Structure-Guided Design and Optimization of Small Molecules Targeting the Protein-Protein Interaction between the von Hippel-Lindau (VHL) E3 Ubiquitin Ligase and the Hypoxia Inducible Factor (HIF) Alpha Subunit with In Vitro Nanomolar Affinities

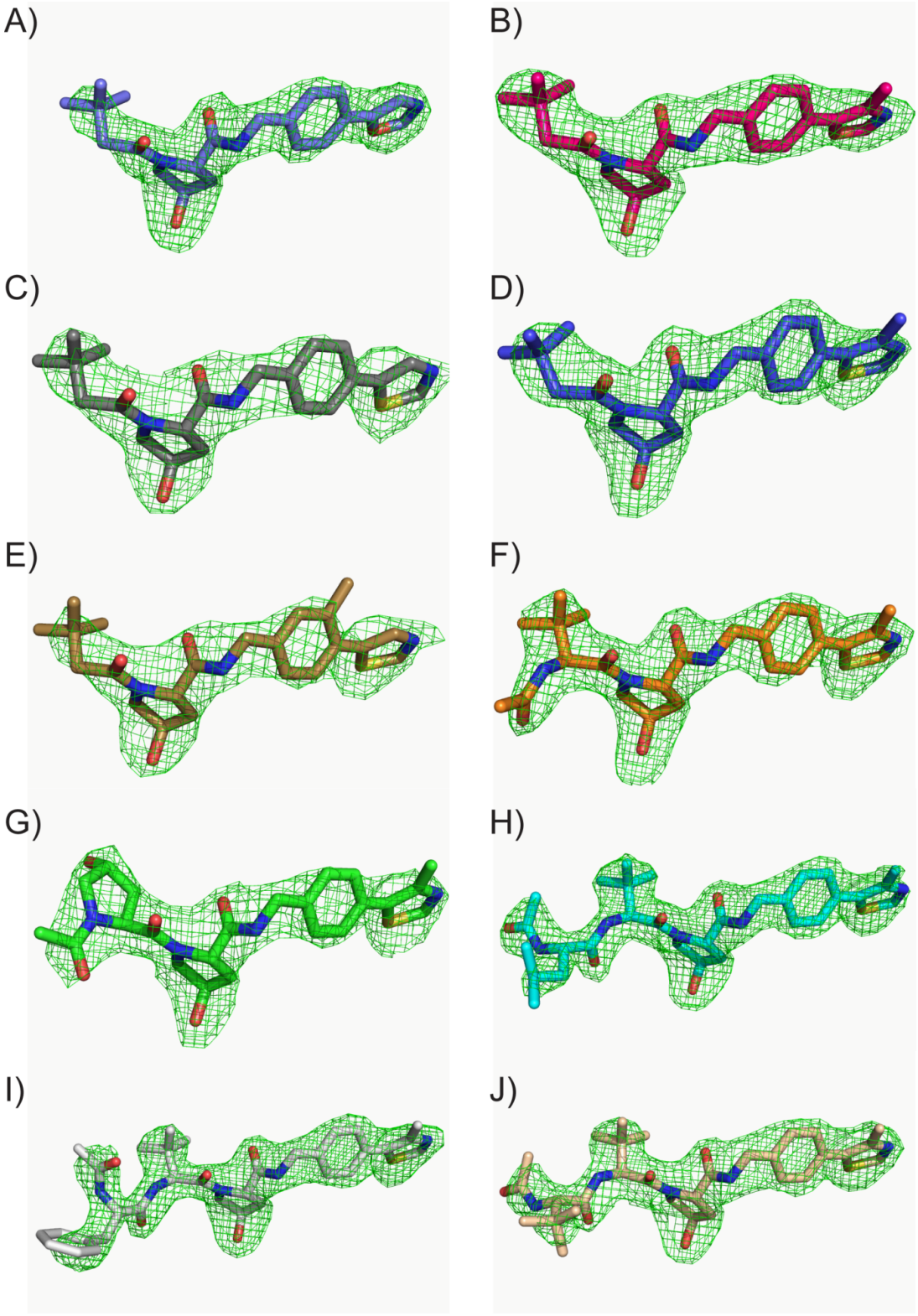
Carles Galdeano[a,b‡], Morgan S. Gadd[a‡], Pedro Soares[a], Salvatore Scaffidi[a], Inge Van Molle[b,†], Ipek Birced[a], Sarah Hewitt[b,†], David M. Dias [b], Alessio Ciulli[a,b,*]

[a] Division of Biological Chemistry and Drug Discovery, College of Life Sciences, University of Dundee, Dundee, DD1 5EH, Scotland, UK

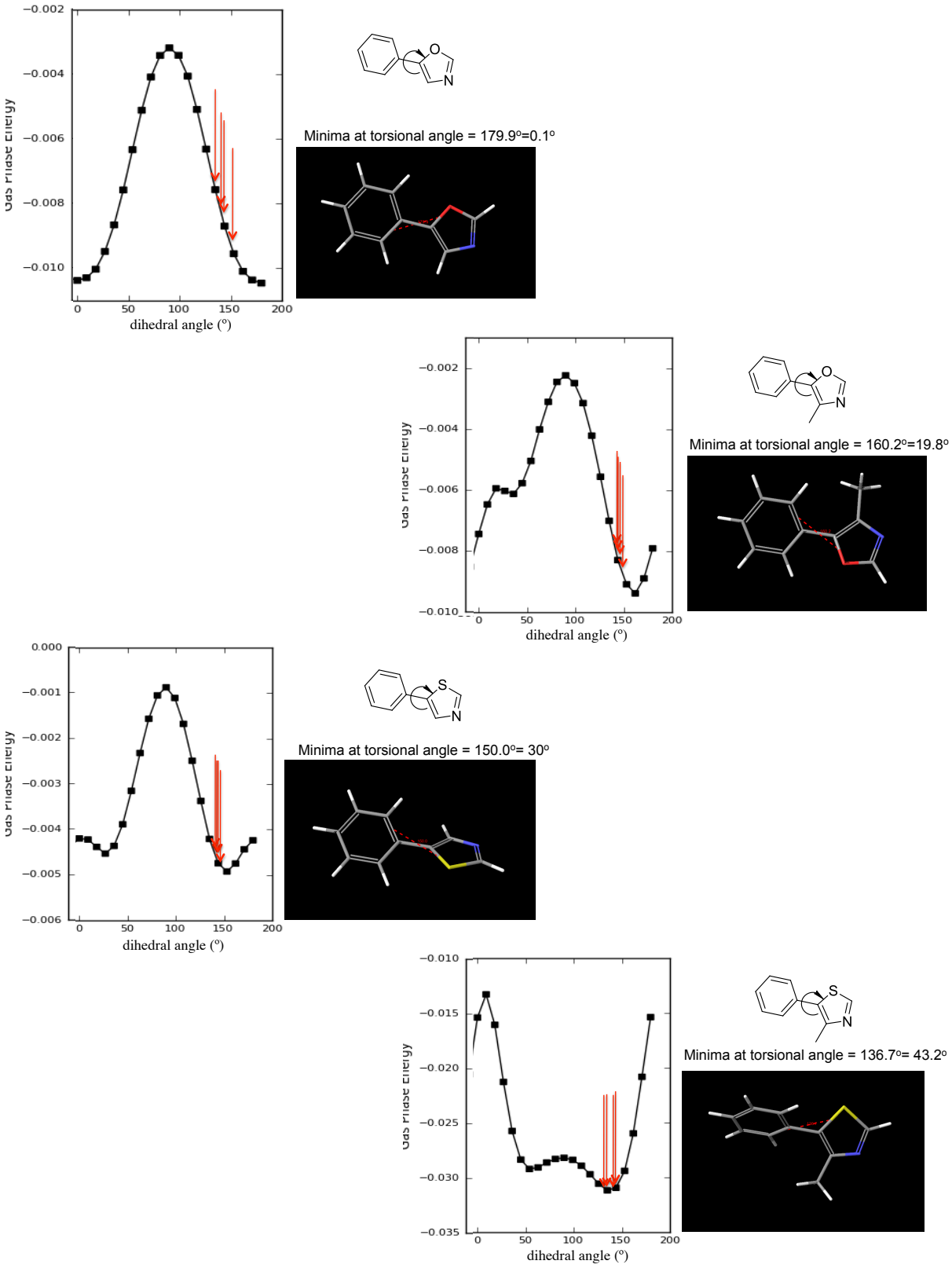
[b] Department of Chemistry, University of Cambridge, Lensfield Road, Cambridge, CB2 1EW, UK

SUPPORTING INFORMATION

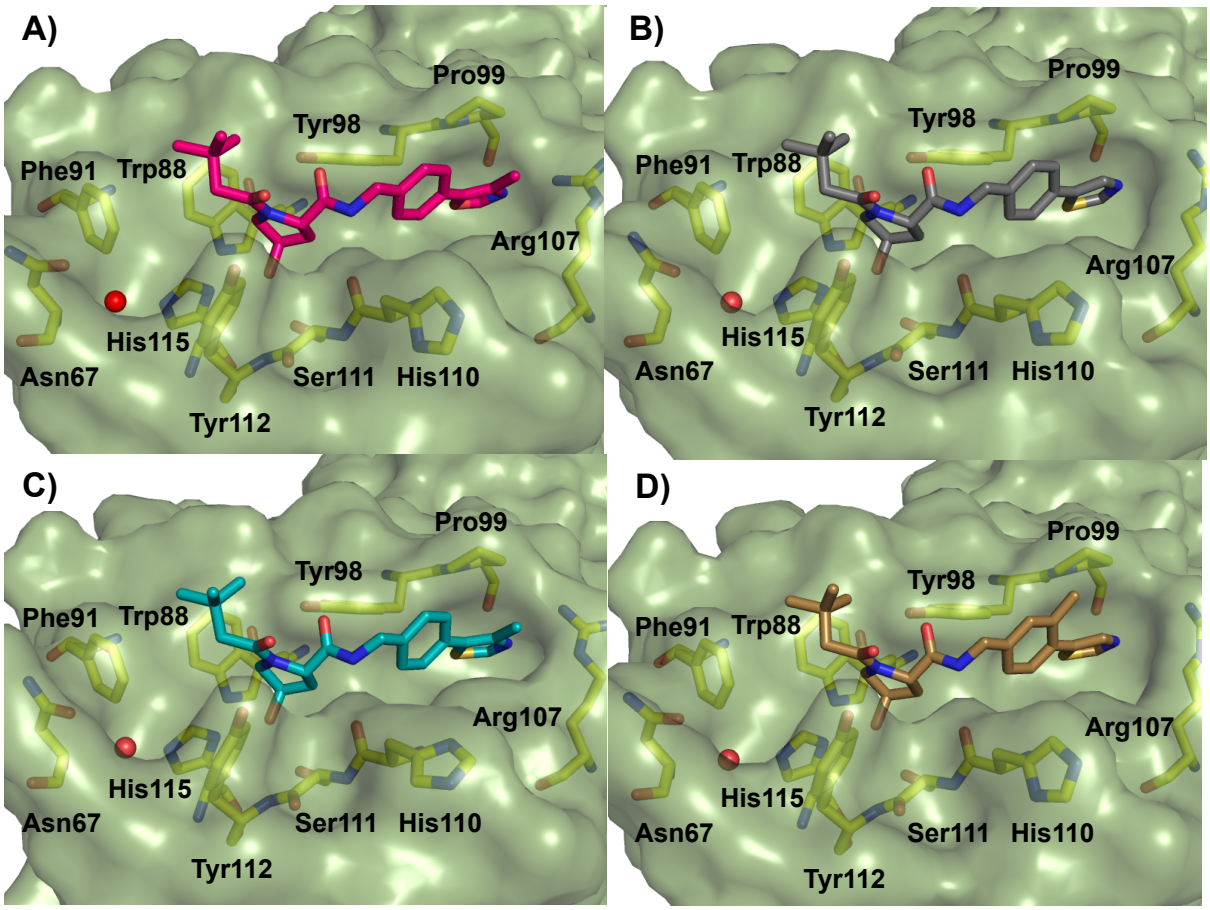
1. SUPPLEMENTARY FIGURES



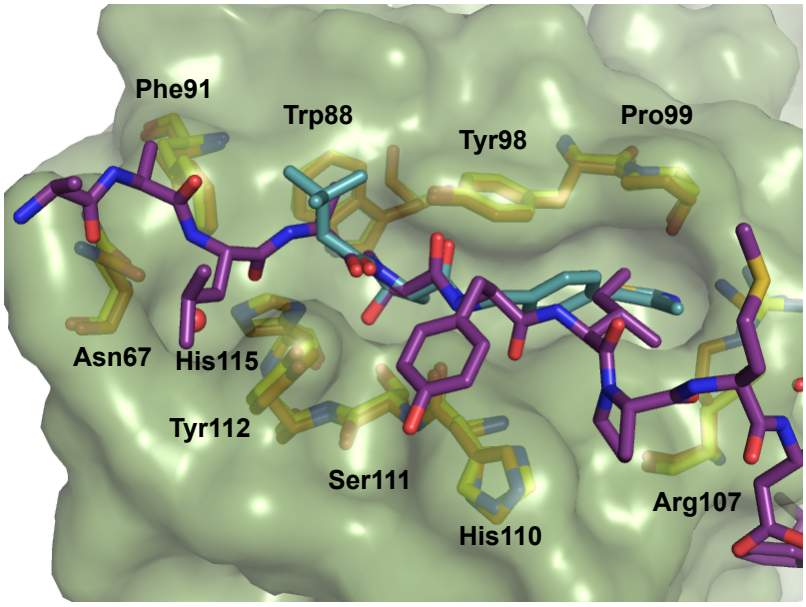
Supplementary Figure 1. Electron density at ligand binding sites. Ligands A) **2**; B) **3**; C) **4**; D) **5**; E) **6**; F) **7**; G) **10**; H) **13**; I) **14**; J) **15** shown as sticks bound to the complex of VBC (not shown). An omit map (F_o–F_c) is shown in green contoured at 3σ around each modelled ligand with a carve radius of 1.6 Å.



