Selective inhibitors of biotin protein ligase from *Staphylococcus aureus*

Supplementary Information

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Supplementary data

Fig S1: Molecular details of biotinol-5´-AMP (2) binding to *Sa*BPL.

A) 2D depiction of inhibitor **2** bound to *Sa*BPL with hydrogen bonding interactions shown in green dashes and hydrophobic interactions in red. *B*) The corresponding 3D depiction obtained using Chimera with black dash indicating the hydrogen bonding interactions.

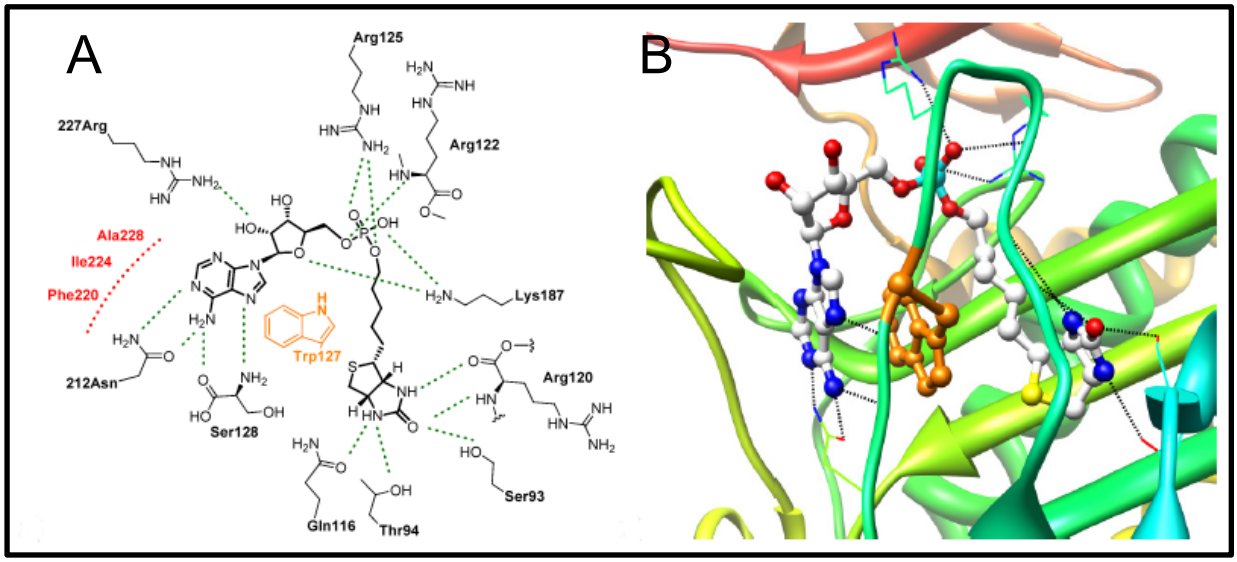


Fig S2: Sequence alignment between structurally determined BPLs and the human homologue.

The amino acid sequences of BPLs for which crystal structures are available in the PDB were aligned using ClustalW. These species are *Staphylococcus aureus* (Sa), *Pyrococcus hirokoshii* (Ph), *Escherichia coli* (Ec), *Mycobacterium tuberculosis* (Mt), *Aquifex aeolicus* (Aa) and *Methanococcus jannaschii* (Mj). Also included in this analysis was the sequence of human BPL, commonly referred to as Holocarboxylase synthetase (HCS). Red triangles indicate hydrogen bonding to biotinol-5'-AMP (**2**), green triangles indicate contact to **2**, orange circles indicate residues involved in salt bridge formation at the dimer interface, black circles indicate dimer hydrogen bonds and blue circles show dimer contact residues for holo-*Sa*BPL.

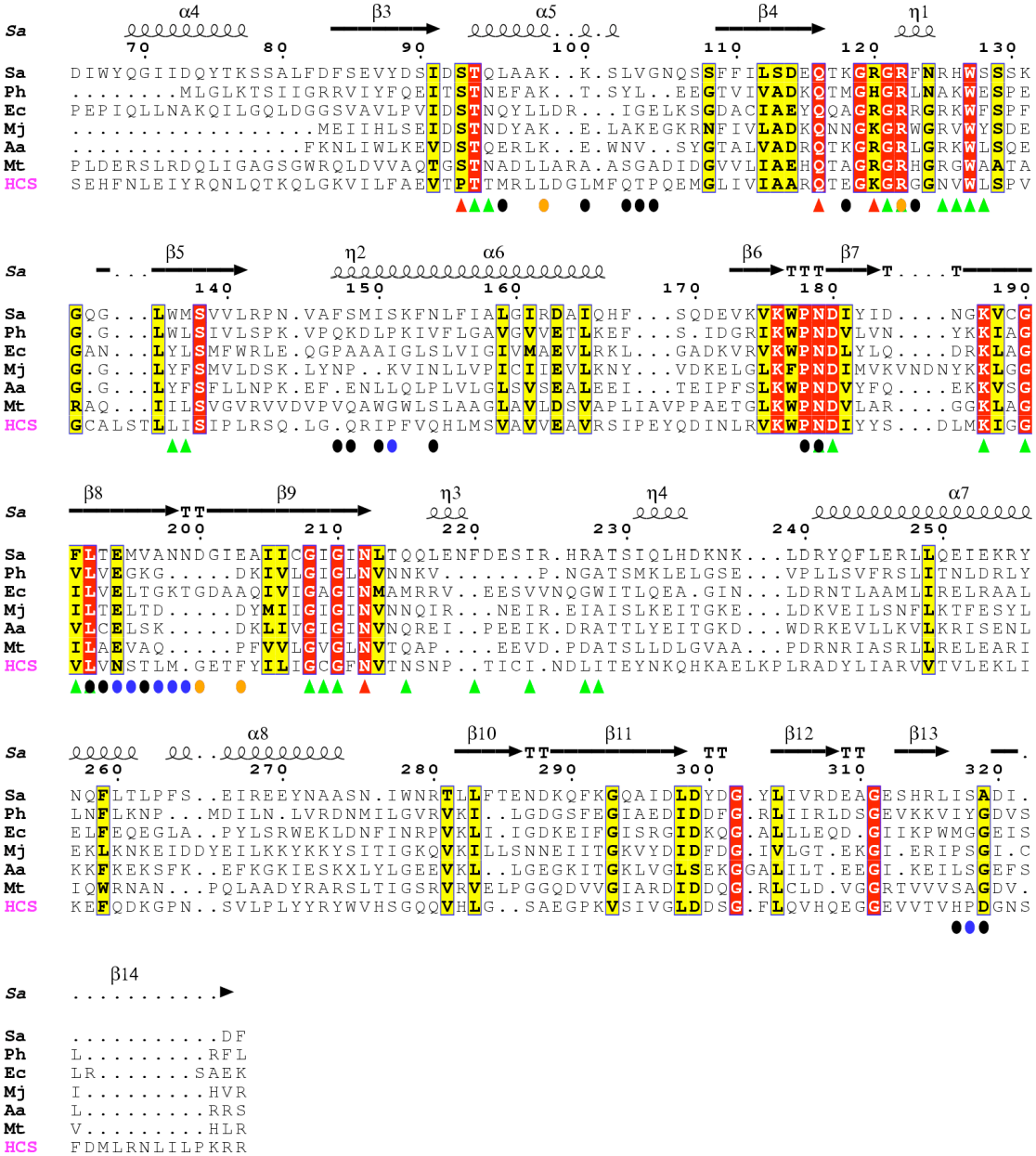


Fig S3: Binding interactions between the ribose ring and Arg 227

The mode of binding of inhibitors (A) biotinol-5´AMP **2** and (B) **7** in the ATP pocket of *Sa*BPL are shown in yellow stick representation. The position of Arg 227 is highlighted in cyan. (*A*) The 2´ hydroxyl group on the ribose ring of **2** hydrogen bonds with the guanidium group on the side chain of Arg 227 (dashed line). (*B*) An isopropylidene protecting group on the diol of the ribose ring cause the side chain of Arg 227 to reposition itself away from the inhibitor, implying the hydrogen bonding interaction is not essential to stabilise the interaction with the inhibitor.

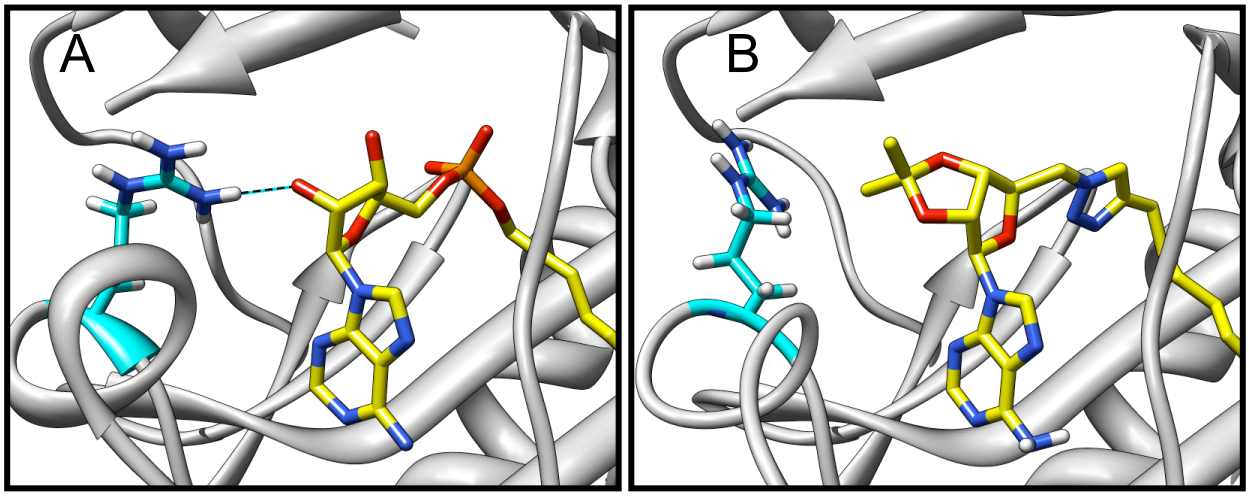


Fig S4: Mechanism of binding for inhibitors 7 and 14.

