SUPPORTING INFORMATION

Targeting *Mycobacterium tuberculosis* Biotin Protein Ligase (MtBPL) with Nucleoside-Based Bisubstrate Adenylation Inhibitors

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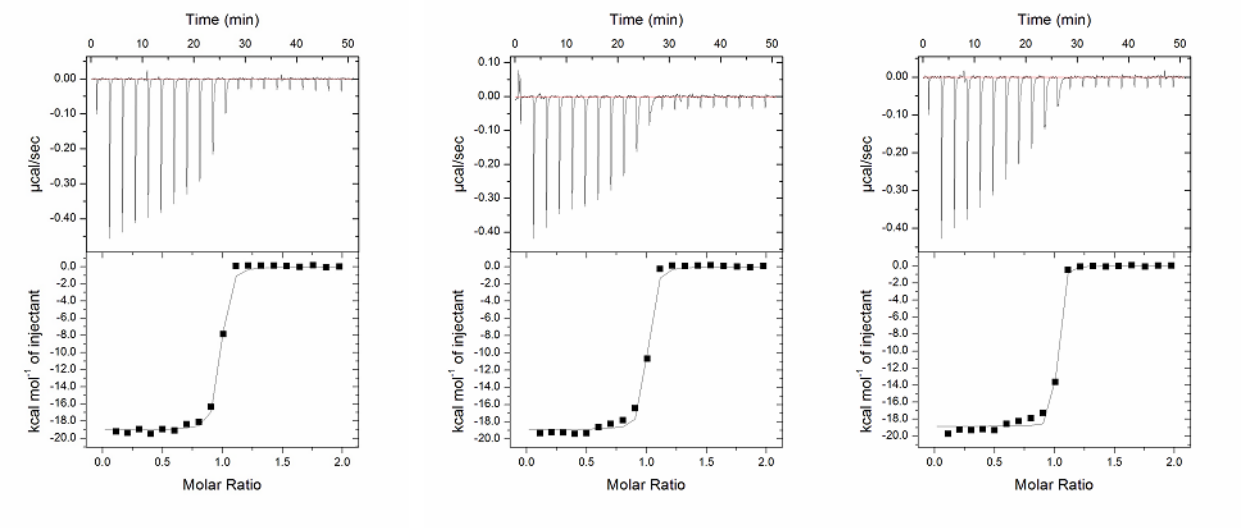

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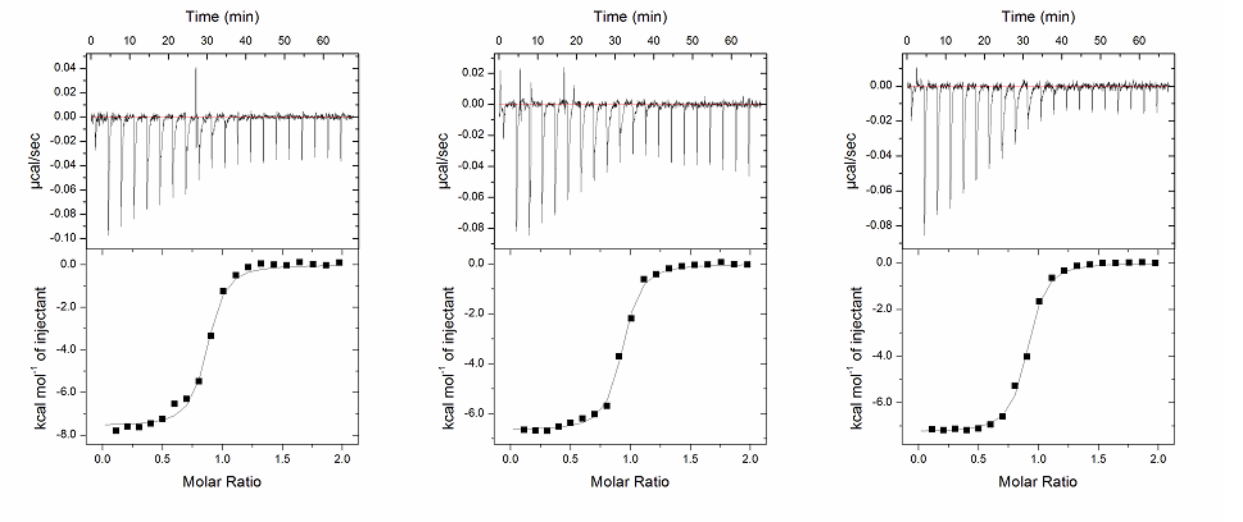

Figure S1: Raw isothermal titration calorimetry (ITC) data of Bio-AMS (**14**). A) Three independent direct binding experiments of Bio-AMS (100 µM) and *Mt*BPL (10 µM). B) Three independent competitive experiments with Biotin (100 µM), Bio-AMS (100 µM) and *Mt*BPL (10 µM).

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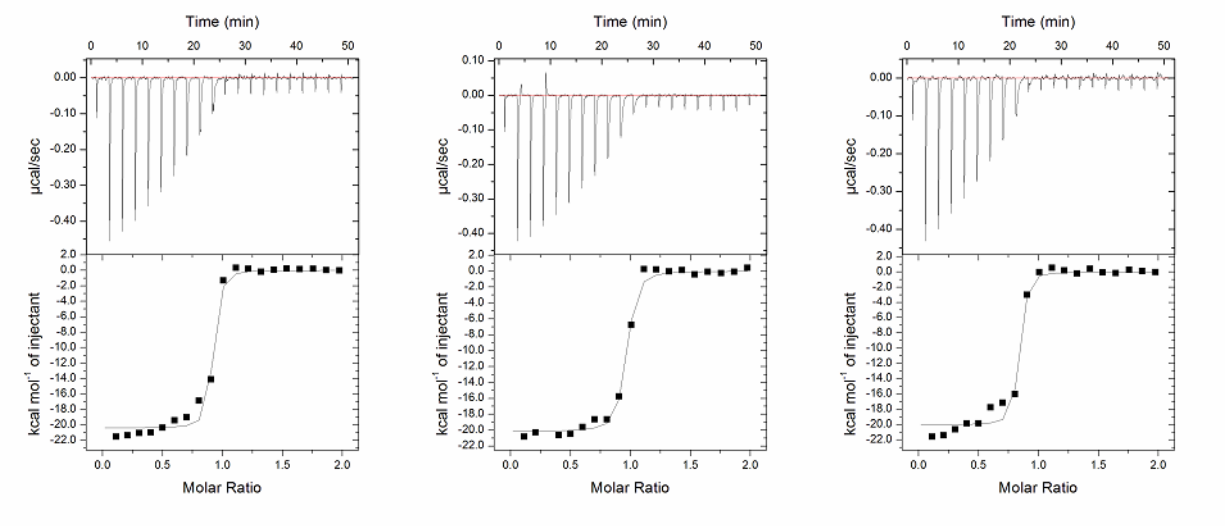

