



HAL
open science

Practical Synthesis and Application of Halogen-Doped Pyrrole Building Blocks

Andrej Emanuel Cotman, Thomas Guérin, Ivana Kovačević, Davide Benedetto Tiz, Martina Durcik, Federica Fulgheri, Štefan Možina, Daniela Secci, Maša Sterle, Janez Ilaš, et al.

► **To cite this version:**

Andrej Emanuel Cotman, Thomas Guérin, Ivana Kovačević, Davide Benedetto Tiz, Martina Durcik, et al.. Practical Synthesis and Application of Halogen-Doped Pyrrole Building Blocks. ACS Omega, 2021, 6 (14), pp.9723-9730. 10.1021/acsomega.1c00331 . hal-03228819

HAL Id: hal-03228819

<https://hal.science/hal-03228819>

Submitted on 18 May 2021

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Practical Synthesis and Application of Halogen-Doped Pyrrole Building Blocks

Andrej Emanuel Čotman, Thomas Guérin, Ivana Kovačević, Davide Benedetto Tiz, Martina Durcik, Federica Fulgheri, Štefan Možina, Daniela Secci, Maša Sterle, Janez Ilaš, Anamarija Zega, Nace Zidar, Lucija Peterlin Mašič, Tihomir Tomašič, Frédéric R. Leroux, Gilles Hanquet, and Danijel Kikelj*



Cite This: <https://doi.org/10.1021/acsomega.1c00331>



Read Online

ACCESS |



Metrics & More

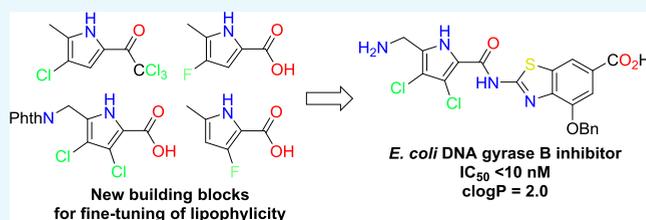


Article Recommendations



Supporting Information

ABSTRACT: A practical access to four new halogen-substituted pyrrole building blocks was realized in two to five synthetic steps from commercially available starting materials. The target compounds were prepared on a 50 mg to 1 g scale, and their conversion to nanomolar inhibitors of bacterial DNA gyrase B was demonstrated for three of the prepared building blocks to showcase the usefulness of such chemical motifs in medicinal chemistry.



1. INTRODUCTION

Halogen-substituted pyrrole-2-carboxamide is an integral molecular fragment of bioactive marine natural products as well as natural and synthetic anti-infectives (Figure 1). In

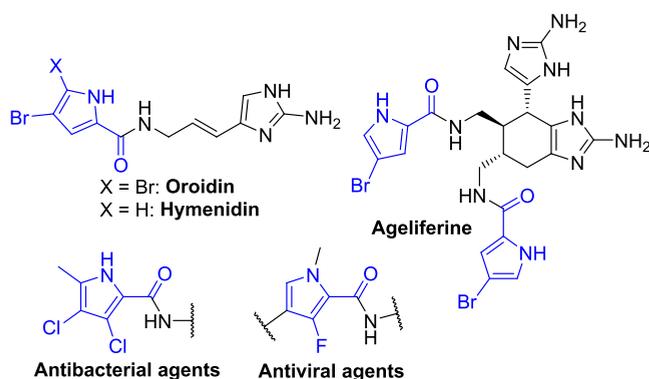


Figure 1. Representative natural products and bioactive compounds with a halogen-doped 1H-pyrrole-2-carboxamide fragment.

particular, mono- and dibromopyrrole-2-carboxamide are found in oroidin¹ and hymenidin,² which are postulated precursors for structurally diverse mono- and oligomeric secondary metabolites involved in the chemical defense of *Agelas* marine sponges. A representative compound ageliferin³ features a complex multichiral scaffold.⁴ Furthermore, 3,4-dichloro-5-methyl-1H-pyrrole-2-carboxamide is a molecular fragment of natural^{5,6} and synthetic⁷ antibacterials, crucial for binding to the active site of bacterial topoisomerases, and the 3-fluoro-1H-pyrrole-2-carboxamide moiety is found in promising preclinical candidates, active against hepatitis B virus (Figure 1).⁸

During our ongoing research in the field of dual bacterial DNA gyrase/topoisomerase IV inhibitors,^{9–15} a promising hit compound **1** (Figure 2) was identified, displaying low

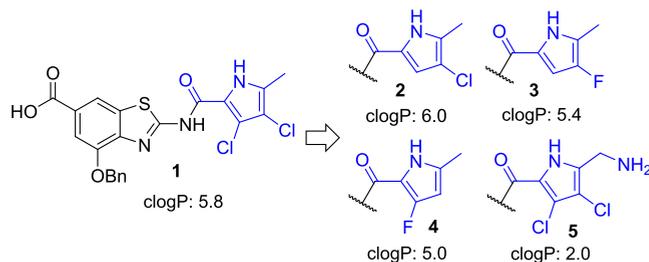


Figure 2. Design of antibacterial hit compound analogues with decreased lipophilicity.

nanomolar inhibition of the target enzymes and broad-spectrum activity against gram-positive bacterial strains.¹⁶ Due to the compound's high lipophilicity, its more polar analogues **2–5** were designed by varying the 3,4-dichloro-5-methyl-1H-pyrrole-2-carboxamide moiety, envisioning improved physical properties (*clogP* was calculated by ChemDraw) of the analogues while retaining the on-target activity (Figure 2).

With no preceding literature on the synthesis of the required pyrrole building blocks for the preparation of compounds **2–5**, we report herein our synthetic endeavors, where the main goal

Received: January 19, 2021

Accepted: March 18, 2021

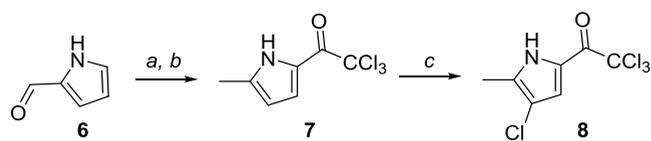
was the timely delivery of at least 100 mg of the sample to be built into the bioactive molecules. The amide bond of the target compounds can be formed using pyrrole-2-carbonyl chloride or 2-trichloroacetylpyrrole; therefore, either would be an acceptable option.

2. RESULTS AND DISCUSSION

The literature procedure for the synthesis of 4-chloro-5-methyl-1*H*-pyrrole-2-carboxylic acid involves chlorination of ethyl 5-methyl-1*H*-pyrrole-2-carboxylate using *N*-chlorosuccinimide at 0 °C and required in our hands laborious chromatographic separation of two barely resolved products.¹⁷

The practical synthesis of an alternative acylating agent **8** for the introduction of the same structural fragment was thus developed (Scheme 1). Trichloroacetylpyrrole **7** was prepared

Scheme 1. Synthesis of 2-Trichloroacetyl-4-chloro-5-methyl-1*H*-pyrrole **8**^a

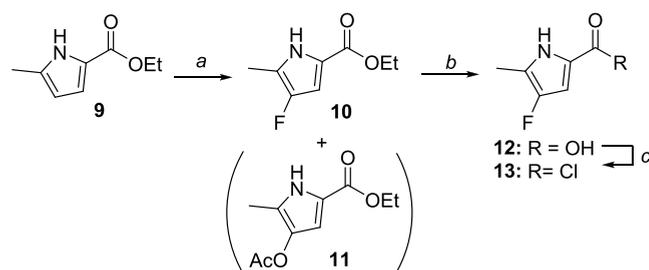


^aReagents and conditions: (a) $\text{NH}_2\text{NH}_2 \cdot \text{H}_2\text{O}$, ethylene glycol, 90 °C, 1 h, then KOH, 90 °C, 2.5 h (70% yield); (b) CCl_3COCl , Et_2O , r.t., 2 h (60% yield); and (c) *N*-chlorosuccinimide, dichloromethane, r.t., 4 h (61% yield).

from pyrrole-2-carbaldehyde **6** employing the Wolff–Kishner reduction and Friedel–Crafts acylation.¹⁸ It was then directly monochlorinated using *N*-chlorosuccinimide at r.t. and the pure product **8** was obtained on the gram scale in 61% isolated yield after convenient crystallization from dichloromethane. Its structure was unambiguously determined by two-dimensional (2D) nuclear magnetic resonance (NMR) experiments (Supporting Information), showing that the electrophilic chlorination was selective for the position next to the electron-donating methyl substituent.