The figure depicts key residues in the ATP pocket of *Sa*BPL required for binding of inhibitors (*A*) **7** and (*B*) **14** (shown in yellow stick mode). Hydrophobic amino acids are highlighted in orange, and other important residues are shown in white stick. The biotin-binding loop (BBL) and ATP-binding loop (ABL) are also shown. *A.* The adenine moiety buttresses against the side chain of Trp127 for a π – π stacking interaction, as well as hydrophobic interactions with the ABL and hydrogen bonding interactions with Asn 212 and Ser 128. *B.* The benzoxazolone moiety adopts a displaced π interaction with Trp 127, and hydrophobic interactions with the ABL.

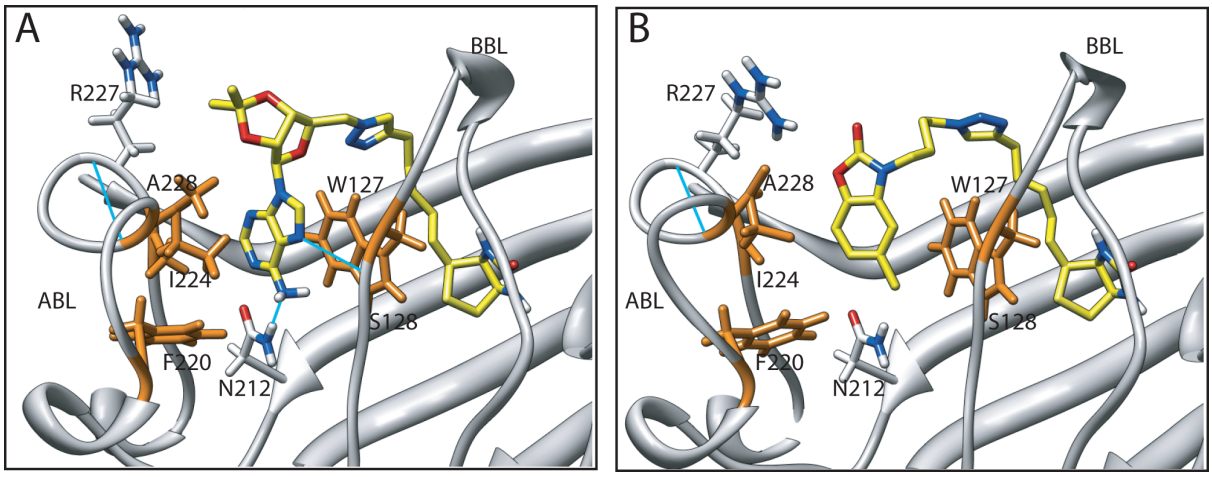


Fig S5: Circular dichroism analysis of *Sa*BPL and Arg 125 muteins.

CD spectra confirmed that wildtype and Arg 125 muteins fold similarly in solution. All three spectra are comprised of double minima at 208 nm and 222 nm, which is characteristic of a predominantly α-helical structure. Therefore, the decrease in catalytic efficiency for the muteins cannot be accounted for by gross perturbations in the secondary structure upon mutation. The chromatographs for wildtype (grey), Arg 125→Ala (red) and Arg 125→Asn (blue) are overlaid.

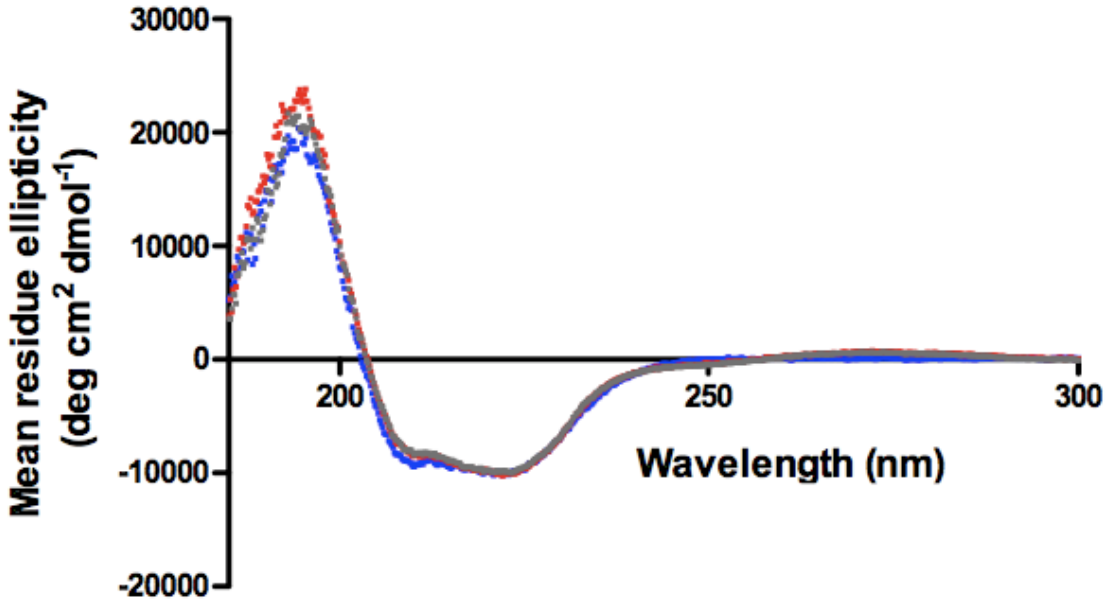


Table S1: Data collection and refinement statistics

	***Sa*BPL with 2**	***Sa*BPL with 7**	***Sa*BPL with 14**
Data collection[a]			
Space group	$P4_22_12$	P 4(2) 2(1) 2	P 4(2) 2(1) 2
Cell dimensions			
a, *b*, *c* (Å)	94.6,94.6, 130.6	93.06 93.06 130.14	93.5 93.5 130.41
a, b, g (°)	90, 90, 90	90 90 90	90 90 90
Resolution (Å)	49.5-2.5 (2.56-2.5)	50-2.77 (2.94-2.77)	58.97 - 3.1 (3.185 – 3.1)
R_{sym} or R_{merge}	4.1 (34.7)	11.7 (71.4)	19.0 (77.9)
I / s*I*	16.6 (2.2)	12.46 (2.60)	7.34 (2.40)
Completeness (%)	99.2 (99.6)	97.9 (99.4)	99.5 (98.0)
Redundancy	11.6 (11.8)	5.46 (5.71)	6.95 (6.94)
Refinement			
Resolution (Å)	49.5-2.5	50 - 2.77	50 - 3.1
No. reflections	20793	14797	10421
R_{work} / R_{free}	21.0/25.5	18.5/22.35	20.284/25.63
No. atoms			
Protein	2614	2602	2613
Ligand/ion	37	39	33
Water	106	85	58
B-factors			
Protein	45.3	46.161	44.183
Ligand/ion	45.1	39.740	33.403
Water	45.2	40.164	32.068
R.m.s. deviations			
Bond lengths (Å)	0.020	0.009	0.0163
Bond angles (°)	1.9	1.543	1.7517

[a] Diffraction data was collected from one crystal for each structure

Table S2: Kinetic analysis of wildtype *S. aureus* BPL and the Arg 125 mutant enzymes

Kinetic properties were determined for the ligands biotin and MgATP.

	Biotin		MgATP	
***Sa*BPL**	k_{cat} (s^{-1}) x 10^3	K_m (μM)	k_{cat} (s^{-1}) x 10^3	K_m (mM)
WT	450 ± 90	1.01 ± 0.16	360 ± 70	0.18 ± 0.03
Arg125 Ala	0.81 ± 0.11	1.08 ± 0.12	0.44 ± 0.06	0.42 ± 0.39
Arg125 Asn	0.84 ± 0.12	1.08 ± 0.14	0.52 ± 0.09	0.35 ± 0.05

Supplementary Experimental Procedures

In vitro biotinylation assays

Quantitation of BPL catalysed ^{3}H-biotin incorporation into the biotin domain substrate was performed as previously described (Polyak et al., 1999; Polyak et al., 2001). The IC_{50} value of each compound was determined from a dose-response curve by varying the concentration of the inhibitor under the same enzyme concentration. The data was analysed with GraphPad Prism software using a non-linear fit of $\log_{10}$ (inhibitor) vs. normalized response. The K_i, the absolute inhibition constant for a compound, was determined using Eq1 (Cheng and Prusoff, 1973):

Eq1. $K_i = IC_{50} / (1 + [S] / K_m)$

where K_m is the affinity of the substrate for the enzyme ([biotin] =1.01 μM, Table S2) and [S] is the substrate concentration ([biotin] =5 μM). The mode of inhibition was investigated by varying the concentrations of inhibitor alongside varying the concentrations of ^{3}H-biotin. The data was plotted as double reciprocal plots and assessed using Lineweaver-Burk analysis. Data reported here are the means of three independent assays (n = 3) ± standard error of the mean. Statistical analysis between two data sets was performed using a two-tailed unpaired t test using GraphPad Prism.

