAP kinase with SB 203580 was used as template (PDB 1a9u).[19] To take the conformational flexibility of the protein into consideration, the assembly of kinase and inhibitor was then minimized again excluding all protein atoms outside a box of 12 Å around the inhibitor from the calculation (Insight II 2000 Accelrys Inc., 9685 Scranton Road, San Diego, CA 92121-3752).

Chemistry. All reagents and solvents were of commercial quality and used without further purification. Melting points were determined on a Buechi Melting Point B-545 apparatus and are thermodynamically corrected. ^{1}H NMR spectra were obtained on a Bruker Advance 200 at 200 MHz in DMSO-d_6 unless otherwise specified. Chemical shifts are reported in parts per million (ppm) relative to TMS as internal standard. TLC analyses were performed on fluorescent silica gel 60 plates (Merck KGaA; Art.-Nr. 1.05554). Spots were visualized under 254-nm UV illumination. SiO_2 60 was used for purifications by column chromatography unless otherwise specified.

GC/MS analyses were carried out on a HP 6890 series GC-system to which a HP 5973 mass selective detector was attached. The GC was equipped with a HP-5MS capillary column (30 m × 0.25-mm i.d.) with a 5% cross-linked phenylmethylsiloxane stationary phase (0.25 mm film thickness). Helium was used as carrier gas and the following temperature programs were employed: (A) initial isothermal period of 1.0 min at 100 °C, an increase at 10.0 °C/min to 160 °C with an isothermal period of 10.0 min at 160 °C, and then an increase at 15.0 °C/min to 200 °C with an isothermal period of 15.0 min at 200 °C; (B) initial isothermal period of 1.0 min at 160 °C, an increase at 10.0 °C/min to 240 °C with an isothermal period of 5.0 min at 240 °C, and then an increase at 10.0 °C/min to 270 °C with an isothermal period of 20.0 min at 270 °C. GC/MS analyses were performed in the single ion monitoring (SIM) mode with the ionization voltage of the mass selective detector set to 70 eV. GC data are presented as retention times (min) and MS results as *m/z* and intensity (%) relative to the base peak. Positive electrospray ionization (ESI) mass spectra were obtained from a ThermoFinnigan TSQ Quantum instrument (electrospray voltage, 3 kV; heated capillary temperature, 300 °C; sheath and auxiliary gas, nitrogen).

Preparation of 6-Substituted Purines. General Procedure A. The appropriate 6-chloropurines were refluxed together with the suitable anilines or thiophenols and triethylamine (2−4 equiv) in 1-butanol for 3−8 h. The volatiles were evaporated in vacuo, and the residue was purified by column chromatography (CH_2Cl_2/EtOH 9:1).

Preparation of 6-Substituted Purines. General Procedure B. The appropriate 6-chloropurines were melted at 110 °C together with the suitable phenols for 3−8 h. The phenols were extracted with diethyl ether, and the residue was purified by column chromatography (CH_2Cl_2/EtOH 9:1).

Phenyl(9*H*-purin-6-yl)amine (1).[39] The compound was prepared from 6-chloropurine[23] (0.62 g, 4.0 mmol) and aniline (0.74 g, 8.0 mmol) according to general procedure A to yield **1** (642 mg; 76%): mp 278 °C; ^{1}H NMR δ 6.98−7.09 (m, 1H, Ph), 7.28−7.41 (m, 2H, Ph), 7.95−7.99 (m, 2H, Ph), 8.28 (s, 1H), 8.37 (s, 1H), 9.78 (s, 1H, N−H), 13.18 (s, 1H, N−H); GC (temperature program B) 11.6 min; MS *m/z* (%) 211 (52, M$^+$), 210 (100), 183 (6), 156 (9), 129 (6), 105 (8), 77 (10).

(4-Fluorophenyl)(9*H*-purin-6-yl)amine (2). The compound was prepared from 6-chloropurine[23] (0.62 g, 4.0 mmol) and 4-fluoroaniline (0.89 g, 8.0 mmol) according to general procedure A to yield **2** (730 mg; 80%): mp 338 °C; ^{1}H NMR δ 7.13−7.22 (m, 2H, 4-F−Ph), 7.94−8.01 (m, 2H, 4-F−Ph), 8.28 (s, 1H), 8.36 (s, 1H), 9.87 (s, 1H, N−H), 13.19 (s, 1H, N−H); GC (temperature program B) 11.6 min; MS *m/z* (%) 229 (82, M$^+$), 228 (100), 147 (9), 95 (12), 83 (10), 75 (10).

6-Phenoxy-9*H*-purine (3).[40] The compound was prepared from 6-chloropurine[23] (0.4 g, 2.6 mmol) and phenol (1.2 g, 12.77 mmol) according to general procedure B to yield **3** (375 mg; 68%): mp 217 °C, ^{1}H NMR δ 7.26−7.52 (m, 5H, Ph), 8.41 (s, 1H), 8.48 (s, 1H), 13.60 (s, 1H, N−H); GC (temperature program B) 10.1 min; MS *m/z* (%) 212 (100, M$^+$), 211 (100), 184 (8), 158 (7), 119 (10), 94 (10), 77 (9).

6-(4-Fluorophenoxy)-9*H*-purine (4). The compound was prepared from 6-chloropurine[23] (0.4 g, 2.6 mmol) and 4-fluorophenol (1.1 g, 9.75 mmol) according to general procedure B to yield **4** (382 mg, 64%): mp 218 °C, ^{1}H NMR δ 7.26−7.40 (m, 4H, 4-F−Ph), 8.43 (s, 1H), 8.53 (s, 1H), 13.60 (s, 1H, N−H); GC (temperature program B) 9.9 min; MS *m/z* (%) 230 (100, M$^+$), 229 (70), 202 (13), 176 (9), 148 (8), 119 (23), 112 (20), 95 (12). Anal. ($C_{11}H_7FN_4O$) C, H, N; C: calcd, 57.39; found, 57.87.

(9-Methyl-9*H*-purin-6-yl)phenylamine (5).[41] The compound was prepared from 6-chloro-9-methyl-9*H*-purine[24] (0.43 g, 2.6 mmol) and aniline (0.9 g, 8.1 mmol) according to general procedure A to yield **5** (430 mg; 73%): mp 169 °C; ^{1}H NMR δ 3.80 (s, 3H, $N^9{-}CH_3$), 6.99−7.37 (m, 3H, Ph), 7.96−7.99 (m, 2H, Ph), 8.28 (s, 1H), 8.42 (s, 1H), 9.84 (s, 1H, N−H, exchangeable); GC (temperature program B) 10.2 min; MS *m/z* (%) 225 (60, M$^+$), 224 (100), 210 (11), 197 (5), 182 (10), 156 (10), 133 (7), 77 (14).

(4-Fluorophenyl)(9-methyl-9*H*-purin-6-yl)amine (6). The compound was prepared from 6-chloro-9-methyl-9*H*-purine[24] (0.43 g, 2.6 mmol) and 4-fluoroaniline (0.58 g, 6.2 mmol) according to general procedure A to yield **6** (474 mg; 75%): mp 182 °C; ^{1}H NMR δ 3.80 (s, 3H, N^9-CH_3), 7.13−7.22 (m, 2H, 4-F−Ph), 7.94−8.01 (m, 2H, 4-F−Ph), 8.28 (s, 1H), 8.40 (s, 1H), 9.91 (s, 1H, N−H, exchangeable); GC (temperature program B) 10.1 min; MS *m/z* (%) 243 (89, M$^+$), 242 (100), 228 (11), 201 (5), 174 (10), 147 (6), 122 (8), 95 (18).