Next, we targeted the 4-fluoro-substituted building block. The screening of different halogen exchange (“Halex”) conditions involving crown ether 18-C-6 and [2.2.2]cryptand, for the conversion of chloropyrrole **8** or ethyl 4-chloro-5-methylpyrrole-2-carboxylate to the corresponding arylfluorides, returned no hits.¹⁹ We thus resorted to electrophilic fluorination of ethyl 5-methyl-1*H*-pyrrole-2-carboxylate **9** (Scheme 2). Initial 0.5 mmol scale screening of the reaction conditions (Table S1 in the Supporting Information) revealed that Selectfluor-mediated fluorination²⁰ outperformed the *N*-fluorobenzenesulfonimide (NFSI)-mediated Lewis acid-catalyzed fluorination,²¹ as the former resulted in somewhat cleaner conversions. When the fluorination was performed at 0 °C in a mixture of acetonitrile and acetic acid (Table S2, entries 11 and 12), the formation of target compound **10**, accompanied by an acetoxy side product **11**, was observed. Their structures were confirmed by single-crystal X-ray diffraction analysis (Figures S1 and S2 in the Supporting Information). Aiming for an efficient med–chem synthetic route, the reaction was performed on a 2 g scale (Scheme 2), delivering **10** in a consistent 4.5–6.5% yield after flash chromatography. Ester **10** was hydrolyzed to acid **12**, requiring rather forcing conditions, and acyl chloride **13** was finally formed using oxalyl chloride in dichloromethane. It is

Scheme 2. Synthesis of 4-Fluoro-5-methyl-1*H*-pyrrole-2-carbonyl Chloride **13**^a

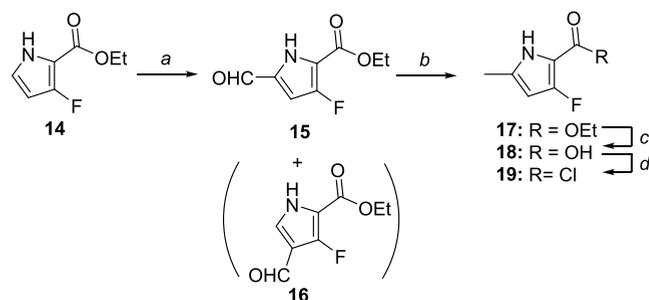


^aReagents and conditions: (a) Selectfluor, MeCN/AcOH 5:1, 0 °C, 2 h (6.5% yield); (b) 10 M NaOH (aq), EtOH, 90 °C, 3 h (76% yield); and (c) oxalyl chloride, dichloromethane, r.t., overnight (quant. yield).

noteworthy that acyl chloride formation using refluxing sulfonyl chloride or oxalyl chloride with the catalytic quantity of dimethylformamide (DMF) resulted in the formation of a significant amount of unidentified side products.

Ethyl 3-fluoro-1*H*-pyrrole-2-carboxylate **14** has recently become commercially available at a reasonable price because it is a key building block for a drug candidate against hepatitis B virus.²² This was a good starting point for the synthesis of 3-fluoro-5-methyl-1*H*-pyrrole-2-carboxylic acid **18** (Scheme 3).

Scheme 3. Synthesis of 3-Fluoro-5-methyl-1*H*-pyrrole-2-carbonyl Chloride **19**^a



^aReagents and conditions: (a) DMF, POCl_3 , 0 °C, 30 min, then **14**, 90 °C, overnight (25% yield); (b) Zn, 4 M HCl/dioxane, r.t., 40 min (20% yield); (c) 10 M NaOH(aq), EtOH, 90 °C, 5 h (82% yield); and (d) oxalyl chloride, dichloromethane, r.t., overnight (quant. yield).

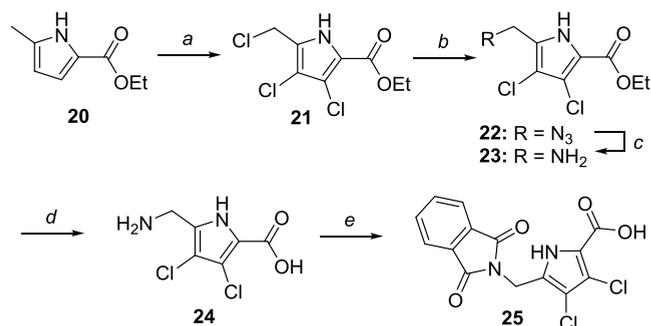
The Vilsmeier–Haack formylation of **14** gave at 68% conversion a 43:57 mixture of 4- and 5-formylated regioisomers **16** and **15**, which were separated by flash chromatography. The regioisomers’ identity was assigned by ¹⁹F NMR as follows: 4-formyl isomer **16** features a singlet and 5-formyl isomer **15** features a doublet, $^3J_{\text{F,H}} = 4$ Hz, confirming the presence of a vicinal proton. Moreover, the ¹³C NMR peak of the formyl carbon of **15** is a singlet and that of **16** is a doublet, $^3J_{\text{C,F}} = 2.8$ Hz.

Based on the literature reports on the reduction of ester-containing formylpyrroles to methylpyrroles,²³ we first attempted a $\text{BH}_3 \cdot \text{THF}$ -mediated reduction of **15**–**17**, which in this case yielded the intermediate alcohol; no full reduction was observed even after several days of stirring with periodical addition of excess $\text{BH}_3 \cdot \text{THF}$. Other literature reports on aldehyde-to-methyl reduction in the presence of ester include a two-step Mozingo protocol via thioketal.²⁴ Aiming to secure a

convenient one-pot procedure, we opted for the modified Clemmensen reduction.²⁵ A dioxane-soluble [$\text{ZnCl}_2(\text{dioxane})_2$] complex²⁶ was prepared by treating zinc dust with anhydrous 4 M HCl in dioxane. This proved to be a very efficient and reasonably selective reduction medium, delivering **17** after 40 min at r.t. in 20% isolated yield. Optimization of the reaction conditions and elucidation of the mechanism is beyond the aim of this study; however, we speculate that a dioxane-soluble Zn(II) species forms a zinc-ylide intermediate more efficiently compared to the classical heterogeneous Clemmensen reduction (Zn/Hg/HCl/H₂O), allowing the reaction to proceed at room temperature.²⁷ The side products are essentially a result of the zinc-ylide reaction with other present electrophiles (ester, aldehyde, and arylfluoride). Using the conditions developed for the synthesis of **13**, ester **17** was readily transformed to acyl chloride **19**.

Ethyl 5-chloromethyl-3,4-dichloro-1*H*-pyrrole-2-carboxylate **21** was prepared from commercially available **20** according to the literature procedure (Scheme 4).¹⁸ After conversion to

Scheme 4. Synthesis of 3,4-Dichloro-5-phthalimidomethyl-1*H*-pyrrole-2-carboxylic Acid **25**^a

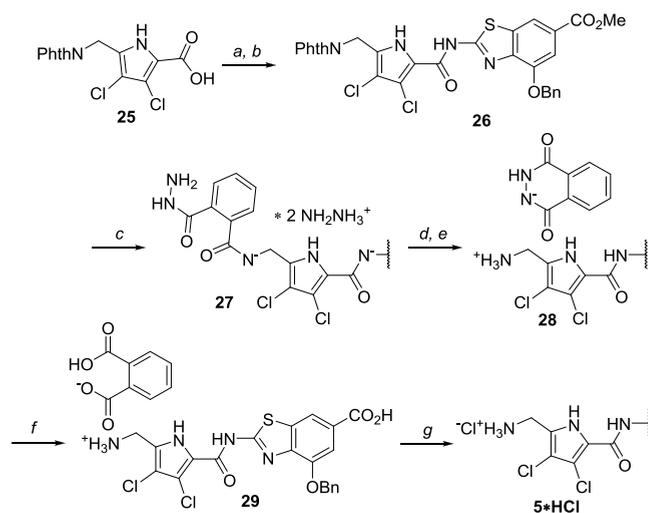


^aReagents and conditions: (a) SO_2Cl_2 , CCl_4 , -5°C , 5 h; (b) NaN_3 , KI (cat.), DMF, r.t., 1.5 h (95% yield); (c) PPh_3 , $\text{H}_2\text{O}/\text{THF}$, r.t., 2.5 h (78% yield); (d) 10 M NaOH, EtOH, 90°C , 4 h (quant. yield); and (e) phthalic anhydride, $130\text{--}180^\circ\text{C}$, 1 h (55% yield).

azide **22** by KI-mediated nucleophilic substitution, the reduction of **22** to amine **23** was first attempted via Pd/C-catalyzed hydrogenation. This resulted in significant side-product formation, possibly via the nucleophilic attack of amine **23** to the electrophilic methylene moiety of **22**, and aryl dehalogenation, as apparent from the ¹H NMR analysis of the crude reaction mixture. Avoiding the coexistence of amine and azide species during the reaction, we resorted to the milder Staudinger reduction,²⁸ which furnished amine **23** in 78% isolated yield. Saponification to **24**, followed by phthalimide protection in neat phthalic anhydride gave **25** with 41% yield over four steps from **21**.