Surface Plasmon Resonance

SPR was performed using a Biacore™ T100 instrument (GE Healthcare). BPL was immobilised on a CM5 sensor chip by amine coupling, as previously described (Mayende et al., 2012). *Sa*BPL (120 μg/mL) in 10 mM sodium acetate buffer (pH 5.8) was coupled onto the surface and 1 M ethanolamine hydrochloride was injected to block any unreacted sites. Typically, 6,500 resonance units of BPL were immobilised on the sensor chip. One channel was left blank which was subtracted from sample channel to allow analysis methods to distinguish between actual and non-specific binding. Experiment was conducted at 25^{o} C at a flow rate of 30 μL/minute with a running buffer containing 10 mM HEPES pH 7.4, 150 mM NaCl and 0.005% (v/v)

surfactant. The concentration of biotin used was 10 μM and the MgATP concentration used was 500 μM.

Circular Dichroism Analysis

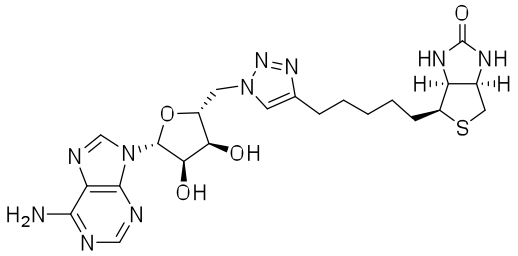
Far UV CD spectra were recorded at 20^{o} C using a Jasco CD J-185 spectrophotometer. Purified wild-type and mutant *Sa*BPL were dialysed overnight against the assay buffer (10 mM $NaPO_4$ pH 8.0) at 4^{o} C prior to analysis. Final concentrations of the proteins used were 0.25 mg/mL (6.5 μM). CD spectra were recorded from 185 nm to 300 nm at 0.2 nm increments, using a 1 mm path length quartz cell. The reported spectra are the average of five scans that were corrected for buffer blanks.

Synthesis and characterisation of chemicals used in this study

General copper azide alkyne cycloaddition procedure:

To a solution of the appropriate azide (1.0 eq) and alkyne (1.0 eq) in acetonitrile (1 mL per 100 mg of alkyne) were added de-ionised water (0.5 mL per 100 mg of alkyne) and copper nano powder (0.2 eq). The reaction mixture was sonicated for 15 min followed by stirring for 4 h at 35 ^{o}C. The reaction mixture was concentrated *in vacuo* and purified by silica gel column chromatography. See individual experiments for details.

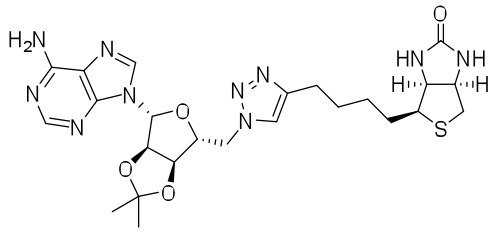
(3a*S*,4*S*,6a*R*)-4-[5-[1-[[(2*R*,3*S*,4*R*,5*R*)-5-(6-Aminopurin-9-yl)-3,4-dihydroxy-tetrahydrofuran-2-yl]methyl]triazol-4-yl]pentyl]-1,3,3a,4,6,6a-hexahydrothieno[3,4-*d*]imidazol-2-one (Compound 5)



To a solution of compound **7** (15 mg, 0.03 mmol) in dichloromethane (1 mL) was added TFA (0.9 mL) and water (0.1 mL) and stirred for 3 h. The reaction mixture was concentrated *in vacuo* and purified by flash chromatography using 20% methanol in dichloromethane to give a white solid (6 mg, 43%).

^{1}H NMR (300 MHz, DMSO-d_6): δ 8.22 (1H, s, Ar**H**), 8.15 (1H, s, Ar**H**), 7.67 (1H, s, Ar^{tri}**H**), 7.37 (2H, bs, ArN**H$_2$**), 6.54 (1H, bs, C(O)N**H**), 6.38 (1H, bs, C(O)N**H**), 5.90 (1H, d, *J* = 5.4 Hz, 1' C**H**), 5.67 (1H, bs, O**H**), 5.54 (1H, bs, O**H**), 4.59-4.73 (3H, m), 4.28-4.33 (1H, m, NHC**H**), 4.20-4.27 (2H, m, 5' C**H$_2$**), 4.11-4.15 (1H, m, NHC**H**), 3.05-3.11 (1H, m, SC**H**), 2.81 (1H, dd, *J* = 5.1, 12.3 Hz, SC**H$_a$**), 2.50-2.59 (3H, m (under DMSO residual peak), ArC^{tri}C**H$_2$**, SC**H$_b$**), 1.65-1.211 (8H, m, 4 x C**H$_2$**); **^{13}C NMR** (150 MHz, DMSO-d_6): δ 162.7, 156.1, 152.6, 149.2, 146.7, 139.8, 122.5, 119.2, 87.7, 82.3, 72.5, 71.1, 70.8, 61.1, 59.2, 55.5, 51.0, 40.0, 28.6, 28.3, 28.2, 24.8; **HRMS** calcd. for (M+ H) $C_{22}H_{31}N_{10}O_4S$: requires 531.2251, found 531.2231.

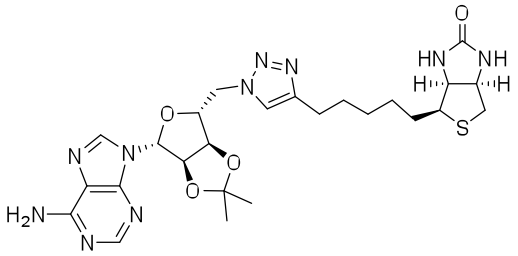
(3a*S*,4*S*,6a*R*)-4-[4-[1-[[(3a*R*,4*R*,6*R*,6a*R*)-4-(6-Aminopurin-9-yl)-2,2-dimethyl-3a,4,6,6a-tetrahydrofuro[3,4-*d*][1,3]dioxol-6-yl]methyl]triazol-4-yl]butyl]-1,3,3a,4,6,6a-hexa hydrothieno[3,4-*d*]imidazol-2-one (Compound 6)



Norbiotin acetylene **17** (15 mg, 0.05 mmol) was reacted with adenosine azide **4** (16 mg, 0.05 mmol) and Cu nanopowder (1 mg, 0.02 mmol) according to CuAAC general procedure. The crude material was purified by flash chromatography eluting with 9% methanol in dichloromethane to yield a white solid, (15 mg, 60%).

^{1}H NMR (600 MHz; 1% CD_3OD, $CDCl_3$): δ 8.17 (1H, s, Ar**H**), 7.81 (1H, s, Ar**H**), 7.12 (1H, s, Ar^{tri}**H**), 5.93 (1H, d, *J* = 3.0 Hz, 1' C**H**), 5.48 (1H, dd, *J* = 3.0, 7.2 Hz, 2' C**H**), 5.02 (0.25H, bs, C(O)N**H**, exchanged with solvent), 4.88 (1H, dd, *J* = 4.2, 15.0 Hz, 5' C$\mathbf{H_a}$), 4.84 (1H, t, *J* = 6.0 Hz, 3' C**H**), 4.57 (1H, dd, *J* = 3.0, 15.0 Hz, 5' C$\mathbf{H_b}$), 4.53-4.54 (1H, m, C**H**NH), 4.43-4.45 (2H, m, 4' C**H**, C**H**NH), 3.18-3.21 (1H, m SC**H**), 2.96 (1H, dd, *J* = 5.4, 13.2 Hz, SC$\mathbf{H_a}$), 2.74 (1H, d, *J* = 13.2 Hz, SC$\mathbf{H_b}$), 2.66-2.70 (2H, m, $ArC^{tri}C\mathbf{H_2}$), 1.70-1.74 (2H, m, C$\mathbf{H_2}$), 1.37 - 1.61 (10H, m); **^{13}C NMR** (150 MHz; 1% CD_3OD, $CDCl_3$): δ 163.9, 156.4, 152.9, 148.7, 148.0, 140.1, 122.9, 115.8, 89.9, 82.8, 82.0, 79.3, 62.2, 59.7, 55.6, 49.9, 49.8, 40.8, 29.6, 28.8, 28.6, 27.2, 25.3, 25.1.

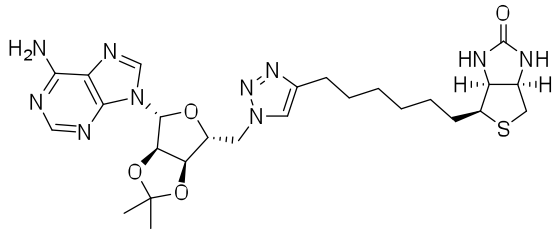
(3a*S*,4*S*,6a*R*)-4-[5-[1-[[(3a*R*,4*R*,6*R*,6a*R*)-4-(6-Aminopurin-9-yl)-2,2-dimethyl-3a,4,6,6a-tetrahydrofuro[3,4-*d*][1,3]dioxol-6-yl]methyl]triazol-4-yl]pentyl]-1,3,3a,4,6,6a-hexa hydrothieno[3,4-*d*]imidazol-2-one (Compound 7)



Biotin acetylene **3** (Corona et al., 2006) (16 mg, 0.066 mmol) was reacted with adenosine azide **4** (20 mg, 0.06 mmol) and Cu nanopowder (1 mg, 0.02 mmol) according to CuAAC general procedure. The reaction mixture was concentrated *in vacuo* and the resulting residue was purified by flash chromatography eluting with 10% methanol in dichloromethane to give a white solid (25 mg, 73%)

^{1}H NMR (300 MHz, $CDCl_3$): δ 8.21 (1H, s, Ar**H**), 7.74 (1H, bs, C(O)N**H**), 7.53 (1H, s, Ar**H**), 7.07 (1H, s, Ar^{tri}**H**), 6.93 (2H, bs, ArN**H$_2$**), 6.00 (1H, d, *J* = 3.6 Hz, 1' C**H**), 5.27 (1H, dd, *J* = 3.6, 8.8 Hz, 2' C**H**), 5.05 (1H, bs, C(O)N**H**), 5.01 (1H, dd, *J* = 3.9, 8.8 Hz, 3' C**H**), 4.91 (1H, dd, *J* = 5.4, 14.4 Hz, 5' C**H$_a$**), 4.52-4.63 (3H, m, 5' C**H$_b$**, 4' C**H**, NHC**H**), 4.35-4.40 (1H, NHC**H**), 3.15-3.22 (1H, SC**H**), 2.93 (1H, dd, *J* = 4.8, 12.9 Hz, SC**H$_a$**), 2.67-2.78 (2H, SC**H$_b$**, ArC^{tri}C**H$_a$**), 2.48-2.50 (1H, m, ArC^{tri}C**H$_b$**), 1.25-1.77 (14H, m); **^{13}C NMR** (75 MHz, $CDCl_3$): δ 164.3, 156.6, 153.4, 149.5, 148.1, 139.5, 123.2, 115.8, 90.1, 83.3, 82.9, 80.6, 63.0, 60.3, 56.3, 50.5, 40.9, 29.5, 29.2, 28.6, 27.5, 25.8, 25.7; **HRMS** calcd. for (M+ H) $C_{25}H_{35}N_{10}O_4S$: requires 571.2564, found 571.2564.

