Synthesis C. Steinebach et al. PSP

# A Facile Synthesis of Ligands for the von Hippel–Lindau E3 Ligase

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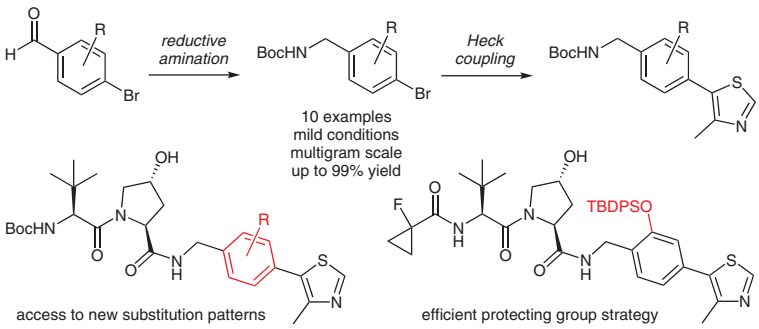


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**Abstract** The proteolysis-targeting chimeras (PROTACs) have become an integral part of different stages of drug discovery. This growing field, therefore, benefits from advancements in all segments of the design of these compounds. Herein, an efficient and optimized synthetic protocol to various von Hippel-Lindau (VHL) ligands is presented, which enables easy access to multigram quantities of these essential PROTAC building blocks. Moreover, the elaborated synthesis represents a straightforward approach to further explore the chemical space of VHL ligands.

**Key words** PROTACs, protein degradation, von Hippel–Lindau, VHL, E3 ligase, reductive amination, protecting group

## Introduction

Proteolysis-targeting chimeras (PROTACs) have received much attention in drug discovery processes in recent years. These bifunctional molecules consist of a target binding unit, a linker, and an E3 ligase binding moiety. The chemically-mediated orchestration of these tripartite binding events ultimately leads to ubiquitination and proteasomal degradation of target proteins by using the cell's ubiquitin-proteasome system.

E3 ubiquitin ligases such as Cereblon (CRBN) and von Hippel–Lindau (VHL) play a central role in this new technique.[1] In addition to the tremendous success in academic PROTAC research,[2] this novel paradigm is also finding its way into clinical and industrial applications.[3] As shown by the example of CRBN, the development of efficient protocols for the synthesis of ligase ligands is of particular importance for further PROTAC advancements.[4] This work aims at the development of a robust and practical method for multigram synthesis of VHL ligands, which is also potentially relevant for industrial applications. Since structural diversity is an essential aspect in the development of new VHL ligands,[5] the development of novel synthetic routes can provide access to advanced chemical entities.

Two access points have been successfully established for the connection of linkers to a VHL ligand.[6] On the one hand, in several prototypical PROTACs, the amino group of ligand VH032 (**1**, Figure 1) was used, and an amide moiety was generated between the linker and the ligand. On the other hand, the central phenolic group in **2** was utilized for linker attachment via an ether bridge. Compound **2** represents an exemplary member of this chemotype of VHL ligands. Affinity to VHL is maintained when the cyano group of **2** is replaced by fluorine or when a 3-methyl-2-(1-oxoisoindolin-2-yl)butanoyl residue is attached to the hydroxyproline (Hyp) nitrogen.[5b,6b]

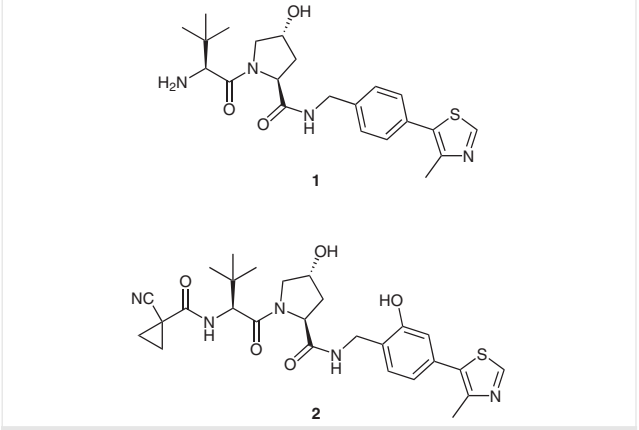


**Figure 1** Selected building blocks for VHL-based PROTACs

Whereas the initially published synthetic route towards VHL ligand **1** started from 4-bromobenzonitrile,[7] recent studies made use of commercially available 4-bromobenzylamine.[5g]

 
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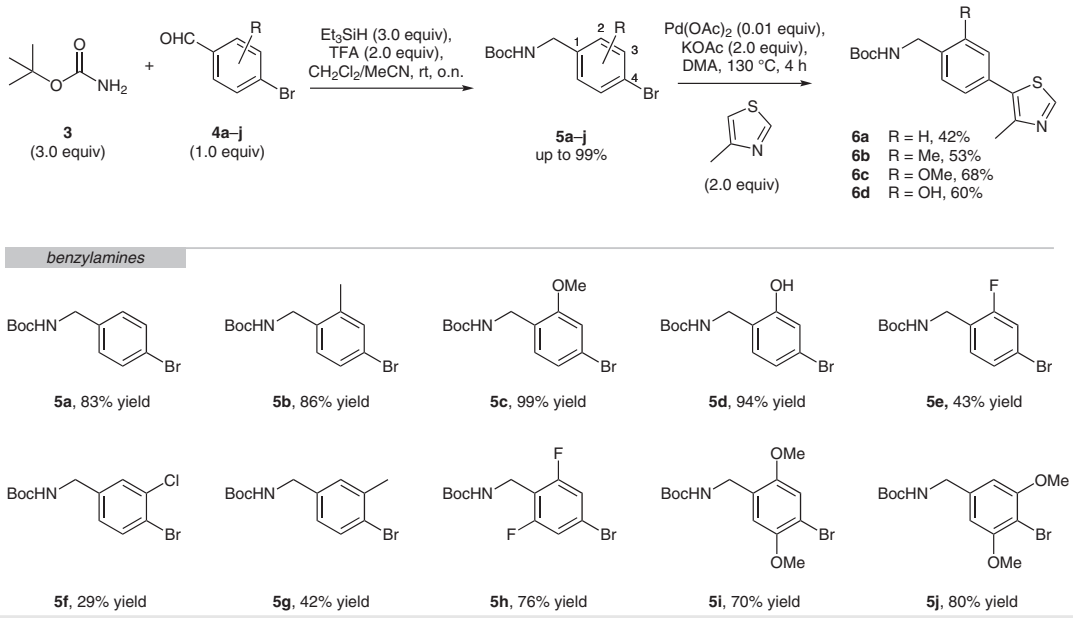
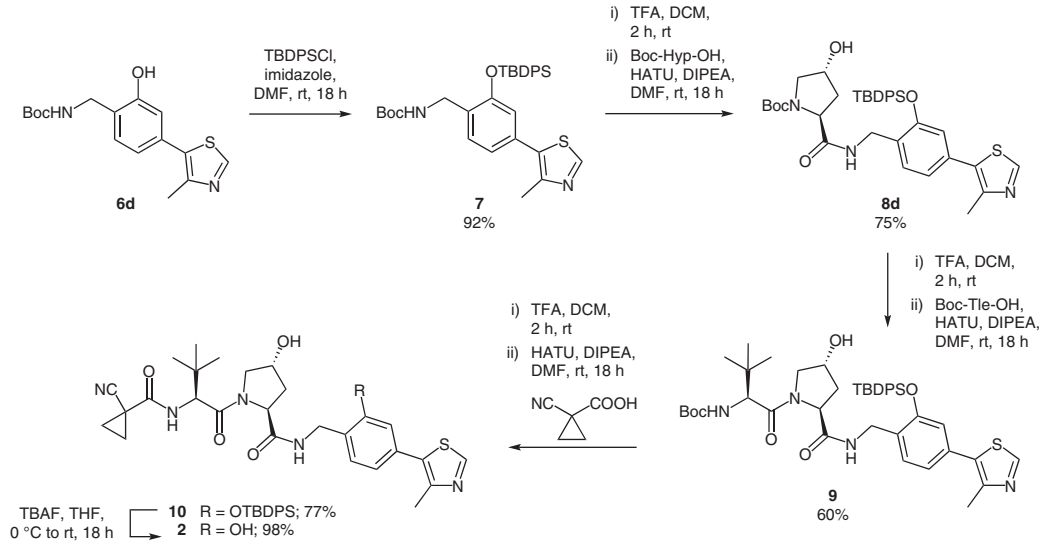



