University of Groningen

Imidazole- and Benzimidazole-Based Inhibitors of the Kinase IspE

Mombelli, Paolo; Le Chapelain, Camille; Munzinger, Noah; Joliat, Evelyne; Illarionov, Boris; Schweizer, W. Bernd; Hirsch, Anna K. H.; Fischer, Markus; Bacher, Adelbert; Diederich, Francois

Published in:
European Journal of Organic Chemistry

DOI:
[10.1002/ejoc.201201467](https://doi.org/10.1002/ejoc.201201467)

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2013

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):

Mombelli, P., Le Chapelain, C., Munzinger, N., Joliat, E., Illarionov, B., Schweizer, W. B., Hirsch, A. K. H., Fischer, M., Bacher, A., & Diederich, F. (2013). Imidazole- and Benzimidazole-Based Inhibitors of the Kinase IspE: Targeting the Substrate-Binding Site and the Triphosphate-Binding Loop of the ATP Site. *European Journal of Organic Chemistry*, 2013(6), 1068-1079. <https://doi.org/10.1002/ejoc.201201467>

Copyright

Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license. More information can be found on the University of Groningen website: <https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment>.

Take-down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): <http://www.rug.nl/research/portal>. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.

Imidazole- and Benzimidazole-Based Inhibitors of the Kinase IspE: Targeting the Substrate-Binding Site and the Triphosphate-Binding Loop of the ATP Site

Paolo Mombelli,^[a] Camille Le Chapelain,^[a] Noah Munzinger,^[a] Evelyne Joliat,^[a]
Boris Illarionov,^[b] W. Bernd Schweizer,^[a] Anna K. H. Hirsch,^[c] Markus Fischer,^{*[b]}
Adelbert Bacher,^{*[b]} and François Diederich^{*[a]}

Keywords: Drug design / Inhibitors / Ligand design / Molecular recognition

The enzymes of the mevalonate-independent biosynthetic pathway to isoprenoids are attractive targets for the development of new drug candidates, in particular against malaria and tuberculosis, because they are present in major human pathogens but not in humans. Herein, the structure-based design, synthesis, and biological evaluation of a series of inhibitors featuring a central imidazole or benzimidazole scaffold for the kinase IspE from *E. coli*, a model for the corresponding malarial enzyme, are described. Optimization of the binding preferences of the hydrophobic sub-pocket at the substrate-binding site allowed IC_{50} values in the lower micromolar range to be reached. Structure–activity relationship studies using a 1,2-disubstituted imidazole central core revealed that alicyclic moieties fit the sub-pocket better than

acyclic aliphatic and aromatic residues. The phosphate-binding region in the ATP-binding site of IspE, a neutral glycine-rich loop, was addressed for the first time by an additional vector attached to the central core. Polar functional groups, such as trifluoromethyl or nitriles, were introduced to undergo orthogonal dipolar interactions with the amide groups in the loop. Alternatively, small hydrogen-bond-accepting heterocyclic residues, capable of binding to the convergent NH groups in the loop, were explored. The biological data showed slightly improved inhibitory potency in some cases and confirmed the challenges in addressing, with gain in binding affinity, the highly water-exposed sections of enzyme active sites, such as the glycine-rich loop of IspE.

Introduction

Discovered in the early 1990s, the non-mevalonate pathway is a biosynthetic route to assemble the universal isoprenoid precursors, isopentenyl diphosphate (IPP; **1**) and dimethylallyl diphosphate (DMAPP; **2**), in seven enzymatic steps starting from pyruvate (**3**) and D-glyceraldehyde 3-phosphate (**4**) (Scheme 1).^[1] The enzymes of this pathway are essential in plants^[2] and many human pathogens, including the tuberculosis-causing *Mycobacterium* (*M.*) *tuberculosis*^[3] and the causative agent of malaria, *Plasmodium* (*P.*) *falciparum*.^[4] In humans, in contrast, isoprenoids are exclusively assembled through the mevalonate-dependent

biosynthetic route, with acetyl coenzyme A serving as the only carbon source. Thus, the enzymes of the non-mevalonate pathway are widely recognized as attractive targets for the generation of lead compounds with potential as selective antibacterial, antimalarial, or agrochemical agents featuring novel modes of action, which is a fundamental issue that needs to be overcome to fight fast-developing resistance.^[5]

The fourth enzyme and only kinase involved in the non-mevalonate pathway, IspE [4-diphosphocytidyl-2C-methyl-D-erythritol (CDP-ME) kinase, EC 2.7.1.148], catalyzes the ATP- and Mg^{2+} -dependent phosphorylation of the tertiary alcohol of CDP-ME (**5**) to give 4-diphosphocytidyl-2C-methyl-D-erythritol 2-phosphate (CDP-ME2P, **6**; Scheme 1).^[6] Due to the lack of structural information on IspE orthologues from pathogens until the recent report of X-ray crystal structures of the *M. tuberculosis* enzyme,^[6h] our initial efforts in mapping the molecular recognition properties of IspE used the enzyme from *Escherichia* (*E.*) *coli* as a model system. In our previous studies, we extensively explored the binding preferences of the small hydrophobic and the ribose sub-pockets of the substrate-binding site.^[6g,7]

Herein, we report the structure-based design, synthesis, and biological evaluation of imidazole- and benzimidazole-based inhibitors targeting the phosphate recognition site –

[a] Laboratorium für Organische Chemie, ETH Zürich, Hönggerberg, HCI, 8093 Zürich, Switzerland
Fax: +41-44-632-1109

E-mail: diederich@org.chem.ethz.ch

Homepage: <http://www.diederich.chem.ethz.ch>

[b] Hamburg School of Food Science, Institut für Lebensmittelchemie, Universität Hamburg, Grindelallee 117, 20146 Hamburg, Germany
Fax: +49-40-42838-4342

E-mail: markus.fischer@hsfs.uni-hamburg.de

Homepage: <http://www.chemie.uni-hamburg.de/lc/fischer.html>

[c] Stratingh Institute for Chemistry, University of Groningen, Nijenborgh 7, 9747 AG Groningen, The Netherlands

Supporting information for this article is available on the WWW under <http://dx.doi.org/10.1002/ejoc.201201467>.

Scheme 1. The non-mevalonate biosynthetic pathway to isoprenoid precursors, i.e., isopentenyl diphosphate (IPP; **1**) and dimethylallyl diphosphate (DMAPP; **2**). DXS: 1-deoxy-D-xylulose 5-phosphate synthase (EC 2.2.1.7); IspC: 1-deoxy-D-xylulose 5-phosphate reductoisomerase (EC 1.1.1.267); IspD: 4-diphosphocytidyl-2C-methyl-D-erythritol 4-phosphate synthase (EC 2.7.7.60); IspE: 4-diphosphocytidyl-2C-methyl-D-erythritol kinase (EC 2.7.1.148); IspF: 2C-methyl-D-erythritol 2,4-cyclodiphosphate synthase (EC 4.6.1.12); IspG: 2C-methyl-D-erythritol 2,4-cyclodiphosphate reductase (EC 1.17.7.1); IspH: (2E)-1-hydroxy-2-methyl-2-butenyl 4-diphosphate reductase (EC 1.17.1.2).

a “neutral” glycine-rich loop – in the ATP-binding pocket, in addition to the cytidine-binding pocket and the hydrophobic sub-pocket of the substrate-binding site of *E. coli* IspE. In addition to expanding our knowledge of the molecular recognition properties of IspE, this approach is interesting in view of the large number of such neutral phosphate recognition sites in biological systems and the lack of systematic studies addressing them.^[8]

Results and Discussion

Ligand Design

Molecular modeling was performed with the software MOLOC^[9] using the X-ray crystal structure of *E. coli* IspE in complex with CDP-ME (**5**) and the ATP-analogue adenosine-5'-(γ -imino)triphosphate (AMP-PNP, PDB accession code 1OJ4, 2.0 Å resolution).^[6c] The active site of *E. coli* IspE can be divided into three main pockets, corresponding to the cytidine- and methylerythritol-binding regions for the recognition of substrate **5**, and the adenosine-binding pocket at the other end of the active site (Figure 1).

In our previous studies, we have addressed the cytidine-binding pocket with a 1,5-disubstituted cytosine scaffold stacking between the aromatic side chains of Tyr25 and Phe185, similar to the natural substrate. Saturated five-membered heterocycles such as 2-tetrahydrothienyl or 2-tetrahydrofuranlyl moieties were shown to address the ribose sub-pocket best.^[6g,7b] Modeling revealed the presence of a small, hydrophobic sub-pocket lined by Leu15, Leu28, and Phe185 adjacent to the cytidine-binding pocket. Appropriate filling, achieved with small lipophilic residues per-

fectly oriented into the cavity by a propargylic sulfonamide moiety in its preferred conformation (staggered with the N-lone pair bisecting the O=S=O moiety), allowed inhibitory constants (K_i) down to the triple-digit nanomolar range to be obtained.^[7a]

The introduction of an additional third vector towards the glycine-rich loop required the installation of a new central core in the ligands. Modeling suggested a 1,2,4-trisubstituted imidazole ring as an appropriate scaffold to direct the three exit vectors, with the heterocycle stacking on the polarized side chain of Asp141 (Figure 2).

We first investigated the appropriate filling of the hydrophobic sub-pocket by introducing different lipophilic substituents at imidazole-C-2. Subsequently, the triphosphate-binding region of *E. coli* IspE was addressed by the introduction of pharmacophores linked to position C-4 of the imidazole moiety by a methylene spacer. In IspE, a glycine-rich loop (Gly101–Ser108; Figure 1) is responsible for binding the ATP-triphosphate through multiple hydrogen bonds with the converging backbone NH groups, without the involvement of basic amino acids or metal cations.^[6c,6f,8] This confers a certain lipophilic character to the loop, which, together with a balance between preorganization and the ability to wrap around a guest, makes it appealing for increasing binding affinity, also taking advantage of a potential bisubstrate inhibition mode.^[10] In addition to hydrogen-bond-accepting heterocycles such as tetrazole or triazole, we considered small and negatively polarized functional groups, such as nitriles and trifluoromethyl groups, as phosphate surrogates suitable for this neutral glycine-rich loop, due to their ability to undergo favorable orthogonal dipolar interactions.^[11]

Figure 1. Schematic view of the active site of *E. coli* IspE depicting the different pockets, of which the inhibitors described here address the cytidine-binding pocket, the hydrophobic sub-pocket, and the phosphate-binding loop.

Figure 2. Design strategy of new ligands bearing a 1,2,4-trisubstituted imidazole central core and proposed binding mode of the imidazole fragment at the active site of *E. coli* IspE (PDB code: 1OJ4)^[6c] as modeled with MOLOC. Distances are given in Å units. Color code: gray C_{enzyme} , green C_{ligand} , blue N, red O, yellow S. If not otherwise stated, this color code is used throughout.