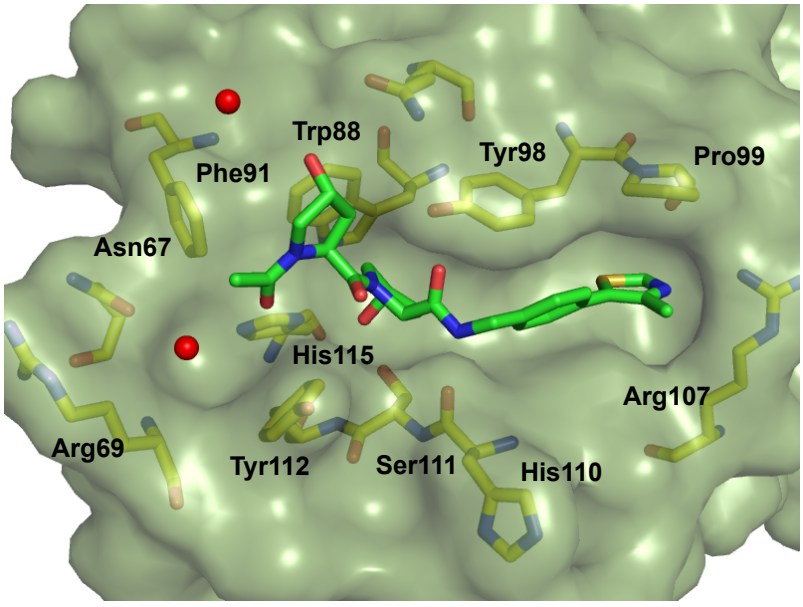
Supplementary Figure 2. Torsional scan energetic profiles (Schrödinger package) of the Right Hand Site (RHS) biaryl of the ligands **2**, **3**, **4** and **5** (top to bottom). Red arrows indicate the dihedral angle found for the ligand molecules in the four protomers in the asymmetric unit of the X-ray structures.



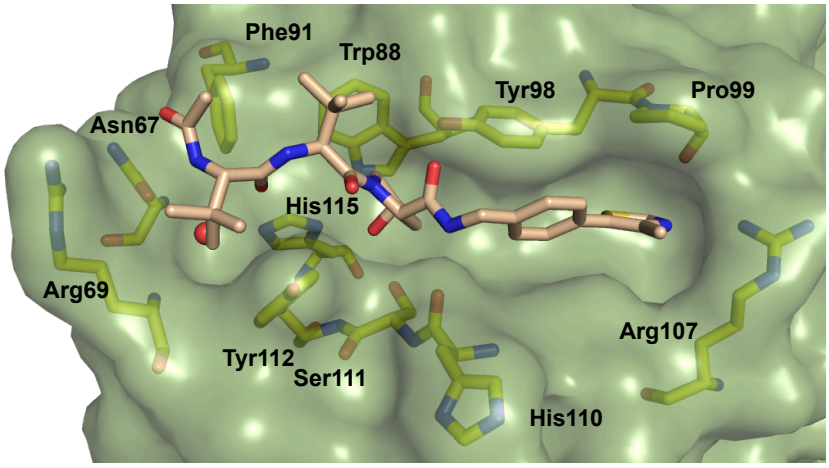
Supplementary Figure 3. Crystal structures of the VBC complex with: A) ligand **3** (PDB 4W9D); B) ligand **4** (PDB 4W9E); C) ligand **5** (PDB 4W9F); D) ligand **6** (PDB 4W9G). pVHL is shown as a pale green surface and the pVHL residues forming the binding pocket as yellow sticks.



Supplementary Figure 4. Crystal structure of the VBC in complex with: ligand **5** (blue teal, (PDB 4W9F) and the HIF-1α peptide (dark purple, PDB 4AJY)[12]. pVHL residues forming the binding pocket are shown as yellow sticks (from complex with ligand **5**) and orange sticks (from complex with HIF-1α peptide). pVHL is shown as a pale green surface.



Supplementary Figure 5. Crystal structure of the VBC in complex with ligand **10** (PDB 4W9I). pVHL is shown as a pale green surface and the pVHL residues forming the binding pocket as yellow sticks.



Supporting Figure 6. Crystal structure of the VBC in complex with ligand **15** (PDB 4W9L). pVHL is shown as a pale green surface and the pVHL residues forming the binding pocket as yellow sticks.

2. BIOCHEMICAL METHODS

VBC complex expression and purification

The numbering of residues from the proteins refers to the following National Center for Biotechnology Information (NCBI) entries for human proteins: pVHL, P40337.2; EloC, Q15369.1; EloB, Q15370.1. A plasmid containing $pVHL_{54–213}$ with an N-terminal His_6 tag and a duet plasmid containing $EloB_{1–104}$ and $EloC_{17–112}$ were used to generate a complex of pVHL:EloB:EloC (VBC) as described previously.[1] All proteins were co-expressed from their respective plasmids in *Escherichia coli* BL21 (DE3) at 24 °C for 16 h. *E. coli* cells were lysed using a pressure cell homogeniser (Stansted Fluid Power) and lysate clarified by centrifugation. His_6-tagged VBC was purified on a HisTrapFF affinity column (GE Healthcare) by elution with an imidazole gradient. The His_6 tag was removed using TEV protease and the untagged complex dialysed into low imidazole concentration buffer. VBC was then flowed through the HisTrapFF column a second time, allowing impurities to bind as the complex eluted without binding. VBC was then additionally purified by anion exchange and size-exclusion chromatography using MonoQ and Superdex-75 columns (GE Healthcare), respectively. All chromatography purification steps were performed using Äkta FPLC purification systems (GE Healthcare) at 4 °C or room temperature. The final purified complex was stored in 20 mM Bis Tris, pH 7, 150 mM sodium chloride and 1 mM dithiothreitol (DTT).

X-ray crystallography

The VBC complex was crystallized as described previously.[1] Liquor solutions contained 0.1 mM sodium cacodylate, pH 6.2–6.5, 16–18% polyethylene glycol 3350, 0.2 M magnesium acetate and 10 mM DTT. VBC complex solution and liquor solution were mixed in equal volumes using the hanging-drop vapour diffusion method at 18 °C. A 2–3 mm layer of Al's Oil (Hampton Research) was applied on top of liquor solutions to slow the vapour diffusion rate. Well diffracting crystals were obtained by streak seeding into the same conditions from initial crystals.

To obtain structures of the ligands **2, 3, 4, 5, 6, 7, 10, 13, 14, 15** bound to VBC, crystals were soaked overnight in 1–10 mM solutions of ligand in 1–10% dimethylsulfoxide, 4–40% isopropanol and 50–95% liquor solution. Crystals were generally not cryo-protected or occasionally in 20% glycerol (ligands **3** and **5**) and flash frozen in liquid nitrogen. Crystals were screened using an in-house Rigaku M007HF X-ray generator and Saturn 944HG+ CCD detector.

All X-ray data were collected at 100 K at the Diamond (beamlines I03 and I04) and ESRF (beamlines ID14-4 and BM14) synchrotron facilities. Indexing and integration of reflections was performed using XDS with the XDSGUI interface,[2] and scaling and merging with POINTLESS and SCALA in CCP4i.[3,4,5] The isomorphous datasets were refined using REFMAC5[6,7] and COOT[8] using a template structure derived from the Protein Data Bank (PDB) entry 1vcb.[9] Ligand structures and restraints were generated using the PRODRG server.[10] The MOLPROBITY server was used to validate the geometry and steric clashes in the structures.[11] The structures were deposited in the PDB and data collection and refinement statistics are presented in Supplementary Table 1.

Supplementary Table 1.
Crystallographic data processing and refinement statistics.

Values in parentheses are for the highest resolution shell.

Dataset	Ligand 2	Ligand 3	Ligand 4	Ligand 5	Ligand 6
Synchrotron	ESRF	Diamond	Diamond	Diamond	Diamond
Beamline	ID14-4	I03	I04	I03	I04
Wavelength (Å)	0.9393	0.9763	0.9795	0.9763	0.9790
Processing statistics					
Space group	$P4_122$	$P4_122$	$P4_122$	$P4_122$	$P4_122$
Unit cell parameters					
a,*b* (Å)	93.8	93.9	93.1	92.7	93.5
c (Å)	366.0	365.9	364.4	364.0	363.9
Resolution limits (Å)	49.2–2.20 (2.32–2.20)	49.2–2.20 (2.32–2.20)	48.9–2.60 (2.74–2.60)	50.0–2.10 (2.15–2.10)	48.9–2.70 (2.85–2.70)
Total reflections	608442 (89034)	1023519 (140759)	442201 (56748)	1217491 (91870)	297722 (44073)
Unique reflections	83149 (12076)	84490 (12124)	50195 (7199)	93841 (6856)	45646 (6532)
Completeness (%)	98.9 (99.9)	100 (100)	99.1 (99.0)	100 (100)	99.9 (100)
Multiplicity	7.3 (7.4)	12.1 (11.6)	8.8 (7.6)	13.0 (13.4)	6.5 (6.7)
R_{merge} (%)	9.8 (68.7)	10.3 (81.8)	10.5 (96.2)	9.5 (130.3)	15.8 (63.8)
$I/\sigma(I)$	12.8 (2.5)	12.7 (2.9)	14.3 (2.0)	18.4 (2.7)	6.9 (2.0)
$CC_{1/2}$ (%)	99.9 (79.9)	99.9 (88.6)	99.9 (72.1)	99.8 (85.0)	99.4 (78.6)
Wilson *B* factor (Å^2)	28.7	39.9	36.3	35.5	38.5
Mosaicity (°)	0.08	0.13	0.29	0.14	0.06
Refinement statistics					
Resolution limits (Å)	93.8–2.20 (2.26–2.20)	93.9–2.30 (2.26–2.20)	93.1–2.60 (2.67–2.60)	92.7–2.10 (2.12–2.10)	93.5–2.70 (2.77–2.70)
R_{work} (%)	20.6 (27.5)	19.7 (25.7)	20.6 (32.8)	20.5 (26.9)	22.2 (31.9)
R_{free} (%)	25.1 (31.0)	23.6 (28.6)	26.1 (36.7)	22.9 (31.1)	26.4 (38.1)
No. reflections	78834 (5773)	80098 (5811)	47544 (3444)	89136 (6510)	43222 (3123)
No. test reflections	4195 (311)	4270 (310)	2553 (189)	4704 (344)	2327 (165)
Model atoms	11174	10920	10819	10937	10839
Protein *B* factor (Å^2)	40.0	52.7	54.2	45.3	46.7
Ligand *B* factor (Å^2)	35.9	40.7	46.6	37.3	42.1
r.m.s.d. bonds (Å)	0.008	0.009	0.006	0.006	0.005
r.m.s.d. angles (°)	1.25	1.35	1.18	1.21	1.05
Ramachandran plot					
Favoured (%)	97.3	97.2	96.1	97.0	96.9
Allowed (%)	2.4	2.6	3.8	2.3	3.0
Disallowed (%)	0.3	0.2	0.1	0.3	0.1
PDB code	4W9C	4W9D	4W9E	4W9F	4W9G

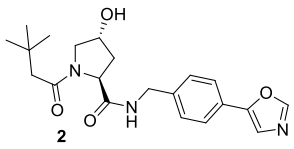
Values in parentheses are for the highest resolution shell.

Dataset	Ligand 7	Ligand 10	Ligand 13	Ligand 14	Ligand 15
Synchrotron	Diamond	Diamond	ESRF	ESRF	Diamond
Beamline	I04	I04	BM14	BM14	I03
Wavelength (Å)	0.9795	0.9795	0.9537	0.9537	0.9763
Processing statistics					
Space group	$P4_122$	$P4_122$	$P4_122$	$P4_122$	$P4_122$
Unit cell parameters					
a,b (Å)	93.0	93.4	93.7	93.7	93.8
c (Å)	364.4	364.3	360.8	361.7	362.6
Resolution limits (Å)	48.8–2.10 (2.21–2.10)	48.9–2.40 (2.53–2.40)	48.8–2.20 (2.32–2.20)	48.9–2.10 (2.21–2.10)	49.0–2.20 (2.32–2.20)
Total reflections	613573 (89745)	614270 (81816)	546027 (40833)	659300 (47141)	533045 (76390)
Unique reflections	94556 (13601)	64415 (9215)	80705 (10162)	81970 (10867)	81578 (11880)
Completeness (%)	99.9 (100)	100 (100)	97.4 (86.2)	98.7 (91.9)	98.3 (99.4)
Multiplicity	6.5 (6.6)	9.5 (8.9)	6.8 (4.0)	7.5 (5.2)	6.5 (6.4)
R_{merge} (%)	10.4 (83.1)	14.6 (73.2)	7.0 (49.3)	6.4 (49.9)	8.2 (91.0)
$I/\sigma(I)$	9.6 (2.0)	9.0 (2.0)	15.4 (2.3)	16.6 (2.8)	13.5 (2.5)
$CC_{1/2}$ (%)	99.8 (72.6)	99.8 (81.9)	99.9 (76.4)	99.9 (80.0)	99.9 (77.5)
Wilson B factor (Å^2)	30.5	29.4	27.2	34.5	38.1
Mosaicity (°)	0.09	0.03	0.14	0.09	0.11
Refinement statistics					
Resolution limits (Å)	93.1–2.10 (2.15–2.10)	93.4–2.40 (2.46–2.40)	93.7–2.20 (2.26–2.20)	93.7–2.10 (2.15–2.10)	93.8–2.20 (2.26–2.20)
R_{work} (%)	21.0 (29.3)	22.5 (29.6)	21.6 (27.5)	21.5 (31.2)	20.4 (28.8)
R_{free} (%)	25.3 (33.8)	27.5 (37.5)	26.5 (30.3)	26.0 (35.3)	24.3 (31.5)
No. reflections	89653 (6492)	61092 (4419)	76517 (4723)	87047 (5133)	77450 (5705)
No. test reflections	4784 (358)	3237 (221)	4079 (258)	4637 (285)	4128 (302)
Model atoms	11272	11065	11148	11022	10937
Protein B factor (Å^2)	41.3	45.4	42.7	52	50.6
Ligand B factor (Å^2)	31.2	30.4	33.7	49.2	41.2
r.m.s.d. bonds (Å)	0.008	0.007	0.008	0.008	0.008
r.m.s.d. angles (°)	1.34	1.2	1.27	1.28	1.28
Ramachandran plot					
Favoured (%)	97	96.8	96.9	96.6	96.8
Allowed (%)	2.8	3	3	3.2	3.1
Disallowed (%)	0.2	0.2	0.1	0.2	0.1
PDB code	4W9H	4W9I	4W9J	4W9K	4W9L

Isothermal titration calorimetry (ITC)

All experiments were carried out using an ITC200 micro-calorimeter (GE Healthcare). The compounds were diluted from a DMSO stock solution to 0.6 mM in a 20 mM Bis-Tris, 150 mM NaCl, 2mM DTT, and pH 7 buffer. The compounds were titrated against 60 μM VBC complex, equilibrated in the same buffer. The final concentration of DMSO in the experiment solution was between 3% and 5%.