**Scheme 1** Synthesis of Boc-protected 4-bromobenzylamines and their use as building blocks for new VHL ligand

In this study, we sought for an easy access to multigram quantities of such benzylamine building blocks useful for the synthesis of established and novel ligands for the E3 ligase VHL, which is a crucial player in the PROTAC field.

As the key step of the newly envisaged strategy, *tert*-butyl carbamate (**3**) was reacted with readily available 4-bromobenzaldehydes **4a–j**. The corresponding reductive amination reactions utilized **3** as the nitrogen and triethylsilane as the hydrogen source.[8] These reactions proceeded under very mild conditions and allowed us to establish a scalable synthetic protocol for the assembly of protected intermediates **5a–j** with attractive substitution patterns (Scheme 1). These include 2-substituted derivatives **5b–e**, 3-substituted derivatives **5f** and **5g**, and highly functionalized arenes such as **5h–j**. The Boc-amino group was compatible with the subsequent Heck coupling, as exemplified by the further conversions to give the biaryl building blocks **6**. Our method proceeded with fair to excellent yields in both steps. The facile access to VHL building blocks **6a–c** allowed us to assemble known and to contrive advanced VHL ligands of




**Scheme 2** New protecting group strategy for the synthesis of phenolic VHL ligands

chemotype **1**. The Boc-amino groups were deprotected quantitatively and employed in the peptide syntheses of VHL ligands (see Supporting Information).

**Scope and Limitations**

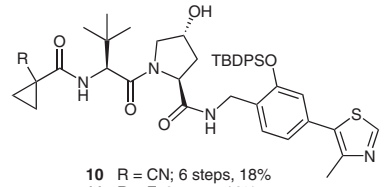
Derivative **6d**, containing a hydroxyl group in position 2, represents an important precursor for the synthesis of phenolic VHL ligands of chemotype **2**. Compound **6d** was synthesized in high yield starting from 4-bromosalicylic aldehyde (**4d**). In the subsequent three amide couplings, we paid attention to a reported side reaction, which occurs as an undesired acylation of the phenolic moiety.[6a,9,10] To circumvent such diacylated side products, we introduced a protecting group, which is stable under a variety of conditions and yet labile for deprotection. Accordingly, **6d** was converted with *tert*-butyldiphenylsilyl chloride (TBDPSCl) into the silyl ether **7** (Scheme 2). After conducting two HATU-promoted amide coupling reactions, the orthogonally protected intermediate **9** was obtained. This compound can be employed in the design of PROTACs using multiple exit vectors.[2f] By introducing the 1-cyanocyclopropyl capping group, the TBDPS-protected precursor **10** was obtained. The silyl protecting group is known to be readily cleavable with fluorine reagents, such as tetrabutylammonium fluoride (TBAF),[11] which we applied with THF as a solvent to obtain the desired VHL ligand **2**. Although including two more steps, this new route is an improvement since it avoids multiple side reactions, and good to excellent yields were achieved throughout the synthesis.

Besides **10**, the protected analogue **11** bearing a fluorine substituent in place of the cyano group was prepared accordingly (Figure 2), which represents a further important precursor for the assembly of VHL-based PROTACs.[6b] Moreover, the isoindolinyl derivative **12** and its non-VHL-binding stereoisomer **13** (Figure 2), which were previously used as precursors for HaloPROTACs,[12] that is, small molecules to induce the degradation of HaloTag fusion proteins, were prepared using this optimized route.

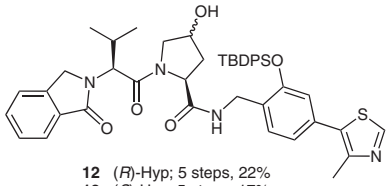
The key entry steps towards VHL ligands are the reductive amination and Heck coupling. They can be reproducibly performed on multigram scales. The synthetic route commenced with 4-halobenzaldehydes, which are commercially available or easily accessible.[14] This allows for the incorporation of further building blocks, to ultimately generate new VHL ligands such as **15** and **16** (Figure 2) and establish novel insights into structure-activity relationships concerning structural diversity at the phenyl part of VHL ligands.

Further investigations are needed to overcome one limitation of the reported process, that is, the lacking opportunity to introduce a substituent at the benzylic methylene position. Such modifications are attractive in the light of the reported increased affinity to VHL.[15]

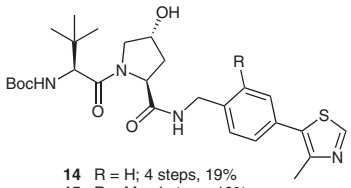
In summary, we have developed a highly efficient protocol for the robust synthesis of multigram quantities of VHL ligands. Several precursors for valuable VHL ligands were successfully prepared through a synthetic route comprising clear advantages over previously reported procedures. In addition to the ease of synthesis and purification, the synthetic strategy can easily be accommodated for a broad scope of new tailored VHL ligands.