To showcase the usefulness of the prepared building blocks in medicinal chemistry, the synthesis of compound **5**, the analogue of antibacterial hit compound **1**, was tackled (Scheme 5). After the smooth coupling of the acyl chloride, prepared from **25** in neat thionyl chloride, with the 2-amino-benzothiazole building block²⁹ in refluxing toluene, the deprotection step required some special attention. The formation of stable hydrazinium salt **27** was observed during the phthalimide deprotection, arguably due to the electron-withdrawing character of dichloropyrrole, which increases the acidity of the neighboring amides. It was crucial to first protonate the nitrogens of **27** to achieve complete

Scheme 5. Synthesis of DNA Gyrase B Inhibitor **5**·HCl^a



^aReagents and conditions: (a) SOCl_2 , 75°C , 1 h (quant. yield); (b) methyl 2-amino-4-benzyloxybenzo[*d*]thiazole-6-carboxylate, toluene, 130°C , 24 h (81% yield); (c) $\text{NH}_2\text{NH}_2 \cdot \text{H}_2\text{O}$, EtOH, 50°C , 40 min; (d) HCl, MeOH, r.t., 15 min; (e) EtOH, 80°C , 18 h (88% yield from **26**); (f) 4 M KOH, EtOH, 50°C , 24 h then 1 M HCl to pH = 9; and (g) HCl, MeOH, r.t. (55% yield from **28**).

deprotection after refluxing in ethanol overnight. Alkaline hydrolysis of phthalic hydrazide salt **28** yielded phthalate salt **29** and the anion was readily exchanged to the chloride salt of **5** by trituration with methanolic HCl.

Antibacterial hit compound **1** ($c \log P = 5.8$) inhibited *Escherichia coli* DNA gyrase with $\text{IC}_{50} < 10$ nM, and compound **5** ($c \log P = 2.0$) inhibited the same enzyme with $\text{IC}_{50} < 10$ nM. Moreover, **5** exhibits activity against *Staphylococcus aureus* (ATCC29213) with a minimal inhibitory concentration of 1 $\mu\text{g}/\text{mL}$. This confirms the hypothesis that the single-digit nanomolar inhibitory on-target activity coupled to the antibacterial activity can be retained while significantly reducing the lipophilicity by the modification of the pyrrole moiety.

To explore the reactivity and bioactivity of the fluorinated pyrroles, two additional analogues of **1** were prepared (Scheme 6) and evaluated for their on-target and antibacterial activities. Thus, compounds **31** and **33** inhibited *E. coli* DNA gyrase with IC_{50} values of 32 and 150 nM, respectively, and possessed weak activity against *S. aureus* (ATCC29213) (**31**: MIC = 64 $\mu\text{g}/\text{mL}$; **33**: MIC > 64 $\mu\text{g}/\text{mL}$).

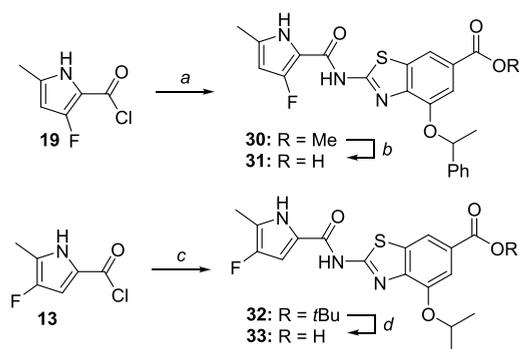
3. CONCLUSIONS

In summary, practical synthetic routes to four new halogen-doped pyrrole building blocks were developed, delivering the target compounds in sufficient quantities for further elaboration. Moreover, the transformation of the building blocks to potent DNA gyrase B inhibitors was demonstrated. Such building blocks are polar alternatives to molecular fragments found in naturally occurring or natural-product-inspired bioactive compounds and are useful in hit-to-lead optimization.

4. EXPERIMENTAL SECTION

4.1. General. Reactions were conducted under an inert atmosphere using anhydrous solvents when required. Analytical thin-layer chromatography (TLC) was performed on silica gel 60 F₂₅₄ plates. Flash column chromatography was

Scheme 6. Synthesis of DNA Gyrase B Inhibitors 31 and 33^a



^aReagents and conditions: (a) methyl 2-amino-4-(1-phenylethoxy)benzo[*d*]thiazole-6-carboxylate, toluene, 130 °C, 24 h (57% yield); (b) MeOH, 2 M NaOH, 40 °C, 48 h; (c) *tert*-butyl 2-amino-4-(2-propyloxy)benzo[*d*]thiazole-6-carboxylate, toluene, 130 °C, 24 h (79% yield); and (d) CF₃COOH, DCM, r.t., 24 h (98% yield).

performed using silica gel 60 (40–63 μm). Melting points were determined on a Kofler apparatus and are uncorrected. ¹H NMR (400 MHz, internal Me₄Si), ¹³C NMR (101 MHz, internal CDCl₃ or DMSO-*d*₆), and ¹⁹F NMR (376 MHz, external CCl₃F) spectra were recorded on a Bruker AVANCE III 400 spectrometer (Bruker Corporation, Billerica, MA) in a DMSO-*d*₆ or CDCl₃ solution. HRMS analysis was performed on a VG Analytical Autospec Q mass spectrometer (Fisons, VG Analytical, Manchester, U.K.).

4.2. Synthetic Procedures. **4.2.1. 2-Trichloroacetyl-4-chloro-5-methyl-1H-pyrrole (8).** A mixture of 2-trichloroacetyl-5-methyl-1H-pyrrole (2.14 g, 9.44 mmol), *N*-chlorosuccinimide (1.26 g, 9.44 mmol), and dichloromethane (9.0 mL) was stirred at r.t. overnight. The reaction mixture was partitioned between EtOAc (50 mL) and water (50 mL), and the organic layer was washed with water and brine, dried (Na₂SO₄), and concentrated. The residue was recrystallized from dichloromethane to get the title compound as white crystals (1.51 g, 61% yield). Mp 140–142 °C (DCM). ¹H NMR (400 MHz, CDCl₃) δ 10.37 (s, 1H), 7.31 (d, *J* = 2.9 Hz, 1H), 2.41 (s, 2H). ¹³C NMR (101 MHz, CDCl₃) δ 172.89, 137.01, 121.03, 119.78, 114.13, 94.87, 11.37. HRMS calcd for C₇H₄ONCl₄ [M - H]⁻ 257.90525, found 257.90521.

4.2.2. Ethyl 4-Fluoro-5-methyl-1H-pyrrole-2-carboxylate (10). To a solution of ethyl 5-methyl-1H-pyrrole-2-carboxylate (2.00 g, 13.1 mmol) in acetonitrile (360 mL) and AcOH (72 mL) at 0 °C was added Selectfluor (9.25 g, 26.1 mmol), and the mixture was stirred at 0 °C for 2 h (full conversion by ¹H NMR). The reaction mixture was partitioned between water (500 mL) and dichloromethane (500 mL), and the organic layer was washed with sat. NaHCO₃ (aq) and brine, dried (Na₂SO₄), and concentrated to get the crude product. Crude products from two 2 g runs were combined and purified by flash chromatography, eluent hexane/EtOAc 4:1, to get the title compound (first eluting) as a colorless amorphous solid (288 mg, 6.5% yield). ¹H NMR (400 MHz, CDCl₃): δ 8.92 (bs, 1H), 6.54 (d, 1H, *J* = 4.0 Hz), 4.30 (q, 2H, *J* = 7.1 Hz), 2.24 (s, 3H), 1.34 (t, 3H, *J* = 7.1 Hz). ¹³C NMR (101 MHz, CDCl₃) δ 161.3 (d, *J* = 3.3 Hz), 148.9 (d, *J* = 238.7 Hz), 117.6, 116.2 (d, *J* = 7.4 Hz), 102.3 (d, *J* = 15.6 Hz), 60.6, 14.6, 9.4 (d, *J* = 2.1 Hz). ¹⁹F NMR (376 MHz, CDCl₃): δ -166.4. HRMS calcd for C₈H₁₁FNO₂ [M + H]⁺ 172.0768, found 172.0769.