6-Phenylsulfanyl-9*H*-purine (7).[42] The compound was prepared from 6-chloropurine[23] (0.5 g, 3.25 mmol) and thiophenol (1.0 g, 9.1 mmol) according to general procedure A to yield **7** (290 mg; 39%): mp 245 °C; ^{1}H NMR δ 7.48−7.68 (m, 5H, Ph), 8.53 (s, 1H), 8.56 (s, 1H), 13.62 (s, 1H, N−H, exchangeable).

6-(4-Fluorophenylsulfanyl)-9*H*-purine (8). The compound was prepared from 6-chloropurine[23] (0.5 g, 3.25 mmol) and 4-fluorothiophenol (1.2 g, 9.4 mmol) according to general procedure A to yield **8** (335 mg; 42%): mp 233 °C; ^{1}H NMR δ 7.29−7.41 (m, 2H, 4-F−Ph), 7.64−7.74 (m, 2H, 4-F−Ph), 8.51 (s, 1H), 8.55 (s, 1H), 13.61 (s, 1H, N−H, exchangeable). Anal. ($C_{11}H_7FN_4S$) C, H, N.

4-Phenylsulfanyl-7*H*-pyrrolo[2,3-*d*]pyrimidine (9). The compound was prepared from 4-chloro-7*H*-pyrrolo[2,3-*d*]pyrimidine[25] (765 mg, 5 mmol) and thiophenol (1.65 g, 15 mmol) according to general procedure A to yield **9** (624 mg; 55%): mp 159 °C; ^{1}H NMR δ 6.03 (1H, HC═CH), 7.45−7.69 (6H, Ph and HC═CH), 8.49 (s, 1H), 12.27 (s, 1H, NH); GC (temperature program B) 13.9 min; MS *m/z* (%) 227 (50, M$^+$), 226 (100), 199 (6), 172 (8), 145 (4), 118 (17), 109 (12), 91 (9), 77 (8). Anal. ($C_{12}H_9N_3S$) C, H, N.

4-(4-Fluorophenylsulfanyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (10). The compound was prepared from 4-chloro-7*H*-pyrrolo[2,3-*d*]pyrimidine[25] (765 mg, 5 mmol) and 4-fluorothiophenol (1.92 g, 15 mmol) according to general procedure A to yield **10** (690 mg; 56%): mp 200 °C; ^{1}H NMR δ 6.12 (d, 1H, ^3J(H,H) = 3.5 Hz, HC═CH), 7.31−7.41 (2H, 4-F−Ph), 7.48 (d, 1H, ^3J(H,H) = 3.5 Hz, HC═CH), 7.66−7.75 (2H, 4-F−Ph), 8.47 (s, 1H), 12.24 (s, 1H, NH); GC (temperature program B)

13.9 min; MS *m/z* (%) 245 (43, M$^+$), 244 (100), 217 (9), 190 (8), 127 (10), 118 (19), 95 (8), 83 (14). Anal. ($C_{12}H_8FN_3S$) C, H, N.

9-Methyl-6-phenylsulfanyl-9*H*-purine (11). The compound was prepared from 6-chloro-9-methyl-9*H*-purine[24] (0.43 g, 2.6 mmol) and thiophenol (0.68 g, 6.2 mmol) according to general procedure A to yield **11** (320 mg; 51%): mp 136 °C; ^{1}H NMR δ 3.83 (s, 3H), 7.48–7.66 (m, 5H, Ph), 8.48 (s, 1H), 8.57 (s, 1H); GC (temperature program B) 10.8 min; MS *m/z* (%) 242 (36, M$^+$), 241 (100), 227 (9), 173 (8), 133 (5), 109 (10), 77 (8). Anal. ($C_{12}H_{10}N_4S$) C, H, N.

6-(4-Fluorophenylsulfanyl)-9-methyl-9*H*-purine (12). The compound was prepared from 6-chloro-9-methyl-9*H*-purine[24] (0.43 g, 2.6 mmol) and 4-fluorothiophenol (0.8 g, 6.2 mmol) according to general procedure A to yield **12** (350 mg; 52%): mp 182 °C; ^{1}H NMR δ 3.83 (s, 3H), 7.31–7.40 (m, 2H, 4-F–Ph), 7.65–7.72 (m, 2H, 4-F–Ph), 8.48 (s, 1H), 8.58 (s, 1H); GC (temperature program B) 10.2 min; MS *m/z* (%) 260 (40, M$^+$), 259 (100), 245 (12), 191 (9), 127 (12), 106 (10), 83 (14). Anal. ($C_{12}H_9FN_4S$) C, H, N.

Preparation of 6-Benzylsulfanylpurines. General Procedure C. The appropriate benzyl chloride was added to a solution of 6-mercaptopurine or 9-methyl-9*H*-purine-6-thiol[24] in 2 N NaOH (10 mL) and stirred for 2 h. The mixture was adjusted to pH 5 with glacial acetic acid, and the precipitate was collected, washed with water and diethyl ether, and purified by column chromatography (CH_2Cl_2/EtOH 9:1).

6-Benzylsulfanyl-9*H*-purine (13).[43] The compound was prepared from 6-mercaptopurine (0.61 g, 3.6 mmol) and benzyl chloride (0.5 g, 3,96 mmol) according to general procedure C to yield **13** (640 mg; 73%): mp 195 °C; ^{1}H NMR δ 4.66 (s, 2H, CH_2), 7.24–7.50 (m, 5H, Ph), 8.47 (s, 1H), 8.75 (s, 2H), 13.52 (s, 1H, N–H, exchangeable); GC (temperature program B) 15.2 min; MS *m/z* (%) 242 (100, M$^+$), 227 (3), 209 (98), 164 (20), 121 (22), 107 (39), 107 (40), 91 (90), 77 (54).

6-(4-Fluorobenzylsulfanyl)-9*H*-purine (14).[42] The compound was prepared from 6-mercaptopurine (0.61 g, 3.6 mmol) and 4-fluorobenzyl chloride (0.57 g, 3,96 mmol) according to general procedure C and to yield **14** (250 mg; 27%): mp 228 °C; ^{1}H NMR δ 4.65 (s, 2H), 7.09–7.20 (m, 2H, 4-F–Ph), 7.46–7.55 (m, 2H, 4-F–Ph), 8.46 (s, 1H), 8.74 (s, 2H), 13.55 (s, 1H, N–H, exchangeable); GC (temperature program B) 15.0 min; MS *m/z* (%) 260 (96, M$^+$), 227 (83), 164 (22), 139 (12), 123 (40), 123 (40), 109 (100), 95 (39), 83 (26).