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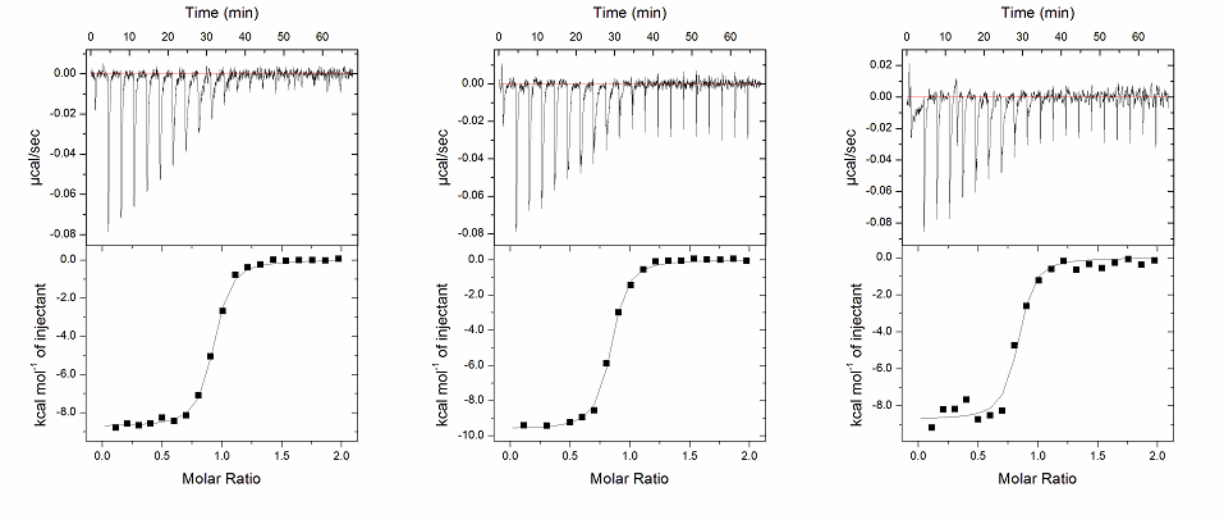

Figure S2: Raw isothermal titration calorimetry (ITC) data of Carbocyclic-Bio-AMS (**28**). A) Three independent direct binding experiments of **28** (100 µM) and *Mt*BPL (10 µM). B) Three independent competitive experiments with Biotin (100 µM), **28** (100 µM) and *Mt*BPL (10 µM).

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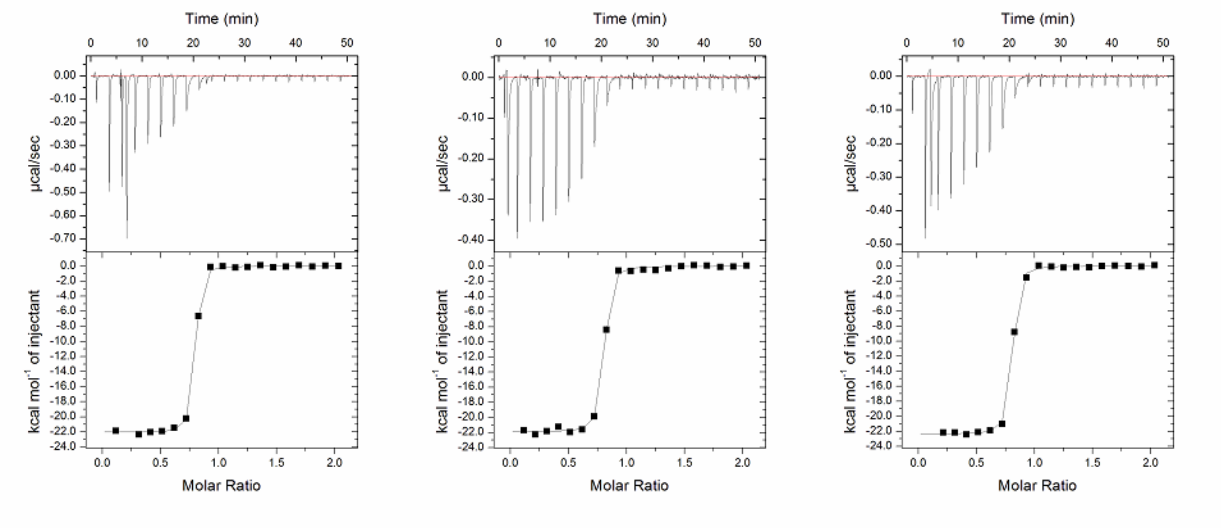

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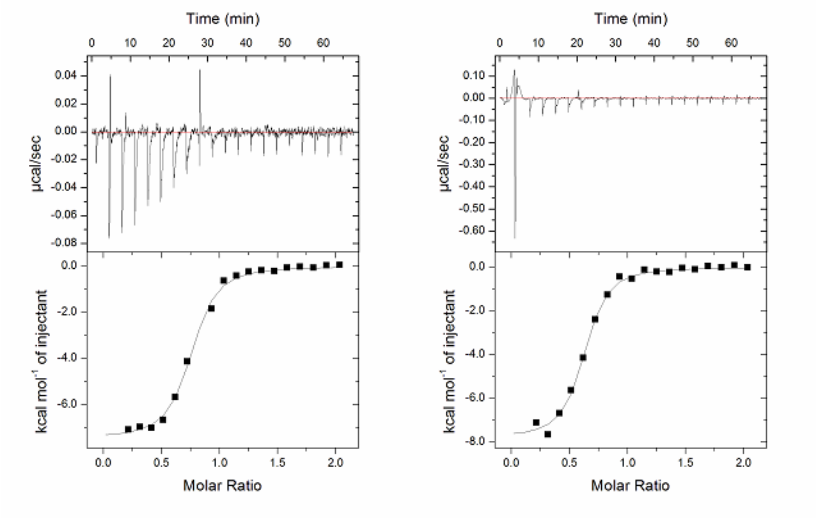

Figure S3: Raw isothermal titration calorimetry (ITC) data of Cyclopentenyl Bio-AMS (**34**). A) Three independent direct binding experiments of **34** (100 µM) and *Mt*BPL (10 µM). B) Three independent competitive experiments with Biotin (100 µM), **34** (100 µM) and *Mt*BPL (10 µM).