This procedure was repeated several times, consistently yielding 4.5–6.5% of the title compound. A monocystal suitable for single-crystal X-ray diffraction analysis was grown from dichloromethane/hexane.

4.2.3. Ethyl 4-Acetoxy-5-methyl-1H-pyrrole-2-carboxylate (11). Ethyl 4-acetoxy-5-methyl-1H-pyrrole-2-carboxylate was isolated as a second eluting product (see purification of 10 above), a white amorphous solid. ¹H NMR (400 MHz, CDCl₃): δ 10.10 (s, 1H), 6.72 (d, *J* = 2.8 Hz, 1H), 4.29 (q, *J* = 7.1 Hz, 1H), 2.24 (s, 1H), 2.16 (s, 1H), 1.32 (t, *J* = 7.1 Hz, 1H). ¹³C NMR (101 MHz, CDCl₃): δ 169.23, 161.46, 134.49, 123.33, 117.80, 108.22, 60.46, 20.85, 14.55, 10.16. A monocystal suitable for single-crystal X-ray diffraction analysis was grown from dichloromethane/hexane.

4.2.4. 4-Fluoro-5-methyl-1H-pyrrole-2-carboxylic Acid (12). A solution of the above ester 10 (280 mg, 1.07 mmol) in abs. EtOH (15 mL) and 10 M NaOH (3.0 mL) was stirred at 90 °C under an argon atmosphere for 3 h, and then the reaction mixture was concentrated under reduced pressure. The residue was acidified to pH = 3 by adding 4 M HCl, and the precipitate was collected, washed with water, and air-dried to get the title compound as a light brown amorphous solid (177 mg, 76% yield). ¹H NMR (400 MHz, DMSO-*d*₆) δ 12.25 (s, 1H), 11.51 (s, 1H), 6.45 (dd, *J* = 2.9, 1.1 Hz, 1H), 2.13 (s, 3H). ¹⁹F NMR (376 MHz, DMSO-*d*₆) δ -167.66 to -167.70 (m). ¹³C NMR (101 MHz, DMSO-*d*₆) δ 161.51 (d, *J* = 3.3 Hz), 147.77 (d, *J* = 235.0 Hz), 116.90 (d, *J* = 25.1 Hz), 116.15 (d, *J* = 7.3 Hz), 100.94 (d, *J* = 14.9 Hz), 8.70 (d, *J* = 2.2 Hz). HRMS calcd for C₆H₅O₂NF [M - H]⁻ 142.03098, found 142.03001.

4.2.5. 4-Fluoro-5-methyl-1H-pyrrole-2-carbonyl Chloride (13). A suspension of the above acid (75 mg, 0.52 mmol) in dry dichloromethane (5.2 mL) and oxalyl chloride (0.45 mL, 5.2 mmol) was stirred at r.t. overnight. The resulting clear solution was concentrated under reduced pressure to get the title compound as a light brown amorphous solid (quant. yield). ¹H NMR (400 MHz, CDCl₃) δ 8.66 (s, 1H), 6.83 (d, *J* = 2.8 Hz, 1H), 2.30 (s, 3H). ¹⁹F NMR shows no clearly identifiable peak.

4.2.6. Ethyl 3-Fluoro-5-formyl-1H-pyrrole-2-carboxylate (15) and Ethyl 3-Fluoro-4-formyl-1H-pyrrole-2-carboxylate (16). To DMF (8.9 mL, 115 mmol) at 0 °C under Ar was added POCl₃ (1.95 mL, 21.0 mmol). After stirring at 0 °C for 30 min, a solution of ethyl 3-fluoro-1H-pyrrole-2-carboxylate 14 (3.00 g, 19.1 mmol) in DMF (29 mL) was added, and the resulting solution was stirred at 90 °C overnight. The reaction mixture was cooled to r.t. and poured onto ice (100 mL), the pH was adjusted to 9 by adding 2 M NaOH (aq) and the product was extracted to Et₂O (3 × 200 mL). The combined organic layers were washed with brine, dried (Na₂SO₄), and concentrated to get the crude product, containing 14, 5-formylated (15), and 4-formylated product (16) in a 42:34:24 ratio (by ¹H NMR). The three compounds were separated by column chromatography, eluent dichloromethane, then hexane/EtOAc 2:1 to yield 14 (983 mg, a white amorphous solid), 15 (869 mg, 25% yield, an orange amorphous solid), and 16 (724 mg, a yellow amorphous solid).

Compound 15: ¹H NMR (400 MHz, CDCl₃) δ 9.88 (s, 1H), 9.42 (br s, 1H), 7.36 (app t, *J* = 3.9 Hz, 1H), 4.39 (q, *J* = 7.1 Hz, 2H), 1.39 (t, *J* = 7.1 Hz, 3H). ¹⁹F NMR (376 MHz, CDCl₃) δ -150.04 (d, *J* = 4 Hz). ¹³C NMR (101 MHz, CDCl₃) δ 183.57, 160.22 (d, *J* = 3.5 Hz), 152.93 (d, *J* = 269.6 Hz), 124.59 (d, *J* = 3.6 Hz), 115.00 (d, *J* = 8.5 Hz), 109.40 (d,

$J = 18.1$ Hz), 61.48, 14.35. HRMS calcd for $C_8H_9O_3NF$ $[M + H]^+$ 186.05610, found 186.05611.

Compound 16: 1H NMR (400 MHz, $CDCl_3$) δ 9.59 (s, 1H), 9.47 (s, 1H), 6.71–6.54 (m, 1H), 4.41 (q, $J = 7.1$ Hz, 2H), 1.40 (t, $J = 7.1$ Hz, 3H). ^{19}F NMR (376 MHz, $CDCl_3$) δ -146.95. ^{13}C NMR (101 MHz, $CDCl_3$) δ 180.38 (d, $J = 2.8$ Hz), 159.67 (d, $J = 3.9$ Hz), 153.06 (d, $J = 260.3$ Hz), 129.65 (d, $J = 4.4$ Hz), 113.90 (d, $J = 20.0$ Hz), 105.40 (d, $J = 14.2$ Hz), 61.75, 14.32.

4.2.7. Ethyl 3-Fluoro-5-methyl-1H-pyrrole-2-carboxylate (17). To a solution of the above aldehyde **15** (500 mg, 2.70 mmol) in 4 M HCl in dioxane (27 mL) was added Zn dust (1.78 g, 27.0 mmol) portionwise at room temperature over 2 min. The reaction is slightly exothermic and performs better if no external cooling is applied. After 40 min (full conversion by TLC), the reaction mixture was concentrated under reduced pressure and filtered through a plug of silica, eluting with EtOAc (200 mL), and concentrated to 5 mL, and the colorless crystals were filtered off and washed with EtOAc (1H NMR analysis revealed only a dioxane peak; it is likely a dioxane-soluble $[ZnCl_2 \cdot (dioxane)_x]$ complex involved in the aldehyde reduction). The filtrate was diluted with EtOAc (200 mL), washed with water (2×100 mL), $NaHCO_3$ (100 mL), and brine, dried (Na_2SO_4), and concentrated to get the crude product (486 mg), containing 30 mol % of the desired product (by ^{19}F NMR). The pure **17** was isolated by flash chromatography on silica, eluent hexane/EtOAc 4:1 (first eluting compound) as a colorless oil containing residual EtOAc (by 1H NMR) (94 mg, 20% yield) and was used as such in the next step. 1H NMR (400 MHz, $CDCl_3$) δ 8.32 (br s, 1H), 6.58 – 6.51 (m, 1H), 4.33 (q, $J = 7.1$ Hz, 2H), 2.02 (s, 3H), 1.36 (t, $J = 7.1$ Hz, 3H). ^{19}F NMR (376 MHz, $CDCl_3$) δ -154.53 (d, $J = 4.7$ Hz). HRMS calcd for $C_8H_{11}O_2NF$ $[M + H]^+$ 172.07683, found 172.07625.

4.2.8. 3-Fluoro-5-methyl-1H-pyrrole-2-carboxylic Acid (18). A solution of the above ester **17** (80 mg, 0.47 mmol) in abs. EtOH (3.5 mL) and 10 M NaOH (0.63 mL) was heated at 90 °C under Ar for 2 h, and then it was concentrated under reduced pressure. The oily residue was dissolved in water (2 mL), and 4 M HCl (1.6 mL) was added. The obtained precipitate was collected, washed with water, and air-dried to get the title compound as a white amorphous powder (55 mg, 82% yield). 1H NMR (400 MHz, $DMSO-d_6$) δ 12.32 (s, 1H), 11.25 (s, 1H), 6.66 (app t, $J = 4.4$ Hz, 1H), 1.93 (s, 3H). ^{19}F NMR (376 MHz, $DMSO-d_6$) δ -156.45. ^{13}C NMR (101 MHz, $DMSO-d_6$) δ 160.49 (d, $J = 3.5$ Hz), 152.39 (d, $J = 252.4$ Hz), 119.46 (d, $J = 6.4$ Hz), 106.77 (d, $J = 18.3$ Hz), 105.46 (d, $J = 13.9$ Hz), 7.26. HRMS calcd. for $C_6H_7O_2NF$ $[M + H]^+$ 144.04553, found 144.04552.