Optimization of the Binding towards the Sub-Pocket

Ligands ($\pm$)-**7**–($\pm$)-**12** (see Table 1 for the structures) were synthesized in two or three steps, as illustrated in Scheme 2 for compound ($\pm$)-**10** [for the syntheses of the other ligands, see the Supporting Information, Scheme S1]. In the first step, cyclohexanecarbaldehyde (**13**) was treated with glyoxal

and ammonium hydrogen carbonate in water, following a literature procedure, to afford the 2-substituted imidazole **14**.^[12] Alkylation of imidazole **14** with propargyl bromide gave alkyne **15**, which was subsequently cross-coupled with the previously described 1-thiolanlylated 5-iodocytosine ($\pm$)-**16**^[7a] under Sonogashira conditions to provide the desired ligand ($\pm$)-**10** in moderate yield.

Scheme 2. Synthesis of ligands (±)-10 and (±)-17. *Reagents and conditions:* (a) Glyoxal, NH_4HCO_3 , H_2O , 25 °C, 18 h, 25%; (b) propargyl bromide, Cs_2CO_3 , DMF, 0 to 25 °C, 9 h, 28%; (c) $[\text{PdCl}_2(\text{PPh}_3)_2]$, CuI, Et_3N , DMF, 25 °C, 3.5 h, 25%; (d) $\text{Me}_2\text{NSO}_2\text{Cl}$, Et_3N , CH_2Cl_2 , 25 °C, 14 h, 62%; (e) i. $n\text{BuLi}$, THF, −78 °C, 45 min; ii. DMF, −78 to 25 °C, 2 h, 83%; (f) Me_3SiCF_3 , $n\text{Bu}_4\text{NF}$, THF, 0 to 25 °C, 2 h, 73%; (g) NaH, MeI, DMF, 0 to 25 °C, 16 h, 85%; (h) 5% aq. HCl, 25 °C, 7 h, 90%; (i) propargyl bromide, Cs_2CO_3 , DMF, 0 to 25 °C, 8 h, 48%; (j) (±)-16, $[\text{PdCl}_2(\text{PPh}_3)_2]$, CuI, Et_3N , DMF, 25 °C, 7 h, 47%.

Table 1. Structures and inhibitory activities of ligands (±)-7–(±)-12 against *E. coli* IspE.

Inhibitor	R	clogD ^[a] at pH 8.0	IC ₅₀ [μM]	K _i ^[b] [μM]
(±)-7	H	1.87	>500	–
(±)-8	<i>n</i> Pr	2.54	83	48
(±)-9	<i>i</i> Bu	2.89	80	46
(±)-10	cyclohexyl	3.50	12	6.9
(±)-11	cyclopentyl	2.97	15	8.6
(±)-12	Ph	3.42	144	83

[a] The clogD values were calculated with the Advanced Chemistry Development (ACD/Labs) Software v. 12.5 (1994–2012, ACD/Labs). [b] K_i values were calculated from IC₅₀ values and K_m = 150 μM using the Cheng–Prusoff equation.^[20]

The half-maximal (50%) inhibitory concentration (IC₅₀) values were determined in an enzyme-coupled assay using pyruvate kinase and lactate dehydrogenase as auxiliary enzymes to enable photometric monitoring (see Scheme S2).^[13] For the phosphorylation of the substrate CDP-ME (5), IspE consumes ATP, which is then regener-

ated by pyruvate kinase dephosphorylating phosphoenolpyruvate. The resulting pyruvate is reduced to L-lactate by the lactate dehydrogenase using NADH as reducing agent. The consumption of NADH is monitored photometrically at 340 nm. This photometric assay has previously been proven to work effectively for structurally related compounds by comparison with a direct NMR spectroscopic assay.^[13]

The inhibitory potency towards *E. coli* IspE was found to be strongly dependent on the substituent filling the hydrophobic sub-pocket (Table 1). Compound (±)-7, unsubstituted at imidazole-C-2, did not display any significant inhibition at 500 μM, whereas an additional substituent at C-2 resulted in a substantial gain in binding affinity. Linear or branched alkyl chains, such as *n*-propyl and isobutyl, led to IC₅₀ values in the double-digit micromolar range for compounds (±)-8 and (±)-9.

Replacement of the alkyl chains with saturated alicyclic moieties in inhibitors (±)-10 and (±)-11 resulted in an additional five- to sixfold enhancement in activity, with IC₅₀ values of 12 and 15 μM, respectively. Interestingly, the phenyl-substituted analogue (±)-12 was found to be substantially less potent (IC₅₀ value = 144 μM), indicating that

the inhibitory activity increase of compounds ($\pm$)-**10** and ($\pm$)-**11** is not solely a result of increasingly favorable partitioning (increasing $\text{clog}D$ values), but also of a better fit of the respective substituents into the sub-pocket (Figure 3).

Figure 3. Close-up of the binding mode of ($\pm$)-**10** in the hydrophobic sub-pocket of *E. coli* IspE as modeled with MOLOC (PDB code: 1OJ4). Distances in Å.

Addressing the Phosphate-Binding Loop

The cyclohexyl substituent was maintained as the moiety filling the hydrophobic sub-pocket, due to the superior binding affinity of ($\pm$)-**10** compared with the other compounds included in Table 1. Therefore, the new series of compounds was based on the structure of inhibitor ($\pm$)-**10** bearing an additional vector at imidazole-C-4 for targeting the glycine-rich P-loop.

The introduction of the additional substituent in ligands ($\pm$)-**17**–($\pm$)-**23** (see Table 2 for the structures) was achieved through the organolithium-mediated formylation of the imidazole moiety and subsequent chain extension by intergroup conversion of the resulting aldehyde. As an example, the synthesis of compound ($\pm$)-**17** is shown in Scheme 2. The 2-substituted imidazole **14** was protected with the *ortho*-directing *N,N*-dimethylsulfamoyl group using the corresponding sulfamoyl chloride and triethylamine as base to give **24** in moderate yield.^[14] Lithiation of imidazole **24** with *n*BuLi, followed by reaction with *N,N*-dimethylformamide (DMF), afforded 5-formylimidazole **25** as a single regioisomer in very good yield.^[15] Aldehyde **25** was then treated with the Ruppert–Prakash reagent^[16] and tetrabutylammonium fluoride to afford alcohol ($\pm$)-**26** as a racemate in good yield. Methylation of the secondary alcohol and subsequent cleavage of the protecting group of ($\pm$)-**27** under acidic conditions gave key intermediate ($\pm$)-**28**. The imidazole moiety was then *N*-alkylated with propargyl bromide using cesium carbonate as base, affording a mixture of regioisomers, which was separated by column chromatography to provide the desired 1,2,4-trisubstituted imidazole ($\pm$)-**29** in moderate yield. Finally, Sonogashira cross-coupling with 5-iodocytosine ($\pm$)-**16** yielded the target compound ($\pm$)-**17** in 47% yield. All other 1,2,4-trisubstituted imidazoles were prepared starting from aldehyde **25** as a common precursor following similar strategies.

The nitrile functional group of ligands ($\pm$)-**18** and ($\pm$)-**19** were introduced by Horner–Wadsworth–Emmons reaction; the common precursor of dinitriles ($\pm$)-**20** and ($\pm$)-**21** was obtained by Knoevenagel condensation (see Scheme S3). Reduction of the aldehyde functional group to give the corresponding alcohol and subsequent activation by treatment with thionyl chloride allowed the introduction by nucleophilic substitution of the 1*H*-tetrazole and 1,2,3-*H*-triazole moieties in ligands ($\pm$)-**22** and ($\pm$)-**23**, respectively (see Scheme S4).

Table 2. Structures and inhibitory activities of ligands ($\pm$)-**17**–($\pm$)-**23** against *E. coli* IspE.

Inhibitor	R	$\text{clog}D^{[a]}$ at pH 8.0	IC_{50} [μM]	$K_i^{[b]}$ [μM]
($\pm$)- 17		4.42	9.9	5.7
($\pm$)-(<i>E</i>)- 18		3.75	32	18
($\pm$)- 19		3.03	37	21
($\pm$)- 20		3.76	38	22
($\pm$)- 21		2.82	22	13
($\pm$)- 22		2.81	87	50
($\pm$)- 23		2.71	107	61

[a] The $\text{clog}D$ values were calculated with the Advanced Chemistry Development (ACD/Labs) Software v. 12.5 (1994–2012, ACD/Labs). [b] K_i values were calculated from IC_{50} values and $K_m = 150 \mu\text{M}$ using the Cheng–Prusoff equation.^[20]

The inhibitory activity of ligands ($\pm$)-**17**–($\pm$)-**23** against *E. coli* IspE is summarized in Table 2. Trifluoromethyl derivative ($\pm$)-**17** was the most active compound of the series, with an IC_{50} value of $9.9 \mu\text{M}$. The more polar nitriles ($\pm$)-(*E*)-**18** and ($\pm$)-**19** and dinitriles ($\pm$)-**20** and ($\pm$)-**21** were less active, with IC_{50} values between 22 and $38 \mu\text{M}$. Tetrazole ($\pm$)-**22** and triazole ($\pm$)-**23** displayed a substantially lower affinity, also compared to reference compound ($\pm$)-**10**.

According to molecular modeling studies, all the compounds in this series should establish favorable orthogonal dipolar interactions with the peptide bonds of the glycine-rich loop (see Figure S1). Only the trifluoromethyl derivative ($\pm$)-**17**, however, was found to be slightly more active than the parent compound ($\pm$)-**10** unsubstituted at imidazole-C-4. This might indicate that the methylene linker carrying the phosphate surrogate is too flexible to efficiently position the pharmacophore in the phosphate-binding re-

gion. This flexibility allows phosphate surrogates, in particular the more polar versions, to orient towards bulk water rather than binding to the loop.

Benzimidazole-Based Ligands

The next series of compounds was designed with the specific aim of establishing a more direct and preorganized access to the glycine-rich loop. For this purpose, the imidazole core was extended to include a benzimidazole moiety. In this way, the methylene linker could be omitted and the tendency of the phosphate mimic to orient toward the bulk solvent should thereby be attenuated. Molecular modeling studies suggested that positions C-4 and C-5 of the benzimidazole core were well-suited for targeting the glycine-rich loop, depending on the phosphate surrogate chosen.