6-Benzylsulfanyl-9-methyl-9*H*-purine (15).[29] The compound was prepared from 9-methyl-9*H*-purine-6-thiol[24] (0.47 g, 2.8 mmol) and benzyl chloride (0.38 g, 3 mmol) according to general procedure C and was purified by column chromatography (CH_2Cl_2/EtOH 95:5) to yield **15** (430 mg; 56%): mp 133 °C; ^{1}H NMR δ 3.82 (s, 3H), 4.66 (s, 2H), 7.24–7.49 (m, 5H, Ph), 8.42 (s, 1H), 8.77 (s, 2H); GC (temperature program B) 13.1 min; MS *m/z* (%) 256 (100, M$^+$), 241 (4), 223 (76), 208 (12), 179 (15), 134 (15), 121 (19), 91 (91), 77 (14).

6-(4-Fluorobenzylsulfanyl)-9-methyl-9*H*-purine (16). The compound was prepared from 9-methyl-9*H*-purine-6-thiol[24] (0.47 g, 2.8 mmol) and 4-fluorobenzyl chloride (0.43 g, 3 mmol) according to general procedure C and was purified by column chromatography (CH_2Cl_2/EtOH 95:5) to yield **16** (450 mg; 58%): mp 152 °C; ^{1}H NMR δ 3.82 (s, 3H), 4.65 (s, 2H), 7.09–7.18 (m, 2H, 4-F–Ph), 7.47–7.54 (m, 2H, 4-F–Ph), 8.43 (s, 1H), 8.77 (s, 2H); GC (temperature program B) 12.5 min; MS *m/z* (%) 274 (100, M$^+$), 259 (3), 241 (70), 226 (10), 178 (20), 134 (22), 123 (20), 109 (95), 95 (22). Anal. ($C_{13}H_{11}FN_4S$) C, H, N.

7-Phenyl-7*H*-purine (17).[27] A solution of phenylaminoacetonitrile (1.65 g, 12.5 mmol) and formamidine acetate (6.24 g, 60 mmol) in *n*-butanol (13 mL) was refluxed for 5 h. The solvent was evaporated in vacuo and the residue taken up in water. This was extracted with CH_2Cl_2. The organic layer was dried over Na_2SO_4 and evaporated in vacuo, and the residue purified was by column chromatography (CH_2Cl_2/EtOH 9:1) to produce **17** (710 mg; 29%): mp 186 °C; ^{1}H NMR δ 7.48–7.83 (m, 5H, Ph), 9.06 (s, 1H), 9.10 (s, 1H), 9.22 (s, 1H); GC (temperature program B) 8.7 min; MS *m/z* (%) 196 (100, M$^+$), 169 (31), 142 (31), 115 (20), 105 (10), 91 (11), 77 (30).

7-(4-Fluorophenyl)-7*H*-purine (18). The compound was prepared from (4-fluorophenylamino)acetonitrile (3 g, 20 mmol) and formamidine acetate (15.61 g, 150 mmol) according to the method described for **17** to yield **18** (1.1 g; 26%): mp 232 °C; ^{1}H NMR δ 7.47–7.56 (m, 2H, 4-F–Ph), 7.83–7.92 (m, 2H, 4-F–Ph), 9.08 (s, 1H), 9.20 (s, 2H); GC (temperature program B) 8.5 min; MS *m/z* (%) 214 (100, M$^+$), 187 (31), 160 (38), 133 (29), 108 (18), 95 (29), 75 (20). Anal. ($C_{11}H_7FN_4$) C, H, N.

7-Benzyl-7*H*-purine (19).[44] A solution of benzylaminoacetonitrile (2.08 g, 14.2 mmol) and formamidine acetate (4.44 g, 43 mmol) in ethylene glycol (7 mL) was stirred for 5 h at 140 °C. The mixture was taken up in water and extracted with CH_2Cl_2. The organic layer was dried over Na_2SO_4 and evaporated in vacuo, and the residue was purified by column chromatography (CH_2Cl_2/EtOH 9:1) to produce **19** (300 mg; 10%): mp 146 °C; ^{1}H NMR δ 5.65 (s, 2H, CH_2), 7.29–7.47 (m, 5H, Ph), 8.94 (s, 1H), 8.99 (s, 1H), 9.14 (s, 1H); GC (temperature program B) 10.0 min; MS *m/z* (%) 210 (35, M$^+$), 182 (4), 155 (3), 128 (4), 120 (4), 106 (3), 91 (100), 77 (20), 65 (40).

7-(4-Fluoro-benzyl)-7*H*-purine (20). The compound was prepared from (4-fluorobenzylamino)acetonitrile (2.5 g, 15 mmol) and formamidine acetate (4.44 g, 43 mmol) according to the method described for **19** and was purified by column chromatography (CH_2Cl_2/EtOH 9:1) to yield **20** (220 mg; 6.5%): mp 138 °C; ^{1}H NMR δ 5.61 (s, 2H, CH_2), 7.17–7.26 (m, 2H, 4-F–Ph), 7.49–7.56 (m, 2H, 4-F–Ph), 8.91 (s, 1H), 8.98 (s, 1H), 9.15 (s, 1H); GC (temperature program B) 15.2 min; MS *m/z* (%) 228 (79, M$^+$), 200 (4), 173 (3), 146 (4), 126 (3), 109 (100), 83 (25), 65 (13). Anal. ($C_{12}H_9FN_4$) C, H, N.

9-Benzyl-6-chloro-9*H*-purine (21) and 7-Benzyl-6-chloro-9*H*-purine (22).[28] K_2CO_3 (4.15 g, 30 mmol) was suspended in a stirred solution of 6-chloropurine (1.55 g, 15 mmol) in DMF (50 mL). To this was added benzyl chloride (1.89 g, 15 mmol) and the mixture stirred for 20 h at room temperature. After filtration, the solvent was evaporated in vacuo, and the isomers were separated by column chromatography (EtOAc/hexane 7:3) with the 9-isomer eluting prior to the 7-isomer to produce **21** (1.11 g; 46%) and **22** (140 mg; 6%). Compound **21**: mp 88 °C; ^{1}H NMR δ 5.56 (s, 2H, CH_2), 7.28–7.38 (m, 5H, Ph), 8.81 (s, 1H), 8.88 (s, 1H); GC (temperature program B) 12.9 min; MS *m/z* (%) 246 (24), 245 (42), 244 (78, M$^+$), 243 (100), 209 (10), 182 (16), 167 (12), 152 (7), 128 (8), 91 (100), 65 (32). Compound **22**: mp 150 °C; ^{1}H NMR δ 5.76 (s, 2H, CH_2), 7.17–7.41 (m, 5H, Ph), 8.82 (s, 1H), 9.01 (s, 1H); GC (temperature program B) 15.1 min; MS *m/z* (%) 246 (20), 245 (15), 244 (62, M$^+$), 243 (23), 209 (9), 182 (7), 153 (5), 126 (5), 91 (100), 65 (29).