**10** R = CN; 6 steps, 18%
**11** R = F; 6 steps, 16%




**12** (*R*)-Hyp; 5 steps, 22%
**13** (*S*)-Hyp; 5 steps, 17%




**14** R = H; 4 steps, 19%
**15** R = Me; 4 steps, 10%
**16** R = OMe; 4 steps, 8%

**Figure 2** Synthesized VHL ligands via the new reaction sequences

The structures of the newly synthesized compounds were in accordance with analytical data (see Supporting Information). Finally, to unambiguously confirm one of our syntheses, we subjected **14** (Figure 2) to an X-ray diffraction analysis (Figure 3). As expected, *tert*-leucine and hydroxyproline are connected via a *trans* peptide bond as indicated by the omega torsion angle of 179.8°. The planes of the phenyl and methylthiazole part are twisted with respect to each other by 29°.

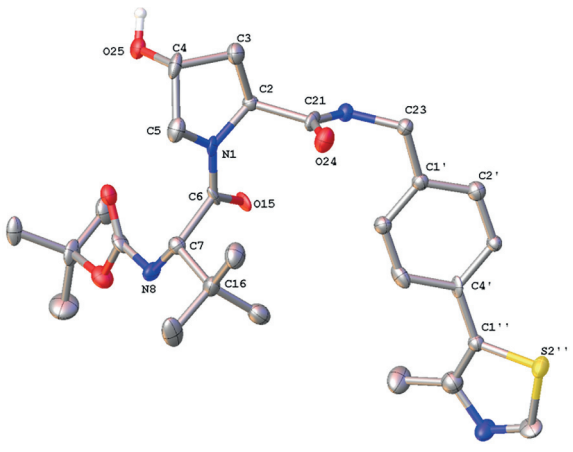



**Figure 3** Molecular plot of the Boc-protected VHL ligand **14** showing the 2*S*,4*R*-stereochemistry of hydroxyproline[13]

Preparative column chromatography was performed using Merck silica gel 60 (63–200 mesh) or using an automated flash chromatography system CombiFlash Rf 200. Petroleum ether (PE) used was a mixture of alkanes boiling between 40–60 °C. Melting points were determined on a Büchi 510 oil bath apparatus or on a Reichelt hot-stage apparatus and are uncorrected. $^1$H NMR and $^{13}$C NMR spectra were recorded on a Bruker Avance 400 MHz NMR spectrometer, Bruker Avance 500 MHz NMR spectrometer or on a Bruker Avance III 600 MHz NMR spectrometer, respectively. NMR spectra were processed and analyzed in MestReNova. Chemical shifts are given in parts per million (ppm), coupling constants *J* are given in hertz (Hz), and standard abbreviations are used to indicate spin multiplicities. All multiplets related with $J_{C,F}$ couplings in $^{13}$C NMR spectra are centered. In case of overlapping extraneous solvent peaks, multiplet analyses in $^1$H NMR spectra were performed using qGSD (quantitative Global Spectral Deconvolution). In the case of rotamers, only the peaks for the major rotamer are given, resonance assignments were made based on one- and two-dimensional NMR techniques, which include $^1$H, $^{13}$C, DEPT, HSQC, and HMBC experiments. HRMS was recorded on a micrOTOF-Q mass spectrometer (Bruker) with ESI-source coupled with an HPLC Dionex UltiMate 3000 (Thermo Scientific). The purity and identity of the compounds were determined by HPLC-UV obtained on an LC-MS instrument (Applied Biosystems API 2000 LC/MS/MS, HPLC Agilent 1100) or separately on an LC instrument (Acquity UPLC) and mass spectrometer (Thermo Scientific Q Exactive Plus). The purity of all the final compounds was confirmed to be ≥95% purity by LC.

### Reductive Amination with Hydrosilanes; General Procedure I

*tert*-Butyl carbamate (**3**; 17.57 g, 150 mmol) and the corresponding benzaldehyde **4** (50 mmol) were dissolved in $CH_2Cl_2$ (100 mL) and MeCN (300 mL). $Et_3SiH$ (17.44 g, 23.96 mL, 150 mmol) was slowly added followed by the dropwise addition of trifluoroacetic acid (11.40 g, 7.65 mL, 100 mmol). After stirring for 18 h at rt, the mixture was carefully quenched by the addition of sat. aq $NaHCO_3$ (100 mL) and the aqueous layer was extracted with $CH_2Cl_2$ (3 × 100 mL). The combined organic phases were washed with brine (100 mL), dried ($Na_2SO_4$), filtered, and concentrated in vacuo.

### Heck Coupling; General Procedure II

The corresponding bromoaryl compound **5** (25 mmol), $Pd(OAc)_2$ (56 mg, 0.25 mmol), and anhyd $K_2CO_3$ (4.91 g, 50 mmol) were dissolved in *N*,*N*-dimethylacetamide (25 mL). 4-Methylthiazole (4.96 g, 4.55 mL, 50 mmol) was added, and the solution was heated to 130 °C under an argon atmosphere for 4 h. Subsequently, the mixture was cooled to rt, diluted with $H_2O$ (100 mL), and extracted with $CH_2Cl_2$ (3 × 100 mL). The combined organic layers were washed with brine (100 mL), dried ($Na_2SO_4$), filtered, and concentrated in vacuo.

### TBDPS-Protection; General Procedure III

The corresponding phenol **6d** (15 mmol) was dissolved in anhyd DMF (30 mL), and imidazole (2.04 g, 30 mmol) was added. Subsequently, *tert*-butyldiphenylsilyl chloride (4.33 g, 4.10 mL, 15.75 mmol) was added dropwise. The mixture was allowed to stir at rt for 18 h, after which it was quenched by the addition of EtOH (1 mL). $H_2O$ (150 mL) was added, and the mixture was extracted with $CH_2Cl_2$ (2 × 150 mL). The combined organic layers were washed with brine (150 mL), dried ($Na_2SO_4$), filtered, and concentrated in vacuo.

### Boc Deprotection and HATU Coupling;[2e] General Procedure IV

The corresponding Boc-protected amine (1 equiv) was dissolved in anhyd $CH_2Cl_2$ (5 mL/mmol), and TFA (5 mL/mmol) was added. The mixture was stirred at rt for 2 h. After removal of the volatiles, the oily residue was further dried under high vacuum. The crude deprotected amine was dissolved in anhyd DMF (5 mL/mmol), and the appropriate acid (1 equiv) was added. While stirring the solution, DIPEA (4 equiv) was added, followed by the addition of HATU (1.1 equiv) after 5 min. The mixture was stirred at rt for 1 h, after which $H_2O$ (50 mL/mmol) was added, and extracted with EtOAc (3 × 25 mL/mmol). The combined organic phases were washed with brine (50 mL/mmol), dried ($Na_2SO_4$), filtered, and concentrated in vacuo.