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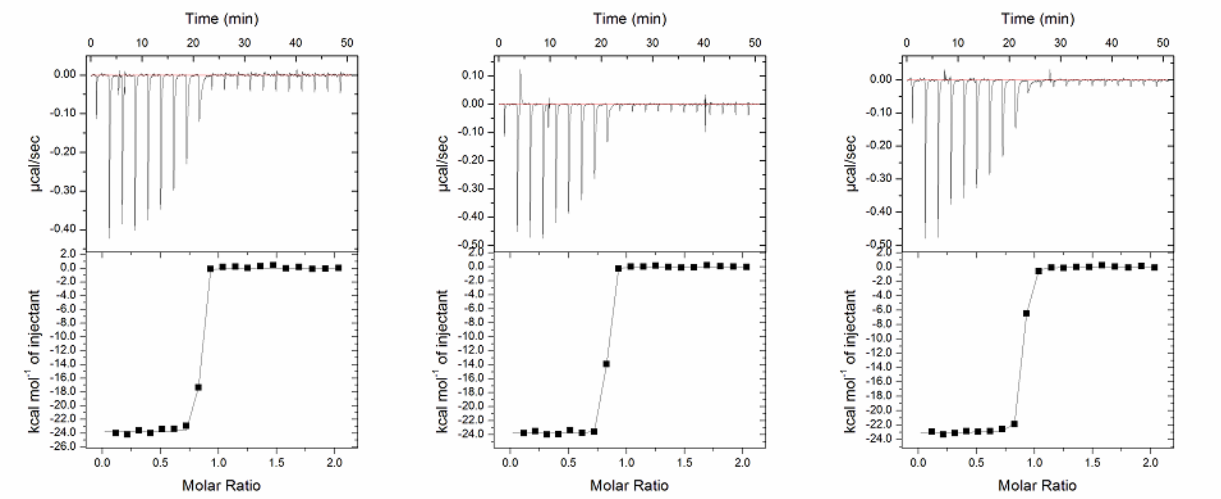

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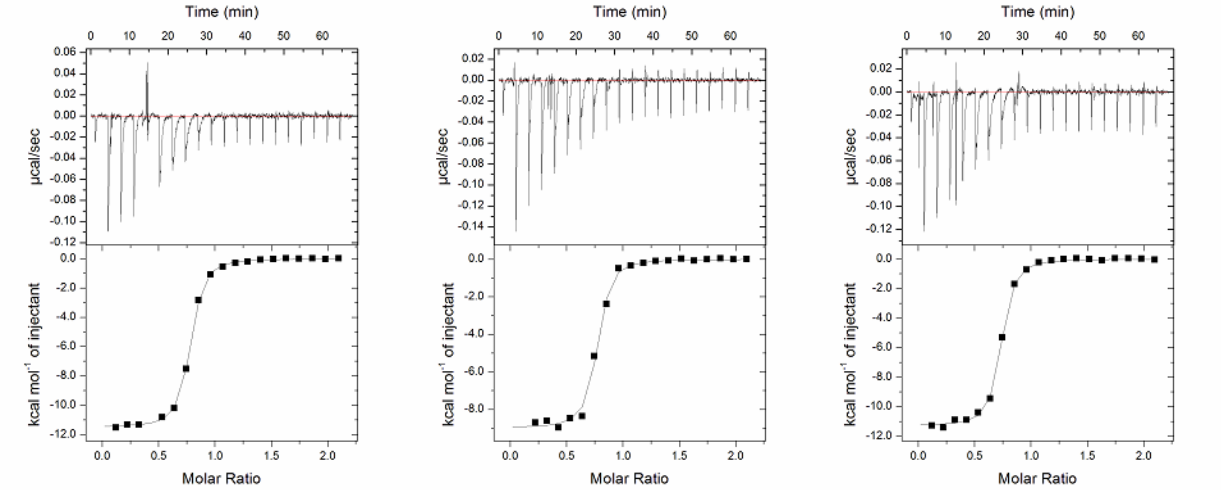

Figure S4: Raw isothermal titration calorimetry (ITC) data of Cyclopentyl Bio-AMS (**36**). A) Three independent direct binding experiments of **36** (100 µM) and *Mt*BPL (10 µM). B) Three independent competitive experiments with Biotin (100 µM), **36** (100 µM) and *Mt*BPL (10 µM).

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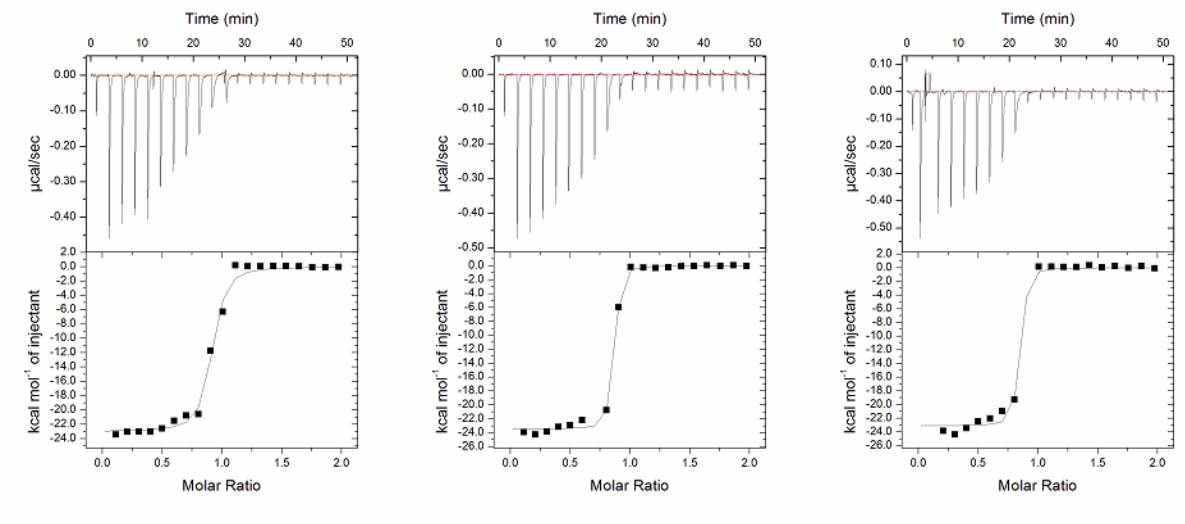