6-Chloro-9-(4-fluorobenzyl)-9*H*-purine[45] (23) and 6-Chloro-7-(4-fluorobenzyl)-7*H*-purine (24). The compounds were prepared from 6-chloropurine (1.55 g, 15 mmol) and 4-fluorobenzyl chloride (2.16 g, 15 mmol) together with K_2CO_3 (4.15 g, 30 mmol) as a base according to the method described for **21** and **22**. The compounds were separated by column chromatography (EtOAc/hexane 7:3) to yield first **23** (1.31 g; 50%) and afterwards **24** (520 mg; 20%). Compound **23**: mp 135 °C; ^{1}H NMR δ 5.54 (s, 2H, CH_2), 7.15–7.23 (m, 2H, 4-F–Ph), 7.42–7.49 (m, 2H, 4-F–Ph), 8.80 (s, 1H), 8.86 (s, 1H); GC (temperature program B) 12.9 min; MS *m/z* (%) 264 (28), 262 (84, M$^+$), 227 (6), 200 (7), 167 (5), 146 (4), 109 (100), 83 (32). Compound **24**: mp 170 °C; ^{1}H NMR δ 5.75 (s, 2H, CH_2), 7.15–7.33 (m, 4H, 4-F–Ph), 8.82 (s, 1H), 9.0 (s, 1H); GC (temperature program B) 15.0 min; MS *m/z* (%) 264 (8), 262 (24, M$^+$), 227 (5), 200 (6), 173 (5), 153 (4), 109 (100), 83 (32). Anal. ($C_{12}H_8ClFN_4$) C, H, N.

9-Benzyl-9*H*-purin-6-ylamine (25).[46] A solution of 9-benzyl-6-chloro-9*H*-purine **21** (400 mg, 1.64 mmol) in 7 N methanolic ammonia (40 mL) was stirred in a sealed bottle at 90 °C for 7 h. The solvent was evaporated in vacuo and the residue washed with diethyl ether to afford **25** (240 mg; 65%): mp 235 °C; ^{1}H NMR δ 5.38 (s, 2H, CH_2), 7.27–7.36 (m, 7H, phenyl, C^6-NH_2 (exchangeable)), 8.17 (s, 1H), 8.28 (s, 1H); GC (temperature program B) 13.3 min; MS *m/z* (%) 225 (44), 224 (100, M$^+$), 207 (7), 182 (11), 148 (13), 91 (94), 65 (52).

9-(4-Fluorobenzyl)-9*H*-purin-6-ylamine (26). The compound was prepared from 6-chloro-9-(4-fluorobenzyl)-9*H*-pu-

rine **23** according to the method described for **25** to yield **26** (350 mg; 76%): mp 215 °C; ^{1}H NMR δ 5.37 (s, 2H, CH_2), 7.13−7.43 (m, 6H, 4-F−Ph, C^6-NH_2, exchangeable), 8.17 (s, 1H), 8.28 (s, 1H); GC (temperature program B) 13.1 min; MS *m/z* (%) 243 (38, M^+), 242 (48), 200 (7), 134 (14), 109 (100), 83 (36). Anal. ($C_{12}H_{10}FN_5$) C, H, N.

7-(4-Fluorobenzyl)-7*H*-purin-6-ylamine (27) and 7-(4-Fluorobenzyl)-6-methoxy-7*H*-purine (28). The compounds were prepared from 6-chloro-7-(4-fluorobenzyl)-7*H*-purine **24** (200 mg, 0.76 mmol) according to the method described for **25**. The compounds were separated by column chromatography (Al_2O_3, CH_2Cl_2/EtOH 9:1) yielding first **28** (80 mg; 41%) as a previously undesired side product and subsequently **27** (20 mg; 11%). Compound **28**: mp 170 °C; ^{1}H NMR δ 4.07 (s, 3H), 5.55 (s, 2H), 7.14−7.42 (m, 4H, 4-F−Ph), 8.54 (s, 1H), 8.70 (s, 1H); GC (temperature program B) 15.5 min; MS *m/z* (%) 258 (70, M^+), 243 (7), 227 (7), 201 (3), 149 (16), 109 (100), 83 (26). Anal. ($C_{12}H_{10}FN_5$) C, H, N. Compound **27**: mp 220 °C; ^{1}H NMR δ 5.69 (s, 2H, CH_2), 7.05 (s, 2H, C^6-NH_2, exchangeable), 7.13−7.22 (m, 4H, 4-F−Ph), 8.23 (s, 1H), 8.47 (s, 1H); GC (temperature program B) 20.9 min; MS *m/z* (%) 243 (47, M^+), 242 (30), 200 (5), 134 (11), 109 (100), 83 (27). Anal. ($C_{13}H_{11}FN_4O$) C, H, N.

6-Benzyl-9*H*-purine (29).[30] Benzyl(tri-*n*-butyl)phosphonium bromide[47] (3.42 g, 9.2 mmol) was suspended in DME (50 mL) at −35 °C. *n*-Butyllithium (4 g, 9.25 mmol, 15% in hexane) was added dropwise and the mixture stirred for 1 h. A solution of 6-chloro-9-(tetrahydro-2-pyranyl)purine[29] (1 g, 4.2 mmol) in DME (10 mL) was added. The mixture was allowed to warm up to room temperature, at which point it was stirred for 6 h. A solution of Na_2CO_3 (0.44 g, 4.2 mmol) in water (10 mL) was added and the mixture refluxed for 3 h. The volatiles were evaporated in vacuo, and the residue was suspended in water. The mixture was extracted with diethyl ether, and the organic phase dried (Na_2SO_4) and evaporated in vacuo. The residue was dissolved in a mixture of ethanol (25 mL) and 1 N HCl (10 mL) and stirred at room temperature for 4 h. The excess acid was neutralized by addition of $NaHCO_3$ (ca. 1 g) and the solvent was removed in vacuo. The residue was purified by column chromatography (CH_2Cl_2/EtOH 95:5) to produce **29** (390 mg; 44%): mp 150 °C; ^{1}H NMR ($CDCl_3$) δ 4.59 (s, 2H, CH_2), 7.19−7.45 (m, 5H, Ph), 8.24 (s, 1H), 8.98 (s, 1H), (N^9-H not visible).

6-(4-Fluorobenzyl)-9*H*-purine (30). The compound was prepared from tributyl(4-fluorobenzyl)phosphonium bromide[47] (3.6 g, 9.2 mmol) and 6-chloro-9-(tetrahydro-2-pyranyl)purine[29] (1 g, 4.2 mmol) according to the method described for **29** and was purified by column chromatography (CH_2Cl_2/EtOH 95:5) to yield **30** (230 mg; 24%): mp 176 °C; ^{1}H NMR ($CDCl_3$) δ 4.55 (s, 2H, CH_2), 6.92−7.01 (m, 2H, 4-F−Ph), 7.39−7.46 (m, 2H, 4-F−Ph), 8.29 (s, 1H), 8.96 (s, 1H), 12.75 (s, 1H, N^9-H, exchangeable). Anal. ($C_{12}H_9FN_4$) C, H, N; C: calcd, 63.15; found, 59.86.