Benzimidazoles ($\pm$)-**30**–($\pm$)-**34** (see Figure 4 for the structures) were synthesized by applying the strategy described previously for the imidazole-based ligands. This involved construction of the heterocyclic core and its eventual modification by intergroup conversion, followed by *N*-alkylation and final Sonogashira cross-coupling. As an example, the synthesis of 4-alkoxybenzimidazole ($\pm$)-**34** is shown in Scheme 3 (for the syntheses of the other benzimidazole-

derived ligands, see Scheme S5). Reductive cyclization of 2-amino-3-nitrophenol (**35**) with aldehyde **13** in the presence of sodium dithionite according to a literature procedure gave 2,4-disubstituted benzimidazole **36** in 80% yield.^[17] Treatment of alcohol **36** with 2-propanol under Mitsunobu conditions provided ether **37** in moderate yield after recrystallization from ethyl acetate. Whereas the alkylation of 2,5-disubstituted benzimidazoles afforded a ca. 1:1 mixture of regioisomers, usually separable with column chromatography with the structural assignment based on 2D NMR spectroscopy, the outcome of the alkylation of 4-alkoxybenzimidazole **37** with sodium hydride and propargyl bromide proved to be strongly dependent on the solvent (see Figure S2). In DMF, the undesired and sterically more hindered 1,2,7-trisubstituted benzimidazole **38** was the major isomer formed (ratio **38/39** = 4:1), indicating electronic rather than steric control of the reaction. The regioselectivity of the alkylation was inverted in tetrahydrofuran (THF), in which a 1:3 ratio of **38/39** was observed. The ratio in favor of the desired isomer was significantly improved (**38/39** = 1:5) in a less polar, noncoordinating solvent, such as dichloromethane. This observation supports a mechanism involving coordination of the Na⁺ cation to the deprotonated nitrogen atom of the benzimid-

Figure 4. Structures and inhibitory activities of ligands ($\pm$)-**30**–($\pm$)-**34** against *E. coli* IspE. [a] clogD values were calculated with Advanced Chemistry Development (ACD/Labs) Software V12.5 (1994–2012 ACD/Labs). [b] K_i values were calculated from IC_{50} values and $K_m = 150 \mu\text{M}$ using the Cheng–Prusoff equation.^[20]

Scheme 3. Synthesis of ligand (±)-**34** and ORTEP plot at 100 K (atomic displacement parameters are shown at the 50% probability level) of the X-ray crystal structure of (±)-**34**. For the sake of clarity, solvent molecules are omitted and only one position of the disordered tetrahydrothienyl ring and isopropyl group are shown. *Reagents and conditions:* (a) 1 M aq. Na₂S₂O₄, EtOH, 70 °C, 5 h, 80%; (b) PPh₃, diisopropyl azodicarboxylate, *i*PrOH, THF, 25 °C, 2 h, 49%; (c) propargyl bromide, NaH, CH₂Cl₂, 0 to 25 °C, 24 h, 44%; (d) (±)-**16**, [PdCl₂(PPh₃)₂], CuI, *i*Pr₂NEt, DMF, 25 °C, 24 h, 36%.

azole moiety assisted by the proximal alkoxy group. As a consequence, the N-3 position is blocked, and alkylation is directed to position N-1. In polar solvents such as DMF, the solvent competes for Na⁺ complexation, and electronic control of the regioselectivity is established. Sonogashira cross-coupling reaction of 1,2,4-isomer **39** with 5-iodocytosine (±)-**16** finally afforded ligand (±)-**34** in moderate yield.

Crystals of benzimidazole (±)-**34** suitable for X-ray crystal structure analysis were grown by slow evaporation of a CH₂Cl₂/MeOH solution (Scheme 3), subsequent measurements confirmed the correct assignment of the regioisomers formed in the alkylation reaction. In the crystal structure, the isopropoxy substituent is disordered and adopts two orientations. In both conformations, however, the O(10)–C(11) bond is almost antiperiplanar to the C(4)–C(9) bond, with torsion angles $|\tau|(\text{C8}–\text{C9}–\text{O10}–\text{C11})$ of 7° and 2°, respectively, allowing conjugation of the oxygen atom with the aromatic system. The propargylic unit of (±)-**34** is perpendicular to the aromatic ring, as expected, with a torsion angle $\tau(\text{C2}–\text{N1}–\text{C20}–\text{C21}) = 100^\circ$. These conformational preferences were corroborated by searches in the Cambridge Structural Database (CSD)^[18] performed on similar molecular fragments (see Figure S3).

The inhibitory activities of ligands (±)-**30**–(±)-**34** against *E. coli* IspE were determined by using the photometric assay described above and are summarized in Figure 4. The unsubstituted control compound (±)-**30** displayed an *IC*₅₀ value of 17 μM, which is in a similar range to that of the corresponding imidazole (±)-**10** (Table 1). Introduction of

a CF₃ group at position C-5 of the benzimidazole core resulted in a slight loss of potency for (±)-**31**, with an *IC*₅₀ value of 25 μM. The more polar nitrile (±)-**32** (*IC*₅₀ = 13 μM) was found to be slightly more potent. Interestingly, the trend is reversed as compared to the imidazole-based inhibitors with a flexible linker, in which activity was lost with the introduction of more polar residues and was highest for the CF₃ derivative (see Table 1). With an *IC*₅₀ value of 85 μM, phosphate (±)-**33** is a weaker inhibitor than control compound (±)-**30**. The dramatic difference in *clogD* values [(±)-**30**: 5.97; (±)-**33**: –0.37], however, suggests that the expected energetic gains from phosphate binding to the glycine-rich loop, in analogy to the β-phosphate of AMP-PNP in the cocrystal structure (see Figure S4), might well be overcompensated for by the highly unfavorable partitioning. The lipophilic isopropoxy counterpart (±)-**34** is inactive at inhibitor concentrations below 500 μM, despite strain-free binding suggested by the modeling. This finding also supports attractive polar interactions of the substituents in (±)-**31**–(±)-**33** with the P-loop.

Conclusions

We have used the structure-based approach to design ligands featuring trisubstituted imidazole or benzimidazole central cores to address the cytidine-binding pocket, the hydrophobic sub-pocket, and the glycine-rich loop at the active site of the kinase IspE from *E. coli*. The syntheses of

the inhibitors relied on the construction of the heterocyclic moieties and their subsequent regioselective functionalization, such as by formylation of imidazoles and alkylation of 4-alkoxybenzimidazoles. Biological evaluation of the ligands demonstrated that appropriate filling of the hydrophobic sub-pocket is essential to gain binding affinity towards IspE, as this cavity represents one of the few lipophilic environments of the otherwise highly polar active site. Addressing the water-exposed phosphate-binding region requires careful choice of the linker. Whereas flexible linkers are not appropriate to orient polar phosphate surrogates into the loop, they seem to be better tolerated for apolar phosphate mimics. We are currently focused on further enhancing the degree of preorganization of the ligands, which should allow additional binding affinity in the glycine-rich loop to be gained. The study has provided valuable new insight into the molecular recognition properties at the active site of IspE. The fact that the best biological activities only reach into the lower micromolar IC_{50} -range, despite convincing molecular modeling predictions, underlines once more the challenges of efficiently addressing highly polar binding sites with synthetic ligands.

Experimental Section

In vitro Assays, Materials: [1,3,4- $^{13}C_3$]4-Diphosphocytidyl-2C-methyl-D-erythritol (CDP-ME, **5**) was prepared as described earlier.^[13] *E. coli* IspE was prepared according to a published procedure.^[6a] NADH and phosphoenolpyruvate potassium salt (PEP) were purchased from Biomol; ATP, the pyruvate kinase, and the lactate dehydrogenase from Sigma–Aldrich.

Enzyme-Coupled Photometric Assay for IC_{50} Determination: Assays were conducted in 96-well plates (Nunc, Cat. No 781602) with transparent flat bottoms. Assay mixtures were prepared as described previously^[13] with some minor modifications: A solution containing 100 mM Tris-HCl (pH 8.0), 10 mM $MgCl_2$, 2 mM dithiothreitol, 2.5 mM potassium phosphoenolpyruvate, 2 mM ATP, 0.46 mM NADH, 1 U of lactate dehydrogenase, 1 U of pyruvate kinase, and 1 U of IspE protein (total volume 60 μ L) was added to the inhibitor solution (60 μ L). Dilution series (1:2) of inhibitors covered the concentration range of 200 to 0.8 μ M. The reaction was started by addition of CDP-ME (60 μ L; final concentration 250 μ M) and monitored at a wavelength of 340 nm at 27 °C. IC_{50} values were evaluated with a nonlinear regression method using the program Dynafit.^[19]

Chemistry. General: For a detailed description of the chemicals, analytical equipment, and experimental methods used, see the Supporting Information. In the following, the general synthetic procedures and experimental details for the syntheses of all compounds shown in Scheme 1 and Scheme 3 are described. All other synthetic and analytical protocols are included in the Supporting Information. For the atom numbering used for the assignment of the 1H NMR spectra, see the spectra appended in the Supporting Information.

N-Alkylation of Imidazoles. General Procedure A: A solution of the imidazole (1.0 equiv.) in DMF (7.7 mL/mmol) was treated with CS_2CO_3 (1.1 equiv.) and the alkyl halide (1.0 equiv.) at 0 °C. The suspension was stirred at 0 °C for 0.5 h and at 25 °C for 6–20 h and concentrated. The residue was treated with water and extracted

twice with CH_2Cl_2 . The combined organic phases were washed with water (3 $\times$) and brine, dried with $MgSO_4$, filtered, and concentrated. The residue was purified by column chromatography (CC).

Sonogashira Cross-Coupling Reaction. General Procedure B: A solution of 5-iodocytosine ($\pm$)-**16** (1.0 equiv.), the acetylene derivative (1.0–3.0 equiv.), and the base (2.0–3.0 equiv.) in anhydrous DMF was deoxygenated thoroughly by bubbling with argon for 45 min. $[PdCl_2(PPh_3)_2]$ (0.01–0.10 equiv.) and CuI (0.02–0.20 equiv.) were added and the suspension was stirred at 25 °C for 1–48 h. The resulting suspension was filtered through a plug of SiO_2 (washing with $CH_2Cl_2/MeOH$, 92:8) and concentrated. The residue was purified by CC. If Et_3N was used in the eluting solvent, the fractions containing the desired product were concentrated, dissolved in CH_2Cl_2 , washed with water (2 $\times$) and brine (2 $\times$), dried with $MgSO_4$, filtered, and the solvents evaporated.

N-Alkylation of Benzimidazoles. General Procedure C: A solution of the benzimidazole (1.0 equiv.) in DMF, THF, or CH_2Cl_2 (7.7 mL/mmol) was treated with NaH (60% dispersion in oil, 1.1 equiv.) and stirred at 25 °C for 1 h. The alkyl halide (1.0 equiv.) was added at 0 °C. The suspension was stirred at 0 °C for 0.5 h and at 25 °C for 6–20 h and the solvents evaporated. The residue was treated with water and extracted twice with CH_2Cl_2 . The combined organic phases were washed with water (3 $\times$) and brine, dried with $MgSO_4$, filtered, and concentrated. The residue was purified by CC.