4.2.9. 3-Fluoro-5-methyl-1H-pyrrole-2-carbonyl Chloride (19). A suspension of the above acid **18** (50 mg, 0.35 mmol) in dry dichloromethane (3.5 mL) and oxalyl chloride (0.3 mL, 3.5 mmol) was stirred at r.t. overnight. The resulting clear solution was concentrated under reduced pressure to get the title compound as a light brown amorphous solid (quant. yield). 1H NMR (400 MHz, $CDCl_3$) δ 8.51 (br s, 1H), 6.81–6.76 (m, 1H), 2.04 (d, $J = 0.9$ Hz, 3H). ^{19}F NMR showed no clearly identifiable peak.

4.2.10. Ethyl 5-(azidomethyl)-3,4-dichloro-1H-pyrrole-2-carboxylate (22). To a solution of ethyl 5-(chloromethyl)-3,4-dichloro-1H-pyrrole-2-carboxylate **21** (2.00 g, 7.80 mmol) in DMF (16 mL) was added NaN_3 (1.00 g, 15.6 mmol), followed by KI (130 mg, 0.78 mmol). The resulting suspension was

vigorously stirred at r.t. for 1.5 h, and then it was poured into water (100 mL). The white precipitate was collected, washed with water, and air-dried to yield the title compound as a white amorphous powder (1.95 g, 95% yield). 1H NMR (400 MHz, $CDCl_3$) δ 9.57 (s, 1H), 4.45 (s, 2H), 4.40 (q, $J = 7.1$ Hz, 2H), 1.41 (t, $J = 7.1$ Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 160.08, 126.48, 118.30, 117.72, 113.26, 61.66, 45.22, 14.45. HRMS calcd for $C_8H_7O_2N_4Cl_2$ $[M - H]^-$ 260.99515, found 260.99529.

4.2.11. Ethyl 5-(aminomethyl)-3,4-dichloro-1H-pyrrole-2-carboxylate (23). To a solution of the above azide **22** (1.70 g, 6.46 mmol) in THF/ H_2O 10:1 (35 mL) was added PPh_3 (3.39 g, 12.9 mmol) at r.t. The resulting amber solution was stirred at r.t. (caution: gas evolution) for 2.5 h, and then it was concentrated under reduced pressure. The oily residue was partitioned between EtOAc (200 mL) and 0.5 M HCl (300 mL). The water layer was brought to pH = 11 by adding 2 M NaOH. The precipitate was collected, washed with water, and dried in vacuo to yield the title compound as a white amorphous powder (1.20 g, 78%). 1H NMR (400 MHz, $CDCl_3$) δ 9.87 (s, 1H), 4.36 (q, $J = 7.1$ Hz, 2H), 3.99 (s, 2H), 1.63 (s, 2H), 1.38 (t, $J = 7.1$ Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 160.16, 133.16, 117.50, 116.76, 110.24, 61.02, 36.85, 14.49. HRMS calcd for $C_8H_9O_2N_2Cl_2$ $[M - H]^-$ 235.00466, found 235.00459.

4.2.12. 5-(Aminomethyl)-3,4-dichloro-1H-pyrrole-2-carboxylic Acid (24). A mixture of the above ester **23** (1.42 g, 6.00 mmol), abs. EtOH (60 mL), and 10 M NaOH (10.8 mL) was stirred at 90 °C under argon for 3 h. The reaction mixture was concentrated under reduced pressure, dissolved in water (20 mL), filtered through cotton, and cooled to 0 °C. Conc. HCl(aq) (7.5 mL) was added, followed by 2 M HCl to adjust the pH to 8. The precipitate was collected, washed with water, and air-dried to yield the title compound as a white amorphous powder (1.3 g, quant. yield). 1H NMR (400 MHz, $DMSO-d_6$) δ 3.91 (s, 2H). HRMS calcd for $C_6H_5O_2N_2Cl_2$ $[M - H]^-$ 206.97336, found 206.97342.

4.2.13. 5-(Phthalimidomethyl)-3,4-dichloro-1H-pyrrole-2-carboxylic Acid (25). A homogeneous mixture of the above amino acid **24** (300 mg, 1.44 mmol) and powdered phthalic anhydride (213 mg, 1.44 mmol) in a 25 mL round-bottom flask was heated on an oil bath under a stream of argon from 150 to 180 °C for 15 min while stirring with a magnetic stirrer at 100 rpm and agitating the flask manually. The temperature was kept at 180 °C for 30 min during which the reaction mixture caked. After cooling to r.t., the crude product was triturated successively with dichloromethane, EtOAc, and 1 M HCl, washed with water, and air-dried to get the title compound as a light gray amorphous solid (270 mg, 55% yield). 1H NMR (400 MHz, $DMSO-d_6$) δ 13.04 (s, 1H), 12.57 (s, 1H), 8.01–7.76 (m, 4H), 4.79 (s, 2H). ^{13}C NMR (101 MHz, $DMSO-d_6$) δ 167.31, 160.04, 134.42, 131.83, 127.18, 123.14, 117.38, 114.98, 109.12, 33.00. HRMS calcd for $C_{14}H_7O_4N_2Cl_2$ $[M - H]^-$ 336.97884, found 336.97929.

4.2.14. Methyl 4-(benzyloxy)-2-(3,4-dichloro-5-((1,3-dioxoisindolin-2-yl)methyl)-1H-pyrrole-2-carboxamido)benzo[d]thiazole-6-carboxylate (26). A suspension of the above carboxylic acid **25** (110 mg, 0.324 mmol) in $SOCl_2$ (1 mL) was refluxed for 1 h and then concentrated under reduced pressure. To the solid residue were added methyl 2-amino-4-(benzyloxy)benzo[d]thiazole-6-carboxylate (102 mg, 0.324 mmol) and normal grade toluene (6.5 mL), and the resulting suspension was refluxed overnight. After cooling to r.t., the

precipitate was collected, washed with toluene, and air-dried to get the title compound as a gray amorphous solid (165 mg, 81% yield). ^1H NMR (400 MHz, DMSO- d_6) δ 12.44 (s, 1H), 12.39 (s, 1H), 8.30 (s, 1H), 7.95–7.85 (m, 4H), 7.61 (s, 1H), 7.55–7.50 (m, 2H), 7.46–7.34 (m, 3H), 5.30 (s, 2H), 4.82 (s, 2H), 3.88 (s, 3H). HRMS calcd for $\text{C}_{30}\text{H}_{19}\text{O}_6\text{N}_4\text{Cl}_2\text{S}$ $[\text{M} - \text{H}]^-$ 633.04078, found 633.04098.