6-Styryl-9*H*-purine (31).[30] Methyltriphenylphosphonium bromide (3.28 g, 9.2 mmol) was suspended in DME (50 mL) at −35 °C. *n*-Butyllithium (4 g, 9.25 mmol, 15% in hexane) was added dropwise and the mixture stirred for 1 h. A solution of 6-chloro-9-(tetrahydro-2-pyranyl)purine[29] (1 g, 4.2 mmol) in DME (10 mL) was added. The mixture was allowed to warm to room temperature, where it was stirred for 4 h. A solution of benzaldehyde (1.78 g, 16.8 mmol) in DME (5 mL) was added and the mixture stirred for 24 h. The precipitated phosphonium salt was removed by filtration and the filtrate evaporated in vacuo. The residue was dissolved in a mixture of ethanol (25 mL) and 1 N HCl (10 mL) and stirred at room temperature for 4 h. Excess acid was neutralized by addition of $NaHCO_3$ (ca. 1 g) and the solvent was removed in vacuo. The residue was purified by column chromatography (CH_2Cl_2/EtOH 95:5) to produce **31** (185 mg; 20%): mp 207 °C; ^{1}H NMR δ 7.37−7.52 (m, 3H), 7.65−7.81 (m, 3H), 8.29−8.37 (m, 1H), 8.63 (s, 1H), 8.86 (s, 1H), 13.56 (s, 1H, N^9-H); ESI-MS *m/z* (%) 323 (46, $[M + 1]^+$), 208 (100), 154 (27), 119 (33), 115 (30), 94 (24).

9-Methyl-6-styryl-9*H*-purine (32). A mixture of 6-chloro-9-methyl-9*H*-purine[24] (144 mg, 0.85 mmol), *trans*-2-phenylvinylboronic acid (190 mg, 1.28 mmol), K_2CO_3 (316 mg, 2.3 mmol), DME (8 mL), and $Pd(PPh_3)_4$ (28 mg, 0.02 mmol) was stirred under argon protected from light at 90 °C for 3 h. The reaction mixture was filtered, the filtrate evaporated in vacuo, and the residue purified by column chromatography (CH_2Cl_2/EtOH 9:1) to produce **32** (32 mg; 15%): mp 167 °C; ^{1}H NMR δ 3.87 (s, 1H, N^9-CH_3), 7.41−7.51 (m, 3H, Ph), 7.65 (d, 1H, ^3J(H,H) = 16.2 Hz, trans), 7.77−7.82 (m, 2H, Ph), 8.42 (d, 1H, ^3J(H,H) = 16.2 Hz, trans), 8.59 (s, 1H), 8.89 (s, 1H); GC (temperature program B) 16.9 min; MS *m/z* (%) 236 (25), 235 (100, M^+), 221 (5), 208 (5), 193 (5), 181 (3), 167 (6), 155 (3), 140 (9), 128 (7). Anal. ($C_{14}H_{12}N_4$) C, H, N.

4-Chloro-5-nitro-6-phenylpyrimidine (33). A mixture of 4,6-dichloro-5-nitropyrimidine (2 g, 10.36 mmol), benzeneboronic acid (1.26 g, 10.36 mmol), Na_2CO_3 (3.29 g, 31 mmol), Pd-$(PPh_3)_4$ (200 mg, 0.17 mmol), water (8 mL), and toluene (10 mL) was stirred under argon protected from light at 90 °C for 4.5 h. After the addition of water, the mixture was extracted with CH_2Cl_2. The organic layer was dried (Na_2SO_4) and evaporated in vacuo, and the residue was purified by column chromatography (CH_2Cl_2) to afford **33** (1.54 g: 63%): mp 65 °C; ^{1}H NMR δ 7.55−7.71 (m, 5H, Ph), 9.35 (s, 1H); GC (temperature program A) 13.5 min; MS *m/z* (%) 237 (6), 253 (18, M^+), 209 (28), 207 (86), 178 (18), 144 (40), 127 (100), 100 (50), 77 (85).

4-Chloro-6-(4-fluorophenyl)-5-nitropyrimidine (34). The compound was prepared according to the procedure for **33** from 4,6-dichloro-5-nitropyrimidine (2 g, 10.36 mmol), 4-fluorobenzeneboronic acid (1.45 g, 10.36 mmol), Na_2CO_3 (3.29 g, 31 mmol), $Pd(PPh_3)_4$ (200 mg, 0.17 mmol), water (10 mL), and toluene (25 mL) and was purified by column chromatography (CH_2Cl_2) to yield **34** (1.58 g; 60%): mp 57 °C; ^{1}H NMR ($CDCl_3$) δ 7.18−7.27 (m, 2H, 4-F−Ph), 7.71−7.81 (m, 2H, 4-F−Ph), 9.11 (s, 1H);); GC (temperature program A) 13.1 min; MS *m/z* (%) 255 (19), 253 (54, M^+), 223 (21), 187 (18), 165 (23), 145 (100), 118 (25), 99 (71), 75 (44), 57 (18).

Methyl-(5-nitro-6-phenylpyrimidin-4-yl)amine (35). A solution of **33** (1.54 g, 6.55 mmol) and methylamine (20 mL, 40% in water) in dioxane (25 mL) was refluxed for 1 h. After evaporation of the volatiles, the residue was dissolved in ethanol and the solution filtered over SiO_2 (CH_2Cl_2/EtOH 9:1). The filtrate was evaporated in vacuo to give **35** (1.43 g; 95%): mp 142 °C; ^{1}H NMR δ 2.97 (d, 3H, CH_3, ^3J(H,H) = 3.9 Hz), 7.50 (s, br, 5H, Ph), 8.14 (s, br, 1H, N−H, exchangeable), 8.67 (s, 1H); GC (temperature program A) 19.7 min; MS *m/z* (%) 230 (95, M^+), 216 (46), 200 (90), 173 (39), 156 (57), 128 (85), 102 (67), 77 (100), 57 (63).

[6-(4-Fluorophenyl)-5-nitropyrimidin-4-yl]methylamine (36). A solution of **34** (1.58 g, 6.25 mmol) and methylamine (20 mL, 40% in water) in dioxane (25 mL) was refluxed for 1 h. After evaporation of the volatiles, the residue was dissolved in ethanol and the solution filtered over SiO_2 (CH_2Cl_2/EtOH 9:1). The filtrate was evaporated in vacuo to give **35** (1.47 g; 95%): mp 114 °C; ^{1}H NMR ($CDCl_3$) δ 3.19 (d, 3H, CH_3, ^3J(H,H) = 4.9 Hz), 7.09−7.18 (m, 2H, 4-F−Ph), 7.36 (s, br, 1H, N−H, exchangeable), 7.49−7.56 (m, 2H, 4-F), 8.66 (s, 1H); GC (temperature program A) 18.8 min; MS *m/z* (%) 248 (100, M^+), 224 (44), 218 (72), 174 (64), 146 (71), 121 (66), 81 (57), 57 (65).

***N*′4′-Methyl-6-phenylpyrimidin-4,5-diamine (37).** A solution of **35** (1.5 g, 6.55 mmol) in a 1:1 mixture of CH_2Cl_2 and ethanol (50 mL) containing Pd/C (100 mg) was stirred for 30 min under a hydrogen pressure of 4 bar. The mixture was filtered and the solvent evaporated in vacuo to produce **37** (1.2 g; 92%): mp 161 °C; ^{1}H NMR δ 2.91 (d, 3H, CH_3, ^3J(H,H) = 4.5 Hz, exchangeable), 4.52 (s, 2H, N−H_2), 6.73 (d, 1H, C^4-N−H, ^3J(H,H) = 4.5 Hz, exchangeable), 7.37−7.67 (m, 5H, Ph), 8.03 (s, 1H, C^2-H); GC (temperature program B) 10.7 min; MS *m/z* (%) 200 (100, M^+), 199 (98), 170 (20), 143 (14), 117 (15), 104 (31), 89 (22), 77 (18).