2-Cyclohexyl-1H-imidazole (14): A suspension of NH_4HCO_3 (2.82 g, 35.6 mmol) and 40% aq. glyoxal (2.0 mL, 17.8 mmol) in water (3.5 mL) was treated dropwise with cyclohexanecarbaldehyde (**13**; 2.2 mL, 17.8 mmol). The mixture was stirred at 25 °C for 18 h, diluted with water (80 mL), and extracted with $CH_2Cl_2/iPrOH$ (3:1, 3 $\times$ 100 mL). The combined organic phases were washed with brine (150 mL), dried with $MgSO_4$, filtered, and concentrated. CC (SiO_2 ; $CH_2Cl_2/MeOH$, 95:5 $\rightarrow$ 90:10) gave **14** (0.66 g, 25%) as a yellow-beige solid, m.p. 136 °C (decomp.). 1H NMR (300 MHz, $CDCl_3$): δ = 1.22–1.44 (m, 3 H, 4'- H_{ax} and 3',5'- H_{ax}), 1.46–1.58 (m, 2 H, 2',6'- H_{ax}), 1.69–1.75 (m, 1 H, 4'- H_{eq}), 1.80–1.86 (m, 2 H, 3',5'- H_{eq}), 2.03–2.10 (m, 2 H, 2',6'- H_{eq}), 2.75 (tt, J = 11.5, 3.6 Hz, 1 H, 1'-H), 6.95 (s, 2 H, 4-H and 5-H) ppm, NH not visible. ^{13}C NMR (100 MHz; $CDCl_3/CD_3OD$, 97:3): δ = 25.78, 25.97 (2 C), 31.77 (2 C), 37.55, 120.76 (2 C), 152.62 ppm. IR (ATR): $\tilde{\nu}$ = 3049 (w), 2928 (s), 2852 (m), 1674 (w), 1571 (m), 1480 (w), 1435 (m), 1384 (m), 1284 (w), 1230 (w), 1182 (w), 1152 (w), 1134 (w), 1098 (s), 1074 (m), 1012 (w), 976 (m), 913 (m), 882 (s), 818 (m), 767 (s), 756 (m), 739 (s), 708 (s), 689 (m) cm^{-1} . HRMS (EI): calcd. for $C_9H_{14}N_2^+$ [M] $^+$ 150.1151, found 150.1151 (25%); calcd. for $C_5H_7N_2^+$ [$M - C_4H_7$] $^+$ 95.0604, found 95.0604 (100%); calcd. for $C_4H_6N_2^+$ [$M - C_5H_8$] $^+$ 82.0525, found 82.0520 (34%).

2-Cyclohexyl-1-(prop-2-yn-1-yl)-1H-imidazole (15): Imidazole **14** (220 mg, 1.47 mmol) was treated with CS_2CO_3 (525 mg, 1.61 mmol) and propargyl bromide (130 μ L, 1.47 mmol) in DMF (11 mL) according to General Procedure A. The suspension was stirred at 25 °C for 9 h. CC (SiO_2 ; pentane/EtOH, 9:1 $\rightarrow$ 7:1) gave **15** (77 mg, 28%) as a yellow solid. R_f = 0.31 (SiO_2 ; pentane/EtOH, 9:1), m.p. 48–52 °C. 1H NMR (300 MHz, $CDCl_3$): δ = 1.31–1.43 (m, 3 H, 4'- H_{ax} and 3',5'- H_{ax}), 1.59–1.74 (m, 3 H, 2',6'- H_{ax} and 4'- H_{eq}), 1.82–1.93 (m, 4 H, 3',5'- H_{eq} and 2',6'- H_{eq}), 2.42 (t, J = 2.6 Hz, 1 H, $HC\equiv C$), 2.64 (tt, J = 11.6, 3.4 Hz, 1 H, 1'-H), 4.64 (d, J = 2.6 Hz, 2 H, NCH_2), 6.93 and 6.96 (2d, J = 1.4 Hz, 2 H, 4-H and 5-H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 25.99, 26.53 (2 C), 31.94 (2 C), 35.14, 35.94, 73.86, 77.30, 118.41, 127.19, 151.63 ppm. IR (ATR): $\tilde{\nu}$ = 3231 (w), 3200 (w), 3104 (w), 2928 (m), 2853 (m), 2116 (w), 1674 (w), 1523 (w), 1484 (m), 1452 (m), 1376 (w), 1337 (w),

1274 (s), 1245 (w), 1159 (m), 1144 (w), 1107 (w), 1097 (m), 1066 (w), 1008 (w), 942 (w), 921 (m), 891 (w), 859 (w), 839 (w), 821 (w), 779 (m), 748 (m), 720 (s) cm^{-1} . HRMS (EI): calcd. for $\text{C}_{12}\text{H}_{16}\text{N}_2^+$ $[\text{M}]^+$ 188.1308, found 188.1298 (20%); calcd. for $\text{C}_{12}\text{H}_{15}\text{N}_2^+$ $[\text{M} - \text{H}]^+$ 187.1230, found 187.1228 (21%); calcd. for $\text{C}_{10}\text{H}_{11}\text{N}_2^+$ $[\text{M} - \text{C}_2\text{H}_5]^+$ 159.0917, found 159.0920 (28%); calcd. for $\text{C}_8\text{H}_9\text{N}_2^+$ $[\text{M} - \text{C}_4\text{H}_7]^+$ 133.0760, found 133.0763 (100%); calcd. for $\text{C}_7\text{H}_8\text{N}_2^+$ $[\text{M} - \text{C}_5\text{H}_8]^+$ 120.0682, found 120.0681 (40%).

($\pm$)-5-[3-(2-Cyclohexyl-1H-imidazol-1-yl)prop-1-yn-1-yl]-1-(tetrahydrothien-2-yl)cytosine [($\pm$)-10]: Alkyne **15** (54 mg, 0.29 mmol) was treated with 5-iodocytosine ($\pm$)-**16** (93 mg, 0.29 mmol), Et_3N (120 μL , 0.86 mmol), $[\text{PdCl}_2(\text{PPh}_3)_2]$ (20 mg, 0.03 mmol), and CuI (11 mg, 0.06 mmol) in DMF (7 mL) according to General Procedure B. The suspension was stirred at 25 °C for 3.5 h. CC (SiO_2 ; $\text{CH}_2\text{Cl}_2/\text{MeOH}$, 95:5 $\rightarrow$ 92:8) gave ($\pm$)-**10** (28 mg, 25%) as an off-white solid. R_f = 0.31 (SiO_2 ; $\text{CH}_2\text{Cl}_2/\text{MeOH}$, 93:7), m.p. 99 °C (decomp.). ^1H NMR (400 MHz, CDCl_3 , assignment based on DQF-COSY experiment): δ = 1.24–1.42 (m, 3 H, 4'''- H_{ax} and 3''', 5'''- H_{ax}), 1.66–1.94 (m, 8 H, 4'''- H_{eq} , 3''', 5'''- H_{eq} , 2''', 6'''- H_2 , and 4'- H_a), 2.04–2.17 (m, 2 H, 3'- H_a and 4'- H_b), 2.29–2.37 (m, 1 H, 3'- H_b), 2.69 (tt, J = 11.7, 3.3 Hz, 1 H, 1'''-H), 2.92–2.98 (m, 1 H, 5'- H_a), 3.16–3.21 (m, 1 H, 5'- H_b), 4.89 (s, 2 H, NCH_2), 5.59–5.68 (br. s, 1 H, NH), 6.30 (dd, J = 6.1, 3.5 Hz, 1 H, 2'-H), 6.96 (d, J = 1.3 Hz, 1 H, 5''-H), 6.99 (d, J = 1.3 Hz, 1 H, 4''-H), 7.67–7.76 (br. s, 1 H, NH), 8.19 (s, 1 H, 6-H) ppm. ^{13}C NMR (100 MHz, CDCl_3): δ = 25.77, 26.36 (2 C), 28.19, 31.89 (2 C), 33.42, 35.82, 35.91, 38.88, 66.11, 76.88, 88.99, 89.23, 118.57, 127.64, 146.27, 151.96, 154.56, 164.18 ppm. IR (ATR): $\tilde{\nu}$ = 3104 (sh. w), 2926 (m), 2851 (w), 2231 (w), 1637 (s), 1482 (s), 1447 (m), 1401 (m), 1326 (m), 1297 (m), 1270 (m), 1226 (m), 1179 (w), 1160 (w), 1100 (m), 970 (w), 923 (w), 890 (w), 778 (s), 721 (m) cm^{-1} . HRMS (MALDI; 3-HPA): calcd. for $\text{C}_{20}\text{H}_{25}\text{KN}_5\text{OS}^+ [\text{M} + \text{K}]^+$ 422.1411, found 422.1402 (2%); calcd. for $\text{C}_{20}\text{H}_{25}\text{N}_5\text{NaOS}^+ [\text{M} + \text{Na}]^+$ 406.1672, found 406.1670 (3%); calcd. for $\text{C}_{20}\text{H}_{26}\text{N}_5\text{OS}^+ [\text{M} + \text{H}]^+$ 384.1853, found 384.1852 (100%); calcd. for $\text{C}_{16}\text{H}_{20}\text{N}_5\text{O}^+ [\text{M} - \text{C}_4\text{H}_6\text{S} + \text{H}]^+$ 298.1662, found 298.1661 (14%).

2-Cyclohexyl-*N,N*-dimethyl-1H-imidazole-1-sulfonamide (24): *N,N*-Dimethylsulfamoyl chloride (6.3 mL, 58.6 mmol) was added to a solution of **14** (8.00 g, 53.3 mmol) and Et_3N (8.5 mL, 61.2 mmol) in CH_2Cl_2 (80 mL). The suspension was stirred at 25 °C for 14 h, filtered (washing with CH_2Cl_2), and the filtrate was concentrated. CC (SiO_2 ; pentane/EtOAc, 2:1 $\rightarrow$ 1:2) gave **24** (8.48 g, 62%) as a white solid. R_f = 0.30 (SiO_2 ; pentane/EtOAc, 1:1), m.p. 95–97 °C. ^1H NMR (300 MHz, CDCl_3): δ = 1.25–1.43 (m, 3 H, 4'- H_{ax} and 3', 5'- H_{ax}), 1.59–1.74 (m, 3 H, 2', 6'- H_{ax} and 4'- H_{eq}), 1.83–1.97 (m, 4 H, 3', 5'- H_{eq} and 2', 6'- H_{eq}), 2.88 (s, 6 H, Me), 3.14 (tt, J = 11.7, 3.4 Hz, 1 H, 1'-H), 6.95 (d, J = 1.7 Hz, 1 H, 4-H), 7.17 (d, J = 1.7 Hz, 1 H, 5-H) ppm. ^{13}C NMR (75 MHz, CDCl_3): δ = 25.84, 26.43 (2 C), 32.65 (2 C), 37.23, 38.17 (2 C), 118.95, 127.22, 153.88 ppm. IR (ATR): $\tilde{\nu}$ = 3102 (w), 2949 (w), 2927 (m), 2853 (m), 1673 (w), 1533 (w), 1482 (m), 1466 (m), 1458 (m), 1446 (m), 1415 (m), 1388 (s), 1345 (w), 1321 (w), 1298 (w), 1261 (m), 1226 (w), 1186 (m), 1174 (s), 1163 (s), 1152 (s), 1133 (s), 1093 (s), 1072 (m), 1047 (s), 999 (m), 961 (s), 909 (m), 887 (m), 856 (w), 817 (w), 765 (m), 737 (m), 721 (s), 708 (s) cm^{-1} . HRMS (EI): calcd. for $\text{C}_{11}\text{H}_{19}\text{N}_3\text{O}_2\text{S}^+ [\text{M}]^+$ 257.1192, found 257.1199 (7%); calcd. for $\text{C}_9\text{H}_{13}\text{N}_2^+ [\text{M} - \text{Me}_2\text{NSO}_2]^+$ 149.1073, found 149.1064 (100%); calcd. for $\text{C}_5\text{H}_7\text{N}_2^+ [\text{M} - \text{Me}_2\text{NSO}_2 - \text{C}_3\text{H}_6]^+$ 95.0604, found 95.0594 (33%). $\text{C}_{11}\text{H}_{19}\text{N}_3\text{O}_2\text{S}$ (257.4): calcd. C 51.34, H 7.44, N 16.33; found C 51.64, H 7.39, N 16.05.