The titrations consisted of 20 injections of 2 μL ligand solution at a rate of 2 sec/μL at 120 s time intervals (with the exemption of the ligand **3**). An initial injection of ligand (0.4 μL) was made and discarded during data analysis. All experiments were performed at 25ºC, whilst stirring at 1000rpm. The data were fitted to a single binding site model using the Microcal LLC ITC200 Origin software provided by the manufacturer. The data from direct binding titrations were fitted to a single binding site model to obtain the stoichiometry *n*, the dissociation constant *K*d and the enthalpy of binding ΔH



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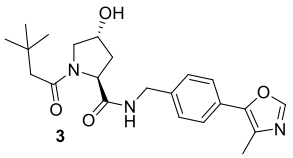

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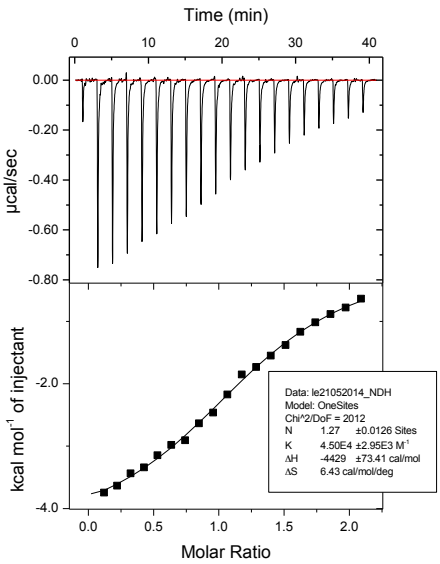

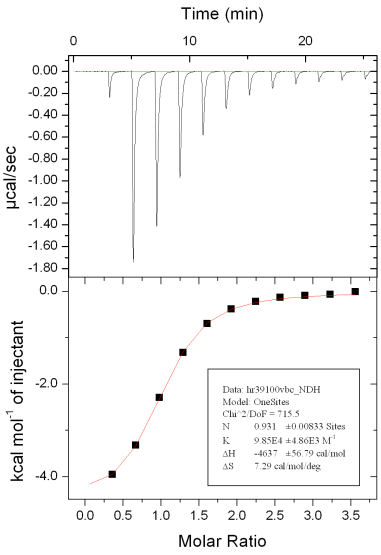

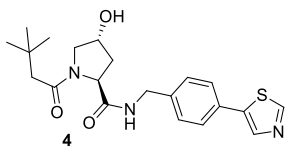
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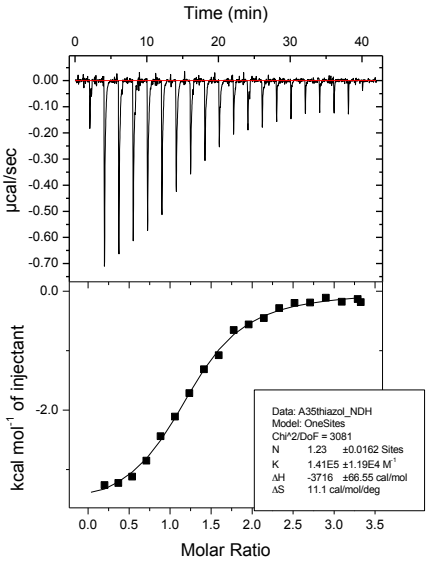
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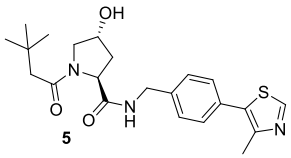
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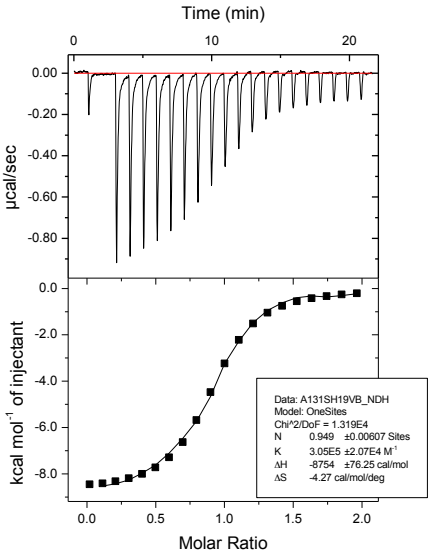
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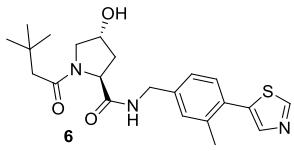
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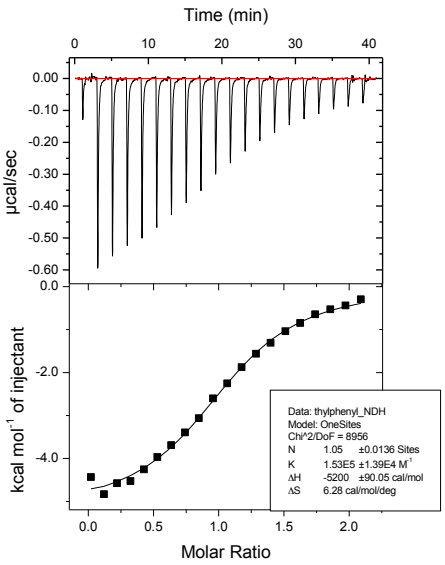
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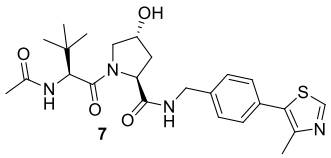
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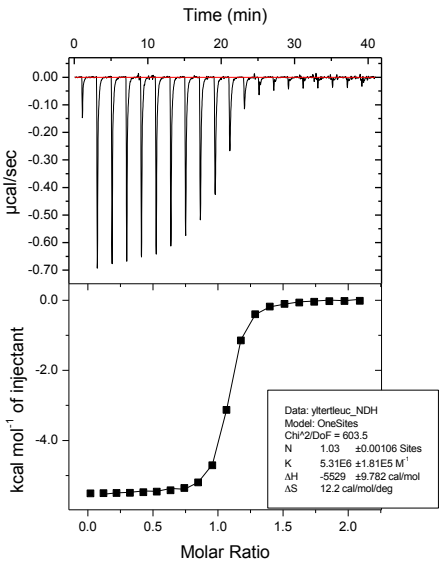
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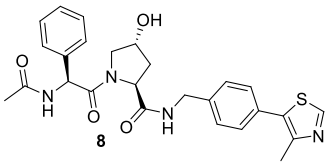
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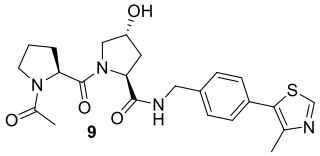
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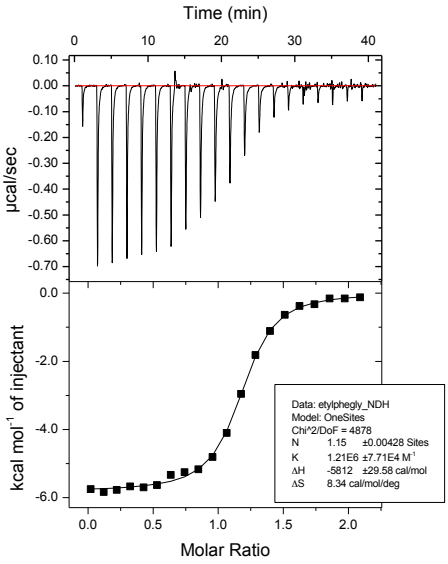
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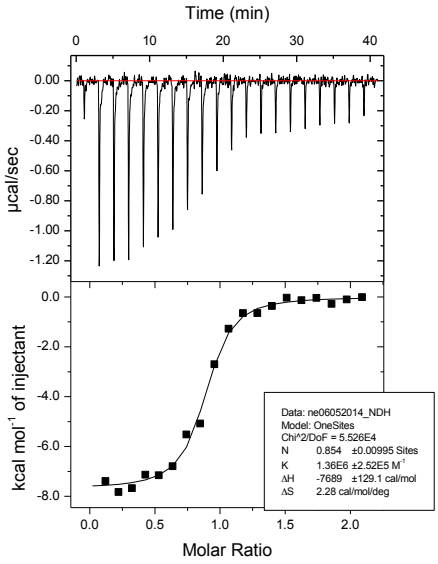
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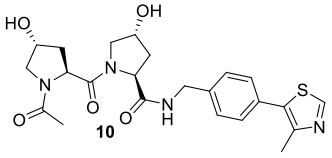
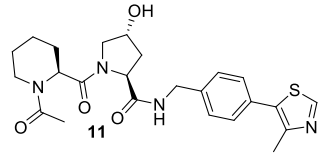
ΔH -7689 ±129.1 cal/mol
ΔS 2.28 cal/mol/deg


HO
OH
N
O
N
O
O
N
H
S
N

10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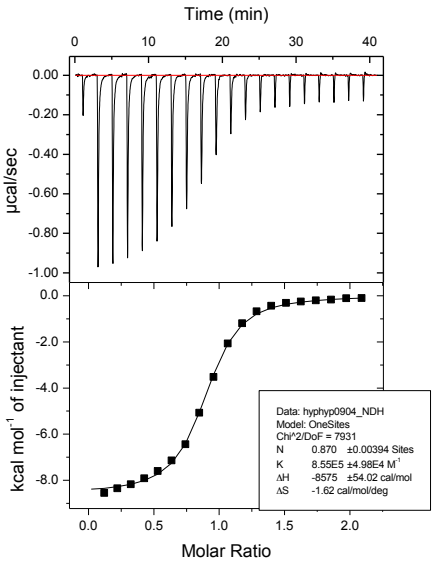
N

11


Time (min)
µcal/sec
kcal mol-1 of injectant
Molar Ratio
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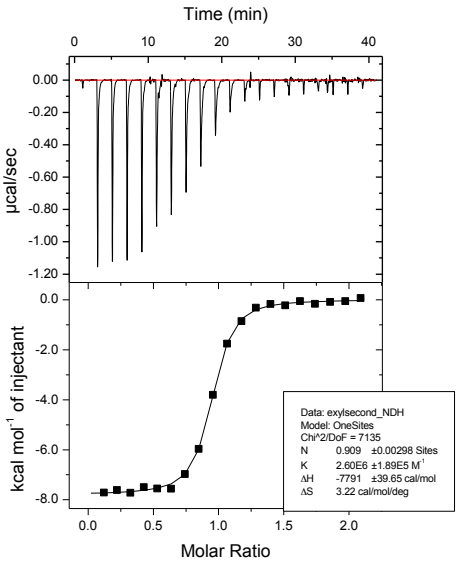
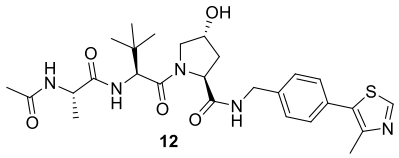
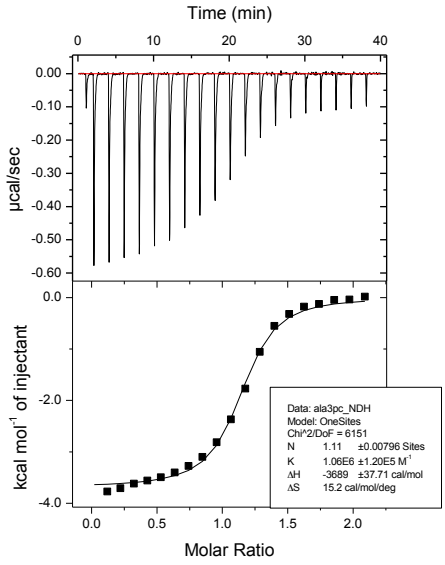
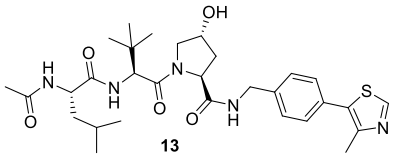
Model: OneSites
Chi^2/DoF = 7931
N 0.870 ±0.00394 Sites
K 8.55E5 ±4.98E4 M-1
ΔH -8575 ±54.02 cal/mol
ΔS -1.62 cal/mol/deg


Time (min)
µcal/sec
kcal mol-1 of injectant
Molar Ratio
Data: exylsecond_NDH
Model: OneSites
Chi^2/DoF = 7135
N 0.909 ±0.00298 Sites
K 2.60E6 ±1.89E5 M-1
ΔH -7791 ±39.65 cal/mol
ΔS 3.22 cal/mol/deg



12


Time (min)
µcal/sec
kcal mol^{-1} of injectant
Molar Ratio
Data: ala3pc_NDH
Model: OneSites
Chi^2/DoF = 6151
N 1.11 ±0.00796 Sites
K 1.06E6 ±1.20E5 M^{-1}
ΔH -3689 ±37.71 cal/mol
ΔS 15.2 cal/mol/deg



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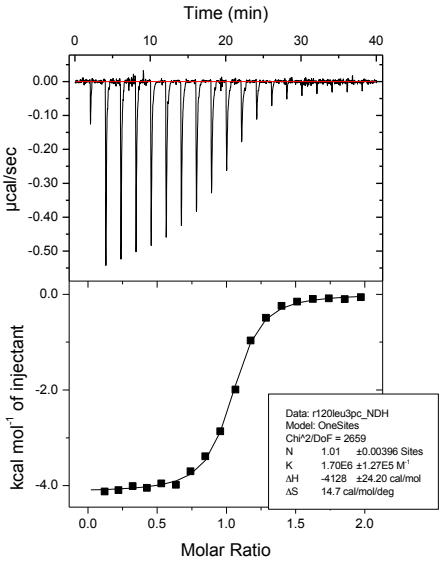
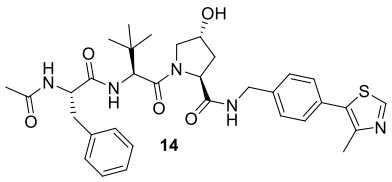