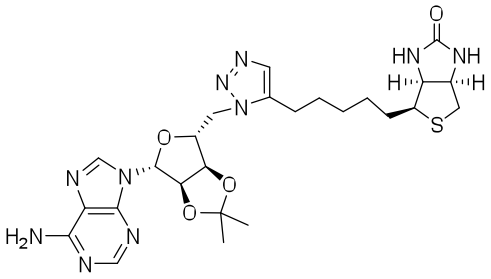
(3a*S*,4*S*,6a*R*)-4-[6-[1-[[(3a*R*,4*R*,6*R*,6a*R*)-4-(6-Aminopurin-9-yl)-2,2-dimethyl-3a,4,6,6a-tetrahydrofuro[3,4-*d*][1,3]dioxol-6-yl]methyl]triazol-4-yl]hexyl]-1,3,3a,4,6,6a-hexa hydrothieno[3,4-*d*]imidazol-2-one (Compound 8)



Homobiotin acetylene **20** (10 mg, 0.04 mmol) was reacted with adenosine azide **4** (14 mg, 0.04 mmol) and Cu nanopowder (1 mg, 0.02 mmol) according to CuAAC general procedure. The crude material was purified by flash chromatography eluting with 9% methanol in dichloromethane to yield a white solid (16 mg, 69%).

MP: 123 - 128 ^{o}C; **^{1}H NMR** (600 MHz; $CDCl_3$): δ 8.29 (1H, s, Ar**H**), 7.93 (1H, s, Ar**H**), 7.42 (1H, bs, ArN**H**), 7.13 (1H, s, Ar^{tri}**H**), 6.80 (1H, bs, ArN**H**), 6.06 (1H, bs, C(O)NH), 5.80 (1H, bs, C(O)NH), 5.37 (1H, d, *J* = 4.2 Hz, 1' C**H**), 5.11 (1H, dd, *J* = 4.8, 6.3 Hz, 3' C**H**), 4.82 (1H, dd, *J* = 6.0, 14.4 Hz, 5' C**H$_a$**), 4.68 (1H, dd, *J* = 3.0, 14.4 Hz, 5' C**H$_b$**), 4.58-4.60 (1H, m, 4' C**H**), 4.53-4.55 (1H, m, NHC**H**), 4.35-4.37 (1H, m, NHC**H**), 3.14-3.17 (1H, m, SC**H**), 2.94 (1H, dd, *J* = 4.8, 12.9 Hz, SC**H$_a$**), 2.75 (1H, d, *J* = 12.9 Hz, SC**H$_b$**), 2.61 (2H, t, *J* = 7.8 Hz, ArC^{tri}C**H$_2$**), 1.46-1.73 (4H, m, 2 x C**H$_2$**), 1.60 (3H, s, CC**H$_3$**), 1.37 (3H, s, CC**H$_3$**), 1.26-1.28 (6H, m, 3 x C**H$_2$**); **^{13}C NMR** (150 MHz; $CDCl_3$): δ 164.4, 156.5, 153.4, 149.3, 148.5, 140.1, 122.6, 120.5, 115.4, 90.7, 84.7, 83.3, 81.3, 77.5, 62.5, 60.3, 56.1, 51.1, 40.9, 29.4, 29.2, 28.9, 28.9, 27.5, 25.7, 25.6; **HRMS** calcd. for (M + H) $C_{26}H_{37}N_{10}O_4S$: requires 585.2720, found 585.2745.

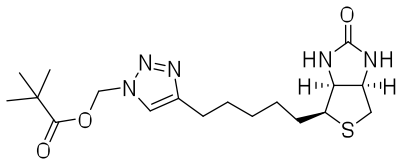
(3a*S*,4*S*,6a*R*)-4-[5-[3-[[(3a*R*,4*R*,6*R*,6a*R*)-4-(6-Aminopurin-9-yl)-2,2-dimethyl-3a,4,6,6a-tetrahydrofuro[3,4-*d*][1,3]dioxol-6-yl]methyl]triazol-4-yl]pentyl]-1,3,3a,4,6,6a-hexahydro thieno[3,4-*d*]imidazol-2-one (Compound 9)



To a solution of biotin acetylene **3** (Corona et al., 2006) (20 mg, 0.08 mmol) and adenosine azide **4** (31 mg, 0.09 mmol) in THF (2.5 mL) was added $Cp^*Ru(PPh_3)_2Cl$ (1 mg, 0.001 mmol) and the solution was refluxed under nitrogen atmosphere for 4h. The reaction mixture was cooled, diluted with dichloromethane (25 mL), washed with water (1 x 25 mL) and brine (1 x 25 mL), dried over Na_2SO_4, filtered and concentrated *in vacuo*. The residue was purified by flash chromatography eluting with 8% methanol in dichloromethane to give a light yellow solid (5 mg, 10%).

^{1}H NMR (600 MHz, $CDCl_3$): δ 8.31 (1H, s, Ar**H**), 7.80 (1H, s, Ar**H**), 7.78 (1H, bs, C(O)N**H**), 7.30 (1H, s, Ar^{tri}**H**), 7.09 (2H, bs, ArN**H$_2$**), 6.04 (1H, s, 1' C**H**), 5.86 and 5.83 (1H, bd, C(O)N**H**), 5.42 (1H, d, *J* = 6.0 Hz, 2' C**H**), 5.33 (1H, dd, *J* = 2.4, 6.0 Hz, 3' C**H**), 4.69-4.84 (3H, m, 5' C**H$_2$**, 4' C**H**), 4.59-4.61 (1H, m, NHC**H**), 4.37-4.39 (1H, m, NHC**H**); 3.12 (1H, m, SC**H**), 2.95 (1H, dd, *J* = 4.8, 12.6 Hz, SC**H$_a$**), 2.71 (1H, d, *J* = 12.6 Hz, SC**H$_b$**), 1.76-1.90 (2H, m), 1.60 (3H, s, CC**H$_3$**), 1.40 (3H, s, CC**H$_3$**), 1.39-1.29 (6H, m, C**H$_2$**, ArC^{tri}C**H$_2$**), 0.87-0.92 (1H, m), 0.47-0.53 (1H, m); **^{13}C NMR** (150 MHz, $CDCl_3$): 164.5, 156.5, 153.0, 148.6, 143.9, 138.2, 131.7, 120.2, 114.6, 110.0, 91.4, 82.3, 84.5, 82.5, 62.2, 60.1, 56.1, 40.7, 30.3, 29.2, 29.1, 28.1, 27.1, 25.4, 23.0; **HRMS** calcd. for (M + H) $C_{25}H_{35}N_{10}O_4S$: requires 571.2564, found 571.2586.

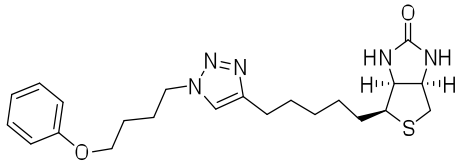
[4-[5-[(3a*S*,4*S*,6a*R*)-2-oxo-1,3,3a,4,6,6a-hexahydrothieno[3,4-*d*]imidazol-4-yl]pentyl]triazol-1-yl]methyl 2,2-dimethylpropanoate (Compound 10)



Biotin acetylene **3** (32 mg, 0.13 mmol) and azidomethylpivalate (Loren et al., 2005) (23 mg, 0.15 mmol) was reacted according to CuAAC general procedure The residue was purified by flash chromatography eluting with 5% methanol in dichloromethane to give a white solid (25 mg, 48%).

^{1}H NMR (300 MHz, $CDCl_3$): δ 7.53 (1H, s, Ar**H**), 6.19 (2H, s, OC**H$_2$**), 5.62 (1H, bs, C(O)N**H**), 5.24 (1H, bs, C(O)N**H**), 4.49-4.53 (1H, m, NHC**H**), 4.28-4.33 (1H, m, NHC**H**), 3.12-3.18 (1H, m, SC**H**), 2.90 (1H, dd, *J* = 4.8, 12.6 Hz, SC**H$_a$**), 2.69-2.74 (3H, m, SC**H$_b$**, ArC**H$_2$**), 1.67-1.76 (4H, m, 2 x C**H$_2$**), 1.34-1.52 (4H, m, 2 x C**H$_2$**), 1.18 (9H, s, CC**H$_3$**); **^{13}C NMR** (75 MHz, $CDCl_3$): δ 178.2, 163.9, 149.0, 122.4, 69.9, 62.3, 60.4, 56.0, 40.8, 39.1, 29.3, 29.1, 29.0, 28.8, 27.1, 25.6.