2-Cyclohexyl-5-formyl-*N,N*-dimethyl-1H-imidazole-1-sulfonamide (25): A solution of *n*BuLi (1.6 M in hexane, 26.3 mL, 42.1 mmol)

was added dropwise over 40 min to a solution of **24** (9.01 g, 35.0 mmol) in THF (160 mL) at –78 °C. The resulting solution was stirred at –78 °C for 45 min, treated dropwise with DMF (13.0 mL, 168.0 mmol), and stirred at –78 °C for 1 h. The solution was slowly warmed to 25 °C over 1 h, stirred at 25 °C for 1 h, cooled to –78 °C, and treated with sat. aq. NaHCO_3 (30 mL). The residue was diluted with water (20 mL) at 25 °C and extracted with CH_2Cl_2 (2 $\times$ 50 mL). The combined organic phases were washed with water (50 mL) and brine (50 mL), dried with MgSO_4 , filtered, and concentrated. CC (SiO_2 ; pentane/EtOAc, 3:1 $\rightarrow$ 1:2) gave **25** (8.31 g, 83%) as a yellow solid. R_f = 0.53 (SiO_2 ; pentane/EtOAc, 1:1), m.p. 94–96 °C. ^1H NMR (300 MHz, CDCl_3): δ = 1.23–1.43 (m, 3 H, 4'- H_{ax} and 3', 5'- H_{ax}), 1.63–1.77 (m, 3 H, 2', 6'- H_{ax} and 4'- H_{eq}), 1.85–1.97 (m, 4 H, 3', 5'- H_{eq} and 2', 6'- H_{eq}), 2.92 (s, 6 H, Me), 3.27 (tt, J = 11.6, 3.3 Hz, 1 H, 1'-H), 7.78 (s, 1 H, 4-H), 10.05 (s, 1 H, CHO) ppm. ^{13}C NMR (100 MHz, CDCl_3): δ = 25.63, 26.19 (2 C), 32.54 (2 C), 37.96, 38.14 (2 C), 132.92, 138.04, 160.63, 180.47 ppm. IR (ATR): $\tilde{\nu}$ = 3102 (w), 2933 (m), 2854 (m), 1671 (m), 1538 (m), 1458 (s), 1414 (s), 1389 (s), 1249 (m), 1164 (s), 1094 (s), 1047 (s), 962 (s), 888 (m), 781 (m), 763 (m), 720 (s), 630 (w) cm^{-1} . HRMS (ESI): 318.1483 (100%); calcd. for $\text{C}_{12}\text{H}_{19}\text{N}_3\text{NaO}_3\text{S}^+ [\text{M} + \text{Na}]^+$ 308.1039, found 308.1038 (13%); calcd. for $\text{C}_{12}\text{H}_{20}\text{N}_3\text{O}_3\text{S}^+ [\text{M} + \text{H}]^+$ 286.1220, found 286.1219 (49%); 206.1646 (30%). $\text{C}_{12}\text{H}_{19}\text{N}_3\text{O}_3\text{S}$ (285.4): calcd. C 50.51, H 6.71, N 14.72; found C 50.52, H 6.67, N 14.61.

($\pm$)-2-Cyclohexyl-*N,N*-dimethyl-5-(2,2,2-trifluoro-1-hydroxyethyl)-1H-imidazole-1-sulfonamide [($\pm$)-26]: A solution of aldehyde **25** (1.20 g, 4.21 mmol) in THF (20 mL) was treated dropwise with Me_3SiCF_3 (0.69 mL, 4.63 mmol) and a solution of *n*Bu₄NF (1 M in THF, 0.42 mL, 0.42 mmol) at 0 °C. The solution was warmed to 25 °C over 0.5 h, stirred at 25 °C for 1 h, treated with water (20 mL), and extracted with CH_2Cl_2 (3 $\times$ 50 mL). The combined organic phases were washed with water (70 mL) and brine (70 mL), dried with MgSO_4 , filtered, and concentrated. CC (SiO_2 ; pentane/EtOAc, 2:1 $\rightarrow$ 1:2) gave additional ($\pm$)-**26** (779 mg, 52%). Total yield: 1.09 g (73%). R_f = 0.44 (SiO_2 ; pentane/EtOAc, 1:1), m.p. 115–117 °C. ^1H NMR (400 MHz, CDCl_3): δ = 1.25–1.38 (m, 3 H, 4'- H_{ax} and 3', 5'- H_{ax}), 1.60–1.73 (m, 3 H, 2', 6'- H_{ax} and 4'- H_{eq}), 1.84–1.90 (m, 4 H, 3', 5'- H_{eq} and 2', 6'- H_{eq}), 2.92 (s, 6 H, Me), 3.12 (tt, J = 11.7, 3.1 Hz, 1 H, 1'-H), 3.98–4.12 (br. s, 1 H, OH), 5.58 [q, $^3J(\text{H},\text{F})$ = 6.7 Hz, 1 H, $\text{HC}-\text{CF}_3$], 7.13 (d, J = 0.7 Hz, 1 H, 4-H) ppm. ^{13}C NMR (100 MHz, CDCl_3): δ = 25.61, 26.38, 26.39, 32.50, 32.58, 37.81 (2 C), 38.03, 64.75 [q, $^2J(\text{C},\text{F})$ = 33.6 Hz], 124.02 [q, $^1J(\text{C},\text{F})$ = 281.7 Hz], 127.11 (br.), 129.01 [q, $^4J(\text{C},\text{F})$ = 3.0 Hz], 157.22 ppm. ^{19}F NMR (376 MHz, CDCl_3): δ = –75.84 [d, $^3J(\text{H},\text{F})$ = 6.9 Hz] ppm. IR (ATR): $\tilde{\nu}$ = 3130 (w), 2932 (w), 2858 (w), 1580 (w), 1497 (w), 1450 (w), 1385 (m), 1367 (m), 1276 (m), 1157 (s), 1128 (s), 1114 (m), 1104 (m), 1080 (s), 1018 (w), 967 (m), 859 (m), 806 (m), 766 (m), 723 (s), 705 (s), 655 (w) cm^{-1} . HRMS (EI): calcd. for $\text{C}_{13}\text{H}_{20}\text{F}_3\text{N}_3\text{O}_3\text{S}^+ [\text{M}]^+$ 355.1172, found 355.1170 (11%); calcd. for $\text{C}_{11}\text{H}_{14}\text{F}_3\text{N}_2\text{O}^+ [\text{M} - \text{Me}_2\text{NSO}_2]^+$ 247.1053, found 247.1060 (100%); 180.0495 (35%); 108.0114 (51%).

($\pm$)-2-Cyclohexyl-*N,N*-dimethyl-5-(2,2,2-trifluoro-1-methoxyethyl)-1H-imidazole-1-sulfonamide [($\pm$)-27]: NaH (60% dispersion in oil, 62 mg, 1.55 mmol) was added in two portions to a solution of ($\pm$)-**26** (500 mg, 1.41 mmol) in DMF (5 mL) at 0 °C. The mixture was stirred at 0 °C for 0.5 h, at 25 °C for 0.5 h, and treated with MeI (96 μL , 1.55 mmol) at 0 °C. The mixture was stirred at 25 °C for 16 h, poured onto ice/water, and extracted with CH_2Cl_2 (3 $\times$

40 mL). The combined organic phases were washed with water (3 × 100 mL) and brine (100 mL), dried with MgSO_4 , filtered, and concentrated. CC (SiO_2 ; pentane/EtOAc, 5:1 → 2:1) gave ($\pm$)-**27** (440 mg, 85%) as an off-white solid. R_f = 0.44 (SiO_2 ; pentane/EtOAc, 7:3), m.p. 76–78 °C. ^1H NMR (300 MHz, CDCl_3): δ = 1.26–1.43 (m, 3 H, 4'- H_{ax} and 3',5'- H_{ax}), 1.59–1.76 (m, 3 H, 2',6'- H_{ax} and 4'- H_{eq}), 1.85–1.93 (m, 4 H, 3',5'- H_{eq} and 2',6'- H_{eq}), 2.92 (s, 6 H, NMe_2), 3.14 (tt, J = 11.5, 3.3 Hz, 1 H, 1'-H), 3.51 (d, J = 0.6 Hz, 3 H, OMe), 5.31 [qd, $^3J(\text{H},\text{F})$ = 6.2, J = 0.6 Hz, 1 H, $\text{HC}-\text{CF}_3$], 7.18 (br. s, 1 H, 4-H) ppm. ^{13}C NMR (100 MHz, CDCl_3): δ = 25.68, 26.39 (2 C), 32.58, 32.64, 37.62 (2 C), 38.02, 58.58, 72.84 [q, $^2J(\text{C},\text{F})$ = 32.1 Hz], 123.84 [q, $^1J(\text{C},\text{F})$ = 283.2 Hz], 125.55 (br.), 130.34 [q, $^4J(\text{C},\text{F})$ = 2.3 Hz], 157.35 ppm. ^{19}F NMR (376 MHz, CDCl_3): δ = -74.31 [d, $^3J(\text{H},\text{F})$ = 6.4 Hz] ppm. IR (ATR): $\tilde{\nu}$ = 3152 (w), 2931 (w), 2859 (w), 1570 (w), 1497 (w), 1475 (w), 1461 (w), 1417 (w), 1389 (m), 1364 (m), 1347 (m), 1262 (m), 1239 (w), 1208 (w), 1159 (s), 1128 (s), 1113 (m), 1099 (m), 1084 (s), 1015 (w), 969 (m), 856 (m), 844 (m), 767 (m), 726 (s), 705 (s), 640 (w) cm^{-1} . HRMS (EI): calcd. for $\text{C}_{14}\text{H}_{22}\text{F}_3\text{N}_3\text{O}_3\text{S}^+$ [M] $^+$ 369.1328, found 369.1330 (16%); calcd. for $\text{C}_{12}\text{H}_{16}\text{F}_3\text{N}_2\text{O}^+$ [$\text{M} - \text{Me}_2\text{NSO}_2$] $^+$ 261.1209, found 261.1215 (100%); calcd. for $\text{C}_{11}\text{H}_{12}\text{F}_3\text{N}_2^+$ [$\text{M} - \text{Me}_2\text{NSO}_2 - \text{MeOH}$] $^+$ 229.0947, found 229.0947 (73%); 194.0669 (45%); 108.0111 (60%). $\text{C}_{14}\text{H}_{22}\text{N}_3\text{O}_3\text{F}_3\text{S}$ (369.4) calcd. C 45.52, H 6.00, N 11.38; found C 45.81, H 6.00, N 11.26.