Time (min)
µcal/sec
kcal mol^{-1} of injectant
Molar Ratio
Data: r120leu3pc_NDH
Model: OneSites
Chi^2/DoF = 2659
N 1.01 ±0.00396 Sites
K 1.70E6 ±1.27E5 M^{-1}
ΔH -4128 ±24.20 cal/mol
ΔS 14.7 cal/mol/deg



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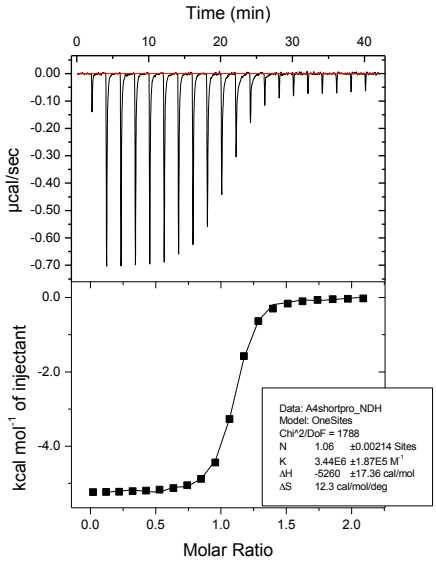
Time (min)
µcal/sec
kcal mol^{-1} of injectant
Molar Ratio
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K 3.44E6 ±1.87E5 M^{-1}
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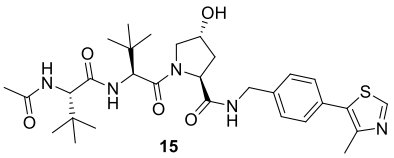
ΔS 12.3 cal/mol/deg



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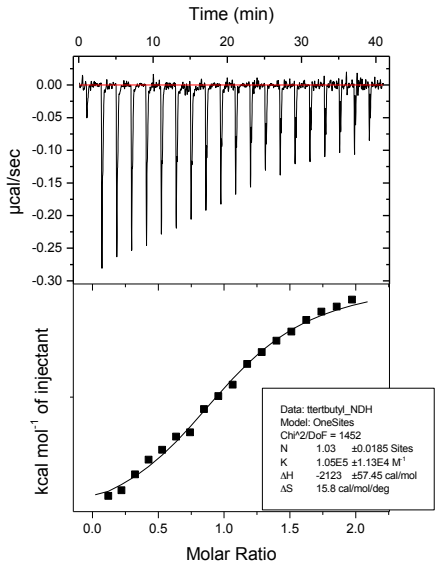
Time (min)
µcal/sec
kcal mol^{-1} of injectant
Molar Ratio
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Chi^2/DoF = 1452
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K 1.05E5 ±1.13E4 M^{-1}
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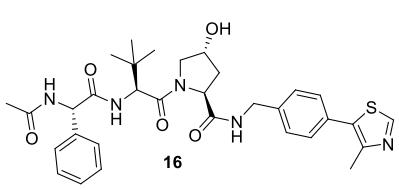
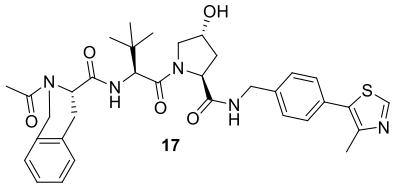
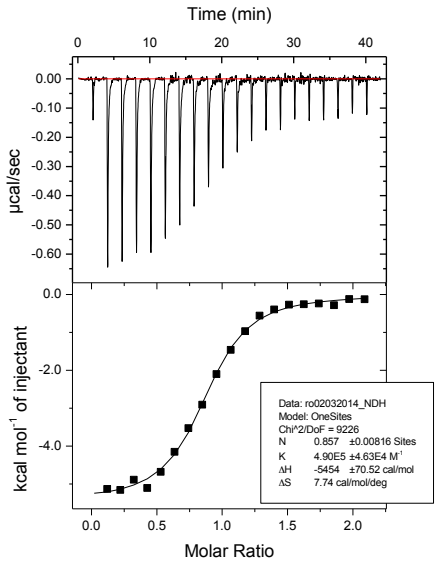
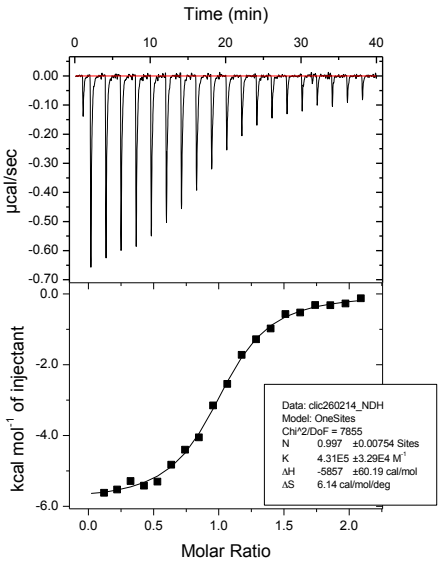
ΔS 15.8 cal/mol/deg









3. COMPUTATIONAL METHODS

Dihedral conformation calculations

Torsional energy profiles of the biaryl system at the right hand site of the ligands **2**, **3**, **4**, **5** and **6** were calculated using Schrödinger package (Version 9.4).

Conformational global minima and posterior minimization of the ligands were performed in vacuum using a MMFF94 force field (Macromodel, Schrödinger). Geometries of the ligands were optimized using Jaguar (Schrödinger) at the B1B95/6-311++G(d,p) level. Single point energies for a 9º step incremental scan between -180º and +180º were subsequently calculated at the B1B95/aug-cc-pvtz(-f) level (Jaguar, Schrödinger). Results were plotted in Maestro (Schrödinger).

4. SYNTHETIC METHODS

General Chemistry

Commercial materials were used as received unless otherwise noted. All reactions were carried out using anhydrous solvents. Analytical thin-layer chromatography (TLC) was performed on pre-coated TLC plates (layer 0.20 mm silica gel 60 with fluorescent indicator UV 254; Merck). Developed plates were air-dried and analysed under a UV lamp (254/365 nm). Flash column chromatography was performed using pre-packed silica gel cartridges (230–400 mesh, 40–63 mm; SiliCycle) using a Teledyne ISCO Combiflash Companion or Combiflash Retrieve using mixtures of DCM and MeOH as a mobile phase.

Liquid chromatography–mass spectrometry (LCMS) analyses were performed with either an Agilent HPLC 1100 series connected to a Bruker Daltonics MicroTOF or an Agilent Technologies 1200 series HPLC connected to an Agilent Technologies 6130 quadrupole spectrometer or a Waters 2795 connected to a Waters ZQ Micromass spectrometer, where all instruments were connected to an diode array detector. All of the final ligands (**2**-**17**) were evaluated after preparative LCMS separations with a Waters X-bridge C18 column (50 mm x 2.1 mm, 3.5 mm particle size), with a mobile phase of water/acetonitrile+0.1% HCOOH, or water/acetonitrile+0.1% NH_3, using a linear gradient from 80:20 to 5:95 over 3.5 min and then held for 1.5 min, at a flow rate of 0.5 mL/min. All ligand samples evaluated had a measured purity of >95% as determined using the analytical LCMS System described above. High-resolution electrospray measuraments were performed on a Bruker Daltonics Micro-TOF mass spectrometer or on a Micromass Quadrapole-Time of Flight (Q-TOF) spectrometer.

^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Avance II 500 spectrometer (^{1}H at 500.1 MHz; ^{13}C at 125.8 MHz) or on a Bruker DPX-400 Cryo spectrometer (^{1}H at 400 MHz: ^{13}C at 101 MHz) or on a Bruker DPX-300 spectrometer (^{1}H at 300.1 MHz). Chemical shifts (δ) are expressed in ppm recorded using the residual solvent as the internal reference in all cases. Signal splitting patterns are described as singlet (s), doublet (d), triplet (t), multiplet (m), broad (br), or a combination thereof. Coupling constants (*J*) are quoted to the nearest 0.1 Hz.

Synthesis and characterization

General method A:
To a solution of amine (1 eq.) in DMF was added the appropriate acid (1 eq.) and the solution was stirred at room temperature. DIPEA (4 eq.) was added dropwise and the mixture was stirred for 5 minutes at room temperature. HATU (1.1 eq.) was added and the mixture was stirred at room temperature for another 30 minutes. Water was added and the mixture was extracted with ethyl acetate (3x). The combined organic phases were washed with brine (x2), dried over $MgSO_4$ and evaporated under reduced pressure to give the corresponding crude, which was purified by flash column chromatography purification to yield the final compound.

General method B:

The deprotected aminoacid derivate (1eq.) was dissolved in DCM, and triethylamine (3 eq.) was added to the solution. After stirring the mixture for 10 minutes at room temperature,

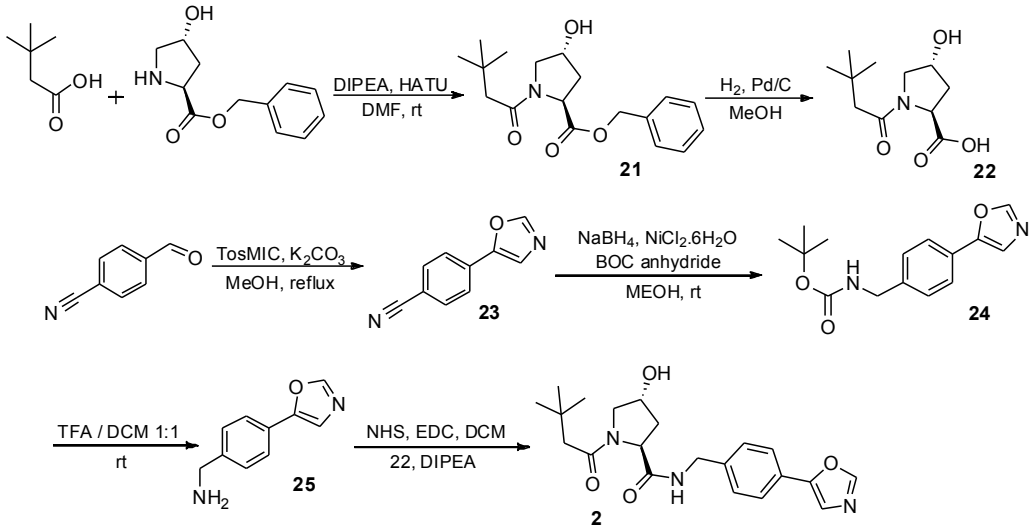
acetic anhydride (1.5 eq.) was added and the reaction was stirred 90 minutes at room temperature. The solvents were evaporated under reduced pressure to give the corresponding crude, which was purified by flash column chromatography to yield the final compound.

General method C:

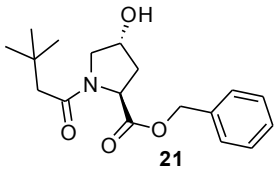
To a solution of acid (1 eq.) in DCM was added NHS (1.3 eq.) and EDC (1.2 eq.). The resulting mixture was stirred under argon atmosphere at room temperature for 3 hours. The amine (1.2 eq.) and DIPEA (3 eq.) were added and the mixture was stirred at room temperature for 20 hours. $NaHCO_3$ was added and the mixture extracted with DCM (x3). The combined organic phases were washed with brine (x2), dried over $MgSO_4$ and evaporated under reduced pressure to give the crude, which was purified by flash chromatography to yield the final compound.

NOTE: All of the final ligands (**2**-**17**) were evaluated after the corresponding flash column chromatography purification, followed by preparative LC-MS purification.

Synthesis of **2**:



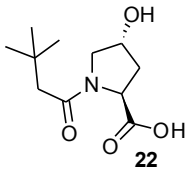
Benzyl (2*S*,4*R*)-1-(3,3-dimethylbutanoyl)-4-hydroxypyrrolidine-2-carboxylate, 21



Following the **general method A**, from *L*-4-hydroxyproline benzylester hydrochoride (1.00 g, 3.90 mmol, 1 eq.) and *tert*-butyl acetic acid (0.45 g, 0.50 mL, 3.9 mmol, 1 eq.), compound **21** was obtained as a white powder (1.13 g, 3.54 mmol, 91 %). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.47 – 7.29 (m, 5H), 5.21 (d, *J* = 12.3 Hz, 1H), 5.14 (d, *J* = 12.3 Hz, 1H), 4.68 (t, *J* = 8.0 Hz, 1H), 4.51 (tt, 1H, *J* = 4.7, 2.7 Hz), 3.75 (dd, 1H, *J* = 10.8, 4.7 Hz), 3.58 (ddd, 1H, *J* = 10.8, 2.7, 1.5 Hz), 2.94 (s, 1H), 2.39 – 2.22 (m, 2H), 2.15 (d, 1H, *J* = 13.6 Hz), 2.10 – 1.99 (m, 1H), 1.05 (s, 9H); ^{13}C NMR ($CDCl_3$, 101 MHz): δ 172.7, 171.9, 136.1, 129.1, 128.9,

128.6, 70.7, 67.2, 58.1, 56.6, 47.2, 38.1, 32.3, 30.3; HRMS (ESI) m/z: [M+1] calculated for $C_{18}H_{26}NO_4$: 320.1862; found: 320.1880**.**

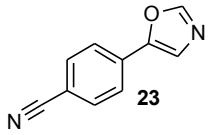
(2*S*,4*R*)-1-(3,3-dimethylbutanoyl)-4-hydroxypyrrolidine-2-carboxylic acid, 22



22

A solution of **21** (1.00 g, 3.13 mmol, 1 eq.) in methanol (14 mL) was degassed under argon atmosphere, and palladium on carbon (160 mg, 0.15 mmol, 0.05 eq.) was added. The solution was stirred for 90 minutes at room temperature under hydrogen. The resulting mixture was filtered twice and evaporated under reduced pressure to give **22** a white solid (655 mg, 2.86 mmol, 91 %). ^{1}H NMR (MeOD, 500 MHz): δ 4.48-4.46 (m, 1H), 3.74-3.70 (m, 1H), 3.65 (d, *J* = 10 Hz, 1H), 3.20 (m, 1H), 2.81 (s, 2H), 2.35 (d, *J* = 15.0, 1H), 2.18 (d, *J* = 15.0, 1H), 1.09 (s, 9H); ^{13}C NMR (MeOD, 125 MHz): δ 175.8, 173.6, 70.8, 59.0, 57.4, 47.5, 38.5, 32.7, 30.2. HRMS (ESI) m/z: [M+1] calculated for $C_{11}H_{20}NO_4$: 230.1392; found: 230.1417**.**