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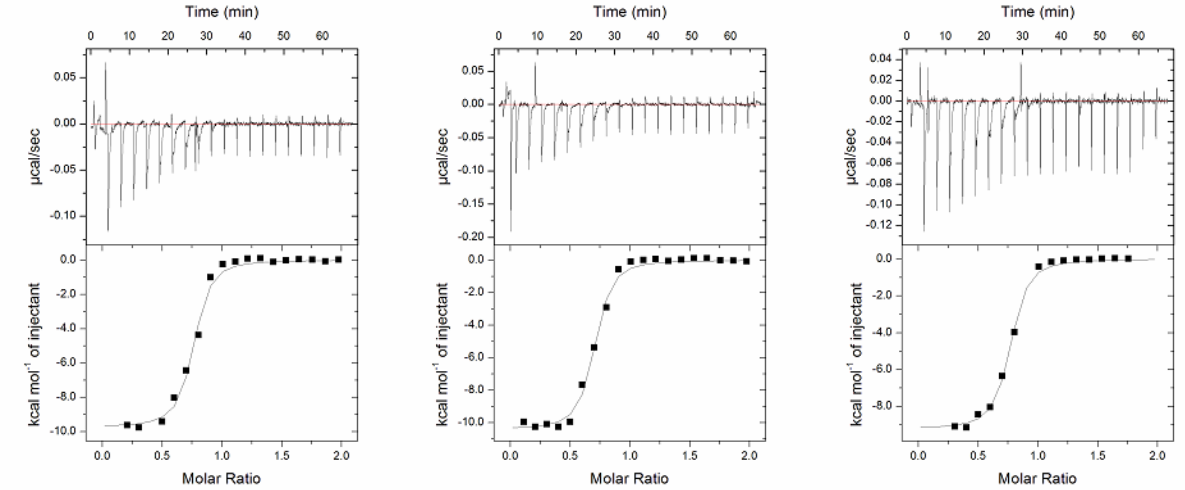

Figure S5: Raw isothermal titration calorimetry (ITC) data of 2′-α-Azido-2′-deoxy-Bio-AMS (**46**). A) Three independent direct binding experiments of **46** (100 µM) and *Mt*BPL (10 µM). B) Three independent competitive experiments with Biotin (100 µM), **46** (100 µM) and *Mt*BPL (10 µM).

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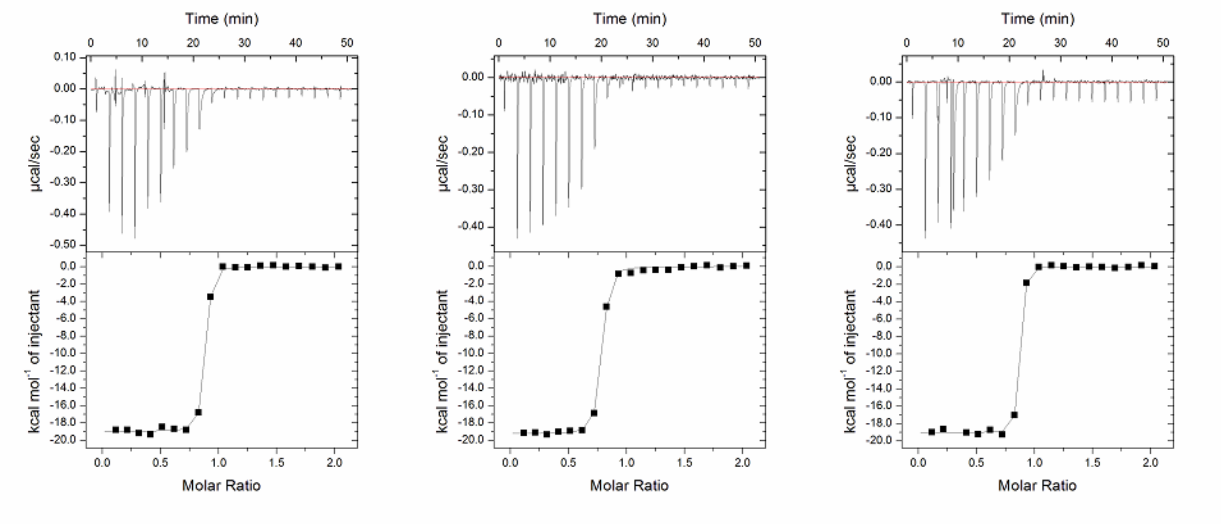

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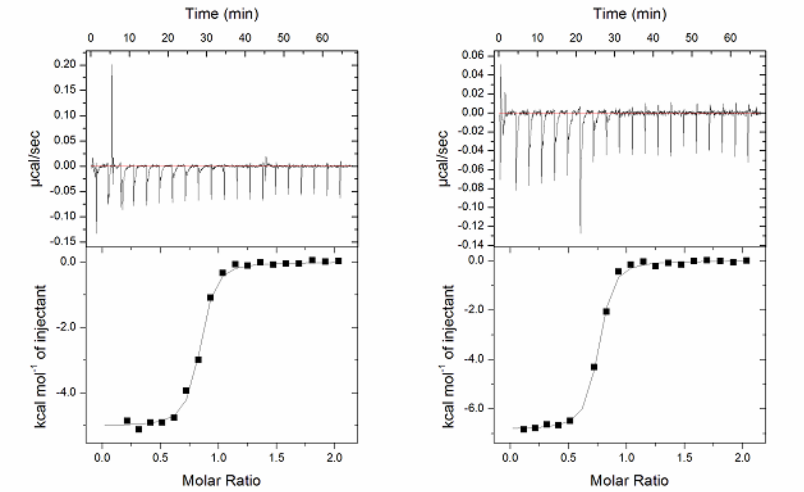

Figure S6: Raw isothermal titration calorimetry (ITC) data of 2′-α-Amino-2′-deoxy-Bio-AMS (**47**). A) Three independent direct binding experiments of **47** (100 µM) and *Mt*BPL (10 µM). B) Two independent competitive experiments with Biotin (100 µM), **47** (100 µM) and *Mt*BPL (10 µM).