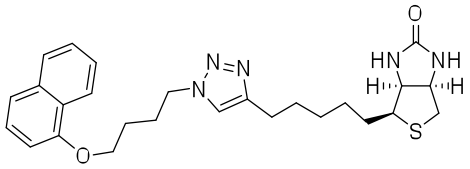
(3a*S*,4*S*,6a*R*)-4-[5-[1-(4-Phenoxybutyl)triazol-4-yl]pentyl]-1,3,3a,4,6,6a-hexa hydrothieno[3,4-*d*]imidazol-2-one (Compound 11)



Biotin acetylene **3** (18 mg, 0.075 mmol) was reacted with 4-azidobutoxybenzene (13, 14) (16 mg, 0.083 mmol) and Cu nanopowder (1 mg, 0.015 mmol) according to CuAAC general procedure. The residue was purified by flash chromatography eluting with 7% methanol in dichloromethane to give a white solid, (21 mg, 64%).

MP: 117 - 120 ^{o}C; **^{1}H NMR** (300 MHz, $CDCl_3$): δ 7.25-7.31 (3H, m, Ar^{tri}**H**, ArH), 6.86-6.97 (3H, m, Ar**H**), 5.55 (1H, bs, C(O)N**H**), 5.18 (1H, bs, C(O)N**H**), 4.47-4.51 (1H, m, NHC**H**), 4.41 (2H, t, *J* = 7.2 Hz, ArN^{tri}C**H$_2$**), 4.27-4.31 (1H, m NHC**H**), 3.98 (2H, t, *J* = 6.0 Hz, ArOC**H$_2$**), 3.11-3.18 (1H, m, SC**H**), 2.90 (1H, dd, *J* = 5.1, 12.9 Hz, SC**H$_a$**), 2.67-2.73 (3H, m, SC**H$_b$**, ArC^{tri}C**H$_2$**), 2.06-2.16 (2H, m, C**H$_2$**), 1.63-1.85 (4H, m, 2 x C**H$_2$**), 1.37-1.50 (4H, m, 2 x C**H$_2$**); **^{13}C NMR** (75 MHz, $CDCl_3$): δ 163.5, 158.9, 129.7, 121.0, 120.8, 114.6, 67.0, 62.2, 60.3, 55.9, 50.0, 40.8, 29.3, 28.9, 28.8, 27.5, 26.5, 25.7; **HRMS** calcd. for (M + H) $C_{22}H_{32}N_5O_2S$: requires 430.2277, found 430.2311.

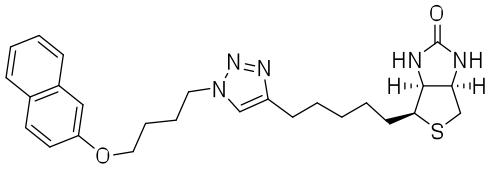
(3a*S*,4*S*,6a*R*)-4-[5-[1-[4-(1-Naphthyloxy)butyl]triazol-4-yl]pentyl]-1,3,3a,4,6,6a-hexahydrothieno[3,4-*d*]imidazol-2-one (Compound 12)



Biotin acetylene **3** (16 mg, 0.07 mmol) was reacted with 1-(4-bromobutoxy)naphthalene (15, 16) (18 mg, 0.07 mmol) and Cu nanopowder (1 mg, 0.02 mmol) according to CuAAC general procedure. The residue was purified by flash chromatography eluting with 8% methanol in dichloromethane to give a white solid, (14 mg, 41%).

MP: 97 - 98 ^{o}C; **^{1}H NMR** (300 MHz, $CDCl_3$): δ 8.21-8.24 (1H, m, Ar**H**), 7.78-7.81 (1H, s, Ar**H**), 7.37-7.50 (4H, m, Ar**H**), 7.29 (1H, s, Ar^{tri}**H**), 6.87 (1H, d, *J* = 7.5 Hz, Ar**H**), 5.63 (1H, bs, C(O)N**H**), 5.24 (1H, bs, C(O)N**H**), 4.45 (3H, m, ArN^{tri}C**H$_2$**, NHC**H**), 4.23-4.27 (1H, m, NHC**H**), 4.15 (2H, t, *J* = 6.0 Hz, ArOC**H$_2$**), 3.08-3.14 (1H, m, SC**H**), 2.87 (1H, dd, *J* = 5.1, 12.6 Hz, SC**H$_a$**), 2.67-2.71 (3H, m, SC**H$_b$**, ARC^{tri}C**H$_2$**), 2.16-2.26 (2H, m, C**H$_2$**), 1.90-2.00 (2H, m, C**H$_2$**), 1.57-1.71 (2H, m, 2 x C**H$_2$**), 1.33-1.48 (2H, m, 2 x C**H$_2$**); **^{13}C NMR** (75 MHz, $CDCl_3$): δ 163.6, 154.6, 134.7, 127.7, 126.6, 126.0, 125.7, 125.4, 122.0, 120.8, 120.5, 104.8, 67.2, 62.2, 55.9, 50.1, 40.7, 29.3, 29.2, 28.9, 28.7, 27.8, 26.5, 25.7; **HRMS** calcd. for (M+ H) $C_{26}H_{34}N_5O_2S$: requires 480.2433, found 480.2458.

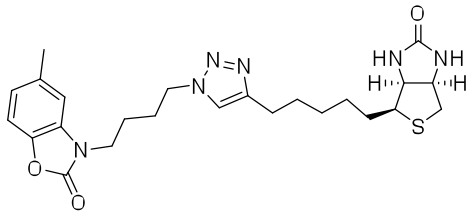
(3a*S*,4*S*,6a*R*)-4-[5-[1-[4-(2-Naphthyloxy)butyl]triazol-4-yl]pentyl]-1,3,3a,4,6,6a-hexahydrothieno[3,4-*d*]imidazol-2-one (Compound 13)



Biotin acetylene **3** (18 mg, 0.07 mmol) was reacted with 2-(4-bromobutoxy)naphthalene (Huang et al., 2010) (20 mg, 0.08 mmol) and Cu nanopowder (1 mg, 0.02 mmol) according to CuAAC general procedure. The residue was purified by flash chromatography eluting with 8% methanol in dichloromethane to give a white solid, (26 mg, 74%).

MP: 96-98 ^{o}C; **^{1}H NMR** (300 MHz, $CDCl_3$): δ 7.70-7.77 (3H, m, Ar**H**), 7.41-7.46 (1H, m, Ar**H**), 7.31-7.35 (1H, m, Ar**H**), 7.30 (1H, Ar^{tri}**H**), 7.10-7.14 (2H, m, Ar**H**), 5.39 (1H, bs, C(O)N**H**), 5.05 (1H, bs, C(O)N**H**), 4.41-4.50 (3H, m, NHC**H**, ArN^{tri}C**H$_2$**), 4.26-4.30 (1H, m, NHC**H**), 4.10 (2H, t, *J* = 6.0 Hz, ArOC**H$_2$**), 3.10-3.16 (1H, m, SC**H**), 2.89 (1H, dd, *J* = 5.4, 12.9 Hz, SC**H$_a$**), 2.67-2.73 (3H, m, SC**H$_b$**, ArC^{tri}C**H$_2$**), 2.11-2.20 (2H, m, C**H$_2$**), 1.83-1.92 (2H, m, C**H$_2$**), 1.63-1.72 (4H, m, 2 x C**H$_2$**), 1.37-1.46 (4H, m, 2 x C**H$_2$**); **^{13}C NMR** (75 MHz, $CDCl_3$): δ 163.8, 156.8, 148.4, 134.7, 129.6, 129.1, 127.8, 126.9, 126.6, 123.9, 120.8, 118.9, 106.8, 67.1, 62.2, 60.3, 56.0, 50.0, 40.7, 29.3, 29.2, 24.9, 28.7, 27.5, 26.4, 25.7; **HRMS** calcd. for (M+ H) $C_{26}H_{34}N_5O_2S$: requires 480.2433, found 480.2454.

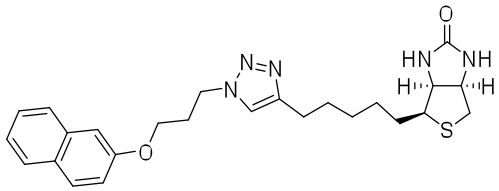
3-[4-[4-[5-[(3a*S*,4*S*,6a*R*)-2-Oxo-1,3,3a,4,6,6a-hexahydrothieno[3,4-*d*]imidazol-4-yl]pentyl]triazol-1-yl]butyl]-5-methyl-1,3-benzoxazol-2-one (Compound 14)



Biotin acetylene **3** (Corona et al., 2006) (21 mg, 0.06 mmol) was reacted with azide **21** (24 mg, 0.06 mmol) and Cu nanopowder (1 mg, 0.02 mmol) according to CuAAC general procedure. The residue was purified by flash chromatography eluting with 8% methanol in dichloromethane to give a white solid, (29 mg, 68%).