($\pm$)-2-Cyclohexyl-4-(2,2,2-trifluoro-1-methoxyethyl)-1H-imidazole [($\pm$)-28**]:** A suspension of ($\pm$)-**27** (300 mg, 0.81 mmol) in 5% aq. HCl (10 mL) was stirred at 25 °C for 7 h. The mixture was cooled to 0 °C, neutralized with sat. aq. NaHCO_3 , and extracted with CH_2Cl_2 (3 × 20 mL). The combined organic phases were washed with brine (30 mL), dried with MgSO_4 , filtered, and concentrated. CC (SiO_2 ; pentane/EtOAc, 2:1 → 1:1) gave ($\pm$)-**28** (191 mg, 90%) as a white crystalline solid, m.p. 154–155 °C. ^1H NMR (400 MHz, CDCl_3): δ = 1.23 (qt, J = 12.2, 3.3 Hz, 1 H, 4'- H_{ax}), 1.36 (qt, J = 12.2, 3.2 Hz, 2 H, 3',5'- H_{ax}), 1.48 (qd, J = 12.2, 2.8 Hz, 2 H, 2',6'- H_{ax}), 1.69–1.74 (m, 1 H, 4'- H_{eq}), 1.79–1.84 (m, 2 H, 3',5'- H_{eq}), 2.02–2.06 (m, 2 H, 2',6'- H_{eq}), 2.74 (tt, J = 11.7, 3.5 Hz, 1 H, 1'-H), 3.44 (s, 3 H, OMe), 4.63 [q, $^3J(\text{H},\text{F})$ = 6.7 Hz, 1 H, $\text{HC}-\text{CF}_3$], 7.03 (s, 1 H, 5-H), 9.40–9.86 (br. s, 1 H, NH) ppm. ^{13}C NMR (100 MHz, CDCl_3): δ = 25.76, 25.94 (2 C), 31.94 (2 C), 37.80, 58.15, 114.26 (br.), 153.32 (br.) ppm, the signals of C(4) and CF_3 are not visible. ^{19}F NMR (376 MHz, CDCl_3): δ = -76.27 [d, $^3J(\text{H},\text{F})$ = 4.8 Hz] ppm. IR (ATR): $\tilde{\nu}$ = 2930 (m), 2856 (w), 1577 (w), 1531 (w), 1451 (w), 1371 (w), 1270 (m), 1240 (m), 1167 (s), 1120 (s), 1104 (s), 1015 (w), 971 (m), 879 (w), 806 (w), 707 (m) cm^{-1} . HRMS (ESI): calcd. for $\text{C}_{12}\text{H}_{18}\text{F}_3\text{N}_2\text{O}^+$ [$\text{M} + \text{H}$] $^+$ 263.1366, found 263.1376 (100%).

($\pm$)-2-Cyclohexyl-1-(prop-2-yn-1-yl)-4-(2,2,2-trifluoro-1-methoxyethyl)-1H-imidazole [($\pm$)-29**]:** Imidazole ($\pm$)-**28** (170 mg, 0.65 mmol) was treated with Cs_2CO_3 (232 mg, 0.71 mmol) and propargyl bromide (56 μL , 0.65 mmol) in DMF (5 mL) according to General Procedure A. The suspension was stirred at 25 °C for 8 h. CC (SiO_2 ; pentane/EtOAc, 4:1 → 1:1) gave ($\pm$)-**29** (94 mg, 48%) as a colorless oil. R_f = 0.34 (SiO_2 ; pentane/EtOAc, 4:1). ^1H NMR (300 MHz, CDCl_3): δ = 1.31–1.44 (m, 3 H, 4'- H_{ax} and 3',5'- H_{ax}), 1.60–1.75 (m, 3 H, 2',6'- H_{ax} and 4'- H_{eq}), 1.84–1.93 (m, 4 H, 3',5'- H_{eq} and 2',6'- H_{eq}), 2.46 (t, J = 2.6 Hz, 1 H, $\text{HC}\equiv\text{C}$), 2.64 (tt, J = 11.7, 3.4 Hz, 1 H, 1'-H), 3.47 (d, J = 0.5 Hz, 3 H, OMe), 4.65 [br. q, $^3J(\text{H},\text{F})$ = 6.8 Hz, 1 H, $\text{HC}-\text{CF}_3$], 4.65 (d, J = 2.6 Hz, 2 H, NCH_2), 7.08 (s, 1 H, 5-H) ppm. ^{13}C NMR (75 MHz, CDCl_3): δ = 25.81, 26.44 (2 C), 31.74, 31.76, 35.55 and 35.98 (1 C), 58.34, 74.50, 74.56, 76.17, 76.65 [q, $^2J(\text{C},\text{F})$ = 34.6 Hz], 117.66, 123.79 [q, $^1J(\text{C},\text{F})$ = 280.7 Hz], 132.48 [br. q, $^4J(\text{C},\text{F})$ = 1.7 Hz], 151.66 ppm. ^{19}F NMR (376 MHz, CDCl_3): δ = -76.25 [d, $^3J(\text{H},\text{F})$ = 6.5 Hz] ppm.

IR (ATR): $\tilde{\nu}$ = 3307 (w), 2932 (m), 2856 (w), 2123 (w), 1504 (w), 1450 (w), 1385 (m), 1378 (w), 1271 (m), 1165 (s), 1120 (s), 1100 (s), 1018 (w), 971 (w), 891 (w), 869 (w), 838 (w), 806 (w), 698 (m) cm^{-1} . HRMS (ESI): calcd. for $\text{C}_{15}\text{H}_{20}\text{F}_3\text{N}_2\text{O}^+$ [$\text{M} + \text{H}$] $^+$ 301.1522, found 301.1528 (100%).

($\pm$)-5-{3-[2-Cyclohexyl-4-(2,2,2-trifluoro-1-methoxyethyl)-1H-imidazol-1-yl]prop-1-yn-1-yl}-1-(tetrahydrothien-2-yl)cytosine [($\pm$)-17**]:** Alkyne ($\pm$)-**29** (56 mg, 0.19 mmol) was treated with 5-iodocytosine ($\pm$)-**16** (60 mg, 0.19 mmol), Et_3N (78 μL , 0.56 mmol), $[\text{PdCl}_2(\text{PPh}_3)_2]$ (13 mg, 0.02 mmol), and CuI (7 mg, 0.04 mmol) in DMF (4.6 mL) according to General Procedure B. The suspension was stirred at 25 °C for 7 h. CC (SiO_2 - NH_2 ; $\text{CH}_2\text{Cl}_2 \rightarrow \text{CH}_2\text{Cl}_2/\text{EtOH}$, 95:5) gave ($\pm$)-**17** (43 mg, 47%) as a light-yellow solid. R_f = 0.34 (SiO_2 ; $\text{CH}_2\text{Cl}_2/\text{MeOH}$, 94:6), m.p. 117–120 °C. ^1H NMR (300 MHz, CDCl_3): δ = 1.25–1.44 (m, 3 H, 4'''- H_{ax} and 3''',5'''- H_{ax}), 1.63–1.94 (m, 8 H, 4'''- H_{eq} , 3''',5'''- H_{eq} , 2''',6'''- H_2 , and 4'- H_a), 2.05–2.20 (m, 2 H, 3'- H_a and 4'- H_b), 2.29–2.41 (m, 1 H, 3'- H_b), 2.68 (tt, J = 11.5, 3.3 Hz, 1 H, 1'''-H), 2.92–3.01 (m, 1 H, 5'- H_a), 3.17–3.26 (m, 1 H, 5'- H_b), 3.50 (s, 3 H, OMe), 4.66 [q, $^3J(\text{H},\text{F})$ = 6.8 Hz, 1 H, $\text{HC}-\text{CF}_3$], 4.91 (s, 2 H, NCH_2), 5.74–5.85 (br. s, 1 H, NH), 6.30 (dd, J = 6.1, 3.5 Hz, 1 H, 2'-H), 7.10 (s, 1 H, 5''-H), 7.71–7.88 (br. s, 1 H, NH), 8.23 (s, 1 H, 6-H) ppm. ^{13}C NMR (100 MHz, CDCl_3): δ = 25.62, 26.27 (2 C), 28.23, 31.74, 31.78, 33.42, 35.94, 36.20, 38.86, 58.51, 66.16, 77.05 [q, $^2J(\text{C},\text{F})$ = 31.9 Hz], 77.25, 88.64, 89.31, 117.68 [br. q, $^4J(\text{C},\text{F})$ = 1.2 Hz], 124.05 [q, $^1J(\text{C},\text{F})$ = 282.0 Hz], 133.11 [br. q, $^3J(\text{C},\text{F})$ = 1.4 Hz], 146.39, 151.96, 154.39, 164.04 ppm. ^{19}F NMR (376 MHz, CDCl_3): δ = -76.12 [t, $^3J(\text{H},\text{F})$ = 7.4 Hz] ppm. IR (ATR): $\tilde{\nu}$ = 3326 (w), 3178 (w), 2931 (m), 2854 (w), 2235 (w), 1639 (s), 1496 (s), 1448 (m), 1402 (m), 1328 (w), 1297 (w), 1271 (m), 1228 (w), 1162 (s), 1120 (s), 1097 (s), 1017 (w), 969 (w), 884 (w), 838 (w), 806 (w), 779 (m), 697 (w) cm^{-1} . HRMS (MALDI; 3-HPA): calcd. for $\text{C}_{23}\text{H}_{28}\text{F}_3\text{KN}_5\text{O}_2\text{S}^+$ [$\text{M} + \text{K}$] $^+$ 534.1547, found 534.1555 (8%); calcd. for $\text{C}_{23}\text{H}_{28}\text{F}_3\text{N}_5\text{NaO}_2\text{S}^+$ [$\text{M} + \text{Na}$] $^+$ 518.1808, found 518.1816 (12%); calcd. for $\text{C}_{23}\text{H}_{29}\text{F}_3\text{N}_5\text{O}_2\text{S}^+$ [$\text{M} + \text{H}$] $^+$ 496.1989, found 496.1993 (100%); calcd. for $\text{C}_{19}\text{H}_{23}\text{F}_3\text{N}_5\text{O}_2^+$ [$\text{M} - \text{C}_4\text{H}_6\text{S} + \text{H}$] $^+$ 410.1798, found 410.1799 (48%).