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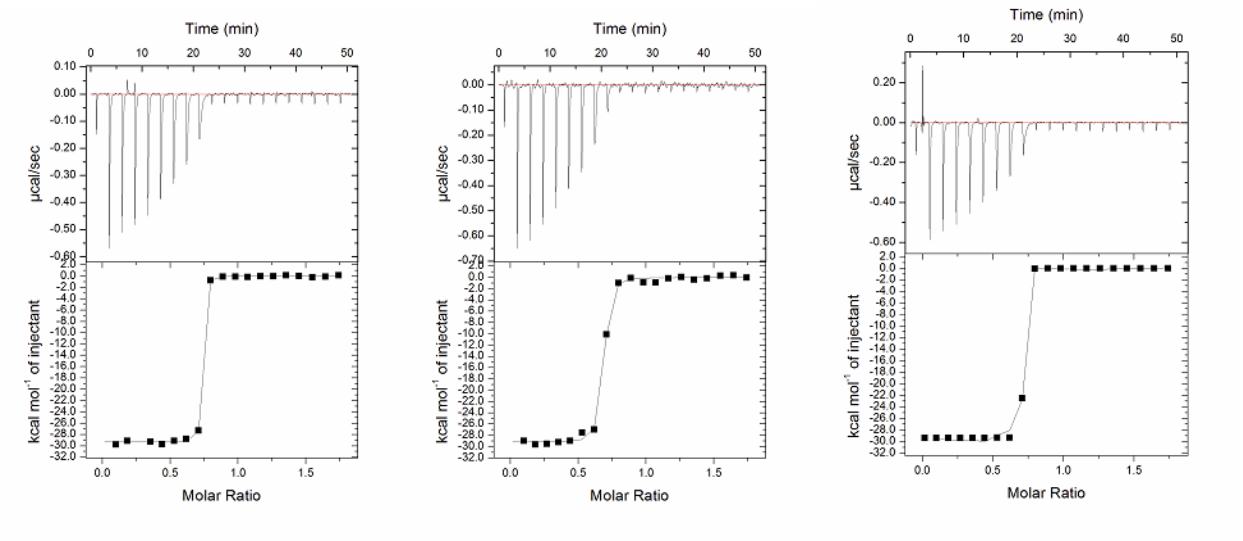

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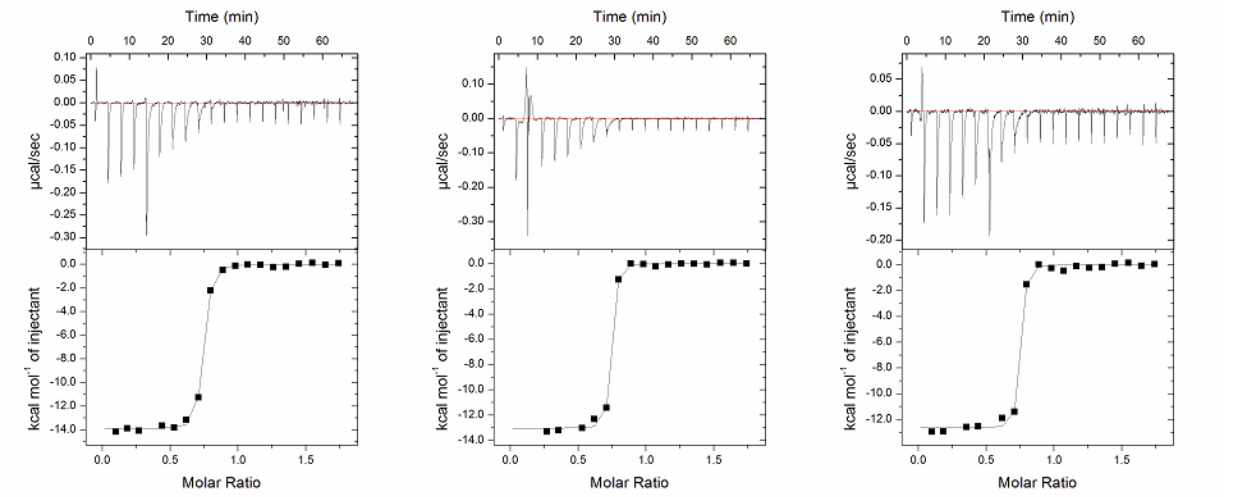

Figure S7: Raw isothermal titration calorimetry (ITC) data of 2′-β-azido-2′-deoxy-Bio-AMS (**57**). A) Three independent direct binding experiments of **57** (100 µM) and *Mt*BPL (10 µM). B) Three independent competitive experiments with Biotin (100 µM), **57** (100 µM) and *Mt*BPL (10 µM).

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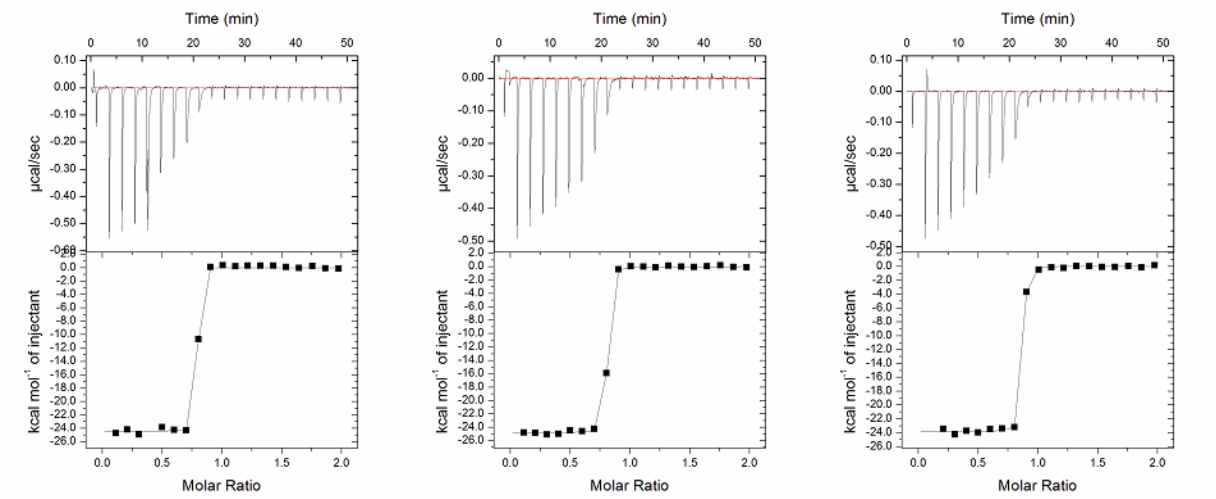