MP: 99 - 101 ^{o}C; **^{1}H NMR** (300 MHz, $CDCl_3$): δ 7.32 (1H, s, Ar^{tri}**H**), 7.06 (1H, d, *J* = 8.1 Hz, Ar**H**), 6.90 (1H, d, *J* = 8.1 Hz, Ar**H**), 6.77 (1H, m, Ar**H**), 6.22 (1H, bs, C(O)N**H**), 5.81 (1H, bs, C(O)N**H**), 4.46-4.50 (1H, m, NHC**H**), 4.39 (2H, t, *J* = 6.9 Hz, ArN^{tri}C**H$_2$**), 4.26-4.31 (1H, m, NHC**H**), 3.83 (2H, t, *J* = 6.9 Hz, NC**H$_3$**), 3.11-3.17 (1H, m, SC**H**), 2.89 (1H, dd, *J* = 5.1, 12.6 Hz, SC**H$_a$**), 2.66-2.73 (3H, m, SC**H$_b$**, ArC^{tri}C**H$_2$**), 2.39 (3H, s, ArC**H$_3$**), 1.93-2.03 (2H, m, C**H$_2$**), 1.65-1.84 (6H, m, 3 x C**H$_2$**), 1.33-1.43 (4H, m, 2 x C**H$_2$**); **^{13}C NMR** (300 MHz, $CDCl_3$): δ 164.0, 155.1, 148.5, 140.8, 134.1, 130.8, 123.1, 121.0, 109.8, 109.0, 62.2, 60.3, 56.0, 49.3, 41.3, 40.7, 29.3, 29.2, 28.9, 28.7, 27.4, 25.7, 24.8, 21.7; **HRMS** calcd. for (M + H) $C_{24}H_{33}N_6O_3S$: requires 485.2335, found 485.2359.

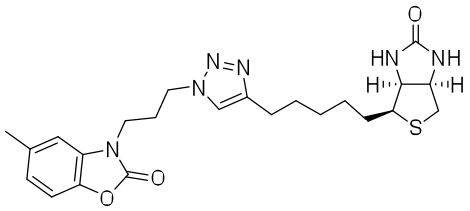
(3a*S*,4*S*,6a*R*)-4-[5-[1-[3-(2-Naphthyloxy)propyl]triazol-4-yl]pentyl]-1,3,3a,4,6,6a-hexahydrothieno[3,4-*d*]imidazol-2-one (Compound 15)



Biotin acetylene **3** (20 mg, 0.08 mmol) was reacted with 2-(3-azidopropoxy)naphthalene (Huang et al., 2010; Shinde et al., 2009) (21 mg, 0.09 mmol) and Cu nanopowder (1 mg, 0.02 mmol) according to CuAAC general procedure. The residue was purified by flash chromatography eluting with 8% methanol in dichloromethane to give a white solid, (20 mg, 51%).

^{1}H NMR (300 MHz, $CDCl_3$): δ 7.77 (1H, d, *J* = 7.5 Hz, Ar**H**), 7.75 (1H, d, *J* = 8.9 Hz, Ar**H**), 7.71 (1H, d, *J* = 7.5 Hz, Ar**H**), 7.42-7.47 (1H, m, Ar**H**), 7.32-7.37 (1H, m, Ar**H**), 7.28 (1H, s, Ar^{tri}**H**), 7.14 (1H, dd, *J* = 2.7, 8.7 Hz, Ar**H**), 7.09 (1H, d, *J* = 2.7 Hz, Ar**H**), 5.50 (1H, bs, C(O)N**H**), 5.15 (1H, bs, C(O)N**H**), 4.59 (2H, t, *J* = 6.6 Hz, ArN^{tri}C**H$_2$**), 4.44-4.49 (1H, m, NHC**H**), 4.23-4.27 (1H, m, NHC**H**), 4.07 (2H, t, *J* = 6.0 Hz, ArOC**H$_2$**), 3.06-3.13 (1H, m, SC**H**), 2.87 (1H, dd, *J* = 5.1, 12.6 Hz, SC**H$_a$**), 2.65-2.72 (3H, m, SC**H$_b$**, ArC^{tri}C**H$_2$**), 2.41-2.49 (2H, m, C**H$_2$**), 1.62-1.69 (4H, m, 2 x C**H$_2$**), 1.34-1.42 (4H, m, 2 x C**H$_2$**); **^{13}C NMR** (75 MHz, $CDCl_3$): δ 163.4, 156.6, 148.3, 134.6, 129.8, 129.3, 127.9, 127.0, 126.7, 124.0, 121.5, 118.8, 106.9, 64.3, 62.2, 60.2, 55.9, 47.1, 40.7, 30.1, 29.2, 29.1, 28.9, 25.7; **HRMS** calcd. for (M+ H) $C_{25}H_{32}N_5O_2S$: requires 466.2277, found 466.2294.

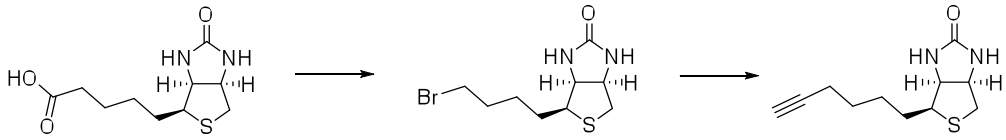
3-[3-[4-[5-[(3a*S*,4*S*,6a*R*)-2-Oxo-1,3,3a,4,6,6a-hexahydrothieno[3,4-*d*]imidazol-4-yl]pentyl]triazol-1-yl]propyl]-5-methyl-1,3-benzoxazol-2-one (Compound 16)



Biotin acetylene **3** (14 mg, 0.06 mmol) was reacted with azide **22** (15 mg, 0.06 mmol) and Cu nanopowder (1 mg, 0.02 mmol) according to CuAAC general procedure. The residue was purified by flash chromatography eluting with 8% methanol in dichloromethane to give a white solid, (17 mg, 61%).

MP: 134-136 ^{o}C; **^{1}H NMR** (300 MHz, $CDCl_3$): δ 7.48 (1H, s, Ar^{tri}**H**), 7.08 (1H, d, *J* = 8.1 Hz, Ar**H**), 6.92 (1H, d, *J* = 8.1 Hz, Ar**H**), 6.78 (1H, s, Ar**H**), 5.70 (1H, bs, C(O)N**H**), 5.30 (1H, bs, C(O)N**H**), 4.48-4.52 (1H, m, NHC**H**), 4.40 (2H, t, *J* = 6.6 Hz, ArN^{tri}C**H$_2$**), 4.29-4.33 (1H, m, NHC**H**), 3.87 (2H, t, *J* = 6.6 Hz, NC**H$_2$**), 3.12-3.19 (1H, m, SC**H**), 2.90 (1H, dd, *J* = 5.1, 12.6 Hz, SC**H$_a$**), 2.70-2.74 (3H, m, SC**H$_b$**, ArC^{tri}C**H$_2$**), 2.39-2.47 (5H, m, C**H$_2$**, ArC**H$_3$**), 1.36-1.78 (8H, m, 4 x C**H$_2$**); **^{13}C NMR** (75 MHz, $CDCl_3$): δ 163.6, 155.28, 148.4, 140.9, 134.4, 130.7, 123.4, 121.9, 110.0, 109.1, 62.2, 60.3, 55.9, 47.3, 40.8, 39.5, 29.2, 28.9, 28.7, 28.67, 25.7, 21.8 **HRMS** calcd. for (M + H) $C_{23}H_{31}N_6O_3S$: requires 471.2178, found 471.2198.

(3a*S*,6a*R*)-4-Hex-5-ynyl-1,3,3a,4,6,6a-hexahydrothieno[3,4-*d*]imidazol-2-one (Compound 17)



To a suspension of biotin (121 mg, 0.50 mmol) in anhydrous dichloromethane (10 mL) were added thionyl chloride (243 mg, 1.98 mmol) and DMF (1 mL) and the solution was stirred under nitrogen atmosphere for 1 h. The reaction mixture was concentrated *in vacuo* and used as crude. The acid chloride and pyrithione sodium salt (82 mg, 0.55 mmol) were suspended in 9:1 DMF and bromotrichloromethane (5 mL) and the mixture was stirred at 80 ^{o}C for 4 h. The mixture was cooled and diluted with de-ionized ice water (1 x 20 mL) and extracted with dichloromethane (6 x 25 mL). The organic layer was washed with 0.25 M aqueous NaOH (1 x 100 mL), 0.25 M aqueous HCl (1 x 100 mL), water (1 x 100 mL) and brine (1 x 100 mL), dried over Na_2SO_4 and filtered. Activated carbon was added to the solution and stirred for 30 min, filtered over Celite® and washed with dichloromethane (3 x 100 mL). The filtrate was concentrate *in vacuo* and the residue was purified by flash chromatography eluting with 6% MeOH in DCM to give norbiotin bromide as a white solid (29 mg, 21%).

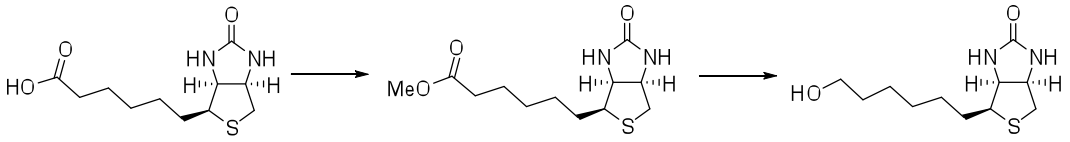
MP: 146 – 148 ^{o}C; **^{1}H NMR (**300 MHz, DMSO-d_6): δ 6.49 (1H, bs, N**H**), 6.42 (1H, bs, N**H**), 4.33-4.38 (1H, m, C**H**NH), 4.17-4.21 (1H, m, C**H**NH), 3.68 (2H, t, *J* = 6.6 Hz, C**H$_2$**Br), 3.13-3.19 (1H, m, C**H**S), 2.89 (1H, dd, *J* = 5.1, 12.6 Hz, C**H$_a$**S), 2.70 (1H, d, *J* = 12.6 Hz, C**H$_b$**S), 1.46-1.84 (6H, m, C**H$_2$**); **^{13}C NMR (**75 MHz, DMSO-d_6): δ 162.7, 61.0, 59.2, 55.3, 45.2, 39.8, 32.0, 27.6, 25.9.