2-Cyclohexyl-1H-benzimidazol-4-ol (36**):** A solution of 2-amino-3-nitrophenol (**35**; 1.10 g, 7.13 mmol) and cyclohexanecarbaldehyde (**13**; 0.86 mL, 7.13 mmol) in EtOH (28 mL) was treated with aq. $\text{Na}_2\text{S}_2\text{O}_4$ (1 M, 21.4 mL, 21.4 mmol) at 25 °C and heated to 70 °C for 5 h. The mixture was cooled to 25 °C, treated dropwise with aq. NH_3 (1 M, 14.3 mL), and cooled to 0 °C. The resulting precipitate was filtered off, washed with H_2O , and dried in a desiccator over CaCl_2 to give **36** (1.24 g, 80%) as a white solid, m.p. 225–227 °C. ^1H NMR [300 MHz, $(\text{CD}_3)_2\text{SO}$]: δ = 1.22–1.43 (m, 3 H, 4'- H_{ax} and 3',5'- H_{ax}), 1.55–1.72 (m, 3 H, 2',6'- H_{ax} and 4'- H_{eq}), 1.77–1.81 (m, 2 H, 3',5'- H_{ax}), 1.96–2.00 (m, 2 H, 2',6'- H_{eq}), 2.80 (tt, J = 11.5, 3.6 Hz, 1 H, 1'-H), 6.45–6.52 (m, 1 H, 5-H), 6.84–6.94 (m, 2 H, 6-H and 7-H), 9.52 (br. s, 1 H, NH), 11.93 (br. s, 1 H, OH) ppm. ^{13}C NMR [100 MHz, $(\text{CD}_3)_2\text{SO}$]: δ = 25.59, 25.63 (2 C), 31.36 (2 C), 37.78, 106.13, 121.74, 157.38 ppm, four aromatic signals are not visible. IR (ATR): $\tilde{\nu}$ = 3211 (w), 3062 (w), 3030 (w), 2925 (m), 2851 (w), 2373 (w), 1896 (w), 1731 (w), 1625 (w), 1597 (m), 1519 (w), 1430 (m), 1381 (m), 1300 (m), 1239 (m), 1194 (m), 1154 (m), 1128 (m), 1064 (s), 1044 (s), 887 (m), 850 (w), 788 (s), 732 (s), 713 (m), 665 (w), 606 (w) cm^{-1} . HRMS (ESI): calcd. for $\text{C}_{13}\text{H}_{17}\text{N}_2\text{O}^+$ [$\text{M} + \text{H}$] $^+$ 217.1335, found 217.1330 (100%).

2-Cyclohexyl-4-isopropoxy-1H-benzimidazole (37**):** A solution of alcohol **36** (700 mg, 3.24 mmol), PPh_3 (1.02 g, 3.89 mmol), and $i\text{PrOH}$ (0.30 mL, 3.89 mmol) in THF (30.8 mL) was treated with DIAD (94% in toluene, 0.82 mL, 3.89 mmol) at 25 °C. The mixture

was stirred at 25 °C for 2 h and concentrated. CC (SiO₂; pentane/EtOAc, 2:1) and recrystallization in EtOAc gave **37** (410 mg, 49%) as a white solid. *R*_f = 0.57 (SiO₂; pentane/EtOAc, 1:1), m.p. 216 °C. ¹H NMR (300 MHz, CDCl₃): δ = 1.18–1.48 (m, 3 H, 4'-H_{ax} and 3',5'-H_{ax}), 1.40 (d, *J* = 6.0 Hz, 6 H, Me), 1.60–1.89 (m, 5 H, 2',6'-H_{ax}, 4'-H_{eq} and 3',5'-H_{eq}), 2.13–2.19 (m, 2 H, 2',6'-H_{eq}), 2.91 (br. s, 1 H, 1'-H), 4.72 (br. s, 1 H, OCH), 6.66 (d, *J* = 8.1 Hz, 1 H, 5-H), 7.10 (t, *J* = 8.0 Hz, 1 H, 6-H), 7.29 (br. s, 1 H, 7-H), 9.07 (br. s, 1 H, NH) ppm. ¹³C NMR (100 MHz, CDCl₃): δ = 22.16 (2 C), 25.83, 26.04 (2 C), 31.93 (2 C), 38.57, 70.30, 104.86, 122.21, 157.85 ppm, four aromatic signals are not visible. IR (ATR): ν̄ = 3675 (w), 2975 (m), 2926 (m), 2852 (m), 1618 (w), 1601 (m), 1539 (m), 1495 (w), 1444 (m), 1416 (m), 1380 (m), 1370 (m), 1357 (m), 1322 (m), 1292 (w), 1265 (m), 1242 (s), 1167 (w), 1140 (w), 1116 (s), 1088 (m), 1061 (m), 1041 (w), 985 (m), 903 (m), 891 (w), 856 (m), 782 (m), 767 (w), 735 (s), 669 (w), 632 (w), 609 (w) cm⁻¹. HRMS (EI): calcd. for C₁₆H₂₂N₂O⁺ [M]⁺ 258.1727, found 258.1729 (39%); calcd. for C₁₃H₁₆N₂O⁺ [M - C₃H₆]⁺ 216.1257, found 216.1251 (25%); calcd. for C₉H₈N₂O⁺ [M - C₃H₆ - C₄H₇]⁺ 161.0709, found 161.0706 (100%).

2-Cyclohexyl-4-isopropoxy-1-(prop-2-yn-1-yl)-1H-benzimidazole (39): Benzimidazole **37** (320 mg, 1.24 mmol) was treated with NaH (60% dispersion in oil, 50 mg, 1.24 mmol) and propargyl bromide (106 μL, 1.24 mmol) in CH₂Cl₂ (24 mL) according to General Procedure C. The suspension was stirred at 25 °C for 24 h. CC (SiO₂; pentane/EtOAc, 95:5→90:10) gave **39** (160 mg, 44%) as a white crystalline solid. *R*_f = 0.85 (SiO₂; pentane/EtOAc, 4:1), m.p. 99–101 °C. ¹H NMR (400 MHz, CDCl₃): δ = 1.32–1.41 (m, 3 H, 4'-H_{ax} and 3',5'-H_{ax}), 1.46 (d, *J* = 6.1 Hz, 6 H, Me), 1.74–2.05 (m, 7 H, 2',6'-H₂, 4'-H_{eq} and 3',5'-H_{eq}), 2.33 (t, *J* = 2.5 Hz, 1 H, HC≡C), 2.87 (tt, *J* = 11.6, 3.7 Hz, 1 H, 1'-H), 4.84 (d, *J* = 2.5 Hz, 2 H, NCH₂), 4.96 (sept., *J* = 6.1 Hz, 1 H, OCH), 6.70 (br. d, *J* = 7.9 Hz, 1 H, 5-H), 6.98 (dd, *J* = 8.1, 0.8 Hz, 1 H, 7-H), 7.15 (t, *J* = 8.0 Hz, 1 H, 6-H) ppm. ¹³C NMR (100 MHz, CDCl₃): δ = 22.20 (2 C), 25.67, 26.31 (2 C), 31.29 (2 C), 32.85, 36.63, 70.82, 73.12, 77.12, 101.69, 106.23, 122.87, 133.23, 136.53, 149.70, 156.55 ppm. IR (ATR): ν̄ = 3155 (m), 2970 (w), 2936 (m), 2916 (m), 2849 (w), 2119 (w), 1863 (w), 1608 (m), 1595 (m), 1566 (w), 1508 (m), 1497 (m), 1467 (w), 1448 (m), 1435 (m), 1407 (m), 1381 (w), 1365 (m), 1339 (m), 1300 (w), 1293 (w), 1270 (m), 1250 (s), 1222 (m), 1197 (w), 1172 (w), 1156 (w), 1134 (m), 1113 (m), 1070 (m), 1060 (m), 1045 (w), 1010 (w), 976 (w), 946 (w), 916 (w), 891 (w), 872 (w), 838 (w), 814 (w), 793 (w), 779 (m), 746 (m), 727 (s), 718 (s), 650 (w) cm⁻¹. HRMS (EI): calcd. for C₁₉H₂₄N₂O⁺ [M]⁺ 296.1883, found 296.1889 (48%); calcd. for C₁₈H₂₁N₂O⁺ [M - CH₃]⁺ 281.1648, found 281.1650 (75%); calcd. for C₁₆H₁₈N₂O⁺ [M - C₃H₆]⁺ 254.1414, found 254.1405 (30%); calcd. for C₁₂H₁₁N₂O⁺ [M - C₃H₆ - C₄H₇]⁺ 199.0867, found 199.0867 (100%). C₁₉H₂₄N₂O (296.2) calcd. C 76.99, H 8.16, N 9.45; found C 76.98, H 8.15, N 9.29.

(±)-5-{3-[2-Cyclohexyl-4-(propan-2-yloxy)-1H-benzimidazol-1-yl]-prop-1-yn-1-yl}-1-(tetrahydrothien-2-yl)cytosine [(±)-34]: Alkyne **39** (64 mg, 0.22 mmol) was treated with 5-iodocytosine (±)-**16** (35 mg, 11 mmol), *i*Pr₂NEt (116 μL, 0.65 mmol), [PdCl₂(PPh₃)₂] (8 mg, 0.01 mmol), and CuI (4 mg, 0.02 mmol) in DMF (2.5 mL) according to General Procedure B. The suspension was stirred at 25 °C for 24 h. CC (SiO₂; CH₂Cl₂/MeOH, 96:4) gave (±)-**34** (19 mg, 36%) as a yellow-brown solid. *R*_f = 0.17 (SiO₂; CH₂Cl₂/MeOH, 96:4), m.p. 165–170 °C (decomp.). ¹H NMR (400 MHz, CDCl₃): δ = 1.35–1.41 (m, 3 H, 4'-H_{ax} and 3',5'-H_{ax}), 1.46 (d, *J* = 6.1 Hz, 6 H, Me), 1.76–2.13 (m, 10 H, 2'',6''-H₂, 4''-H_{eq}, 3'',5''-H_{eq}, 3'-H_a, and 4'-H₂), 2.27–2.34 (m, 1 H, 3'-H_b), 2.87–2.96 (m, 2 H, 1''-H and 5'-H_a), 3.13–3.19 (m, 1 H, 5'-H_b), 4.95 (sept., *J* = 6.1 Hz, 1 H,