6-(4-Fluorophenyl)-*N*′4′-methylpyrimidine-4,5-diamine (38). A solution of **36** (1.55 g, 6.25 mmol) in a 1:1 mixture of CH_2Cl_2 and ethanol (50 mL) containing Pd/C (100 mg) was stirred for 30 min under a hydrogen pressure of 4

bar. The mixture was filtered and the solvent evaporated in vacuo to give **38** (1.23 g; 90%): mp 169 °C; ^{1}H NMR δ 2.90 (d, 3H, CH_3, ^3J(H,H) = 4.5 Hz, exchangeable), 4.57 (s, 2H, N−H_2), 6.76 (d, 1H, C^4-N−H, ^3J(H,H) = 4.5 Hz, exchangeable), 7.23−7.32 (m, 2H, 4-F−Ph), 7.65−7.72 (m, 2H, 4-F−Ph), 8.01 (s, 1H); GC (temperature program B) 10.5 min; MS *m/z* (%) 218 (100, M^+), 217 (93), 188 (26), 161 (19), 139 (21), 122 (42), 107 (32), 95 (18).

9-Methyl-6-phenyl-9*H*-purine (39).[48] A solution of **37** (600 mg, 3 mmol) and *p*-toluenesulfonic acid (10 mg) in a mixture of ethanol (10 mL) and triethylorthoformate (20 mL) was refluxed for 5 h. The volatiles were evaporated in vacuo, and the residue was purified by column chromatography (CH_2Cl_2/EtOH 9:1) to yield **39** (300 mg; 48%): mp 120 °C; ^{1}H NMR δ 3.88 (s, 3H), 7.56−7.63 (m, 3H, Ph), 8.64 (s, 1H), 8.83−8.88 (m, 2H, Ph), 9.00 (s, 1H); GC (temperature program B) 11.7 min; MS *m/z* (%) 210 (100, M^+), 191 (20), 183 (19), 182 (19), 141 (21), 159 (12), 114 (18), 77 (23).

6-(4-Fluorophenyl)-9-methyl-9*H*-purine (40). The compound was prepared from **38** (500 mg, 2.29 mmol) according to the method described for **39** and was purified by column chromatography (EtOAc/hexane 7:3) to yield **40** (190 mg; 36%): mp 140 °C; ^{1}H NMR δ 3.88 (s, 3H), 7.39−7.48 (m, 2H, 4-F−Ph), 8.64 (s, 1H), 8.89−8.95 (m, 2H), 8.98 (s, 1H); GC (temperature program B) 11.2 min; MS *m/z* (%) 228 (100, M^+), 223 (26), 200 (17), 188 (25), 159 (12), 132 (11), 95 (10). Anal. ($C_{12}H_9FN_4$) C, H, N.

6-(4-Fluorophenyl)-9-methyl-8-phenyl-9*H*-purine (41). The compound was prepared from **38** (600 mg, 2.75 mmol) and orthobenzoic acid trimethyl ester (10 mL) according to the method described for **39** and was purified by column chromatography (CH_2Cl_2/EtOH 95:5) to yield **41** (310 mg; 37%): mp 154 °C; ^{1}H NMR δ 3.93 (s, 3H), 7.38−7.67 (m, 5H, Ph), 7.98−8.02 (m, 2H, 4-F−Ph), 8.92−8.99 (m, 3H, 4-F−Ph, C^2-H); GC (temperature program B) 23.1 min; MS *m/z* (%) 304 (100, M^+), 303 (98), 289 (7), 276 (6), 247 (4), 145 (11), 118 (14), 95 (8), 77 (18). Anal. ($C_{18}H_{13}FN_4$) C, H, N; C: calcd, 71.04; found, 69.57.

8-Bromo-6-chloro-9-methyl-9*H*-purine (42). A stirred solution of diisopropylamine (1.70 g, 16.80 mmol) in dry THF (15 mL) was cooled to −78 °C. *n*-Butyllithium (7.17 g, 16.88 mmol, 15% in hexane) was added dropwise and the mixture stirred for 1 h. At this point, a solution of 6-chloro-9-methyl-9*H*-purine[24] (2.02 g, 12 mmol) in dry THF (20 mL) was added slowly and the mixture stirred for 1 h at −78 °C. Subsequently, a solution of dibromotetrachloroethane (7.81 g, 24 mmol) in dry THF (20 mL) was added and the mixture stirred for 1 h at −78 °C. A saturated aqueous solution of ammonium (10 mL) was added. The mixture was allowed to warm to room temperature and THF was evaporated in vacuo. Water (50 mL) was added to the residue and extracted with CH_2Cl_2. The organic phase was dried (Na_2SO_4) and evaporated in vacuo. The residue was extracted with boiling hexane, which was filtered hot. The remainder then was purified by column chromatography (CH_2Cl_2/EtOAc 6:4) to afford **42** (1.96 g; 66%): mp 187 °C; ^{1}H NMR δ 3.7 (s, 3H), 8.7 (s, 1H); GC (temperature program B) 7.2 min; MS *m/z* (%) 250 (25), 249 (8), 248 (100), 247 (8, M^+), 246 (70), 213 (21), 211 (23), 169 (11), 167 (33), 132 (25), 105 (35), 77 (25), 52 (12).

6-Chloro-9-methyl-8-phenyl-9*H*-purine (43). A mixture of **42** (820 mg, 3.33 mmol), benzeneboronic acid (440 mg, 3.60 mmol), K_2CO_3 (550 mg, 4.0 mmol), $Pd(PPh_3)_4$ (120 mg, 0.1 mmol), and toluene (15 mL) was stirred under argon protected from light at 90 °C for 7 h. The mixture was filtered, the filtrate evaporated in vacuo, and the residue purified by column chromatography (CH_2Cl_2/EtOH 975:25) to yield **43** (530 mg; 65%): mp 157 °C; ^{1}H NMR ($CDCl_3$) δ 3.99 (s, 3H), 7.56−7.62 (m, 5H, Ph), 8.76 (s, 1H); GC (temperature program B) 8.8 min; MS *m/z* (%) 245 (33), 244 (82, M^+), 243 (100), 207 (52), 141 (7), 103 (10), 77 (11).

9-Methyl-8-phenyl-6-phenylsulfanyl-9*H*-purine (44). The compound was prepared from **43** (350 mg, 1.43 mmol) and thiophenol (380 mg, 6.2 mmol) according to general procedure A and was purified by column chromatography (CH_2Cl_2/EtOH 975:25) to yield **44** (200 mg; 44%): mp 147 °C; ^{1}H NMR δ 3.90 (s, 3H), 7.50−7.64 (m, 8H, Ph), 7.92−7.97 (m, 2H, Ph), 8.60 (s, 1H); GC (temperature program B) 21.3 min; MS *m/z* (%) 318 (57, M^+), 317 (100), 303 (7), 173 (8), 141 (11), 103 (15), 77 (27). Anal. ($C_{18}H_{14}N_4S$) C, H, N.