To a suspension of 90% lithium acetylide ethylene diamine complex (1.5 equiv) in dry DMSO (0.5 mL) cooled at 15 ^{o}C was added dropwise a solution of norbiotin bromide (25 mg, 0.09 mmol) in dry DMSO (0.25 mL) and stirred at ambient temperature for 3 h. The reaction mixture was poured into ice-water and extracted with dichloromethane. The organic layer was collected and washed with water and brine, dried over Na_2SO_4, filtered, concentrated *in vacuo* and purified by flash

chromatography eluting with 5% methanol in dichloromethane to give a white solid (9 mg, 44%).

^{1}H NMR (300 MHz, $CDCl_3$): δ 5.16 (1H, bs, C(O)N**H**), 5.11 (1H, bs, C(O)N**H**), 4.49-4.53 (1H, m, NHC**H**), 4.30-4.33 (1H, m, NHC**H**), 3.13-3.20 (1H, m, SC**H**), 2.94 (1H, dd, *J* = 5.1, 12.9 Hz, SC**H$_a$**), 2.73 (1H, d, *J* = 12.9 Hz, SC**H$_b$**), 2.21 (2H, dt, *J* = 2.7, 6.75 Hz, C**H$_2$**C≡CH), 1.97 (1H, t, *J* = 2.7 Hz, CH_2C≡C**H**), 1.51-1.70 (6H, m, 3 x C**H$_2$**); **^{13}C NMR** (300 MHz, DMSO-d_6): δ 163.2, 84.4, 68.9, 62.2, 60.3, 55.5, 40.8, 28.3 (x2), 28.1, 18.4; **HRMS** calcd. for (M + H) $C_{11}H_{17}N_2OS$: requires 225.1062, found 225.1065.

(3a*S*,6a*R*)-4-(6-Hydroxyhexyl)-1,3,3a,4,6,6a-hexahydrothieno[3,4-*d*]imidazol-2-one (Compound 18)



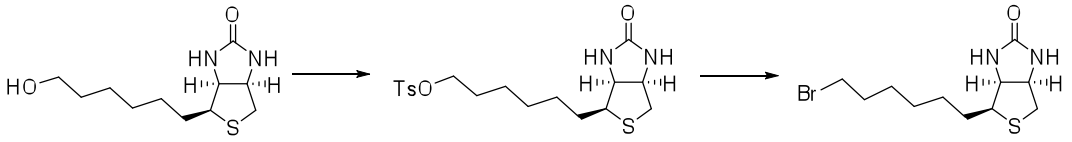
A suspension of homobiotin (Wilbur et al., 2001) (210 mg, 0.81 mmol) in dry methanol (5 mL) was added thionyl chloride (290 mg, 0.211 mL, 2.43 mmol) and stirred for 0.75 h. The reaction mixture was concentrated *in vacuo* and purified by flash chromatography eluting with 5% methanol in dichloromethane to give the methyl ester as a off white solid (221 mg, 100%).

MP: 168 – 170 ^{o}C; **^{1}H NMR** (300 MHz, 2% CD_3OD, $CDCl_3$)**:** δ 4.46-4.50 (1H, m, C**H**NH), 4.24-4.30 (1H, m, C**H**NH), 3.64 (1H, s, COOC**H$_3$**), 3.10-3.16 (1H, m, SC**H**), 2.92 (1H, dd, *J* = 4.8, 12.6 Hz, SC**H$_a$**), 2.70 (1H, d, *J* = 12.6 Hz, SC**H$_b$**), 2.29 (2H, t, *J* = 7.5 Hz, C**H$_2$**COOCH$_3$), 1.30-1.65 (8H, m, 4 x C**H$_2$**); **^{13}C NMR** (75 MHz, 2% CD_3OD, $CDCl_3$)**:** δ 174.5, 164.0, 62.2, 60.3, 56.0, 51.7, 40.8, 34.2, 29.2, 28.9, 28.6, 24.7.

A suspension of the methyl ester (141 mg, 0.52 mmol) in dry THF (5 mL) was added $LiAlH_4$ (78 mg, 2.04 mmol) and stirred for 8 h under a nitrogen atmosphere. The reaction mixture was slowly quenched with methanol (1 mL) and water (1 mL). To the mixture was added saturated sodium sulphate, stirred for 20 min and concentrated *in vacuo*. The residue was dissolved in 1:4 methanol and dichloromethane (25 mL), stirred for 30 min, filtered and washed with 1:4 methanol and dichloromethane (25 mL). The filtrate was concentrated *in vacuo* and purified by flash chromatography eluting with 8% methanol in dichloromethane to give a white solid (115 mg, 91%).

MP: 158 -161 ^{o}C; **^{1}H NMR** (300 MHz, CD_3OD): δ 4.49 (1H, ddd, *J* = 0.9, 4.8, 6.9 Hz, C**H**NH), 4.29 (1H, dd, *J* = 4.5, 7.8 Hz, C**H**NH), 3.55 (2H, t, *J* = 6.6 Hz, C**H$_2$**COOCH$_3$), 3.17-3.24 (1H, m, SC**H**), 2.93 (1H, dd, *J* = 5.1, 12.8 Hz, SC**H$_a$**), 2.70 (1H, d, *J* = 12.8 Hz, SC**H$_b$**), 1.29-1.78 (8H, m, 4 x C**H$_2$**); **^{13}C NMR** (75 MHz, CD_3OD): δ 156.1, 54.0, 53.4, 52.1, 47.7, 31.5, 24.0, 21.0, 20.9, 20.3, 17.3; **HRMS** calcd. for (M + H) $C_{11}H_{21}N_2O_2S$: requires 245.1324, found 245.1357.

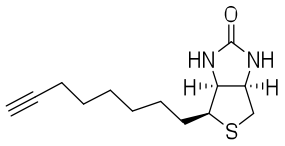
(3a*S*,6a*R*)-4-(6-Bromohexyl)-1,3,3a,4,6,6a-hexahydrothieno[3,4-*d*]imidazol-2-one (Compound 19)



To a suspension of homobiotinol **18** (122 mg, 0.50 mmol) in dry pyridine (1 mL) was added dropwise a solution of tosyl chloride (105 mg, 0.55 mmol) in dry pyridine (1 mL), and the solution was stirred in an ice bath for 1 h and subsequently placed in a 4 ^{o}C fridge for 5 h. The reaction mixture was diluted with dichloromethane (25 mL) and washed with 0.5 M aqueous HCl (1 x 25 mL), aqueous saturated sodium bicarbonate (1 x 25 mL), water (1 x 25 mL) and brine (1 x 25 mL). The organic layer was dried over Na_2SO_4, filtered, and concentrated *in vacuo* to give the crude tosylate as a white solid that was used with no further purification. To a suspension of the tosylate in dry methyl ethyl ketone (2 mL) was added lithium bromide (87 mg, 1.00 mmol) and stirred at 80 ^{o}C for 3 h. The reaction mixture was cooled, diluted with dichloromethane (1 x 25 mL) and washed with water (1 x 25 mL) and brine (1 x 25 mL). The organic layer was dried over Na_2SO_4, filtered and concentrated *in vacuo* and purified by flash chromatography eluting with 4% methanol in dichloromethane to give a white solid (78 mg, 51% over two steps).

MP: 159 – 161 ^{o}C; **^{1}H NMR** (300 MHz, $CDCl_3$)**:** δ 5.30 (1H, s, CHN**H**), 5.15 (1H, s, CHN**H**), 4.50-4.54 (1H, m, C**H**NH), 4.30-4.34 (1H, m, C**H**NH), 3.41 (2H, t, *J* = 6.9 Hz, C**H$_2$**Br), 3.14-3.21 (1H, m, SC**H**), 2.94 (1H, dd, *J* = 5.1, 12.8 Hz, SC**H$_a$**), 2.74 (1H, d, *J* = 12.8 Hz, SC**H$_b$**), 1.83-1.90 (2H, m, C**H$_2$**), 1.30-1.68 (4H, m, 2 x C**H$_2$**); **^{13}C NMR** (300 MHz, $CDCl_3$): δ 163.4, 62.2, 60.3, 55.7, 40.8, 34.2, 32.8, 29.1, 28.9, 28.8, 28.1; **HRMS** calcd. for (M + H) $C_{11}H_{20}BrN_2OS$: requires 307.0480, found 307.0478.

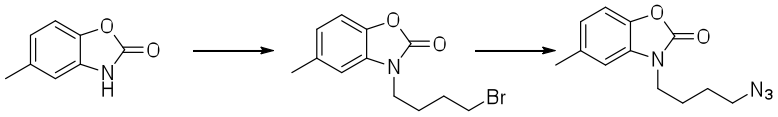
(3a*S*,6a*R*)-4-Oct-7-ynyl-1,3,3a,4,6,6a-hexahydrothieno[3,4-*d*]imidazol-2-one (Compound 20)



To a suspension of 90% lithium acetylide ethylene diamine complex (23 mg, 23 mmol) in anhydrous DMSO (0.5 mL) cooled at 15 ^{o}C was added dropwise a solution of homobiotin bromide **19** (46 g, 0.15 mmol) in dry DMSO (0.25 mL) and the mixture was stirred at ambient temperature for 3 h. The reaction mixture was poured into ice-water and extracted with dichloromethane (1 x 50 mL). The organic layer was collected and washed with water (1 x 50 mL) and brine (1 x 50 mL), dried over Na_2SO_4, filtered, concentrated *in vacuo* and purified by flash chromatography eluting with 3% methanol in dichloromethane to give a white solid (14 mg, 37%).