OCH), 5.08 (s, 2 H, NCH₂), 5.50 (br. s, 1 H, NH), 6.27–6.30 (m, 1 H, 2'-H), 6.72 (d, *J* = 7.9 Hz, 1 H, 5''-H), 6.92 (br. s, 1 H, NH), 6.99 (d, *J* = 8.0 Hz, 1 H, 7''-H), 7.18 (t, *J* = 8.0 Hz, 1 H, 6''-H), 8.13 (s, 1 H, 6-H) ppm. ¹³C NMR (100 MHz, CDCl₃): δ = 22.20 (2 C), 25.63, 26.33 (2 C), 28.17, 31.47 (2 C), 33.40, 33.77, 36.74, 38.88, 66.11, 70.88, 76.32, 88.93, 89.15, 101.45, 106.20, 123.24, 133.16, 136.38, 146.18, 149.82, 154.53, 156.58, 163.95 ppm. IR (ATR): ν̄ = 3602 (w), 3427 (w), 3366 (w), 3059 (w), 2931 (m), 2852 (w), 2224 (w), 1670 (s), 1637 (s), 1611 (m), 1598 (m), 1495 (s), 1449 (m), 1403 (s), 1385 (m), 1372 (w), 1343 (m), 1330 (m), 1296 (m), 1271 (s), 1247 (m), 1222 (s), 1197 (m), 1178 (m), 1160 (w), 1138 (w), 1116 (s), 1066 (m), 1010 (w), 976 (m), 921 (w), 892 (w), 838 (w), 817 (w), 777 (s), 740 (s), 719 (w), 676 (w), 661 (w) cm⁻¹. HRMS (MALDI): calcd. for C₂₇H₃₄N₅O₂S⁺ [M + H]⁺ 492.2428, found 492.2450 (100%); calcd. for C₂₃H₂₈N₅O₂⁺ [M - C₄H₆S + H]⁺ 406.2238, found 406.2231 (66%).

Supporting Information (see footnote on the first page of this article): Figures (modeling, regioselective alkylation, CSD searches) and schemes (biological assay, syntheses not shown in the main manuscript) referred to in this article, materials and general methods, synthetic and analytical protocols, X-ray diffraction data, NMR spectra of new compounds.

Acknowledgments

This research was supported by the Swiss National Science Foundation and the Hans-Fischer Gesellschaft, Germany. The authors thank Dr. Daniel Zimmerli (Hoffmann-La Roche AG, Basel) for providing the amino-modified silica and for advice on purification of compounds.

- [1] a) M. Rohmer, M. Knani, P. Simonin, B. Sutter, H. Sahm, *Biochem. J.* **1993**, 295, 517–524; b) M. K. Schwarz, ETH-Dissertation Nr. 10951, ETH Zürich, **1994**; c) S. T. J. Broers, ETH-Dissertation Nr. 10978, ETH Zürich, **1994**; d) W. Eisenreich, B. Menhard, P. J. Hylands, M. H. Zenk, A. Bacher, *Proc. Natl. Acad. Sci. USA* **1996**, 93, 6431–6436; e) W. Eisenreich, M. Schwarz, A. Cartayrade, D. Arigoni, M. H. Zenk, A. Bacher, *Chem. Biol.* **1998**, 5, R221–R233; f) T. Gräwert, M. Groll, F. Rohdich, A. Bacher, W. Eisenreich, *Cell. Mol. Life Sci.* **2011**, 68, 3797–3814.
- [2] a) J. M. Estévez, A. Cantero, A. Reindl, S. Reichler, P. León, *J. Biol. Chem.* **2001**, 276, 22901–22909; b) C. S. Ahn, H.-S. Pai, *Plant Mol. Biol.* **2008**, 66, 503–517.
- [3] a) L. Buetow, A. C. Brown, T. Parish, W. N. Hunter, *BMC Struct. Biol.* **2007**, 7, 68–78; b) A. C. Brown, T. Parish, *BMC Microbiol.* **2008**, 8, 78–86.
- [4] a) H. Jomaa, J. Wiesner, S. Sanderbrand, B. Altincicek, C. Weidemeyer, M. Hintz, I. Türbachova, M. Eberl, J. Zeidler, H. K. Lichtenthaler, D. Soldati, E. Beck, *Science* **1999**, 285, 1573–1576; b) M. B. Cassera, F. C. Gozzo, F. L. D'Alexandri, E. F. Merino, H. A. del Portillo, V. J. Peres, I. C. Almeida, M. N. Eberlin, G. Wunderlich, J. Wiesner, H. Jomaa, E. A. Kimura, A. M. Katzin, *J. Biol. Chem.* **2004**, 279, 51749–51759.
- [5] a) I. Hale, P. M. O'Neill, N. G. Berry, A. Odom, R. Sharma, *Med. Chem. Commun.* **2012**, 3, 418–433; b) J.-Y. van der Meer, A. K. H. Hirsch, *Nat. Prod. Rep.* **2012**, 29, 721–728.
- [6] a) H. Lüttgen, F. Rohdich, S. Herz, J. Wungsintaweeikul, S. Hecht, C. A. Schuhr, M. Fellermeier, S. Sagner, M. H. Zenk, A. Bacher, W. Eisenreich, *Proc. Natl. Acad. Sci. USA* **2000**, 97, 1062–1067; b) T. Kuzuyama, M. Takagi, K. Kaneda, H. Watanabe, T. Daiiri, H. Seto, *Tetrahedron Lett.* **2000**, 41, 2925–2928; c) L. Miallau, M. S. Alphey, L. E. Kemp, G. A. Leonard, S. M. McSweeney, S. Hecht, A. Bacher, W. Eisenreich, F. Rohdich, W. N. Hunter, *Proc. Natl. Acad. Sci. USA* **2003**, 100, 9173–9178; d) T. Wada, T. Kuzuyama, S. Satoh, S. Kuramitsu,

- S. Yokoyama, S. Unzai, J. R. H. Tame, S.-Y. Park, *J. Biol. Chem.* **2003**, 278, 30022–30027; e) C. M. Crane, A. K. H. Hirsch, M. S. Alphey, T. Sgraja, S. Lauw, V. Illarionova, F. Rohdich, W. Eisenreich, W. N. Hunter, A. Bacher, F. Diederich, *ChemMedChem* **2008**, 3, 91–101; f) T. Sgraja, M. S. Alphey, S. Ghilagaber, R. Marquez, M. N. Robertson, J. L. Hemmings, S. Lauw, F. Rohdich, A. Bacher, W. Eisenreich, V. Illarionova, W. N. Hunter, *FEBS J.* **2008**, 275, 2779–2794; g) A. K. H. Hirsch, M. S. Alphey, S. Lauw, M. Seet, L. Barandun, W. Eisenreich, F. Rohdich, W. N. Hunter, A. Bacher, F. Diederich, *Org. Biomol. Chem.* **2008**, 6, 2719–2730; h) S. Shan, X. H. Chen, T. Liu, H. C. Zhao, Z. H. Rao, Z. Y. Lou, *FASEB J.* **2011**, 25, 1577–1584.
- [7] a) A. K. H. Hirsch, S. Lauw, P. Gersbach, W. B. Schweizer, F. Rohdich, W. Eisenreich, A. Bacher, F. Diederich, *ChemMedChem* **2007**, 2, 806–810; b) A. P. Schütz, S. Osawa, J. Mathis, A. K. H. Hirsch, B. Bernet, B. Illarionov, M. Fischer, A. Bacher, F. Diederich, *Eur. J. Org. Chem.* **2012**, 3278–3287; c) A. P. Schütz, S. Locher, B. Bernet, B. Illarionov, M. Fischer, F. Diederich, *Eur. J. Org. Chem.*, DOI:10.1002/ejoc.201201454.
- [8] A. K. H. Hirsch, F. R. Fischer, F. Diederich, *Angew. Chem.* **2007**, 119, 342–357; *Angew. Chem. Int. Ed.* **2007**, 46, 338–352.
- [9] a) P. R. Gerber, K. Müller, *J. Comput.-Aided Mol. Des.* **1995**, 9, 251–268; b) P. R. Gerber, *J. Comput.-Aided Mol. Des.* **1998**, 12, 37–51.
- [10] a) A. D. Broom, *J. Med. Chem.* **1989**, 32, 2–7; b) B. Masjost, P. Ballmer, E. Borroni, G. Zürcher, F. K. Winkler, R. Jakob-Roetne, F. Diederich, *Chem. Eur. J.* **2000**, 6, 971–982.
- [11] a) R. Paulini, K. Müller, F. Diederich, *Angew. Chem.* **2005**, 117, 1820–1839; *Angew. Chem. Int. Ed.* **2005**, 44, 1788–1805; b) J. A. Olsen, D. W. Banner, P. Seiler, U. Obst-Sander, A. D'Arcy, M. Stihle, K. Müller, F. Diederich, *Angew. Chem.* **2003**, 115, 2611–2615; *Angew. Chem. Int. Ed.* **2003**, 42, 2507–2511; c) H.-J. Böhm, D. Banner, S. Bendels, M. Kansy, B. Kuhn, K. Müller, U. Obst-Sander, M. Stahl, *ChemBioChem* **2004**, 5, 637–643.
- [12] M. Zhong, I. Nowak, J. F. Cannon, M. J. Robins, *J. Org. Chem.* **2006**, 71, 4216–4221.
- [13] V. Illarionova, J. Kaiser, E. Ostrozhenskova, A. Bacher, M. Fischer, W. Eisenreich, F. Rohdich, *J. Org. Chem.* **2006**, 71, 8824–8834.
- [14] a) D. J. Chadwick, R. I. Ngochindo, *J. Chem. Soc. Perkin Trans. 1* **1984**, 481–486; b) V. Snieckus, *Chem. Rev.* **1990**, 90, 879–933.
- [15] a) S. Sahli, B. Stump, T. Welti, W. B. Schweizer, F. Diederich, D. Blum-Kaelin, J. D. Aebi, H.-J. Böhm, *Helv. Chim. Acta* **2005**, 88, 707–730; b) C. Madsen, A. A. Jensen, T. Liljefors, U. Kristiansen, B. Nielsen, C. P. Hansen, M. Larsen, B. Ebert, B. Bang-Andersen, P. Krogsgaard-Larsen, B. Frølund, *J. Med. Chem.* **2007**, 50, 4147–4161.
- [16] G. K. S. Prakash, A. K. Yudin, *Chem. Rev.* **1997**, 97, 757–786.
- [17] D. Yang, D. Fokas, J. Li, L. Yu, C. M. Baldino, *Synthesis* **2005**, 47–56.
- [18] F. H. Allen, *Acta Crystallogr., Sect. B: Struct. Sci.* **2002**, 58, 380–388.
- [19] P. Kuzmič, *Anal. Biochem.* **1996**, 237, 260–273.
- [20] Y. Cheng, W. H. Prusoff, *Biochem. Pharmacol.* **1973**, 22, 3099–3108.

Received: November 2, 2012
Published Online: January 4, 2013