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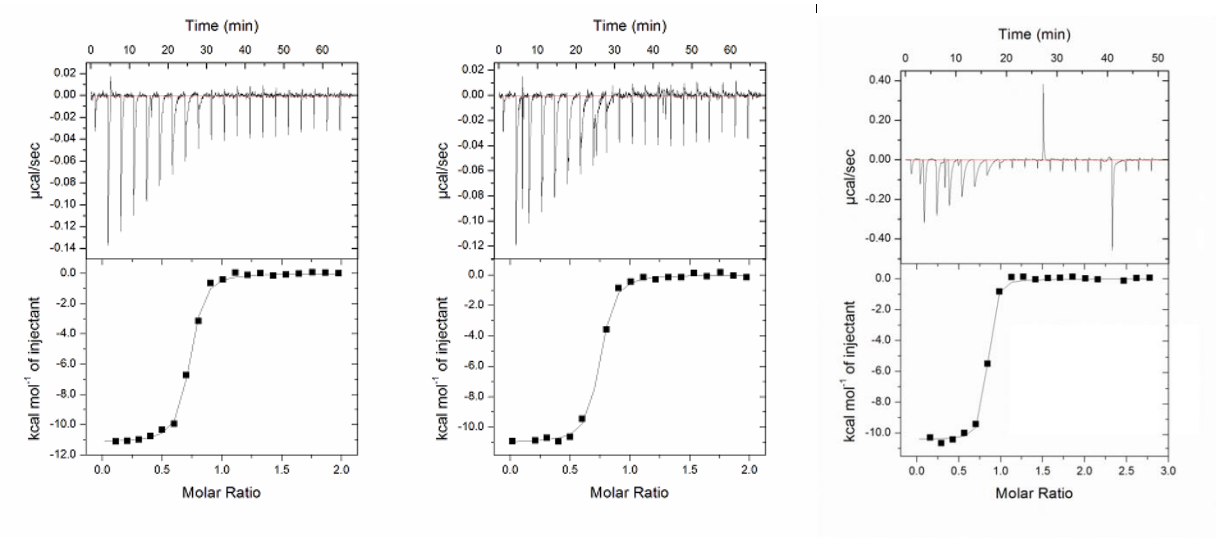

Figure S8: Raw isothermal titration calorimetry (ITC) data of 2′-Deoxy-2′-α-fluoro-Bio-AMS (**63**). A) Three independent direct binding experiments of **63** (100 µM) and *Mt*BPL (10 µM). B) Three independent competitive experiments with Biotin (100 µM), **63** (100 µM) and *Mt*BPL (10 µM).

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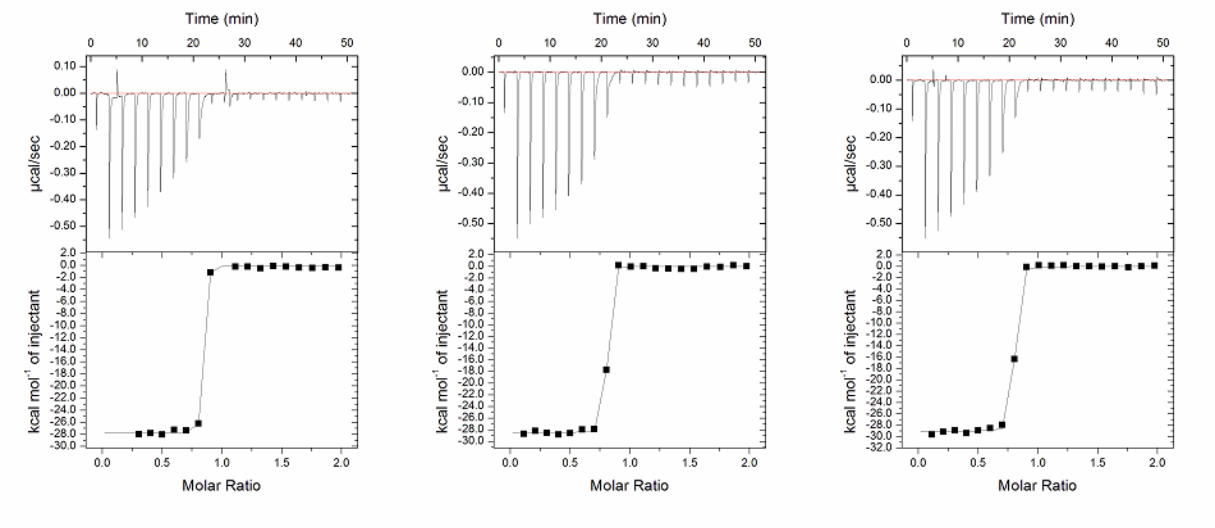

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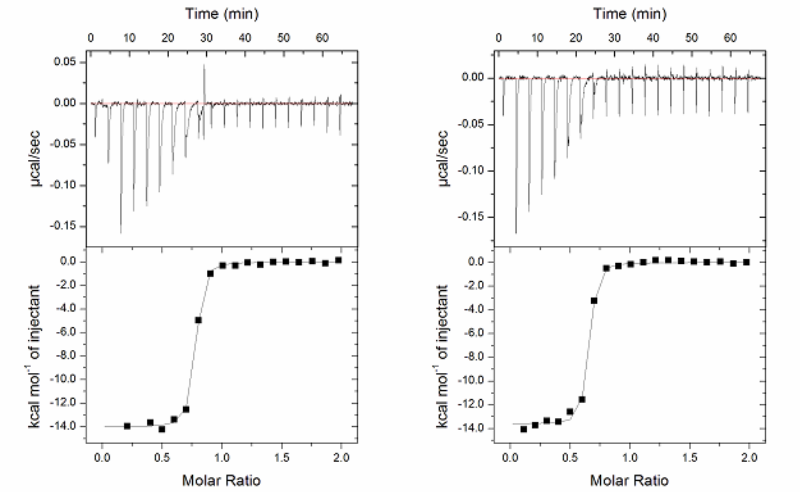
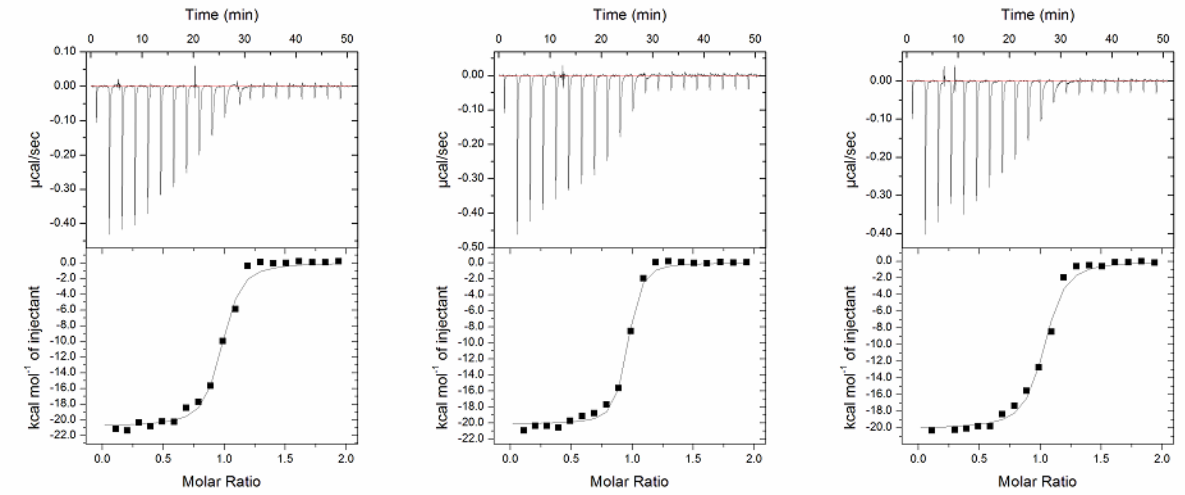


Figure S9: Raw isothermal titration calorimetry (ITC) data of 2′-Deoxy-2′-β-fluoro-Bio-AMS (**69**). A) Three independent direct binding experiments of **69** (100 µM) and *Mt*BPL (10 µM). B) Two independent competitive experiments with Biotin (100 µM), **69** (100 µM) and *Mt*BPL (10 µM).