### (2*S*,4*R*)-4-Hydroxy-*N*-{[2-hydroxy-4-(4-methylthiazol-5-yl)phenyl]methyl}-1-{(2*S*)-2-[(1-isocyanocyclopropanecarbonyl)amino]-3,3-dimethylbutanoyl}pyrrolidine-2-carboxamide (2)

[CAS Reg. No. 2244684-43-1]

Silyl ether **10** (0.78 g, 1.0 mmol) was dissolved in anhyd THF (10 mL) and cooled to 0 °C. TBAF (1 M in THF, 3 mL) was added and the mixture was stirred for 18 h at rt. It was quenched by the addition of sat. aq $NH_4Cl$ (100 mL) and extracted with $CH_2Cl_2$ (3 × 50 mL). The combined organic layers were washed with aq 1 N HCl and brine (each 100 mL), dried ($Na_2SO_4$), filtered, and concentrated in vacuo. The crude product was purified by column chromatography ($CH_2Cl_2$/MeOH 19:1) to obtain a colorless solid; yield: 0.53 g (98%); mp 128–130 °C (lit. mp: no report found); $R_f$ = 0.38 ($CH_2Cl_2$/MeOH 9:1).

$^1$H NMR (600 MHz, DMSO-$d_6$): δ = 0.94 [s, 9 H, C($CH_3$)$_3$], 1.45–1.52 (m, 2 H), 1.57–1.65 (m, 2 H, 2‴-H), 1.87–1.93 (m, 1 H), 2.03–2.09 (m, 1 H, 3-H), 2.43 (s, 3 H, $CH_3$), 3.55 (d, *J* = 10.8 Hz, 1 H), 3.60–3.65 (m, 1 H, 5-H), 4.15–4.27 (m, 2 H), 4.31–4.37 (m, 1 H), 4.47–4.53 (m, 2 H, 2-H, 4-H, NHC*H*, NHC*H*$_2$), 5.13 (d, *J* = 3.6 Hz, 1 H, OH), 6.82 (d, *J* = 7.8 Hz, 1 H), 6.87–6.93 (m, 1 H), 7.28–7.36 (m, 2 H, ArH, CONH), 8.49 (t, *J* = 6.0 Hz, 1 H, CONH), 8.94 (s, 1 H, 2″-H), 9.78 (s, 1 H, ArO*H*).

$^{13}$C NMR (151 MHz, DMSO-$d_6$): δ = 13.90 (C-1‴), 16.22 ($CH_3$), 16.77, 16.95 (C-2‴), 26.25 [C(*C*H$_3$)$_3$], 36.39 [*C*(CH$_3$)$_3$], 37.48 (C-3), 38.02 (NHCH$_2$), 56.78, 57.52, 58.93 (C-2, C-5, NHCH), 69.05 (C-4), 115.19 (C-3′), 119.49 (C-5′), 120.28 (CN), 125.39 (C-1′), 128.71 (C-6′), 130.83, 131.47 (C-4′, C-5″), 147.63 (C-4″), 151.40 (C-2″), 154.97 (C-2′), 164.59, 168.87, 172.07 (C=O).

LC-MS (ESI): *m*/*z* [M + H]$^+$ calcd for $C_{27}H_{33}N_5O_5S$: 540.22; found: 540.3.

HRMS (ESI): *m*/*z* [M + H]$^+$ calcd for $C_{27}H_{33}N_5O_5S$: 540.2236; found: 540.2264.

### *tert*-Butyl *N*-[(4-Bromophenyl)methyl]carbamate (5a)

[CAS Reg. No. 68819-84-1]

This compound was prepared using General Procedure I and 4-bromobenzaldehyde (**4a**; 9.25 g). The crude product was purified by column chromatography (PE/EtOAc 10:1) to obtain a colorless solid; yield: 11.88 g (83%); mp 86–88 °C (Lit.[16] mp 86–88 °C); $R_f$ = 0.39 (PE/EtOAc 10:1).

$^1$H NMR (600 MHz, DMSO-$d_6$): δ = 1.37 [s, 9 H, C($CH_3$)$_3$], 4.07 (d, *J* = 6.2 Hz, 2 H, $CH_2$), 7.13–7.22 (m, 2 H, ArH), 7.39 (t, *J* = 6.3 Hz, 1 H, NH), 7.45–7.54 (m, 2 H, ArH).

$^{13}$C NMR (151 MHz, DMSO-$d_6$): δ = 28.39 [C(*C*H$_3$)$_3$)], 42.96 (NHCH$_2$), 78.08 [*C*(CH$_3$)$_3$], 119.79 (C-4), 129.34, 131.25 (C-2, C-3), 139.85 (C-1), 155.95 (C=O).

LC-MS (ESI) (90% $H_2O$ to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 200–400 nm): $t_R$ = 11.34 min, 99% purity; *m*/*z* [M + H]$^+$ calcd for $C_{12}H_{16}{}^{81}BrNO_2$: 286.04; found. 285.9.

HRMS (ESI): *m*/*z* [M + H]$^+$ calcd for $C_{12}H_{16}{}^{81}BrNO_2$: 286.0437; found: 286.0430.

### *tert*-Butyl *N*-{[4-(4-Methylthiazol-5-yl)phenyl]methyl}carbamate (6a)

[CAS Reg. No. 2308507-34-6]

This compound was prepared using General Procedure II and compound **5a** (7.15 g). The crude product was purified by column chromatography (gradient of PE/EtOAc 10:1 to 2:1) to obtain a colorless solid; yield: 3.20 g (42%); mp 112–114 °C (lit. mp: no report found); $R_f$ = 0.50 (PE/EtOAc 1:1).

$^1$H NMR (500 MHz, DMSO-$d_6$): δ = 1.39 [s, 9 H, C($CH_3$)$_3$], 2.44 (s, 3 H, $CH_3$), 4.16 (d, *J* = 6.3 Hz, 2 H, $CH_2$), 7.29–7.35 (m, 2 H, ArH), 7.40 (t, *J* = 6.2 Hz, 1 H, NH), 7.41–7.46 (m, 2 H, ArH), 8.96 (s, 1 H, 2′-H).

$^{13}$C NMR (126 MHz, DMSO-$d_6$): δ = 16.08 ($CH_3$), 28.39 [C($CH_3$)$_3$], 43.20 ($CH_2$), 78.00 [$C$($CH_3$)$_3$], 127.58, 128.97 (C-2, C-3), 129.96, 131.24 (C-1, C-5′), 140.24 (C-4), 147.94 (C-4′), 151.54 (C-2′), 155.96 (C=O).

LC-MS (ESI) (90% $H_2O$ to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 220–400 nm): $t_R$ = 10.85 min, 96% purity; *m*/*z* [M + H]$^+$ calcd for $C_{16}H_{20}N_2O_2S$: 305.13; found: 304.9.

HRMS (ESI): *m*/*z* [M + H]$^+$ calcd for $C_{16}H_{20}N_2O_2S$: 305.1318; found: 305.1311.