4.2.15. (5-((4-(Benzyloxy)-6-(methoxycarbonyl)benzo[d]thiazol-2-yl)carbamo-yl)-3,4-dichloro-1H-pyrrol-2-yl)methanaminium 1,4-Dioxo-3,4-dihydro-1H-phthalazin-2-ide (28). To a suspension of **26** (100 mg, 0.157 mmol) in abs. EtOH (3.2 mL) was added 80% aqueous hydrazine hydrate (0.1 mL, 10 equiv). After stirring at 50 °C for 30 min, full conversion to **27** was achieved; ^1H NMR (400 MHz, DMSO- d_6) δ 8.66 (t, $J = 5.6$ Hz, 1H), 7.95 (d, $J = 1.6$ Hz, 1H), 7.56–7.52 (m, 2H), 7.51–7.45 (m, 4H), 7.45–7.39 (m, 3H), 7.37–7.31 (m, 1H), 5.29 (s, 2H), 4.43 (d, $J = 5.6$ Hz, 2H), 3.83 (s, 3H). The reaction mixture was concentrated under reduced pressure, suspended in MeOH (5 mL), treated with 37% HCl(aq) (three drops), and concentrated to get a white residue. ^1H NMR (400 MHz, DMSO- d_6) δ 12.50–12.36 (m, 2H), 11.41 (s, 1H), 10.63 (s, 2H), 9.10 (t, $J = 5.4$ Hz, 1H), 8.30 (d, $J = 1.4$ Hz, 1H), 7.76–7.71 (m, 1H), 7.67–7.58 (m, 3H), 7.57–7.48 (m, 3H), 7.47–7.41 (m, 2H), 7.40–7.35 (m, 1H), 5.33 (s, 2H), 4.46 (d, $J = 5.3$ Hz, 2H), 3.89 (s, 3H). This reprotonated compound was suspended in 96% EtOH and stirred at 80 °C overnight, and then the reaction mixture was concentrated and triturated with acetone to get the title compound **28** as a gray amorphous solid (90 mg, 88% yield). ^1H NMR (400 MHz, DMSO- d_6) δ 12.66 (s, 1H), 12.51 (s, 1H), 11.64 (s, 1H), 8.42–8.28 (m, 4H), 8.19–7.98 (m, 2H), 7.89 (dd, $J = 5.9, 3.3$ Hz, 2H), 7.64 (d, $J = 1.5$ Hz, 1H), 7.58–7.49 (m, 2H), 7.48–7.41 (m, 2H), 7.41–7.35 (m, 1H), 5.34 (s, 2H), 4.06 (q, $J = 5.2$ Hz, 2H), 3.89 (s, 3H). HRMS calcd for $\text{C}_{22}\text{H}_{19}\text{O}_4\text{N}_4\text{Cl}_2\text{S}$ $[\text{M}]^+$ 505.04986, found 505.04879; calcd for $\text{C}_8\text{H}_5\text{O}_2\text{N}_2$ 161.03565 $[\text{M}]^-$, found 161.03471.

4.2.16. (5-((4-(Benzyloxy)-6-carboxybenzo[d]thiazol-2-yl)carbamo-yl)-3,4-dichloro-1H-pyrrol-2-yl)methanaminium 2-Carboxybenzoate (29). A mixture of the above ester **28** (50 mg, 0.075 mmol), abs. EtOH (1.0 mL), and 4 M KOH (0.15 mL) was stirred at 80 °C for 2 h and then at 50 °C overnight. The reaction mixture was concentrated, water (1 mL) was added, and the solids were filtered off. The filtrate was brought to pH = 6 by adding 1 M HCl and cooled to 0 °C. The amorphous precipitate was collected, washed with water, and air-dried. ^1H NMR (400 MHz, DMSO- d_6) δ 8.18 (s, 1H), 8.08 (s, 2H), 7.96–7.82 (m, 3H), 7.60–7.50 (m, 3H), 7.48–7.31 (m, 3H), 5.30 (s, 2H), 3.94 (s, 2H), exchangeable protons were not observed.

4.2.17. (5-((4-(Benzyloxy)-6-carboxybenzo[d]thiazol-2-yl)carbamo-yl)-3,4-dichloro-1H-pyrrol-2-yl)methanaminium Chloride (5·HCl). The above phthalate salt **29** was triturated with a fresh solution of HCl in MeOH (2 × 1 mL) (prepared by adding two drops of 37% HCl (aq) to absolute methanol (5 mL) (methanol was chosen as a solvent because of the known good solubility of phthalic acid in methanol)) to get the title compound as a brown amorphous powder (22 mg, 55% yield). ^1H NMR (400 MHz, DMSO- d_6) δ 13.05 (s, 1H), 12.64 (s, 1H), 12.47 (s, 1H), 8.39–8.20 (m, 4H), 7.64 (d, $J = 1.3$ Hz, 1H), 7.55 (d, $J = 7.2$ Hz, 2H), 7.47–7.42 (m, 2H), 7.41–7.36 (m, 1H), 5.33 (s, 2H), 4.07 (q, $J = 5$ Hz, 2H). ^{13}C NMR (101 MHz, DMSO- d_6) representative peaks: δ 167.46, 137.04, 128.91, 128.76, 128.58, 127.47, 125.72, 116.82, 115.03, 112.68,

109.63, 70.58, 33.42. HRMS calcd for $\text{C}_{21}\text{H}_{17}\text{O}_4\text{N}_4\text{Cl}_2\text{S}$ $[\text{M} - \text{Cl}]^+$ 491.0348, found 491.0334.

4.2.18. Methyl 2-(3-fluoro-5-methyl-1H-pyrrole-2-carboxamido)-4-(1-phenylethoxy)benzo[d]thiazole-6-carboxylate (30). A suspension of **19** (56 mg, 0.35 mmol) and methyl 2-amino-4-(1-phenylethoxy)benzo[d]thiazole-6-carboxylate (114 mg, 0.349 mmol) in toluene (7 mL) was refluxed overnight, equipped with a CaCl_2 tube. After cooling to r.t., the precipitate was collected and washed with toluene to get the title compound as a light gray amorphous powder (91 mg, 57% yield). ^1H NMR (400 MHz, DMSO- d_6) δ 12.23 (s, 1H), 11.37 (s, 1H), 8.17 (d, $J = 1.5$ Hz, 1H), 7.49–7.42 (m, 2H), 7.40–7.31 (m, 3H), 7.29–7.25 (m, 1H), 6.91 (app t, $J = 4.2$ Hz, 1H), 5.80 (q, $J = 6.1$ Hz, 1H), 3.80 (s, 3H), 1.99 (s, 3H), 1.64 (d, $J = 6.3$ Hz, 3H). ^{19}F NMR (376 MHz, DMSO- d_6) δ -153.97 (d, $J = 4$ Hz). HRMS calcd for $\text{C}_{23}\text{H}_{19}\text{O}_4\text{N}_3\text{FS}$ 452.10858; found 452.10924 $[\text{M} - \text{H}]^-$.

4.2.19. 2-(3-Fluoro-5-methyl-1H-pyrrole-2-carboxamido)-4-(1-phenylethoxy)benzo[d]thiazole-6-carboxylic Acid (31). A solution of the above ester **30** (70 mg, 0.154 mmol) in MeOH (3.0 mL) and 2 M NaOH (0.40 mL) was stirred at 40 °C overnight. NaOH (2 M, 0.40 mL) was added and stirred another night, and then the reaction mixture was concentrated. The residue was suspended in water (2 mL), the pH was adjusted to 2 by adding 4 M HCl, and the precipitate was collected, washed with water, air-dried, and triturated with MeOH to get the title compound as a beige amorphous solid (55 mg, 81% yield). ^1H NMR (400 MHz, DMSO- d_6) δ 12.82 (s, 1H), 12.20 (s, 1H), 11.37 (s, 1H), 8.13 (s, 1H), 7.52–7.40 (m, 2H), 7.40–7.31 (m, 3H), 7.31–7.20 (m, 1H), 6.91 (app t, $J = 4.2$ Hz, 1H), 5.79 (q, $J = 6.3$ Hz, 1H), 1.99 (s, 3H), 1.64 (d, $J = 6.3$ Hz, 3H). ^{19}F NMR (376 MHz, DMSO- d_6) δ -154.06 (d, $J = 4$ Hz). ^{13}C NMR (101 MHz, DMSO- d_6) δ 166.92, 159.75, 156.78, 154.36, 151.82, 148.98, 142.57, 132.83, 128.61, 127.55, 126.31, 125.53, 120.98 (d, $J = 5.4$ Hz), 115.90, 110.44, 107.83 (d, $J = 15.0$ Hz), 106.18 (d, $J = 13.7$ Hz), 75.48, 24.33, 7.17. HRMS calcd for $\text{C}_{22}\text{H}_{17}\text{O}_4\text{N}_3\text{FS}$ 438.09293; found 438.09338 $[\text{M} - \text{H}]^-$.

4.2.20. tert-Butyl 2-(4-fluoro-5-methyl-1H-pyrrole-2-carboxamido)-4-isopropoxybenzo[d]thiazole-6-carboxylate (32). A suspension of **13** (58 mg, 0.36 mmol) and methyl 2-amino-4-(2-propyloxy)benzo[d]thiazole-6-carboxylate (93 mg, 0.30 mmol) in toluene (7 mL) was stirred at 130 °C overnight. The gray amorphous precipitate was collected and washed with toluene. Yield: 79% (104 mg). ^1H NMR (400 MHz, DMSO- d_6) δ 12.66 (s, 1H), 11.85 (s, 1H), 8.13 (d, $J = 1$ Hz, 1H), 7.44 (d, $J = 1$ Hz, 1H), 7.26 (d, $J = 2.3$ Hz, 1H), 4.95–4.84 (m, 1H), 2.20 (s, 3H), 1.36 (d, $J = 6.0$ Hz, 6H). MS 432.2 $[\text{M} - \text{H}]^-$.