6-(4-Fluorophenylsulfanyl)-9-methyl-8-phenyl-9*H*-purine (45). The compound was prepared from **43** (300 mg, 1.23 mmol) and 4-fluorothiophenol (400 mg, 3 mmol) according to general procedure A and was purified by column chromatography (CH_2Cl_2/EtOH 975:25) to yield **45** (310 mg; 75%): mp 157 °C; ^{1}H NMR δ 3.90 (s, 3H), 7.32−7.40 (m, 2H, Ph), 7.61−7.74 (m, 5H, Ph), 7.92−7.97 (m, 2H, Ph), 8.60 (s, 1H); GC (temperature program B) 29.0 min; MS *m/z* (%) 336 (62), 335 (100, M^+), 321 (8), 308 (7), 141 (9), 126 (4). Anal. ($C_{18}H_{13}FN_4S$) C, H, N.

6-Chloro-9-methyl-8-(4-methylsulfanylphenyl)-9*H*-purine (46). A mixture of **42** (500 mg, 2 mmol), 4-methylthiobenzeneboronic acid (370 mg, 2.2 mmol), K_2CO_3 (345 mg, 2.5 mmol), $Pd(PPh_3)_4$ (120 mg, 0.1 mmol), and toluene (15 mL) was stirred under argon protected light at 90 °C for 7.5 h. The mixture was filtered, and the filtrate evaporated in vacuo. The residue was purified by column chromatography (hexane/EtOAc 1:1) to produce **45** (350 mg; 60%): mp 135 °C; ^{1}H NMR δ 2.58 (s, 3H, S−CH_3), 3.95 (s, 3H, N^9-CH_3), 7.45−7.49 (m, 2H, Ph), 7.45−7.49 (m, 2H, Ph), 8.78 (s, 1H); GC (temperature program B) 32.2 min; MS *m/z* (%) 292 (33), 290 (100, M^+), 274 (12), 253 (12), 243 (18), 149 (23).

6-Chloro-8-(4-methansulfinylphenyl)-9-methyl-9*H*-purine (47). To a solution of **46** (660 mg, 2.27 mmol) in glacial acetic acid (6 mL) was added H_2O_2 (1.9 g, 30 mmol, 30% in water) dropwise and the solution stirred for 3 h. Approximately 30 g of ice was added and the solution made basic by the addition of ammonia (25% in water). The mixture was extracted with CH_2Cl_2. The organic phase was washed with water, dried (Na_2SO_4), and evaporated to yield **47** (620 mg; 90%): mp 192 °C; ^{1}H NMR δ 2.87 (s, 3H, SO−CH_3), 3.98 (s, 3H, N^9-CH_3), 7.93−7.97 (m, 2H, Ph), 8.17−8.21 (m, 2H, Ph), 8.53 (s, 1H); GC (temperature program B) 32.4 min; MS *m/z* (%) 308 (18), 306 (54, M^+), 291 (100), 291 (33), 274 (20), 259 (34), 242 (19).

6-Chloro-8-(4-methanesulfonylphenyl)-9-methyl-9*H*-purine (48). A solution of **46** (1.1 g, 3.8 mmol) and 3-chloroperbenzoic acid (2.0 g, 8.14 mmol) in CH_2Cl_2 (20 mL) was refluxed for 4 h. The mixture was washed with a saturated solution of $NaHCO_3$ and water, dried (Na_2SO_4), and evaporated in vacuo to yield **48** (1.16 g; 95%): mp 65 °C; ^{1}H NMR δ 3.34 (s, 3H, SO−CH_3), 3.97 (s, 3H, N^9-CH_3), 8.21 (q, 4H, Ph), 8.85 (s, 1H); GC (temperature program B) 34.0 min; MS *m/z* (%) 317 (51), 322 (100, M^+), 321 (91), 243 (44), 207 (54), 79 (36).

9-Methyl-8-(4-methylsulfanylphenyl)-6-phenylsulfanyl-9*H*-purine (49). The compound was prepared from **46** (320 mg, 1.1 mmol) and thiophenol (288 mg, 2.6 mmol) according to general procedure A and was purified by column chromatography (CH_2Cl_2/EtOH 975:25) to yield **49** (223 mg; 56%): mp 161 °C; ^{1}H NMR δ 2.57 (s, 3H, S−CH_3), 3.90 (s, 3H, N^9-CH_3), 7.44−7.52 (m, 5H, Ph), 7.63−7.68 (m, 2H, Ph), 7.87−7.91 (m, 2H, Ph), 8.58 (s, 1H); GC (temperature program B) 32.8 min; MS *m/z* (%) 364 (65, M^+), 363 (100), 349 (9), 348 (17), 334 (4), 316 (5), 214 (4), 181 (18), 149 (12), 77 (10). Anal. ($C_{19}H_{16}N_4S_2$) C, H, N.

6-(4-Fluorophenylsulfanyl)-9-methyl-8-(4-methylsulfanylphenyl)-9*H*-purine (50). The compound was prepared from **46** (320 mg, 1.1 mmol) and 4-fluorothiophenol (333 mg, 2.6 mmol) according to general procedure A and was purified by column chromatography (CH_2Cl_2/EtOH 975:25) to yield **50** (280 mg; 67%): mp 179 °C; ^{1}H NMR δ 2.57 (s, 3H, S−CH_3), 3.90 (s, 3H, N^9-CH_3), 7.31−7.48 (m, 4H, Ph), 7.67−7.74 (m, 2H, Ph), 7.86−7.91 (m, 2H, Ph), 8.58 (s, 1H). Anal. ($C_{19}H_{15}FN_4S_2$) C, H, N.

8-(4-Methanesulfinylphenyl)-9-methyl-6-phenylsulfanyl-9*H*-purine (51). The compound was prepared from **47** (300 mg, 0.98 mmol) and thiophenol (288 mg, 2.6 mmol) according to general procedure A and was purified by column chromatography (CH_2Cl_2/EtOH 95:5) to yield **51** (180 mg; 50%): mp 196 °C; ^{1}H NMR δ 2.87 (s, 3H, SO−CH_3), 3.95 (s,

3H, N^9-CH_3), 7.50−7.70 (m, 5H, Ph), 7.92−7.96 (m, 2H, Ph), 8.15−8.19 (m, 2H, Ph), 8.64 (s, 1H); ESI-MS *m/z* (%) 381 (100, [M + 1]$^+$), 366 (20). Anal. ($C_{19}H_{16}N_4OS_2$) C, H, N.

6-(4-Fluorophenylsulfanyl)-8-(4-methanesulfinylphenyl)-9-methyl-9*H*-purine (52). The compound was prepared from **47** (300 mg, 0.98 mmol) and 4-fluorothiophenol (333 mg, 2.6 mmol) according to general procedure A and was purified by column chromatography (CH_2Cl_2/EtOH 95:5) to yield **52** (74 mg; 19%): mp 213 °C; ^{1}H NMR δ 2.85 (s, 3H, SO−CH_3), 3.93 (s, 3H, N^9-CH_3), 7.32−7.17 (m, 8H, Ph), 8.62 (s, 1H); ESI-MS *m/z* (%) 399 (100, [M + 1]$^+$), 384 (15). Anal. ($C_{19}H_{15}FN_4OS_2$) C, H, N.