### *tert*-Butyl *N*-[(4-Bromo-2-hydroxyphenyl)methyl]carbamate (5d)

[CAS Reg. No. 1402664-46-3]

This compound was prepared using General Procedure I and 4-bromo-2-hydroxybenzaldehyde (**4d**; 10.05 g). The crude product was purified by column chromatography (gradient of PE/EtOAc 8:1 to 6:1) to obtain a colorless solid; yield: 14.20 g (94%); mp 110–112 °C (lit. mp: no report found); $R_f$ = 0.28 (PE/EtOAc 8:1).

$^1$H NMR (600 MHz, DMSO-$d_6$): δ = 1.38 [s, 9 H, C($CH_3$)$_3$], 4.01 (d, *J* = 6.1 Hz, 2 H, $CH_2$), 6.91–6.96 (m, 2 H), 7.00 (d, *J* = 8.3 Hz, 1 H, 3-H, 5-H, 6-H), 7.16 (t, *J* = 6.1 Hz, 1 H, NH), 9.94 (br s, 1 H, OH).

$^{13}$C NMR (151 MHz, DMSO-$d_6$): δ = 28.40 [C($CH_3$)$_3$], 38.25 ($CH_2$), 78.03 [$C$($CH_3$)$_3$], 117.48, 119.61, 121.64, 125.87, 129.39 (C-1, C-3, C-4, C-5, C-6), 155.84, 156.06 (C-2, C=O).

LC-MS (ESI): (90% $H_2O$ to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 220–400 nm): $t_R$ = 11.01 min, 99% purity; *m*/*z* [M + H]$^+$ calcd for $C_{12}H_{16}{}^{81}BrNO_3$: 304.04; found: 303.9.

HRMS (ESI): *m*/*z* [M + H]$^+$ calcd for $C_{12}H_{16}{}^{79}BrNO_3$: 302.0386; found: 302.0366.

### *tert*-Butyl *N*-{[2-Hydroxy-4-(4-methylthiazol-5-yl)phenyl]methyl}carbamate (6d)

[CAS Reg. No. 2086300-37-8]

This compound was prepared using General Procedure II and compound **5d** (7.55 g). The crude product was purified by column chromatography (gradient of PE/EtOAc 2:1 to 1:1) to obtain a colorless solid; yield: 4.81 g (60%); mp 142–144 °C (lit. mp: no report found); $R_f$ = 0.36 (PE/EtOAc 1:1).

$^1$H NMR (600 MHz, DMSO-$d_6$): δ = 1.39 [s, 9 H, C($CH_3$)$_3$], 2.44 (s, 3 H, $CH_3$), 4.10 (d, *J* = 6.1 Hz, 2 H, $CH_2$), 6.93–6.87 (m, 2 H), 7.24–7.12 (m, 2 H, ArH, NH), 8.94 (s, 1 H, 2″-H), 9.72 (s, 1 H, OH).

$^{13}$C NMR (151 MHz, DMSO-$d_6$): δ = 16.07 ($CH_3$), 28.24 [C($CH_3$)$_3$], 38.26 ($CH_2$), 77.81 [$C$($CH_3$)$_3$], 115.03 (C-3′), 119.42 (C-5″), 125.98, 127.96, 130.59, 131.25 (ArC), 147.46 (C-4″), 151.16 (C-2″), 154.66 (C-2′), 155.94 (C=O).

LC-MS (ESI): (90% $H_2O$ to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 200–400 nm): $t_R$ = 10.68 min, 98% purity; *m*/*z* [M + H]$^+$ calcd for $C_{16}H_{20}N_2O_3S$: 321.12; found: 320.9.

HRMS (ESI): *m*/*z* [M + H]$^+$ calcd for $C_{16}H_{20}N_2O_3S$: 321.1228; found: 321.1285.

### *tert*-Butyl *N*-({2-[*tert*-Butyl(diphenyl)silyl]oxy-4-(4-methylthiazol-5-yl)phenyl}methyl)carbamate (7)

This compound was prepared using General Procedure III and compound **6d** (4.81 g). The crude product was purified by column chromatography (gradient of PE/EtOAc 4:1 to 2:1) to obtain a colorless solid; yield: 7.71 g (92%); mp 110–112 °C; $R_f$ = 0.28 (PE/EtOAc 8:1).

$^1$H NMR (600 MHz, DMSO-$d_6$): δ = 1.06 [s, 9 H, SiC($CH_3$)$_3$], 1.44 [s, 9 H, OC($CH_3$)$_3$], 1.82 (s, 3 H, $CH_3$), 4.41 (d, *J* = 6.2 Hz, 2 H, $CH_2$), 6.38 (s, 1 H, NH), 6.98 (dd, *J* = 1.9, 8.0 Hz, 1 H), 7.26 (d, *J* = 7.9 Hz, 1 H), 7.35 (t, *J* = 6.0 Hz, 1 H), 7.52–7.41 (m, 6 H), 7.73–7.68 (m, 4 H, ArH, SiArH), 8.79 (s, 1 H, 2″-H).

$^{13}$C NMR (151 MHz, DMSO-$d_6$): δ = 15.34 ($CH_3$), 18.94 [Si$C$($CH_3$)$_3$], 26.21 [SiC($CH_3$)$_3$)], 28.27 [OC($CH_3$)$_3$)], 38.70 ($CH_2$), 77.92 [O$C$($CH_3$)$_3$], 117.97 (C-3′), 121.66 (C-5″), 128.00, 128.19, 129.43, 130.12, 130.37, 130.52, 131.44, 134.95 (ArC), 147.36 (C-4″), 151.39 (C-2″), 152.20 (C-2′), 155.87 (C=O).

LC-MS (ESI) (90% $H_2O$ to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 200-400 nm): $t_R$ = 13.28 min, 99% purity; *m*/*z* [M + H]$^+$ calcd for $C_{32}H_{38}N_2O_3SSi$: 559.24; found: 559.1.

HRMS (ESI): *m*/*z* [M + H]$^+$ calcd for $C_{32}H_{38}N_2O_3SSi$: 559.2456; found: 559.2435.

### *tert*-Butyl (2*S*,4*R*)-2-({2-[*tert*-Butyl(diphenyl)silyl]oxy-4-(4-methylthiazol-5-yl)phenyl}methylcarbamoyl)-4-hydroxypyrrolidine-1-carboxylate (8d)

This compound was prepared using General Procedure IV, compound **7** (5.59 g, 10 mmol), and Boc-Hyp-OH (2.31 g, 10 mmol). The crude product was purified by column chromatography (gradient of PE/EtOAc 1:1 to EtOAc) to obtain a colorless solid; yield: 5.06 g (75%); mp 98–100 °C; $R_f$ = 0.46 (EtOAc).