^{1}H NMR (300 MHz, $CDCl_3$): δ 4.79 (2H, bs, 2 x C(O)N**H**), 4.51-4.55 (1H, m C**H**NH), 4.30-4.34 (1H, m, C**H**NH), 3.14-3.20 (1H, m, SC**H**), 2.94 (1H, dd, *J* = 5.1, 12.9 Hz, SC**H$_a$**), 2.73 (1H, d, *J* = 12.6 Hz, SC**H$_b$**), 2.19 (2H, dt, *J* = 2.7, 6.9 Hz, C**H$_2$**C≡CH), 1.95 (1H, d, *J* = 2.7 Hz, CH_2C≡C**H**), 1.38 – 1.68 (8H, m, 4 x C**H$_2$**); **^{13}C NMR** (150 MHz, $CDCl_3$): δ 162.6, 84.5, 68.3, 61.9, 60.1, 55.4, 40.6, 29.7, 29.4, 28.9, 28.6, 28.4, 18.3; **HRMS** calcd. for (M + H) $C_{13}H_{21}N_2OS$: requires 253.1375, found 253.1373.

3-(4-Azidobutyl)-5-methyl-1,3-benzoxazol-2-one (Compound 21)



To a dissolved solution of 2-benzoxazolone (Shankaran et al., 1997) (195 mg, 1.31 mmol) in anhydrous DMF (5 mL) was added K_2CO_3 (271 mg, 1.96 mmol) and the reaction mixture was stirred at 50 ^oC for 1 h under nitrogen atmosphere. The reaction mixture was cooled to ambient temperature and was added 1,4-dibromobutane (420 mg, 1.96 mmol) and stirred at 50 ^oC for a further 6 h under a nitrogen atmosphere. The reaction mixture was cooled and poured into water (1 x 100 mL) and extracted with ethyl acetate (2 x 100 mL). The organic layers were pooled, washed with brine (1 x 100 mL), dried over Na_2SO_4, concentrated *in vacuo* and purified by flash chromatography eluting with 20% ethyl acetate in petroleum ether to yield the bromide as an off white solid (310 mg, 88%).

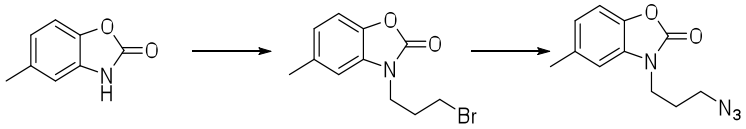
FT-IR (ATR) ν_{max}: 3068, 2931, 1760, 1623, 1383 cm^{-1}; ^{1}**H NMR** (300 MHz; $CDCl_3$): δ 7.04 (1H, d, *J* = 8.7 Hz, ArH), 6.89 (1H, d, *J* = 8.7 Hz, Ar**H**), 6.87 (1H, s, Ar**H**), 3.94 (2H, t, *J* = 6.6 Hz, NC**H$_2$**), 3.43 (2H, t, *J* = 6.6 Hz, C**H$_2$**Br), 2.38 (3H, s, ArC**H$_3$**), 2.32 (2H, tt, *J* = 6.6, 6.6 Hz, C**H$_2$**) ^{13}**C NMR** (75 MHz; $CDCl_3$): δ 154.8, 140.7, 134.1, 131.1, 123.0, 109.7, 109.0, 40.6, 30.9, 30.0, 21.6.

To a solution of the bromide (256 mg, 0.90 mmol) in DMF (5 mL) was added sodium azide (74 mg, 1.1 mmol) and stirred under nitrogen for 12 h. The reaction mixture was diluted with ethyl acetate (50 mL) and washed with water (1 x 50 mL) and brine (1 x 50 mL). The organic layer was dried over Na_2SO_4, filtered, concentrated *in vacuo* and purified by flash chromatography eluting with15% ethyl acetate in petroleum ether to yield a clear oil (205 mg, 91%).

FT-IR (ATR) ν_{max}: 2937, 2093, 1762, 1622, 1494 cm^{-1}; ^{1}**H NMR** (300 MHz; $CDCl_3$): δ 7.08 (1H, d, *J* = 8.1 Hz, Ar**H**), 6.91 (1H, d, *J* = 8.1 Hz, Ar**H**), 6.79 (1H, s, Ar**H**), 3.83 (2H, t, *J* = 6.9 Hz, NC**H$_2$**), 3.36 (2H, t, *J* = 6.9 Hz, C**H$_2$**N_3), 2.40 (3H, s, ArC**H$_3$**), 1.83-1.93 (2H, m, C**H$_2$**), 1.63-1.72 (2H, m, C**H$_2$**); ^{13}**C NMR** (75 MHz; $CDCl_3$): δ 155.0,

140.9, 134.0, 131.1, 123.0, 109.8, 108.9, 51.0, 41.7, 26.2, 25.2, 21.7; **HRMS** calcd. for (M + Na) $C_{12}H_{14}N_4NaO_2$: requires 269.1015, found 269.1006.

3-(3-Azidopropyl)-5-methyl-1,3-benzoxazol-2-one (Compound 22)



2-Benzoxazolone (366 mg, 2.46 mmol) was reacted with 1,2-dibromoethane (788 mg, 3.68 mmol) according to procedure outlined for compound **21** and was purified by flash chromatography eluting with 20% ethyl acetate in petroleum ether to yield the bromide as an off white solid (647 mg, 93%)

FT-IR (ATR) ν_{max}: 2946, 1765, 1623, 1493 cm^{-1}; **^{1}H NMR** (300 MHz; $CDCl_3$): δ 7.07 (1H, d, *J* = 8.1 Hz, Ar**H**), 6.90 (1H, d, *J* = 8.1 Hz, Ar**H**), 6.78 (1H, s, Ar**H**), 3.84 (2H, t, *J* = 6.6 Hz, NC**H$_2$**), 3.43 (2H, t, *J* = 6.6 Hz, C**H$_2$**Br), 2.39 (3H, s, ArC**H$_3$**), 1.92-1.97 (4H, m, C**H$_2$**); **^{13}C NMR** (75 MHz; $CDCl_3$): δ 155.0, 140.9, 134.1, 131.0, 123.0, 109.8, 108.9, 41.4, 32.9, 29.6, 26.5, 21.7

The bromide (112 mg, 0.41 mmol) was reacted according to procedure outlined for compound **21** and purified by silica gel chromatography eluting with 20% ethyl acetate in petroleum ether to yield a white solid (81 mg, 83%).

FT-IR (ATR) ν_{max}: 2945, 2100, 1754, 1495, 1240 cm^{-1}; **^{1}H NMR** (300 MHz; $CDCl_3$): δ 7.08 (1H, d, *J* = 8.4 Hz, ArH), 6.90 (1H, d, *J* = 8.1 Hz, Ar**H**), 6.83 (1H, s, Ar**H**), 3.90 (2H, t, *J* = 6.9 Hz, NC**H$_2$**), 3.43 (2H, t, *J* = 6.9 Hz, C**H$_2$**N_3), 2.40 (3H, s, ArC**H$_3$**), 2.03 (2H, m, C**H$_2$**); **^{13}C NMR** (75 MHz; $CDCl_3$): δ 154.9, 140.8, 134.2, 131.1, 123.1, 109.9, 108.2, 48.6, 39.5, 27.4, 21.7; **HRMS** calcd. for (M+ Na) $C_{11}H_{12}N_4NaO_2$: requires 255.0858, found 255.0851.

Supplementary References

Cheng, Y., and Prusoff, W.H. (1973). Relationship between the inhibition constant (K1) and the concentration of inhibitor which causes 50 per cent inhibition (I50) of an enzymatic reaction. Biochem Pharmacol *22*, 3099-3108.

Corona, C., Bryant, B.K., and Arterburn, J.B. (2006). Synthesis of a biotin-derived alkyne for pd-catalyzed coupling reactions. Org Lett *8*, 1883-1886.

Huang, L., Luo, Z., He, F., Lu, J., and Li, X. (2010). Synthesis and biological evaluation of a new series of berberine derivatives as dual inhibitors of acetylcholinesterase and butyrylcholinesterase. Bioorg Med Chem *18*, 4475-4484.

Loren, J.C., Kransinski, A., Forkin, V.V., and Sharpless, K.B. (2005). NH-1,2,3-Triazoles from Azidomethyl Pivalate and Carbamates: Base-Labile N-Protecting Groups. Synlett *2005*, 2847-2850.

Mayende, L., Swift, R.D., Bailey, L.M., Soares da Costa, T.P., Wallace, J.C., Booker, G.W., and Polyak, S.W. (2012). A novel molecular mechanism to explain biotin-unresponsive holocarboxylase synthetase deficiency. J Mol Med (Berl) *90*, 81-88.

Polyak, S.W., Chapman-Smith, A., Brautigan, P.J., and Wallace, J.C. (1999). Biotin protein ligase from Saccharomyces cerevisiae. The N-terminal domain is required for complete activity. J Biol Chem *274*, 32847-32854.

Polyak, S.W., Chapman-Smith, A., Mulhern, T.D., Cronan, J.E., Jr., and Wallace, J.C. (2001). Mutational analysis of protein substrate presentation in the post-translational attachment of biotin to biotin domains. J Biol Chem *276*, 3037-3045.

Shankaran, K., Donnelly, K.L., Shan, S.K., Humes, J.L., Pacholok, S.G., Grant, S.K., Green, B.G., and MacCoss, M. (1997). Inhibition of nitric oxide synthase by benzoxazolones Bioorganic & Medicinal Chemistry Letters *7*, 2887-2892.

Shinde, S.S., Chi, H.M., Lee, B.S., and Chi, D.Y. (2009). tert-Alcohol-functionalized imidazolium ionic liquid: catalyst for mild nucleophilic substitution reactions at room temperature. Tetrahedron Letters *50*, 6654-6657.

Wilbur, D.S., Hamlin, D.K., Chyan, M.K., Kegley, B.B., and Pathare, P.M. (2001). Biotin reagents for antibody pretargeting. 5. Additional studies of biotin conjugate design to provide biotinidase stability. Bioconjug Chem *12*, 616-623.