8-(4-Methanesulfonylphenyl)-9-methyl-6-phenylsulfanyl-9*H*-purine (53). The compound was prepared from **48** (500 mg, 1.55 mmol) and thiophenol (444 mg, 4.0 mmol) according to general procedure A and was purified by column chromatography (CH_2Cl_2/EtOH 975:25) to yield **53** (260 mg; 42%): mp 241 °C; ^{1}H NMR δ 3.34 (s, 3H, SO−CH_3), 3.94 (s, 3H, N^9-CH_3), 7.51−7.69 (m, 5H, Ph), 8.20 (q, 4H, Ph), 8.64 (s, 1H); ESI-MS *m/z* (%) 397 (22, [M + 1]$^+$), 318 (100). Anal. ($C_{19}H_{16}N_4O_2S_2$) C, H, N.

6-(4-Fluorophenylsulfanyl)-8-(4-methanesulfonylphenyl)-9-methyl-9*H*-purine (54). The compound was prepared from **48** (500 mg, 1.55 mmol) and 4-fluorothiophenol (512 mg, 4.0 mmol) according to general procedure A and was purified by column chromatography (CH_2Cl_2/EtOH 975:25) to yield **54** (150 mg; 23%): mp 250 °C; ^{1}H NMR δ 3.13 (s, 3H, SO−CH_3), 3.99 (s, 3H, N^9-CH_3), 7.14−7.26 (m, 2H, 4-F−Ph), 7.62−7.69 (m, 2H, 4-F−Ph), 8.12 (q, 4H, Ph), 8.66 (s, 1H); ESI-MS *m/z* (%) 415 (30, [M + 1]$^+$), 336 (100). Anal. ($C_{19}H_{15}FN_4O_2S_2$) C, H, N.

8-Bromo-9-methyl-6-phenylsulfanyl-9*H*-purine (55). The compound was prepared from **11** (2.42 g, 10 mmol) according to the procedure described for **42** and was purified by column chromatography (CH_2Cl_2/EtOH 95:5) to yield **55** (1.76 g; 55%): mp 166 °C; ^{1}H NMR δ 3.82 (s, 3H), 7.44−7.67 (m, 5H, Ph), 8.56 (s, 1H); GC (temperature program B) 12.6 min; MS *m/z* (%) 322 (34), 321 (100, M$^+$), 320 (34), 319 (96), 241 (19), 199 (7), 173 (8), 132 (10), 109 (13), 105 (12), 77 (16). Anal. ($C_{12}H_9BrN_4S$) C, H, N.

4-(9-Methyl-6-phenylsulfanyl-9*H*-purin-8-yl)but-3-yn-1-ol (56). A mixture of **55** (482 mg, 1.5 mmol), but-3-yn-1-ol (158 mg, 2.25 mmol), triethylamine (505 mg, 5 mmol), $PdCl_2(PPh_3)_2$ (81 mg, 0.12 mmol), CuI (40 mg, 0.23 mmol), and CH_2Cl_2 (10 mL) was refluxed for 7 h under argon protected from light. The mixture was filtered and the filtrate evaporated in vacuo. The residue was purified by column chromatography (CH_2Cl_2/EtOH/EtOAc 9:2:1) to afford **56** (30 mg; 6.5%): mp 231 °C (dec); ^{1}H NMR δ 2.76 (t, 2H, CH_2, ^3J(H,H) = 6.2 Hz), 3.69 (q, 2H, CH_2, ^3J(H,H) = 6.2 Hz), 3.80 (s, 3H), 5.10 (t, 1H, OH, ^3J(H,H) = 6.2 Hz), 7.51−7.64 (m, 5H, Ph), 8.58 (s, 1H). Anal. ($C_{16}H_{14}N_4OS$) C, H, N.

9-Benzyl-6-phenylsulfanyl-9*H*-purine (57). The compound was prepared from **21** (423 mg, 1.73 mmol) and thiophenol (490 mg, 4.46 mmol) according to general procedure A and was purified by column chromatography (EtOAc/hexane 7:3) to yield **57** (383 mg; 70%): mp 121 °C; ^{1}H NMR δ 5.41 (s, 2H, CH_2), 7.29−7.49 (m, 8H, Ph), 7.46−7.66 (m, 2H, Ph), 7.98 (s, 1H), 8.65 (s, 1H)); GC (temperature program B) 21.1 min; MS *m/z* (%) 318 (90, M$^+$), 317 (100), 227 (44), 91 (96). Anal. ($C_{18}H_{14}N_4S$) C, H, N.

9-Benzyl-6-(4-fluorophenylsulfanyl)-9*H*-purine (58). The compound was prepared from **21** (423 mg, 1.73 mmol) and 4-fluorothiophenol (570 mg, 4.46 mmol) according to general procedure A and was purified by column chromatography (EtOAc/hexane 7:3) to yield **58** (500 mg; 86%): mp 163 °C; ^{1}H NMR δ 5.42 (s, 2H, CH_2), 7.12−7.37 (m, 7H, Ph), 7.60−7.67 (m, 2H, Ph), 7.98 (s, 1H), 8.65 (s, 1H); GC (temperature program B) 19.3 min; MS *m/z* (%) 336 (55, M$^+$), 335 (76), 245 (33), 91 (100). Anal. ($C_{18}H_{13}FN_4S$) C, H, N.

9-(4-Fluorobenzyl)-6-phenylsulfanyl-9*H*-purine (59). The compound was prepared from **23** (453 mg, 1.73 mmol) and thiophenol (490 mg, 4.46 mmol) according to general procedure A and was purified by column chromatography (EtOAc/hexane 7:3) to yield **59** (505 mg; 87%): mp 128 °C; ^{1}H NMR δ 5.38 (s, 2H, CH_2), 6.99−7.08 (m, 2H, Ph), 7.26−7.33 (m, 2H, Ph), 7.45−7.49 (m, 3H, Ph), 7.64−7.69 (m, 2H, Ph), 7.97 (s, 1H), 8.64 (s, 1H); GC (temperature program B) 19.3 min; MS *m/z* (%)337 (8), 336 (28, M$^+$), 227 (17), 110 (9), 109 (100), 83 (18), 77 (7). Anal. ($C_{18}H_{13}FN_4S$) C, H, N.

9-(4-Fluorobenzyl)-6-(4-fluorophenylsulfanyl)-9*H*-purine (60). The compound was prepared from **23** (453 mg, 1.73 mmol) and 4-fluorothiophenol (570 mg, 4.46 mmol) according to general procedure A and was purified by column chromatography (EtOAc/hexane 7:3) to yield **60** (480 mg; 78%): mp 188 °C; ^{1}H NMR δ 5.39 (s, 2H, CH_2), 7.00−7.33 (m, 6H, Ph), 7.60−7.67 (m, 2H, Ph), 7.98 (s, 1H), 8.64 (s, 1H); GC (temperature program B) 19.3 min; MS *m/z* (%) 354 (45, M$^+$), 245 (25), 109 (100), 83 (25). Anal. ($C_{18}H_{12}F_2N_4S$) C, H, N.

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Supporting Information Available: Analysis data for the novel described test compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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