$^1$H NMR (600 MHz, DMSO-$d_6$): δ (major rotamer) = 1.06 [s, 9 H, SiC($CH_3$)$_3$], 1.35 [s, 9 H, OC($CH_3$)$_3$], 1.84 (s, 3 H, $CH_3$), 1.99–1.90 (m, 1 H), 2.16–2.07 (m, 1 H, 3-H), 3.34 (m, 1 H), 3.51–3.40 (m, 1 H, 2-H, 4-H), 4.25–4.34 (m, 2 H, $CH_2$), 4.41 (dd, *J* = 5.4, 16.0 Hz, 1 H, 5-H), 4.65 (dd, *J* = 6.2, 16.0 Hz, 1 H, 5-H), 5.00–5.05 (m, 1 H, OH), 6.39 (dd, *J* = 1.7, 8.9 Hz, 1 H), 6.96 (d, *J* = 7.8 Hz, 1 H), 7.27 (d, *J* = 7.9 Hz, 1 H), 7.52–7.42 (m, 6 H), 7.71 (t, *J* = 6.4 Hz, 4 H, ArH, SiArH), 8.45–8.38 (m, 1 H, CONH), 8.80 (s, 1 H, 2″-H).

$^{13}$C NMR (151 MHz, DMSO-$d_6$): δ = 15.32 ($CH_3$), 18.96 [Si$C$($CH_3$)$_3$], 26.20 [SiC($CH_3$)$_3$], 27.95 [OC($CH_3$)$_3$], 37.30 (C-3), 38.66 ($NHCH_2$), 54.83, 58.97 (C-2, C-5), 67.88 (C-4), 78.59 [O$C$($CH_3$)$_3$], 118.10 (C-3′), 121.54 (C-5″), 128.19, 128.80, 130.38, 130.46, 131.42, 134.93 (ArC), 147.41 (C-4″), 151.44 (C-2″), 152.37 (C-2′), 153.57 (COO), 172.84 (CONH).

LC-MS (ESI) (90% $H_2O$ to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 200–400 nm): $t_R$ = 12.58 min, 99% purity; *m*/*z* [M + H]$^+$ calcd for $C_{37}H_{45}N_3O_5SSi$: 672.29; found: 672.4.

HRMS (ESI): *m*/*z* [M + H]$^+$ calcd for $C_{37}H_{45}N_3O_5SSi$: 672.2922; found: 672.2948.

**tert-Butyl *N*-{(1*S*)-1-[(2*S*,4*R*)-2-({2-[*tert*-butyl(diphenyl)silyl]oxy-4-(4-methylthiazol-5-yl)phenyl}methylcarbamoyl)-4-hydroxypyrrolidine-1-carbonyl]-2,2-dimethylpropyl}carbamate (9)**

This compound was prepared using General Procedure IV, compound **8d** (1.34 g, 2.0 mmol), and Boc-Tle-OH (0.46 g, 2.0 mmol). The crude product was purified by flash chromatography on silica gel (0% to 5% MeOH in $CH_2Cl_2$) to yield the title compound as a colorless solid; yield: 0.94 g (60%); mp 108–110 °C; $R_f$ = 0.43 ($CH_2Cl_2$/MeOH 9:1).

$^1$H NMR (600 MHz, DMSO-$d_6$): δ = 0.94 [s, 9 H, C($CH_3$)$_3$], 1.06 [s, 9 H, SiC($CH_3$)$_3$], 1.39 [s, 9 H, OC($CH_3$)$_3$], 1.84 (s, 3 H, $CH_3$), 1.94–2.01 (m, 1 H), 2.05–2.12 (m, 1 H, 3-H), 3.59–3.72 (m, 2 H, 5-H), 4.13–4.20 (m, 1 H), 4.36–4.46 (m, 2 H), 4.50–4.59 (m, 2 H, 2-H, 4-H, NHC*H*, NHC*H*$_2$), 5.15 (d, *J* = 2.5 Hz, 1 H, OH), 6.37 (d, *J* = 1.3 Hz, 1 H), 6.88 (dd, *J* = 1.7, 7.9 Hz, 1 H), 7.43–7.52 (m, 8 H), 7.69–7.73 (m, 4 H, ArH, CONH), 8.54 (t, *J* = 6.3 Hz, 1 H, CONH), 8.80 (s, 1 H, 2″-H).

$^{13}$C NMR (151 MHz, DMSO-$d_6$): δ = 15.35 ($CH_3$), 18.96 [SiC($CH_3$)$_3$], 26.25 [SiC(*C*H$_3$)$_3$, CHC(*C*H$_3$)$_3$], 28.18 [OC(*C*H$_3$)$_3$], 35.36 [CH*C*(CH$_3$)$_3$], 37.60 (C-3), 38.22 (NHCH$_2$), 56.36, 58.42, 58.83 (C-2, C-5, NHCH), 69.92 (C-4), 78.08 [OC(CH$_3$)$_3$], 117.76 (C-3′), 121.43 (C-5″), 128.21, 128.77, 130.01, 130.39, 131.44, 134.94 (C-Ar), 147.30 (C-4″), 151.38 (C-2″), 152.18 (C-2′), 155.35, 169.93, 172.17 (C=O).

LC-MS (ESI) (90% $H_2O$ to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 200–350 nm): $t_R$ = 12.82 min, 95% purity; *m*/*z* [M + H]$^+$calcd for $C_{43}H_{57}N_4O_6SSi$: 785.38; found: 785.5.

HRMS (ESI): *m*/*z* [M + H]$^+$ calcd for $C_{43}H_{57}N_4O_6SSi$: 785.3763; found: 785.3739.

**(2*S*,4*R*)-*N*-({2-[*tert*-Butyl(diphenyl)silyl]oxy-4-(4-methylthiazol-5-yl)phenyl}methyl)-1-{(2*S*)-2-[(1-cyanocyclopropanecarbonyl)-amino]-3,3-dimethylbutanoyl}-4-hydroxypyrrolidine-2-carboxamide (10)**

This compound was prepared using General Procedure IV, compound **9** (0.79 g, 1.0 mmol), and 1-cyano-1-cyclopropanecarboxylic acid (0.11 g, 1.0 mmol). The crude product was purified by flash chromatography on silica gel (0% to 5% MeOH in $CH_2Cl_2$) to afford the title compound as a slight yellow solid; yield: 0.60 g (77%); mp 112–114 °C; $R_f$ = 0.37 ($CH_2Cl_2$/MeOH 9:1).