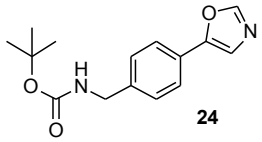
4-(Oxazol-5-yl)benzonitrile, 23



23

23 was synthesized as described previously.[12]

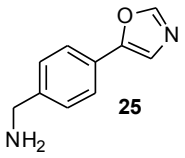
***tert*-Butyl 4-(oxazol-5-yl)benzylcarbamate, 24**



24

24 was synthesized as described previously.[12]

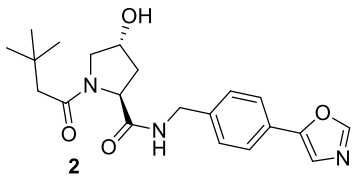
(4-(Oxazol-5-yl)phenyl)methanamine, 25



25

25 was synthesized as described previously.[12]

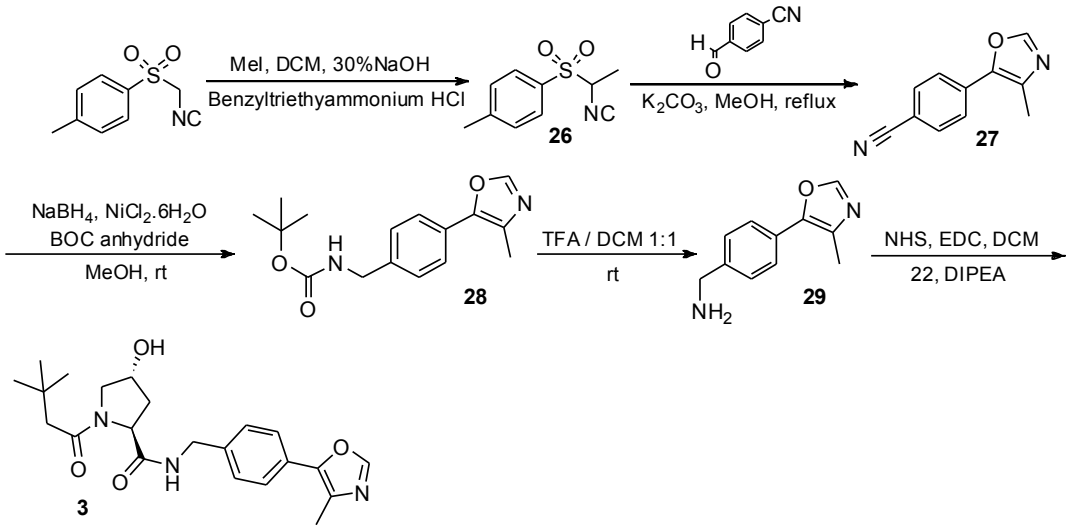
(2*S*,4*R*)-1-(3,3-dimethylbutanoyl)-4-hydroxy-*N*-(4-(oxazol-5-yl)benzyl)pyrrolidine-2-carboxamide, 2



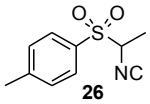
2

Following **general method C**, from **22** (60 mg, 0.26 mmol, 1 eq.) and **25** (66 mg, 0.31 mmol, 1.2 eq.), ligand **2** was obtained as white crystals (77 mg, 0.20 mmol, 76 %). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.83 (s, 1H), 7.57 (t, *J* = 8.0, 1H), 7.55 – 7.43 (m, 2H), 7.24 (d, 2H, *J* = 8.1 Hz), 4.69 (dd, 1H, *J* = 8.3, 5.0 Hz), 4.56 (qu, 1H, *J* = 5.6 Hz), 4.39 (dd, 1H, *J* = 15.2, 5.6 Hz), 4.29 (dd, 1H, *J* = 15.2, 5.6 Hz), 3.60 (dd, 1H, *J* = 10.7, 5.6 Hz), 3.43 (dd, 1H, *J* = 10.7, 5.6 Hz), 2.76 (s, 1H), 2.55 (dt, 1H, *J* = 13.5, 5.3 Hz), 2.20 (d, 1H, *J* = 13.4 Hz), 2.16 – 2.01 (m, 2H), 1.92 (ddd, 1H, *J* = 13.5, 8.3, 5.4 Hz), 0.93 (s, 9H); ^{13}C NMR ($CDCl_3$, 101 MHz): δ 173.2, 171.6, 151.8, 150.8, 139.4, 128.6, 127.1, 125.0, 121.8, 70.6, 58.7, 56.2, 47.3, 43.6, 36.3, 32.1, 30.3; HRMS (ESI) m/z: [M+1] calculated for $C_{21}H_{28}N_3O_4$: 386.2080; found: 386.2086.

Synthesis of **3**:



1-((1-isocyanoethyl) sulfonyl)-4-methylbenzene, 26

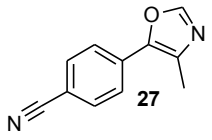


26

To toluenesulfonylmethyl isocyanide (TosMIC) (600 mg, 3.09 mmol, 1 eq.) dissolved in CH_2Cl_2 (10 mL), under argon, was added methyl iodide (877 mg, 6.18 mmol, 2 eq.) and benzyl triethylammonium chloride (140 mg, 0.62 mmol, 0.2 eq.). The resulting mixture was cooled to 0°C and 30 % NaOH solution (10 mL) was added. The reacting biphasic mixture was stirred vigorously for 1 hour, after this methyl iodide (439 mg, 3.09 mmol, 1 eq.) was added and the mixture was stirred vigorously. After 2 hours methyl iodide was added (439

mg, 3.09 mmol, 1 eq.) and was stirred vigorously for a further 3 hours. The reaction mixture was diluted with water (40 mL) and extracted with DCM (3 × 25 mL). The combined organic phases were dried over $MgSO_4$ and evaporated under reduced pressure to give a brown oil **(26)**, solvated with CH_2Cl_2 (794 mg), which was used directly for the next step.

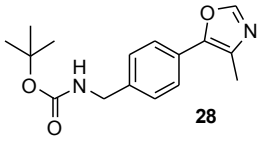
4-(4-methyloxazol-5-yl) benzonitrile, 27



27

To a solution of **26** (645 mg 3.07 mmol, 1.5 eq.) in MeOH (4 mL), 4-cyanobenzaldehyde (268 mg, 2.0 mmol, 1 eq.) and K_2CO_3 (600 mg, 4.30 mmol, 2 eq.) were added. The resulting mixture was heated under reflux for 4 hours, then 5 % HCl (10 mL) was added. This mixture was extracted with DCM (3 × 25 mL), and the combined organic phases were dried over $MgSO_4$ and evaporated under reduced pressure to give a brown solid (306 mg) which was purified by flash column chromatography to give the product as a yellow solid (262 mg, 1.22 mmol, 61 %) ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.82 (d, *J* = 0.6 Hz, 1H), 7.65 (m, 4H), 2.42 (d, *J* = 0.6 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 150.30, 144.35, 134.76, 133.36, 133.03, 125.83, 118.95, 111.43, 14.04. HRMS (ESI) m/z: [M+1] calculated for $C_{11}H_9N_2O$: 185.0719; found: 185.0715.

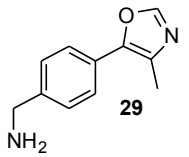
***Tert*-butyl (4-(4-methyloxazol-5-yl) benzyl) carbamate, 28**



28

To a solution of **27** (68 mg, 0.37 mmol, 1 eq) in anhydrous MeOH (3 mL) was added $NiCl_2$ (9 mg, 0.036 mmol, 0.01 eq) and di *tert*-butyl dicarbonate (160 mg, 0.73 mmol, 2 eq). The resulting mixture was cooled to 0°C, and $NaBH_4$ (110 mg, 2.93 mmol, 8 eq) was added portionwise. The mixture was then warmed up to room temperature and stirred for 1.5 hours, filtered through celite and the solvent removed under reduced pressure to give the product **28**, solvated in methanol, as a pale yellow solid (380 mg) which was used directly for the next step.

(4-(4-methyloxazol-5-yl) phenyl) methanamine, 29



29

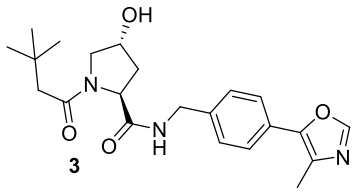
Method 1

To **28** (380 mg, solvated in MeOH) was added 10 % (v/v) TFA in DCM (5 mL) and the mixture was stirred for 16 hours. The solvent was then evaporated, 5 % NaOH (5 mL) was added and this was extracted with CH_2Cl_2 (3 × 10 mL). The combined organic phases were dried over $MgSO_4$ and evaporated under reduced pressure to give **29** as a yellow solid (41 mg, 0.22 mmol, 59% over 2 steps).

Method 2

A solution of **27** (80 mg, 0.44 mmol, 1 eq.) in anhydrous MeOH (3 mL) was cooled to 0°C under argon. $CoCl_2$ (155 mg, 0.65 mmol, 1.5 eq.) was added, followed by portionwise addition of $NaBH_4$ (82 mg, 2.17 mmol, 5 eq.). The mixture was stirred for 1.5 h at room temperature, and then the reaction was quenched with water (10 mL) and ammonium hydroxide (5 mL). The mixture was extracted with chloroform (3 × 25 mL) and the combined organic phases were dried over $MgSO_4$ and evaporated under reduced pressure to give a brown oil, which was purified by flash column chromatography to yield the product **29** as a yellow solid (38 mg, 0.20 mmol, 46 %) that matched the reported spectral data.[12]

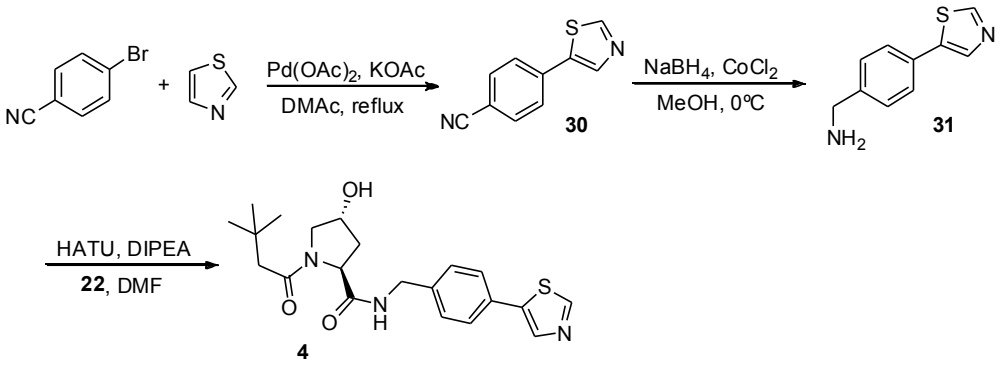
(2*S*,4*R*)-1-(3,3-dimethylbutanoyl)-4-hydroxy-*N*-(4-(4-methyloxazol-5-yl)benzyl) pyrrolidine-2-carboxamide, 3



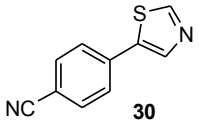
3

Following **general method C**, from **22** (43 mg, 0.19 mmol, 1 eq.) and **29** (39 mg, 0.21 mmol, 1.2 eq.), ligand **3** was obtained as a white solid (25 mg, 0.063 mmol, 33 %). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.75 (s, 1H), 7.46 (d, *J* = 8.0 Hz, 2H), 7.26 (d, *J* = 8.0 Hz, 2H), 4.69 (dd, *J* = 8.3, 5.0 Hz, 1H), 4.66 – 4.48 (*m*, 1H), 4.40 (dd, *J* = 15.2, 6.2 Hz, 1H), 4.30 (dd, 1H, J = 15.2, 5.6 Hz), 3.61 (dd, *J* = 10.7, 5.3 Hz, 1H), 3.45 (m, 1H), 2.56 (dt, *J* = 13.1, 5.3 Hz, 2H), 2.35 (d, *J* = 2.8 Hz, 3H), 2.21 (d, *J* = 13.8 Hz, 1H), 2.11 (m, 2H), 0.93 (s, 9H); ^{13}C NMR ($CDCl_3$, 101 MHz,): δ 173.2, 171.6, 149.3, 145.9, 138.4, 131.6, 128.4, 126.1, 70.7, 58.7, 56.2, 47.3, 43.6, 36.3, 32.1, 30.4, 13.6; HRMS (ESI) m/z: [M+1] calculated for $C_{22}H_{30}N_3O_4$: 400.2236; found: 400.2280.

Synthesis of **4**:

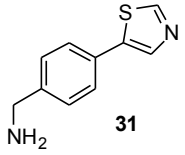


4-(thiazol-5-yl)benzonitrile, 30



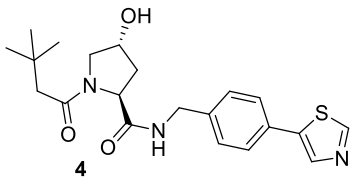
To a solution of 4-bromobenzonitrile (0.75 g, 4.1 mmol, 1 eq.) and $P(OAc)_2$ (4 mg, 0.016 mmol, 0.4 mol%) in dimethylacetamide (4 mL) were added KOAc (0.81 g, 8.2 mmol, 2 eq.) and thiazole (0.70 g, 0.6 mL, 8.2 mmol, 2 eq.). The resulting mixture was heated to 130°C and stirred overnight. The mixture was diluted with water and extracted with DCM (3x). The combined organic phases were dried over $MgSO_4$ and evaporated under reduced pressure to give the corresponding crude, which was purified by flash column chromatography the product **30** as a lime solid (0.40 g, 2.15 mmol, 53%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.78 (d, J = 0.7 Hz, 1H), 8.11 (d, J = 0.7 Hz, 1H), 7.64 (d, J = 8.9 Hz, 2H), 7.61 (d, J = 8.9 Hz, 2H); ^{13}C NMR ($CDCl_3$, 101 MHz,): δ 154.1, 141.1, 137.8, 136.0, 133.3, 128.3, 118.8, 112.3; HRMS (ESI) m/z: [M+1] calculated for $C_{10}H_7N_2S$: 187.0330; found: 187.0330.