A



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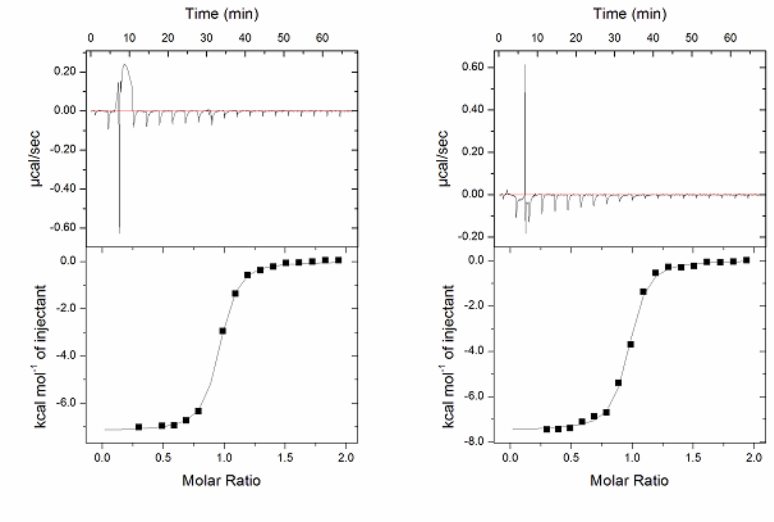
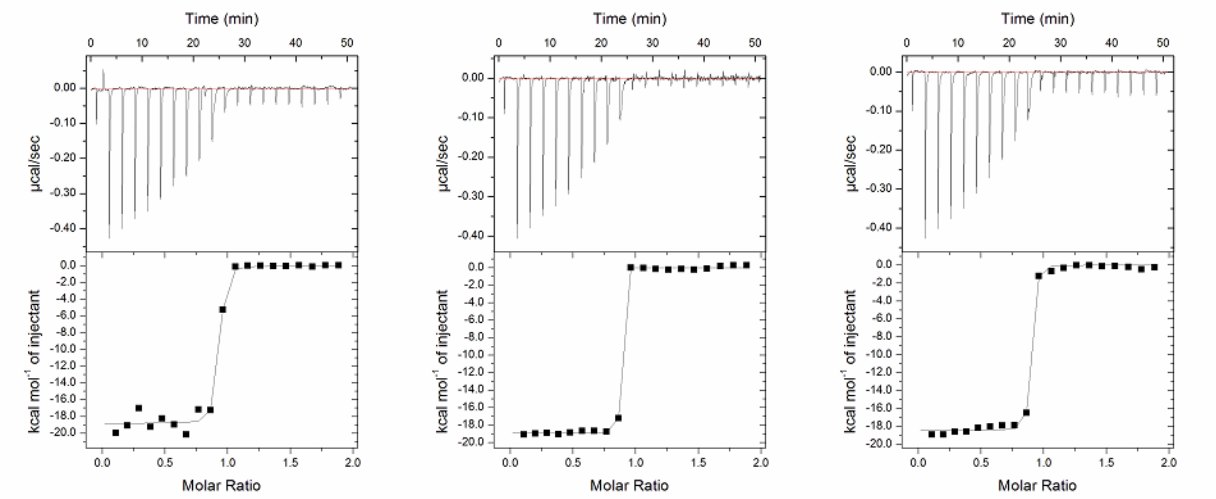


Figure S10: Raw isothermal titration calorimetry (ITC) data of C-2′-α-methyl-Bio-AMS (**73**). A) Three independent direct binding experiments of **73** (100 μM) and *Mt*BPL (10 μM). B) Two independent competitive experiments with Biotin (100 μM), **73** (100 μM) and *Mt*BPL (10 μM).

A



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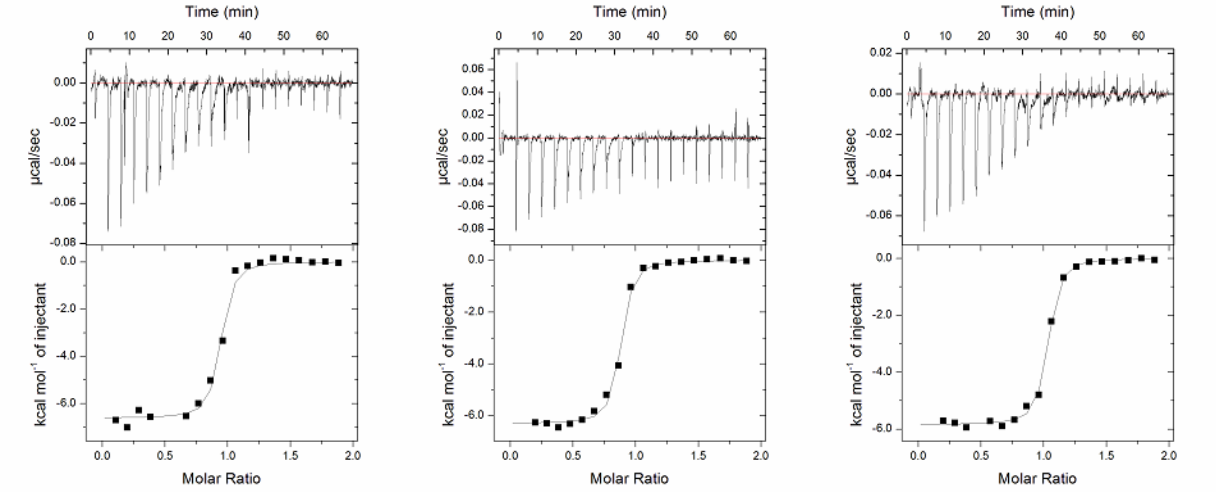
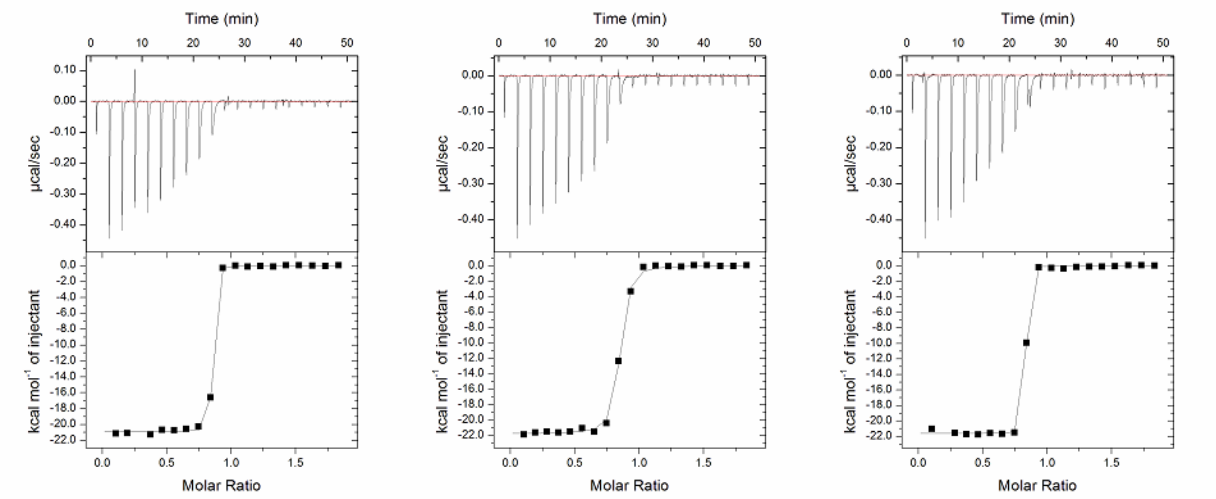