$^1$H NMR (600 MHz, DMSO-$d_6$): δ = 0.97 [s, 9 H, C($CH_3$)$_3$], 1.07 [s, 9 H, SiC($CH_3$)$_3$], 1.45–1.56 (m, 2 H), 1.58–1.66 (m, 2 H, 2‴-H), 1.84 (s, 3 H, $CH_3$), 1.94–2.00 (m, 1 H), 2.09–2.15 (m, 1 H, 3-H), 3.59 (d, *J* = 11.0 Hz, 1 H), 3.64–3.68 (m, 1 H, 5-H), 4.27–4.40 (m, 1 H), 4.42–4.61 (m, 4 H, 2-H, 4-H, NHC*H*, NHC*H*$_2$), 5.17 (d, *J* = 3.7 Hz, 1 H, OH), 6.38 (d, *J* = 1.7 Hz, 1 H), 6.90 (dd, *J* = 1.7, 7.9 Hz, 1 H), 7.37–7.53 (m, 8 H), 7.69–7.73 (m, 4 H, ArH, CONH), 8.59 (t, *J* = 5.9 Hz, 1 H, CONH), 8.80 (s, 1 H, 2″-H).

$^{13}$C NMR (151 MHz, DMSO-$d_6$): δ = 13.72 (C-1‴), 15.33 ($CH_3$), 16.58, 16.77 (C-2‴), 18.96 [C($CH_3$)$_3$], 26.05 [*C*(CH$_3$)$_3$], 26.24 [C(*CH*$_3$)$_3$], 36.21 [*C*(CH$_3$)$_3$], 37.60 (C-3), 37.90 (NHCH$_2$), 56.65, 57.36, 58.87 (C-2, C-5, NHCH), 69.89 (C-4), 117.86 (C-3′), 120.10 (CN), 121.43 (C-5″), 128.22, 128.65, 130.12, 130.38, 130.52, 131.39, 134.94 (C-Ar, C-1′, C-4′, C-5′, C-6′), 147.33 (C-4″), 151.40 (C-2″), 152.24 (C-2′), 164.42, 168.75, 171.87 (C=O).

LC-MS (ESI) (90% $H_2O$ to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 200–330 nm): $t_R$ = 12.51 min, 100% purity; *m*/*z* [M + H]$^+$calcd for $C_{43}H_{52}N_5O_5SSi$: 779.05; found: 778.7.

HRMS (ESI): *m*/*z* [M + H]$^+$ calcd for $C_{43}H_{52}N_5O_5SSi$: 778.3453; found: 778.3431.

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## Supporting Information

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## References

(1) (a) Churcher, I. *J. Med. Chem.* **2018**, *61*, 444. (b) An, S.; Fu, L. *EBioMedicine* **2018**, *36*, 553. (c) Maniaci, C.; Ciulli, A. *Curr. Opin. Chem. Biol.* **2019**, *52*, 145. (d) Burslem, G. M.; Crews, C. M. *Cell* **2020**, *181*, 102. (e) Verma, R.; Mohl, D.; Deshaies, R. J. *Mol. Cell* **2020**, *77*, 446.

(2) (a) Zengerle, M.; Chan, K.-H.; Ciulli, A. *ACS Chem. Biol.* **2015**, *10*, 1770. (b) Lai, A. C.; Toure, M.; Hellerschmied, D.; Salami, J.; Jaime-Figueroa, S.; Ko, E.; Hines, J.; Crews, C. M. *Angew. Chem. Int. Ed.* **2016**, *55*, 807. (c) Gadd, M. S.; Testa, A.; Lucas, X.; Chan, K.-H.; Chen, W.; Lamont, D. J.; Zengerle, M.; Ciulli, A. *Nat. Chem. Biol.* **2017**, *13*, 514. (d) Schiedel, M.; Herp, D.; Hammelmann, S.; Swyter, S.; Lehotzky, A.; Robaa, D.; Oláh, J.; Ovádi, J.; Sippl, W.; Jung, M. *J. Med. Chem.* **2018**, *61*, 482. (e) Steinebach, C.; Kehm, H.; Lindner, S.; Vu, L. P.; Köpff, S.; López Mármol, Á.; Weiler, C.; Wagner, K. G.; Reichenzeller, M.; Krönke, J.; Gütschow, M. *Chem. Commun.* **2019**, *55*, 1821. (f) Testa, A.; Hughes, S. J.; Lucas, X.; Wright, J. E.; Ciulli, A. *Angew. Chem. Int. Ed.* **2020**, *59*, 1727. (g) Steinebach, C.; Ng, Y. L. D.; Sosič, I.; Lee, C.-S.; Chen, S.; Lindner, S.; Vu, L. P.; Bricelj, A.; Haschemi, R.; Monschke, M.; Steinwarz, E.; Wagner, K. G.; Bendas, G.; Luo, J.; Gütschow, M.; Krönke, J. *Chem. Sci.* **2020**, *11*, 3474.

(3) (a) Khan, S.; Zhang, X.; Lv, D.; Zhang, Q.; He, Y.; Zhang, P.; Liu, X.; Thummuri, D.; Yuan, Y.; Wiegand, J. S.; Pei, J.; Zhang, W.; Sharma, A.; McCurdy, C. R.; Kuruvilla, V. M.; Baran, N.; Ferrando, A. A.; Kim, Y.; Rogojina, A.; Houghton, P. J.; Huang, G.; Hromas, R.; Konopleva, M.; Zheng, G.; Zhou, D. *Nat. Med.* **2019**, *25*, 1938. (b) Farnaby, W.; Koegl, M.; Roy, M. J.; Whitworth, C.; Diers, E.; Trainor, N.; Zollman, D.; Steurer, S.; Karolyi-Oezguer, J.; Riedmueller, C.; Gmaschitz, T.; Wachter, J.; Dank, C.; Galant, M.; Sharps, B.; Rumpel, K.; Traxler, E.; Gerstberger, T.; Schnitzer, R.; Petermann, O.; Greb, P.; Weinstabl, H.; Bader, G.; Zoephel, A.; Weiss-Puxbaum, A.; Ehrenhöfer-Wölfer, K.; Wöhrle, S.; Boehmelt, G.; Rinnenthal, J.; Arnhof, H.; Wiechens, N.; Wu, M.-Y.; Owen-Hughes, T.; Ettmayer, P.; Pearson, M.; McConnell, D. B.; Ciulli, A. *Nat. Chem. Biol.* **2019**, *15*, 672.

(4) (a) Lohbeck, J.; Miller, A. K. *Bioorg. Med. Chem. Lett.* **2016**, *26*, 5260. (b) Papatzimas, J.; Gorobets, E.; Brownsey, D.; Maity, R.; Bahlis, N.; Derksen, D. *Synlett* **2017**, *28*, 2881. (c) Wurz, R. P.; Dellamaggiore, K.; Dou, H.; Javier, N.; Lo, M.-C.; McCarter, J. D.; Mohl, D.; Sastri, C.; Lipford, J. R.; Cee, V. J. *J. Med. Chem.* **2018**, *61*, 453. (d) Qiu, X.; Sun, N.; Kong, Y.; Li, Y.; Yang, X.; Jiang, B. *Org. Lett.* **2019**, *21*, 3838. (e) Steinebach, C.; Sosič, I.; Lindner, S.;

 

Bricelj, A.; Kohl, F.; Ng, Y. L. D.; Monschke, M.; Wagner, K. G.; Krönke, J.; Gütschow, M. *Med. Chem. Commun.* **2019**, *10*, 1037.