(4-(thiazol-5-yl)phenyl)methanamine, 31



A solution of **30** (270 mg, 1.4 mmol, 1 eq.) in methanol (15mL) was cooled to 0°C. $CoCl_2$ (282 mg, 2.2 mmol, 1.5 eq) was added, followed by portionwise addition of $NaBH_4$ (274 mg, 7.2 mmol, 5 eq). The resulting mixture was stirred for 90 minutes, the reaction was quenched with water and ammonium hydroxide and the mixture extracted with chloroform (6X). The combined organic phases were dried over $MgSO_4$ and evaporated under reduced pressure to give a dark brown oil which was purified by flash column chromatography to yield the product **31** as a yellow oil (76.5 mg, 0.40 mmol, 29 %). ^{1}H NMR (MeOD, 500 MHz): δ 8.88 (s, 1H), 8.09 (s, 1H), 7.57 (d, J = 10.0 Hz, 2H), 7.35 (d, J = 10 Hz, 2H), 3.78 (s, 2H); ^{13}C NMR (MeOD, 125 MHz): δ 154.3, 144.2, 140.9, 139.4, 130.9, 129.4, 128.1, 46.3; HRMS (ESI) m/z: [M+1] calculated for $C_{10}H_{11}N_2S$: 191.0643; found: 191.0640.

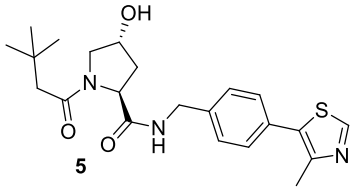
(2*S*,4*R*)-1-(3,3-dimethylbutanoyl)-4-hydroxy-*N*-(4-(thiazol-5-yl)benzyl)pyrrolidine-2-carboxamide, 4



4

Following the **general method A**, from **31** (99 mg, 0.52 mmol, 1 eq.) and **22** (119 mg, 0.52 mmol, 1 eq.) ligand **4** was obtained as a yellow solid (115 mg, 0.29 mmol, 55%). ^{1}H NMR (MeOD, 400 MHz): δ 8.90 (s, 1H), 8.10 (s, 1H), 7.57 (d, *J* = 8.0 Hz, 2H), 7.38 (d, *J* = 8.0 Hz, 2H), 4.51 (t, *J* = 8.0 Hz, 1H), 4.44-4.34 (m, 2H), 3.73 (dd, *J* = 12.0, 4.0 Hz, 1H), 3.60 (d, *J* = 12.0, 1H), 3.27 (s, 4H), 2.31-2.19 (m, 3H), 1.03 (s, 9H); ^{13}C NMR (MeOD, 101 MHz): δ 175.0, 173.7, 154.4, 140.9, 139.4, 131.0, 130.0, 129.3, 128.0, 71.0, 60.5, 57.7, 47.7, 43.6, 39.2, 32.7, 30.4; HRMS (ESI) m/z: [M+1] calculated for $C_{21}H_{28}N_3O_3S$: 402.1851; found: 402.1860.

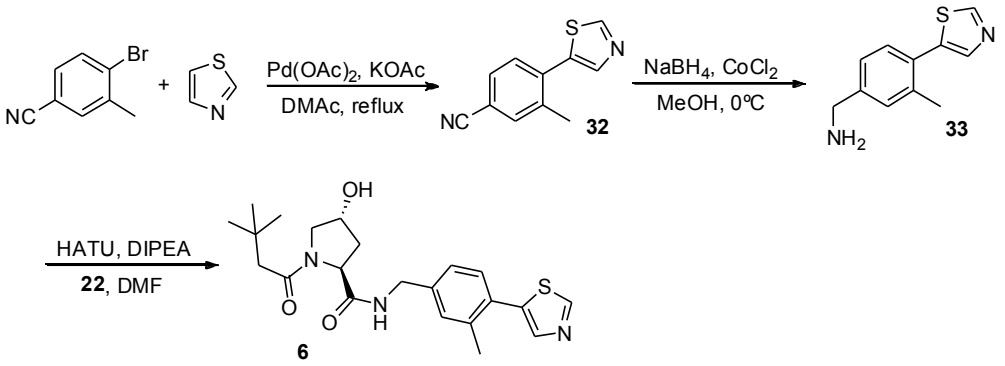
(2*S*,4*R*)-1-(3,3-dimethylbutanoyl)-4-hydroxy-*N*-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide, 5



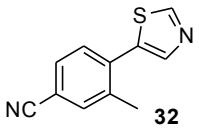
5

Following **general method C**, from **22** (91 mg, 0.40 mmol, 1 eq.) and **18** [synthesis described in the main text] (105 mg, 0.51 mmol, 1.2 eq.) ligand **5** was obtained as pale yellow crystal (109 mg, 0.26 mmol, 66%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.60 (s, 1H), 7.36 – 7.18 (m, 4H), 4.69 (dd, 1H, *J* = 8.2, 5.2 Hz), 4.56 (m, 1H,), 4.41 (dd, 1H, *J* = 15.1, 6.3 Hz), 4.30 (dd, 1H, *J* = 15.1, 5.6 Hz), 3.61 (dd, 1H, *J* = 10.5, 5.2 Hz), 3.49 – 3.37 (m, 1H), 3.08 (s, 1H), 2.54 (dt, 1H *J* = 13.2, 5.3 Hz), 2.44 (s, 3H), 2.21 (d, 1H, *J* = 13.8 Hz), 2.08 (d, 1H, *J* = 13.8 Hz), 1.92 (m, 1H), 0.93 (s, 9H); ^{13}C NMR ($CDCl_3$, 101 MHz): δ 173.2, 171.7, 150.7, 148.8, 138.6, 132.1, 131.3, 129.9, 128.3, 70.6, 58.8, 56.2, 47.3, 43.5, 36.4, 32.1, 30.3, 16.4; HRMS (ESI) m/z: [M+1] calculated for $C_{22}H_{30}N_3O_3S$: 416.2000; found: 416.1920.

Synthesis of **6**:

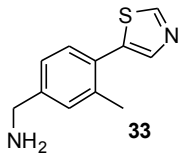


3-methyl-4-(thiazol-5-yl)benzonitrile, 32.



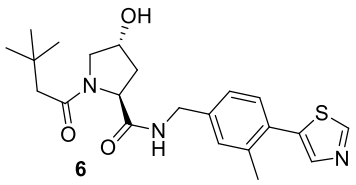
To a solution of 4-bromo-3-methylbenzonitrile (0.62 g, 3.2 mmol, 1 eq.) and $Pd(OAc)_2$ (3 mg, 0.013 mmol, 0.4 mol%) in dimethylacetamide (3 mL) were added KOAc (0.62 g, 6.3 mmol, 2 eq.), and thiazole (0.54 g, 0.45 mL, 6.3 mmol, 2 eq.). The resulting mixture was heated to 130°C and stirred overnight. Then the mixture was diluted with water and extracted with DCM (3x). The combined organic phases were dried over $MgSO_4$ and evaporated under reduced pressure to give the product **32** as a beige solid (0.58 g, 2.90 mmol, 91%). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 8.91 (s, 1H), 7.92 (s, 1H), 7.9 (s, 1H), 7.54 (dd, *J* = 10, 5 Hz, 1H), 7.48 (d, 1H, *J* = 5 Hz), 2.44 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz): 153.2, 142.8, 137.9, 135.5, 134.4, 131.4, 129.9, 118.6, 112.4, 21.2; HRMS (ESI) m/z: [M+1] calculated for $C_{11}H_9N_2S$: 201.0486; found: 201.0487.

(3-methyl-4-(thiazol-5-yl)phenyl)methanamine, 33



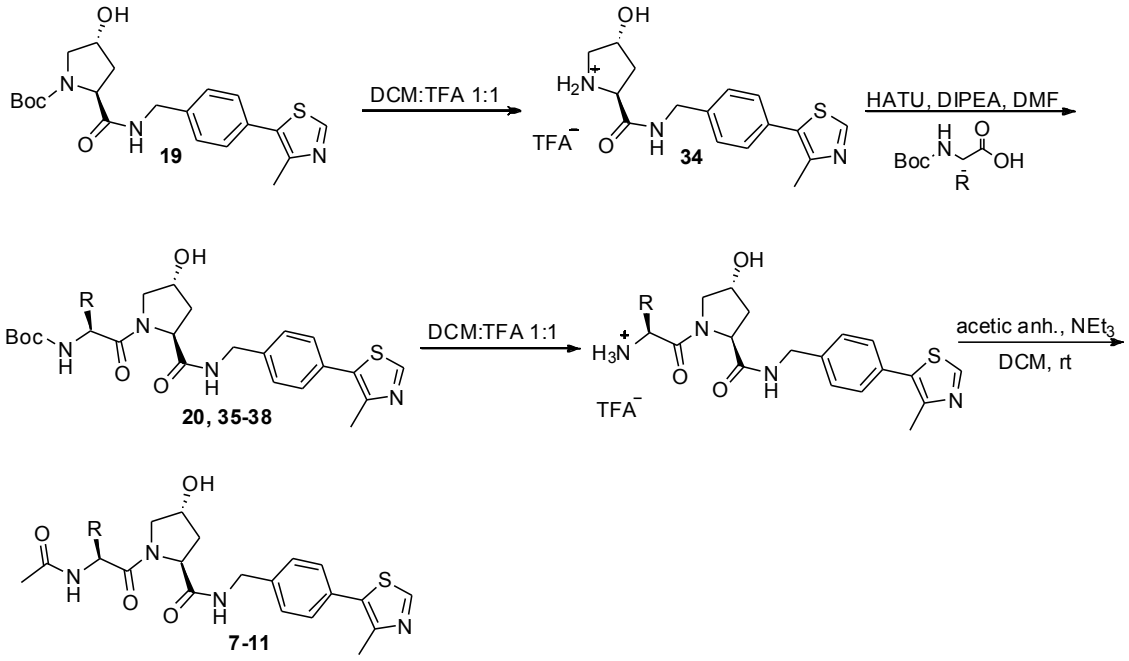
A solution of **32** (545 mg, 2.7 mmol, 1 eq.) in methanol (25mL) was cooled to 0°C. $CoCl_2$ (530 mg, 4.1 mmol, 1.5 eq.) was added, followed by portionwise addition of $NaBH_4$ (516 mg, 7.2 mmol, 5 eq.). The resulting mixture stirred for 90 minutes, then the reaction was quenched with water and ammonium hydroxide and the mixture extracted with chloroform (6X). The combined organic phases were dried over $MgSO_4$ and evaporated under reduced pressure to give a dark brown oil which was purified by flash column chromatography sto yield the product **33** as a dark yellow oil (214 mg, 1.05 mmol, 39 %). ^{1}H NMR (MeOD, 500 MHz): δ 8.97 (s, 1H), 7.80 (s, 1H), 7.31-7.18 (m, 3H), 3.76 (s, 2H), 2.34 (s, 3H); ^{13}C NMR (MeOD, 125 MHz): δ 155.1, 144.5, 142.1, 139.2, 137.8, 132.0, 131.9, 131.1, 129.8 126.4, 46.3, 21.2. HRMS (ESI) m/z: [M+1] calculated for $C_{11}H_{13}N_2S$: 205.0799; found: 205.0797.

(2*S*,4*R*)-1-(3,3-dimethylbutanoyl)-4-hydroxy-*N*-(3-methyl-4-(thiazol-5-yl)benzyl)pyrrolidine-2-carboxamide, 6

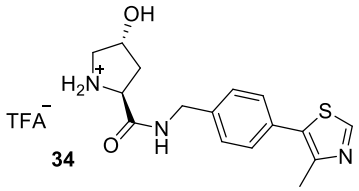


Following the **general method A**, from **33** (120 mg, 0.59 mmol, 1 eq.) and **22** (135 mg, 0.59 mmol, 1 eq.) ligand **6** was obtained as a yellow solid (140 mg, 0.34 mmol, 58%). ^{1}H NMR (MeOD, 400 MHz): δ 8.98 (s, 1H), 7.88 (s, 1H), 7.30-7.28 (m, 2H), 7.19 (d, *J* = 8.0 Hz, 1H), 4.51 (t, *J* = 8.0 Hz, 1H), 4.43-4.31 (m, 2H), 3.73 (dd, *J* = 8.0, 4.0 Hz, 1H), 3.59 (d, *J* = 12 Hz, 1H), 3.27 (s, 2H), 2.32 (s, 3H), 2.30 (d, *J* = 16.0 Hz, 1H), 2.20 (d, *J* = 16.0 Hz, 2H), 1.03 (s, 9H); ^{13}C NMR (MeOD, 101 MHz): δ 175.0, 173.7, 155.1, 142.1, 140.9, 139.2, 137.7, 131.8, 131.0, 129.9, 126.3, 80.0, 60.5, 57.7, 47.7, 43.6, 39.2, 32.7, 30.4, 21.2; HRMS (ESI) m/z: [M+1] calculated for $C_{22}H_{30}N_3O_3S$: 416.2008; observed: 416.1997.

General synthesis of **7-11**:

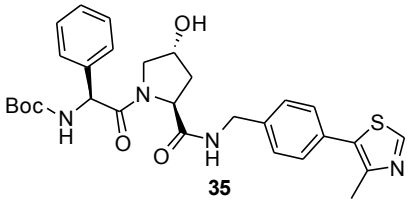


(2*S*,4*R*)-4-hydroxy-*N*-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide, 34



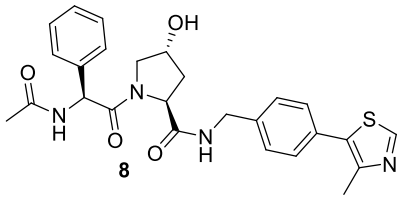
A solution of compound **19** [synthesis described in the main text] (340 mg, 0.81 mmol) in 1:1 TFA:DCM (8 mL) was stirred at room temperature for 30 minutes. The mixture was evaporated under reduced pressure to give the intermediate **34** (TFA salt) without futher purification as a brown oil (330 mg, 0.77 mmol, 98%) that matched the reported spectral data.[13]

***tert*-butyl((*S*)-2-((2*S*,4*R*)-4-hydroxy-2-((4-(4-methylthiazole-5-yl)benzyl)carbamoyl)pyrrolidin-1-yl)-2-oxo-1-phenylethyl)carbamate, 35**



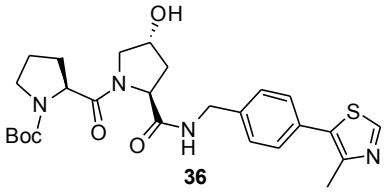
Following the **general method A**, from **34** (338 mg, 0.79 mmol, 1 eq.) and Boc-*L*-phenylglycine (197 mg, 0.79 mmol, 1 eq.), compound **35** was obtained as a yellow solid (431 mg, 0.79 mmol, quantitative yield), which was used directly for the next step.