4.2.21. 2-(4-Fluoro-5-methyl-1H-pyrrole-2-carboxamido)-4-isopropoxybenzo[d]thiazole-6-carboxylic Acid (33). The above tert-butyl ester **32** (75 mg, 0.173 mmol) was suspended in dichloromethane (3 mL). Trifluoroacetic acid (0.13 mL, 1.73 mmol) was added and the suspension turned into a brown solution. The reaction mixture was stirred overnight at room temperature. The solvent was removed under reduced pressure and the residue was triturated with methanol to give **33** as an amorphous solid. Yield: 98% (64 mg). ^1H NMR (400 MHz, DMSO- d_6) δ 12.92 (s, 1H), 12.65 (s, 1H), 11.84 (s, 1H), 8.17 (d, $J = 1.2$ Hz, 1H), 7.48 (s, 1H), 7.26 (d, $J = 2.7$ Hz, 1H), 4.89 (hept, $J = 6$ Hz, 1H), 2.19 (s, 3H), 1.36 (d, $J = 6$ Hz, 6H). HRMS calcd for $\text{C}_{17}\text{H}_{17}\text{O}_4\text{N}_3\text{FS}$ $[\text{M} + \text{H}]^+$ 378.09183, found 378.09203.

4.3. Biological Assays. 4.3.1. *Determination of Inhibitory Activity on E. coli DNA Gyrase.* The supercoiling assay for the determination of IC₅₀ values was performed according to previously reported procedures.³⁰

■ ASSOCIATED CONTENT

SI Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acsomega.1c00331>.

Additional experimental data; copies of ¹H and ¹³C and 2D NMR spectra; and single-crystal X-ray diffraction analysis (PDF)

Compound 10 (CIF)

Compound 11 (CIF)

checkCIF/PLATON report (PDF)

Accession Codes

CCDC 2022198 and 2022199 contain the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/data_request/cif, or by emailing data_request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB21EZ, UK; fax: +44 1223 336033.

■ AUTHOR INFORMATION

Corresponding Author

Danijel Kikelj – Faculty of Pharmacy, University of Ljubljana, 1000 Ljubljana, Slovenia; Email: danijel.kikelj@ffa.uni-lj.si

Authors

Andrej Emanuel Cotman – Faculty of Pharmacy, University of Ljubljana, 1000 Ljubljana, Slovenia; orcid.org/0000-0003-2528-396X

Thomas Guérin – Université de Strasbourg, CNRS, UMR 7042-LIMA, Strasbourg 67087, France

Ivana Kováčević – Faculty of Sciences, Department of Chemistry, Biochemistry and Environmental Protection, University of Novi Sad, 21000 Novi Sad, Serbia

Davide Benedetto Tiz – Faculty of Pharmacy, University of Ljubljana, 1000 Ljubljana, Slovenia

Martina Durcik – Faculty of Pharmacy, University of Ljubljana, 1000 Ljubljana, Slovenia; orcid.org/0000-0002-9218-1771

Federica Fulgheri – Faculty of Pharmacy, University of Ljubljana, 1000 Ljubljana, Slovenia

Štefan Možina – Faculty of Pharmacy, University of Ljubljana, 1000 Ljubljana, Slovenia; orcid.org/0000-0002-7416-6981

Daniela Secci – Faculty of Pharmacy, University of Ljubljana, 1000 Ljubljana, Slovenia; orcid.org/0000-0002-1628-1850

Maša Sterle – Faculty of Pharmacy, University of Ljubljana, 1000 Ljubljana, Slovenia; orcid.org/0000-0003-3898-5194

Janez Ilaš – Faculty of Pharmacy, University of Ljubljana, 1000 Ljubljana, Slovenia; orcid.org/0000-0002-0124-0474

Anamarija Zega – Faculty of Pharmacy, University of Ljubljana, 1000 Ljubljana, Slovenia; orcid.org/0000-0003-4065-0019

Nace Zidar – Faculty of Pharmacy, University of Ljubljana, 1000 Ljubljana, Slovenia; orcid.org/0000-0003-1905-0158

Lucija Peterlin Mašič – Faculty of Pharmacy, University of Ljubljana, 1000 Ljubljana, Slovenia

Tihomir Tomašič – Faculty of Pharmacy, University of Ljubljana, 1000 Ljubljana, Slovenia; orcid.org/0000-0001-5534-209X

Frédéric R. Leroux – Université de Strasbourg, CNRS, UMR 7042-LIMA, Strasbourg 67087, France; orcid.org/0000-0001-8900-5753

Gilles Hanquet – Université de Strasbourg, CNRS, UMR 7042-LIMA, Strasbourg 67087, France

Complete contact information is available at:

<https://pubs.acs.org/doi/10.1021/acsomega.1c00331>

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

Some of the research leading to these results was conducted as part of the ND4BB ENABLE Consortium and has received support from the Innovative Medicines Initiative Joint Undertaking under Grant No. 115583, resources of which comprise financial contributions from the European Union's seventh framework program (FP7/2007-2013) and EFPIA companies' in-kind contribution. We gratefully acknowledge the Slovenian Research Agency (Grant No. P1-0208), COST Action CA15135 MuTaLig, the French Agence Nationale pour la Recherche (ANR) (grant number ANR-17-CE07-0008-01, DEFIS), CNRS, and Université de Strasbourg for financial support. The French Fluorine Network (GIS Fluor) is also acknowledged. T.G. is much grateful to the French Ministry of Education and Research for funding. The authors thank L. Karmazin and C. Bailly from the Service de radiocristallographie de la Fédération de Chimie Le Bel FR 2010 for the SCXRD analyses. Maja Frelj is acknowledged for the acquisition of HRMS spectra and Dr. Žiga Skok for performing the enzyme inhibition assay.

■ REFERENCES

- (1) Kovalerchik, D.; Singh, R. P.; Schlesinger, P.; Mahajni, A.; Shefer, S.; Fridman, M.; Ilan, M.; Carmeli, S. Bromopyrrole Alkaloids of the Sponge *Agelas oroides* Collected Near the Israeli Mediterranean Coastline. *J. Nat. Prod.* **2020**, *83*, 374–384.
- (2) de Souza, R. T. M. P.; Freire, V. F.; Gubiani, J. R.; Ferreira, R. O.; Trivella, D. B. B.; Moraes, F. C.; Paradas, W. C.; Salgado, L. T.; Pereira, R. C.; Amado Filho, G. M.; Ferreira, A. G.; Williams, D. E.; Andersen, R. J.; Molinski, T. F.; Berlinck, R. G. S. Bromopyrrole Alkaloid Inhibitors of the Proteasome Isolated from a *Dictyonella* Sp. Marine Sponge Collected at the Amazon River Mouth. *J. Nat. Prod.* **2018**, *81*, 2296–2300.
- (3) Ma, Z.; Wang, X.; Wang, X.; Rodriguez, R. A.; Moore, C. E.; Gao, S.; Tan, X.; Ma, Y.; Rheingold, A. L.; Baran, P. S.; Chen, C. Asymmetric Syntheses of Scepterin and Massadine and Evidence for Biosynthetic Enantiodivergence. *Science* **2014**, *346*, 219–224.
- (4) Cotman, A. E. Escaping from Flatland: Stereoconvergent Synthesis of 3-Dimensional Scaffolds via Ruthenium(II)-Catalyzed Noyori-Ikariya Transfer Hydrogenation. *Chem. - Eur. J.* **2021**, *27*, 39–53.
- (5) Singh, S. B. Discovery and Development of Kibdelomycin, a New Class of Broad-Spectrum Antibiotics Targeting the Clinically Proven Bacterial Type II Topoisomerase. *Bioorg. Med. Chem.* **2016**, *24*, 6291–6297.
- (6) Sawa, R.; Takahashi, Y.; Hashizume, H.; Sasaki, K.; Ishizaki, Y.; Umekita, M.; Hatano, M.; Abe, H.; Watanabe, T.; Kinoshita, N.; Homma, Y.; Hayashi, C.; Inoue, K.; Ohba, S.; Masuda, T.; Arakawa, M.; Kobayashi, Y.; Hamada, M.; Igarashi, M.; Adachi, H.; Nishimura,