(5) (a) Frost, J.; Galdeano, C.; Soares, P.; Gadd, M. S.; Grzes, K. M.; Ellis, L.; Epemolu, O.; Shimamura, S.; Bantscheff, M.; Grandi, P.; Read, K. D.; Cantrell, D. A.; Rocha, S.; Ciulli, A. *Nat. Commun.* **2016**, *7*, 13312. (b) Soares, P.; Gadd, M. S.; Frost, J.; Galdeano, C.; Ellis, L.; Epemolu, O.; Rocha, S.; Read, K. D.; Ciulli, A. *J. Med. Chem.* **2018**, *61*, 599. (c) Testa, A.; Lucas, X.; Castro, G. V.; Chan, K.-H.; Wright, J. E.; Runcie, A. C.; Gadd, M. S.; Harrison, W. T. A.; Ko, E.-J.; Fletcher, D.; Ciulli, A. *J. Am. Chem. Soc.* **2018**, *140*, 9299. (d) Soares, P.; Lucas, X.; Ciulli, A. *Bioorg. Med. Chem.* **2018**, *26*, 2992. (e) Lucas, X.; Van Molle, I.; Ciulli, A. *J. Med. Chem.* **2018**, *61*, 7387. (f) de Castro, G. V.; Ciulli, A. *Chem. Commun.* **2019**, *55*, 1482. (g) Han, X.; Wang, C.; Qin, C.; Xiang, W.; Fernandez-Salas, E.; Yang, C.-Y.; Wang, M.; Zhao, L.; Xu, T.; Chinnaswamy, K.; Delproposto, J.; Stuckey, J.; Wang, S. *J. Med. Chem.* **2019**, *62*, 941.

(6) (a) Maniaci, C.; Hughes, S. J.; Testa, A.; Chen, W.; Lamont, D. J.; Rocha, S.; Alessi, D. R.; Romeo, R.; Ciulli, A. *Nat. Commun.* **2017**, *8*, 830. (b) Zoppi, V.; Hughes, S. J.; Maniaci, C.; Testa, A.; Gmaschitz, T.; Wieshofer, C.; Koegl, M.; Riching, K. M.; Daniels, D. L.; Spallarossa, A.; Ciulli, A. *J. Med. Chem.* **2019**, *62*, 699. (c) Smith, B. E.; Wang, S. L.; Jaime-Figueroa, S.; Harbin, A.; Wang, J.; Hamman, B. D.; Crews, C. M. *Nat. Commun.* **2019**, *10*, 131.

(7) Galdeano, C.; Gadd, M. S.; Soares, P.; Scaffidi, S.; Van Molle, I.; Birced, I.; Hewitt, S.; Dias, D. M.; Ciulli, A. *J. Med. Chem.* **2014**, *57*, 8657.

(8) Johnson, C. N.; Adelinet, C.; Berdini, V.; Beke, L.; Bonnet, P.; Brehmer, D.; Calo, F.; Coyle, J. E.; Day, P. J.; Frederickson, M.; Freyne, E. J. E.; Gilissen, R. A. H. J.; Hamlett, C. C. F.; Howard, S.; Meerpoel, L.; Mevellec, L.; McMenamin, R.; Pasquier, E.; Patel, S.; Rees, D. C.; Linders, J. T. M. *ACS Med. Chem. Lett.* **2015**, *6*, 31.

(9) Buckley, D. L.; Raina, K.; Darricarrere, N.; Hines, J.; Gustafson, J. L.; Smith, I. E.; Miah, A. H.; Harling, J. D.; Crews, C. M. *ACS Chem. Biol.* **2015**, *10*, 1831.

(10) For the characterization of the *O*-acyl side product occurring during the coupling of phenol **6d** with Boc-Hyp-OH, see Supporting Information.

(11) Kaburagi, Y.; Kishi, Y. *Org. Lett.* **2007**, *9*, 723.

(12) (a) Buckley, D. L.; Van Molle, I.; Gareiss, P. C.; Tae, H. S.; Michel, J.; Noblin, D. J.; Jorgensen, W. L.; Ciulli, A.; Crews, C. M. *J. Am. Chem. Soc.* **2012**, *134*, 4465. (b) Tovell, H.; Testa, A.; Maniaci, C.; Zhou, H.; Prescott, A. R.; Macartney, T.; Ciulli, A.; Alessi, D. R. *ACS Chem. Biol.* **2019**, *14*, 882.

(13) The X-ray crystallographic data collection for compounds **14** was performed on a Bruker X8-Kappa ApexII diffractometer at 100(2) K. The diffractometer was equipped with a low-temperature device (Kryoflex I, Bruker AXS) and used Mo-$K_\alpha$ radiation ($\lambda$ = 0.71073 Å). Intensities were measured by fine-slicing $\phi$- and $\omega$-scans and corrected for background, polarization, and Lorentz effects. Semiempirical absorption corrections were applied for all data sets by using Bruker's SADABS program. The structures were solved by direct methods and refined anisotropically by the least-squares procedure implemented in the ShelX-2014/7 program system. Hydrogen atoms were included isotopically using the riding model on the bound carbon atoms. CCDC 1986177 contains the supplementary crystallographic data for this paper. The data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/getstructures.

(14) For the feasible synthesis of aldehyde **4d** from 3-bromophenol or 4-bromosalicylic acid, see Supporting Information.

(15) (a) Raina, K.; Lu, J.; Qian, Y.; Altieri, M.; Gordon, D.; Rossi, A. M. K.; Wang, J.; Chen, X.; Dong, H.; Siu, K.; Winkler, J. D.; Crew, A. P.; Crews, C. M.; Coleman, K. G. *Proc. Natl. Acad. Sci. U. S. A.* **2016**, *113*, 7124. (b) Hu, J.; Hu, B.; Wang, M.; Xu, F.; Miao, B.; Yang, C.-Y.; Wang, M.; Liu, Z.; Hayes, D. F.; Chinnaswamy, K.; Delproposto, J.; Stuckey, J.; Wang, S. *J. Med. Chem.* **2019**, *62*, 1420. (c) Wei, J.; Hu, J.; Wang, L.; Xie, L.; Jin, M. S.; Chen, X.; Liu, J.; Jin, J. *J. Med. Chem.* **2019**, *62*, 10897.

(16) Yamazaki, Y.; Kohno, K.; Yasui, H.; Kiso, Y.; Akamatsu, M.; Nicholson, B.; Deyanat-Yazdi, G.; Neuteboom, S.; Potts, B.; Lloyd, G. K.; Hayashi, Y. *ChemBioChem* **2008**, *9*, 3074.



 