(2*S*,4*R*)-1-((*S*)-2-acetamido-2-phenylacetyl)-4-hydroxy-*N*-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide, 8



A solution of compound **35** (257 mg, 0.48 mmol) in 1:1 TFA:DCM (5 mL) was stirred at room temperature for 30 minutes. The mixture was evaporated under reduced pressure to give the corresponding intermediate (TFA salt) which part of it was used directly for the next step. Following the **general method B**, from the corresponding deprotected amine (TFA salt, 0.081 mg, 0.15 mmol, 1 eq.), ligand **8** was obtained as a yellow solid (50.5 mg, 0.10 mmol, 72%). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 8.66 (s, 1H), 7.38-7.25 (m, 9H), 7.01-6.99 (t, 1H, *J* = 5.0 Hz), 5.72-5.70 (d, 1H, *J* =10.0 Hz), 4.74-4.71 (t, 1H, *J* = 5.0Hz), 4.43-4.40 (t, 2H, *J* = 10.0 Hz), 3.70-3.67 (d, 1H, *J* = 15 Hz), 3.21-3.18 (q. 1H), 3.13-3.08 (m, 5H), 2.51 (s, 3H), 1.95 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 170.5, 170.1, 169.3, 150.3, 148.6, 137.9, 136.2, 131.2, 129.5, 129.2, 128.6, 127.9, 70.2, 59.2, 55.4, 55.2, 45.6, 43.2, 36.4, 23.1, 16.11, 8.5. HRMS (ESI) m/z: [M+1] calculated for $C_{26}H_{29}N_4O_4S$: 493.1909; observed: 493.1902.

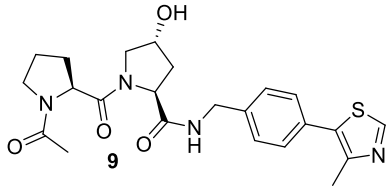
(*S*)-*tert*-butyl-2-((2*S*,4*R*)-4-hydroxy-2-((4-(4-methylthiazole-5-yl)benzyl)carbamoyl)pyrrolidin-1-carbonyl)-pyrrolidine-1-carboxylate, 36



36

Following the **general method A**, from **34** (163 mg, 0.38 mmol, 1 eq.) and Boc-*L*-proline (81.4 mg, 0.38 mmol, 1 eq.), compound **36** was obtained as a yellow solid (194 mg, 0.38 mmol, quantitative yield), which was used directly for the next step.

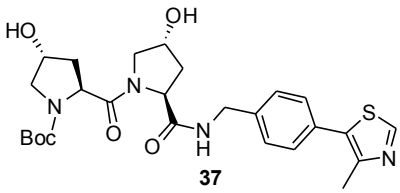
(2*S*,4*R*)-1-(1-acetylpyrrolidine-2-carbonyl)-4-hydroxy-*N*-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide, 9



9

A solution of compound **36** (194 mg, 0.38 mmol) in 1:1 TFA:DCM (4 mL) was stirred at room temperature for 30 minutes. The mixture was evaporated under reduced pressure to give the corresponding intermediate (TFA salt), which part of it was used directly for the next step. Following the **general method B**, from the corresponding deprotected amine (TFA salt, 176 mg, 0.34 mmol, 1 eq.), ligand **9** was obtained as a colourless oil (94 mg, 0.20 mmol, 61%). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 8.66 (s, 1H), 7.68-7.66 (t, 1H, *J* = 5.0 Hz), 7,33-7-28 (dd, 4H, *J* = 5.0 Hz), 4.68-4.65 (t, 1H, *J* = 5.0 Hz), 4.58-4.55 (m, 1H), 4.46-4.35 (m, 4H), 3.99-3.97 (d, 1H, *J* = 10.0 Hz), 3.59-3.45 (m, 4H), 2.48 (s, 3H), 2.28-2.08 (m, 5 H), 1.97 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 172.1, 171.5, 169.7, 150.3, 148.3, 138.6, 131.7, 130.7, 129.4, 127.8 , 70.5, 58.8, 57.8, 55.6, 48.3, 43.0, 36.9, 29.1, 24.9, 22.1, 15.9; HRMS (ESI) m/z: [M+1] calculated for $C_{23}H_{29}N_4O_4S$: 457.1909; observed: 457.1902.

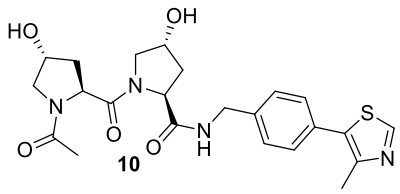
(2*S*,4*R*)-*tert*-butyl-4-hydroxy-2-((2*S*,4*R*)-4-hydroxy-2-((4-(4-methylthiazol-5-yl)carbamoyl)pyrrolidine-1-carbonyl)pyrrolidine-1-carboxylate, 37



37

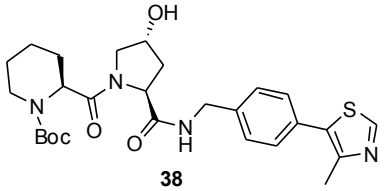
Following the **general method A**, from **34** (220 mg, 0.53 mmol, 1 eq.) and Boc-*L*-4-hydroxyproline (123 mg, 0.53 mmol, 1 eq.), compound **37** was obtained as a yellow solid (120 mg, 0.23 mmol, 43%), which was used directly for the next step.

(2*S*,4*R*)-1-((2*S*,4*R*)-1-acetyl-4-hydroxypyrrolidine-2-carbonyl)-4-hydroxy-*N*-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide, 10



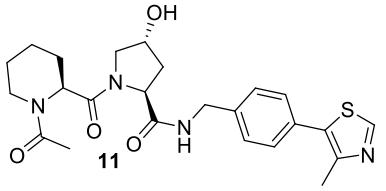
A solution of compound **37** (120 mg, 0.23 mmol) in 1:1 TFA: DCM (3 mL) was stirred at room temperature for 30 minutes. The mixture was evaporated under reduced pressure to give the corresponding intermediate (TFA salt), which was used directly for the next step. Following the **general method B**, from the corresponding deprotected amine (TFA salt, 100 mg, 0.23 mmol, 1 eq.), ligand **10** was obtained as a white solid (55 mg, 0.12 mmol, 62%). ^{1}H NMR (MeOD, 500 MHz): δ 8.87 (s, 1H), 7.43 (q, 4H, *J* = 10.0, 5.0 Hz), 4.76 (t, 1H, *J* = 10.0 Hz), 4.59 (t, 1H, *J* = 10.0 Hz), 4.52-4.46 (m, 3H), 4.43-4.38 (m, 1H), 3.82-3.74 (m, 3H), 3.55-3.52 (m, 1H), 2.48 (s, 3H), 2.27-2.21 (m, 2H), 2.09-2.04 (m, 4H); ^{13}C NMR (MeOD, 125 MHz): δ 174.4, 173.1, 172.3, 152.9, 149.2, 140.3, 133.4, 131.7, 130.5, 129.0, 71.2, 60.9, 58.4, 57.4, 56.4, 43.7, 39.0, 38.3, 22.2, 15.9; HRMS (ESI) m/z: [M+1] calculated for $C_{23}H_{29}N_4O_5S$: 473.1859; observed: 473.1851.

(*S*)-*tert*-butyl-2-((2*S*,4*R*)-4-hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidine-1-carbonyl)piperidine-1-carboxylate, 38



Following the **general method A**, from **34** (162 mg, 0.38 mmol, 1 eq.) and (*S*)-1-(*tert*-butoxycarbonyl) piperidine-2-carboxylic acid (86.3 mg, 0.38 mmol, 1 eq.), compound **38** was obtained as a yellow solid (198 mg, 0.38 mmol, quantitative yield), which was used directly for the next step.

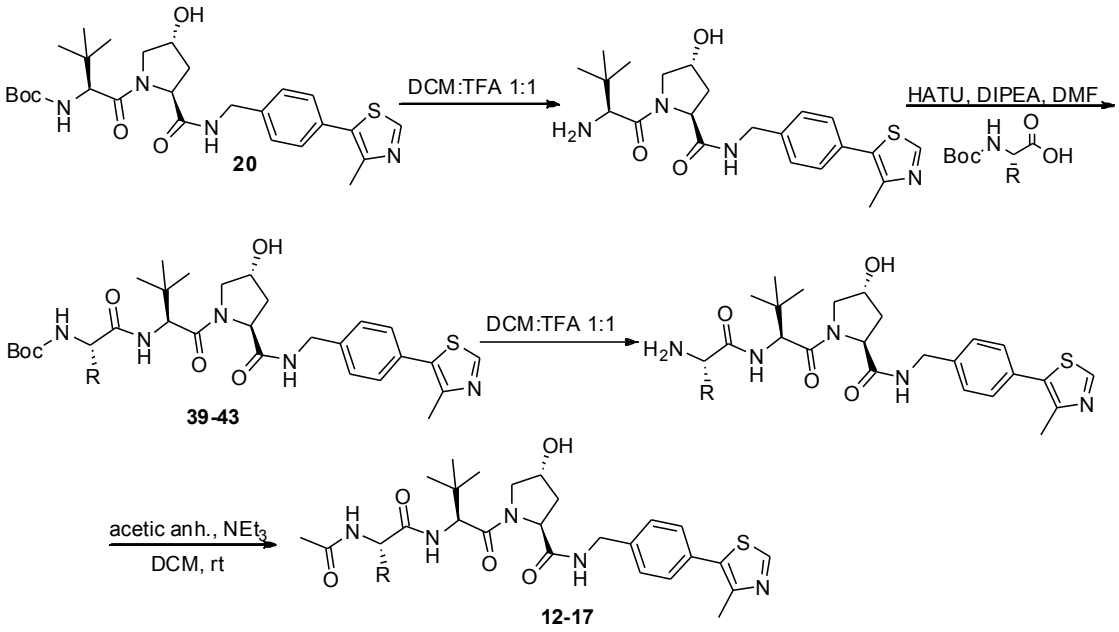
(2*S*,4*R*)-1-((*S*)-1-acetylpiperidine-2-carbonyl)-4-hydroxy-*N*-(4-(4-methylthiazol-5-yl)phenyl)pyrrolidine-2-carboxamide, 11



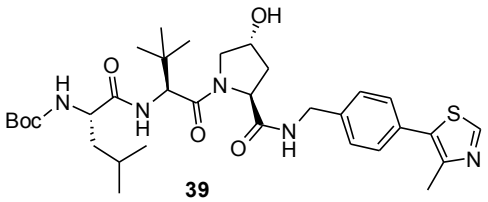
A solution of compound **38** (198 mg, 0.38 mmol, 1 eq.) in 1:1 TFA: DCM (4 mL) was stirred at room temperature for 30 minutes. The mixture was evaporated under reduced pressure to give the corresponding intermediate (TFA salt), which was used directly for the next step. Following the **general method B**, from the corresponding deprotected amine (TFA salt, 203 mg, 0.38 mmol, 1 eq.), ligand **11** was obtained as a brown solid (391.1 mg, 0.27 mmol, 73%).

^{1}H NMR (MeOD, 500 MHz): δ. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 8.64 (s, 1H), 7.63-7.61 (t, 1H, *J* =10.0 Hz), 7,34-7-28 (dd, *J* = 10.0 Hz, 4H), 5.09-5.08 (q, 1H), 4.66-4.63 (t, 1H, *J* = 10.0Hz), 4.47 (s, 1H), 4.40-4.42 (d, 2H, *J* = 5 Hz), 3.83-3.81 (d, 1H, *J* = 10.0 Hz), 3.64-3.54 (m, 4H), 2.48 (s, 3H), 2.32-2.27 (m, 1H), 2.2.13-2.09 (m, 1H) 2.01 (s, 3H), 1.92-1.90 (d, 1H, *J* = 15.0 Hz) 1.67-1.46 (m,5H); ^{13}C NMR ($CDCl_3$, 125 MHz,): δ 172.6, 171.9, 171.5, 150.2, 148.5, 138.5, 131.6, 131.0, 129.4, 128.0, 70.5, 58.7, 55.7, 51.0, 44.3, 43.0, 36.5, 26.0, 24.8, 22.0, 19.1, 16.0; HRMS (ESI) m/z: [M+1] calculated for $C_{24}H_{31}N_4O_4S$: 471.2066; observed: 471.2053.

General Synthesis of **12**-**17**:

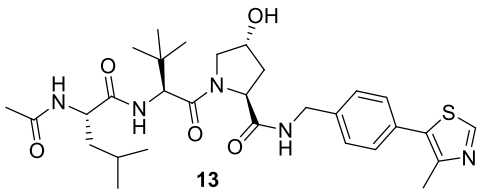


***tert*-butyl-(1-(((S)-1-((2S,4R)-4-hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)amino)-4-methyl-1-oxopentan-2-yl)carbamate, 39**



A solution of compound **20** [synthesis described in the main text] (1.2 g, 2.26 mmol) in 1:1 TFA:DCM (20 mL) was stirred at room temperature for 30 minutes. The mixture was evaporated under reduced pressure to give the corresponding intermediate (TFA salt) which part of it was used directly for the next step. Following the **general method A**, from the deprotected amine (TFA salt, 462 mg, 0.85 mmol, 1 eq.) and Boc-*L*-leucine (196 mg, 0.85 mmol, 1 eq.), compound **39** was obtained as brown solid (392 mg, 0.61 mmol, 70%), which was used directly for the next step.