- Y.; Akamatsu, Y. Amycolamicin: A Novel Broad-Spectrum Antibiotic Inhibiting Bacterial Topoisomerase. *Chem.–Eur. J.* **2012**, *18*, 15772–15781.
- (7) Basarab, G. S.; Hill, P. J.; Garner, C. E.; Hull, K.; Green, O.; Sherer, B. A.; Dangel, P. B.; Manchester, J. I.; Bist, S.; Hauck, S.; Zhou, F.; Uria-Nickelsen, M.; Illingworth, R.; Alm, R.; Rooney, M.; Eakin, A. E. Optimization of Pyrrolamide Topoisomerase II Inhibitors Toward Identification of an Antibacterial Clinical Candidate (AZD5099). *J. Med. Chem.* **2014**, *57*, 6060–6082.
- (8) Vandyck, K.; HACHE, G. Y. P.; Last, S. J.; Gowan, D. C. M.; Rombouts, G.; Verschuere, W. G.; Raboisson, P. J.-M. B. Sulphamoylpyrrolamide Derivatives and the Use Thereof as Medicaments for the Treatment of Hepatitis b. WO2014184350A1 November 20, 2014.
- (9) Gjorgjieva, M.; Tomašič, T.; Kikelj, D.; Mašič, L. P. Benzothiazole-Based Compounds in Antibacterial Drug Discovery. *Curr. Med. Chem.* **2019**, *25*, 5218–5236.
- (10) Zidar, N.; Macut, H.; Tomašič, T.; Mašič, L. P.; Ilaš, J.; Zega, A.; Tammela, P.; Kikelj, D. New N-Phenyl-4,5-Dibromopyrrolamides as DNA Gyrase B Inhibitors. *MedChemComm* **2019**, *10*, 1007–1017.
- (11) Tiz, D. B.; Skok, Ž.; Durcik, M.; Tomašič, T.; Mašič, L. P.; Ilaš, J.; Zega, A.; Draskovits, G.; Révész, T.; Nyerges, A.; Pál, C.; Cruz, C. D.; Tammela, P.; Žigon, D.; Kikelj, D.; Zidar, N. An Optimised Series of Substituted N-Phenylpyrrolamides as DNA Gyrase B Inhibitors. *Eur. J. Med. Chem.* **2019**, *167*, 269–290.
- (12) Lamut, A.; Cruz, C. D.; Skok, Ž.; Barančoková, M.; Zidar, N.; Zega, A.; Mašič, L. P.; Ilaš, J.; Tammela, P.; Kikelj, D.; Tomašič, T. Design, Synthesis and Biological Evaluation of Novel DNA Gyrase Inhibitors and Their Siderophore Mimic Conjugates. *Bioorg. Chem.* **2020**, *95*, No. 103550.
- (13) Lamut, A.; Skok, Ž.; Barančoková, M.; Gutierrez, L. J.; Cruz, C. D.; Tammela, P.; Draskovits, G.; Szili, P. É.; Nyerges, A.; Pál, C.; Molek, P.; Bratkovič, T.; Ilaš, J.; Zidar, N.; Zega, A.; Enriz, R. D.; Kikelj, D.; Tomašič, T. Second-Generation 4,5,6,7-Tetrahydrobenzo[d]Thiazoles as Novel DNA Gyrase Inhibitors. *Future Med. Chem.* **2020**, *12*, 277–297.
- (14) Jakopin, Ž.; Ilaš, J.; Barančoková, M.; Brvar, M.; Tammela, P.; Sollner Dolenc, M.; Tomašič, T.; Kikelj, D. Discovery of Substituted Oxadiazoles as a Novel Scaffold for DNA Gyrase Inhibitors. *Eur. J. Med. Chem.* **2017**, *130*, 171–184.
- (15) Cotman, A. E.; Trampuž, M.; Brvar, M.; Kikelj, D.; Ilaš, J.; Peterlin-Mašič, L.; Montalvão, S.; Tammela, P.; Frlan, R. Design, Synthesis, and Evaluation of Novel Tyrosine-Based DNA Gyrase B Inhibitors. *Arch. Pharm.* **2017**, *350*, No. 1700087.
- (16) Nyerges, A.; Tomašič, T.; Durcik, M.; Revesz, T.; Szili, P.; Draskovits, G.; Bogar, F.; Skok, Ž.; Zidar, N.; Ilaš, J.; Zega, A.; Kikelj, D.; Daruka, L.; Kintses, B.; Vasarhelyi, B.; Foldesi, I.; Kata, D.; Welin, M.; Kimbung, R.; Focht, D.; Mašič, L. P.; Pal, C. Rational Design of Balanced Dual-Targeting Antibiotics with Limited Resistance. *PLoS Biol.* **2020**, *18*, No. e3000819.
- (17) Sherer, B. A.; Hull, K.; Green, O.; Basarab, G.; Hauck, S.; Hill, P.; Loch, J. T.; Mullen, G.; Bist, S.; Bryant, J.; Boriack-Sjodin, A.; Read, J.; DeGrace, N.; Uria-Nickelsen, M.; Illingworth, R. N.; Eakin, A. E. Pyrrolamide DNA Gyrase Inhibitors: Optimization of Antibacterial Activity and Efficacy. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 7416–7420.
- (18) Basarab, G. S.; Hill, P.; Sherer, B.; Zhou, F. 2-(Piperidin-1-Yl)-4-Heterocyclyl-Thiazole-5-Carboxylic Acid Derivatives against Bacterial Infections. WO2010067123A1 June 17, 2010.
- (19) Worrell, B. T.; Hein, J. E.; Fokin, V. V. Halogen Exchange (Halex) Reaction of 5-Iodo-1,2,3-Triazoles: Synthesis and Applications of 5-Fluorotriazoles. *Angew. Chem., Int. Ed.* **2012**, *51*, 11791–11794.
- (20) Heeran, D.; Sandford, G. Fluorination of Pyrrole Derivatives by Selectfluor. *Tetrahedron* **2016**, *72*, 2456–2463.
- (21) Zhang, Y.; Shibatomi, K.; Yamamoto, H. Lewis Acid Catalyzed Highly Selective Halogenation of Aromatic Compounds. *Synlett* **2005**, 2005, 2837–2842.
- (22) René, A.; Quilan, M.; Deng, Y.; Cheng, Y.; Teleha, C. A.; Raboisson, P.; Bonfanti, J.-F.; Fortin, J.; Charette, André. B.; Pannecoucke, X.; Poisson, T.; Jubault, P. Practical Synthesis of Ethyl 3-Fluoro-1-Pyrrole-2-Carboxylate: A Key Fragment of a Potent Drug Candidate against Hepatitis B Virus. *Org. Process Res. Dev.* **2020**, *24*, 792–801.
- (23) Smith, K. M.; Minnetian, O. M. Novel Porphyrins from Copper(II)-Mediated Cyclizations of 1',8'-Dimethyl-A,C-Biladiene Salts: Mechanism of the Cyclization Reaction. *J. Org. Chem.* **1985**, *50*, 2073–2080.
- (24) Carey, F. A.; Sundberg, R. J. *Advanced Organic Chemistry: Part B: Reaction and Synthesis*; 5th ed.; Springer: US, 2007.
- (25) Yamamura, S.; Toda, M.; Hirata, Y. Modified Clemmensen Reduction: Cholestane. *Org. Synth.* **2003**, *53*, 86.
- (26) Boardman, A.; Small, R. W. H.; Worrall, I. J. Structure of Catena-Dichloro- μ -(1,4-Dioxane-O,O')-(1,4-Dioxane)Zinc(II), [Zn-(C₄H₈O₂)₂Cl₂]. *Acta Crystallogr., Sect. C: Cryst. Struct. Commun.* **1983**, *39*, 1005–1007.
- (27) Brewster, J. H. Reductions at Metal Surfaces. II. A Mechanism for the Clemmensen Reduction. *J. Am. Chem. Soc.* **1954**, *76*, 6364–6368.
- (28) Staudinger, H.; Meyer, J. Ü. New Organic Compounds of Phosphorus. III. Phosphine-Methylene Derivatives and Phosphinimines. *Helv. Chim. Acta* **1919**, *2*, 635–646.
- (29) Durcik, M.; Toplak, Ž.; Zidar, N.; Ilaš, J.; Zega, A.; Kikelj, D.; Mašič, L. P.; Tomašič, T. Efficient Synthesis of Hydroxy-Substituted 2-Aminobenzo[d]Thiazole-6-Carboxylic Acid Derivatives as New Building Blocks in Drug Discovery. *ACS Omega* **2020**, *5*, 8305–8311.
- (30) Zidar, N.; Tomašič, T.; Macut, H.; Sirc, A.; Brvar, M.; Montalvão, S.; Tammela, P.; Ilaš, J.; Kikelj, D. New N-Phenyl-4,5-Dibromopyrrolamides and N-Phenylindolamides as ATPase Inhibitors of DNA Gyrase. *Eur. J. Med. Chem.* **2016**, *117*, 197–211.