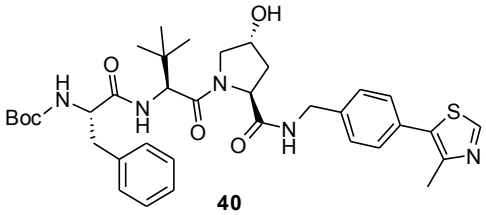
(2*S*,4*R*)-1-((*S*)-2-((*S*)-2-acetamido-4-methylpentanamido)-3,3-dimethylbutanoyl)-4-hydroxy-*N*-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide, 13



13

A solution of compound **39** (247 mg, 0.38 mmol) in 1:1 TFA:DCM (4 mL) was stirred at room temperature for 30 minutes. The mixture was evaporated under reduced pressure to give the corresponding intermediate (TFA salt) which part of it was used directly for the next step. Following the **general method B**, from the corresponding deprotected amine (TFA salt, 100 mg, 0.16 mmol, 1 eq.), ligand **13** was obtained as a white solid (69 mg, 0.18 mmol, 76%). ^{1}H NMR (MeOD, 500 MHz): δ 8.88 (1H, s), 7.43 (dd, *J* = 20.0, 10.0 MHz, 4H), 4.62-4.35 (complex signal, 7H), 3.82 (dd, *J* = 20.0, 10.0 MHz, 2H), 2.47 (s, 3H), 2.22 (m, 1H), 2.08 (m, 1H), 1.98 (s, 3H), 1.65 (m, 1H), 1.56 (t, *J* = 5.0 MHz, 2H), 1.28 (broad s, 1H), 1.03 (s, 9H), 0.92 (dd, *J* = 20.0, 10.0 MHz, 6H). ^{13}C NMR (MeOD, 125 MHz,): δ 174.6, 174.5, 173.6, 172.1, 153.0, 149.1, 140.3, 133.5, 131.6, 130.5, 129.1, 71.2, 60.9, 58.9, 58.2, 53.4, 43.9, 41.5, 39.0, 37.2, 27.1, 26.0, 23.6, 22.5, 22.0, 15.9. HRMS (ESI) m/z: [M+1] calculated for $C_{30}H_{44}N_5O_5S$: 586.3063; observed: 586.3070.

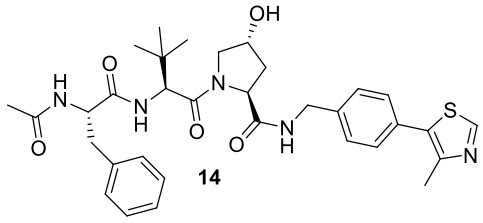
***tert*-butyl-((S)-1-(((S)-1-((2*S*,4*R*)-4-hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)carbamate, 40**



40

A solution of compound **20** [synthesis described in the main text] (1.2 g, 2.26 mmol) in 1:1 TFA:DCM (20 mL) was stirred at room temperature for 30 minutes. The mixture was evaporated under reduced pressure to give the corresponding intermediate (TFA salt) which part of it was used directly for the next step. Following the **general method A**, from the deprotected amine (TFA salt, 419 mg, 0.77 mmol, 1 eq.) and Boc-*L*-phenylalanine (204 mg, 0.77 mmol, 1eq.), compound **40** was obtained as brown solid (492 mg, 0.73 mmol, 94%), which was used directly for the next step.

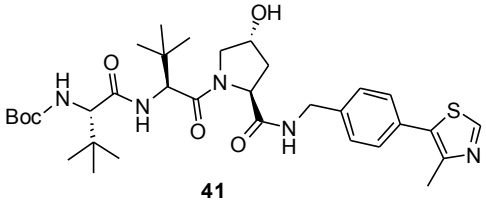
(2*S*,4*R*)-1-((*S*)-2-((*S*)-2-acetamido-3-phenylpropanamido)-3,3-dimethylbutanoyl)-4-hydroxy-*N*-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide, 14



14

A solution of compound **40** (247 mg, 0.38 mmol) in 1:1 TFA: DCM (4 mL) was stirred at room temperature for 30 minutes. The mixture was evaporated under reduced pressure to give the corresponding intermediate (TFA salt) which part of it was used directly for the next step. Following the **general method B**, from the corresponding deprotected amine (TFA salt, 248 mg, 0.36 mmol, 1 eq.), ligand **14** was obtained as a white solid (150 mg, 0.24 mmol, 69%). ^{1}H NMR (MeOD, 500 MHz): δ 8.90 (1H, s), 7.46 (dd, *J* = 20.0, 10.0 MHz, 4H), 7.30-7.21 (m, 5H), 4.66-4.53 (m, 5H), 4.39 (d, *J* = 15.0 MHz, 1H), 3.88-3.83 (m, 2H), 3.12 (dd, *J* =10.0, 5.0 MHz, 1H), 2.88 (dd, *J* = 15.0 MHz, 10.0 MHz, 1H), 2.50 (s, 3H), 2.25 (m, 1H), 2.12 (m, 1H), 1.92 (s, 3H), 1.28 (broad s, 1H), 1.05 (s, 9H). ^{13}C NMR (MeOD, 125 MHz,): δ 174.9, 173.8, 173.7, 172.1, 153.3, 149.5, 140.8, 138.9, 133.9, 132.0, 130.9, 130.7, 130.0, 129.5, 128.2, 71.5, 61.2, 59.3, 58.5, 56.7, 44.2, 39.4, 39.0, 37.5, 27.5, 22.8, 16.3. HRMS (ESI) m/z: [M+1] calculated for $C_{33}H_{42}N_5O_5S$: 620.2907; observed: 620.2909.

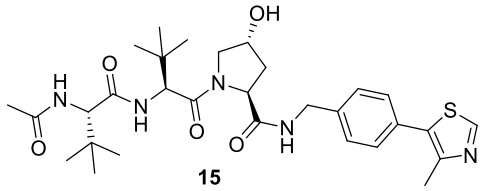
***tert*-butyl-((S)-1-(((S)-1-((2S,4R)-4-hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)amino)-3,3-dimethyl-1-oxobutan-2-yl)carbamate, 41**



41

A solution of compound **20** [synthesis described in the main text] (1.2 g, 2.26 mmol) in 1:1 TFA:DCM (20 mL) was stirred at room temperature for 30 minutes. The mixture was evaporated under reduced pressure to give the corresponding intermediate (TFA salt) which part of it was used directly for the next step. Following the **general method A**, from the deprotected amine (TFA salt, 420 mg, 0.77 mmol, 1 eq.) and Boc-*L-tert*-leucine (179 mg, 0.77 mmol, 1eq.), compound **41** was obtained as brown solid (408 mg, 0.63 mmol, 80%), which was used directly for the next step.

(2*S*,4*R*)-1-((*S*)-2-((*S*)-2-acetamido-3,3-dimethylbutanamido)-3,3-dimethylbutanoyl)-4-hydroxy-*N*-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide, 15

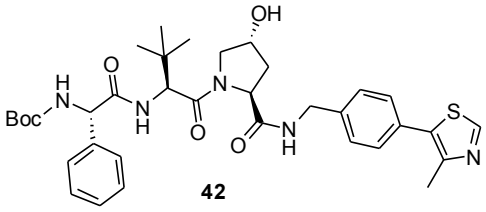


15

A solution of compound **41** (408 mg, 0.63 mmol) in 1:1 TFA:DCM (6 mL) was stirred at room temperature for 30 minutes. The mixture was evaporated under reduced pressure to give the corresponding intermediate (TFA salt) which part of it was used directly for the next step. Following the **general method B**, from the corrsponding deprotected amine (TFA salt, 188 mg, 0.29 mmol, 1 eq.), ligand **15** was obtained as a white solid (132 mg, 0.23 mmol, 79%). ^{1}H NMR (MeOD, 500 MHz): δ 8.80 (1H, s), 7.34 (dd, *J* = 20.0, 10.0 MHz, 4H), 4.55-4.19 (complex signal, 7H), 3.89 (d, *J* = 15.0 MHz, 1H), 3.79 (dd, *J* = 10.0, 5.0 MHz, 1H), 2.48 (s, 3H), 2.20 (m, 1H), 2.08 (m, 1H), 2.01 (s, 3H), 1.28 (broad s, 1H), 1.03 (s, 9H), 0.98 (s, 9H). ^{13}C NMR (MeOD, 125 MHz,): δ 175.0, 173.8, 173.3, 172.2, 153.4, 149.4, 140.8, 134.1,

132.0, 130.9, 129.5, 71.5, 62.9, 61.2, 59.3, 58.6, 44.21, 39.36, 37.22, 35.67, 27.7, 27.50, 22.88, 16.22. HRMS (ESI) m/z: [M+1] calculated for $C_{30}H_{44}N_5O_5S$: 586.3063; observed: 586.3087.

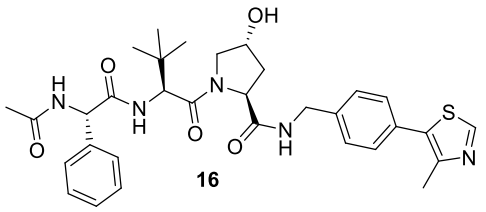
tert-butyl-((S)-2-(((S)-1-((2S,4R)-4-hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)amino)-2-oxo-1-phenylethyl)carbamate, 42



42

A solution of compound **20** [synthesis described in the main text] (400 mg, 0.75 mmol) in 1:1 TFA:DCM (7 mL) was stirred at room temperature for 30 minutes. The mixture was evaporated under reduced pressure to give the corresponding intermediate (TFA salt), which was used directly for the next step. Following the **general method A**, from the deprotected amine (TFA salt, 407 mg, 0.75 mmol, 1 eq.) and Boc-*L*-phenylglycine (251 mg, 0.75 mmol, 1eq.), compound **42** was obtained as brown solid (497 mg, 0.75 mmol, quantitative yield), which was used directly for the next step.

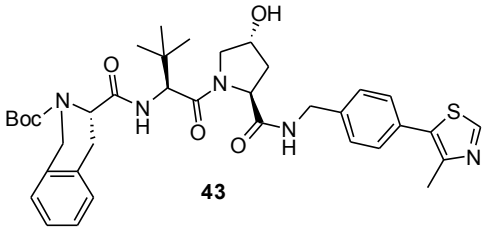
(2*S*,4*R*)-1-((S)-2-((S)-2-acetamido-2-phenylacetamido)-3,3-dimethylbutanoyl)-4-hydroxy-N-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide, 16



16

A solution of compound **42** (408 mg, 0.75 mmol) in 1:1 TFA:DCM (7 mL) was stirred at room temperature for 30 minutes. The mixture was evaporated under reduced pressure to give the corresponding intermediate (TFA salt) which part of it was used directly for the next step. Following the **general method B**, from the corresponding deprotected amine (TFA salt, 501 mg, 0.75 mmol, 1 eq.), ligand **16** was obtained as a white solid (350 mg, 0.58 mmol, 78%). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 8.68 (s, 1H), 7.36-7.19 (m, 10H), 6.69 (d, 1H, *J* = 10.0 Hz), 5.61 (d, 1H, *J* = 10.0 Hz), 4.67 (t, 1H, *J* = 10.0 Hz), 4.58 (d, 1H, *J* = 10.0 Hz), 4.52-4.48 (m, 2H), 4.31 (dd, 1H, *J* = 15.0, 10.0 Hz), 3.91 (d, 1H, *J* = 10 Hz), 3.63 (dd, 1H, *J* = 10.0, 5.0 Hz), 2.50 (s, 3H), 2.44-2.40 (m, 1H), 2.06-2.02 (m, 1H), 1.96 (s, 3H), 0.94 (s, 9H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 171.2, 170.8, 170.7, 170.1, 150.5, 148.6, 138.2, 137.0, 129.6, 129.1, 128.6, 128.3, 127.2, 70.3, 58.5, 58.1, 57.3, 56.9, 45.8, 43.4, 36.5, 35.8, 26.6, 23.2, 16.2, 8.7; HRMS (ESI) m/z: [M+1] calculated for $C_{32}H_{40}N_5O_5S$: 606.2750; observed: 606.2729.

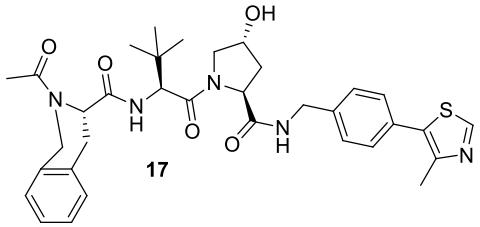
***Tert*-butyl-3-(((*S*)-2-((2*S*,4*R*)-4-hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)carbamoyl)-3,4-dihydroisoquinoline-2(1H)-carboxylate, 43**



43

A solution of compound **20** [synthesis described in the main text] (250 mg, 0.47 mmol) in 1:1 TFA:DCM (5 mL) was stirred at room temperature for 30 minutes. The mixture was evaporated under reduced pressure to give the corresponding intermediate (TFA salt), which it was used directly for the next step. Following the **general method A**, from the deprotected amine (TFA salt, 253 mg, 0.47 mmol, 1 eq.) and 2-(*tert*-butoxycarbonyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (129 mg, 0.47 mmol, 1eq.), compound **43** was obtained as brown solid (135 mg mg, 0.20 mmol, 41%), which was used directly for the next step.

2-acetyl-*N*-((*S*)-1-((2*S*,4*R*)-4-hydroxy-2-((4-(4-methylthiazo-5-yl)benzyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)-1,2,3,4-tetrahydroisoquinoline-3-carboxamide, 17



17

A solution of compound **43** (118 mg, 0.17 mmol) in 1:1 TFA: DCM (2 mL) was stirred at room temperature for 30 minutes. The mixture was evaporated under reduced pressure to give the corresponding intermediate (TFA salt) which part of it was used directly for the next step. Following the **general method B**, from the corresponding deprotected amine (TFA salt, 80 mg, 0.11 mmol, 1 eq.), ligand **17** was obtained as a white solid (51.6 mg, 0.08 mmol, 62%). ^{1}H NMR (MeOD, 500 MHz): δ 8.90 (1H, s), 7.46 (dd, *J* = 20.0, 10.0 MHz, 4H), 7.30-7.21 (m, 5H), 4.66-4.53 (m, 5H), 4.39 (d, *J* = 15.0 MHz, 1H), 3.88-3.83 (m, 2H), 3.12 (dd, *J* =10.0, 5.0 MHz, 1H), 2.88 (dd, *J* = 15.0 MHz, 10.0 MHz, 1H), 2.50 (s, 3H), 2.25 (m, 1H), 2.12 (m, 1H), 1.92 (s, 3H), 1.28 (broad s, 1H), 1.05 (s, 9H). ^{13}C NMR (MeOD, 125 MHz,): δ 174.9, 173.8, 173.7, 172.1, 153.3, 149.5, 140.8, 138.9, 133.9, 132.0, 130.9, 130.7, 130.0, 129.5, 128.2, 71.5, 61.2, 59.3, 58.5, 56.7, 44.2, 39.4, 39.0, 37.5, 27.5, 22.8, 16.3. HRMS (ESI) m/z: [M+1] calculated for $C_{34}H_{42}N_5O_5S$: 632.2907; observed: 632.2928.

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