Figure S11: Raw isothermal titration calorimetry (ITC) data of Morpholinyl-Bio-AMS (**82**). A) Three independent direct binding experiments of **82** (100 µM) and *Mt*BPL (10 µM). B) Three independent competitive experiments with Biotin (100 µM), **82** (100 µM) and *Mt*BPL (10 µM).

A



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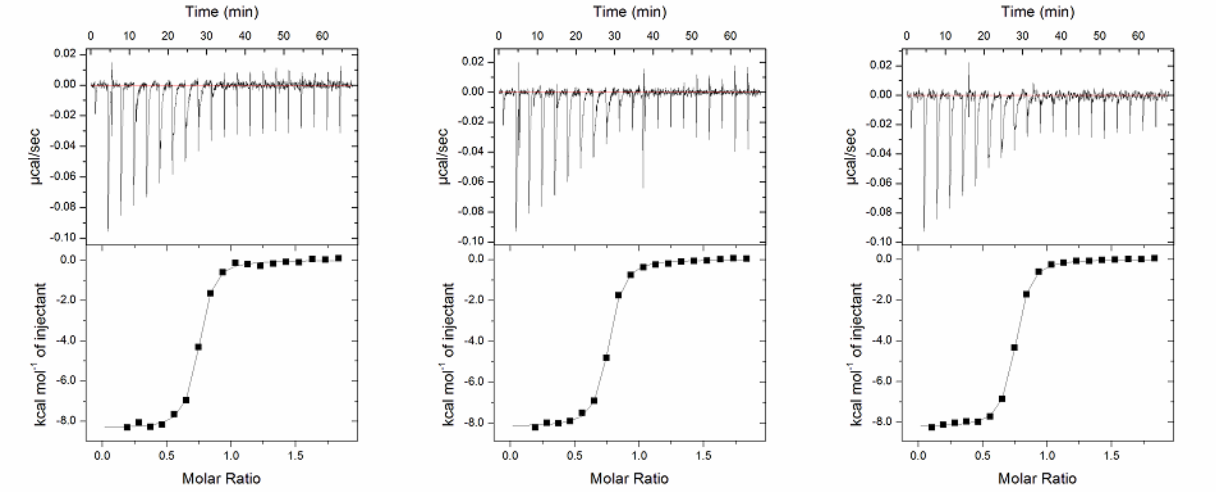

Figure S12: Raw isothermal titration calorimetry (ITC) data of 3′-Deoxy-Bio-AMS (**87**). A) Three independent direct binding experiments of **87** (100 µM) and *Mt*BPL (10 µM). B) Three independent competitive experiments with Biotin (100 µM), **87** (100 µM) and *Mt*BPL (10 µM).

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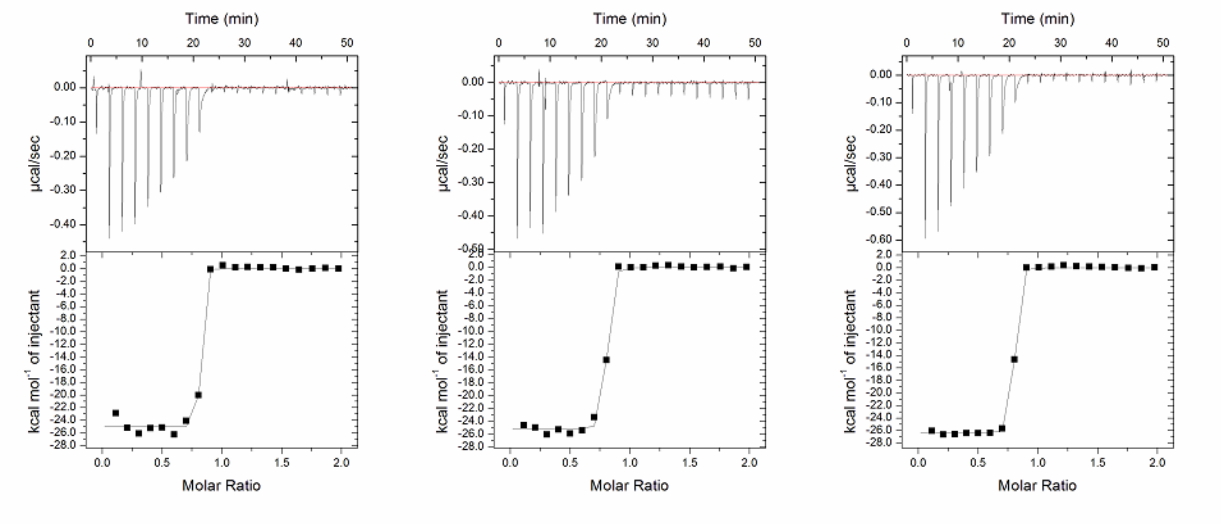

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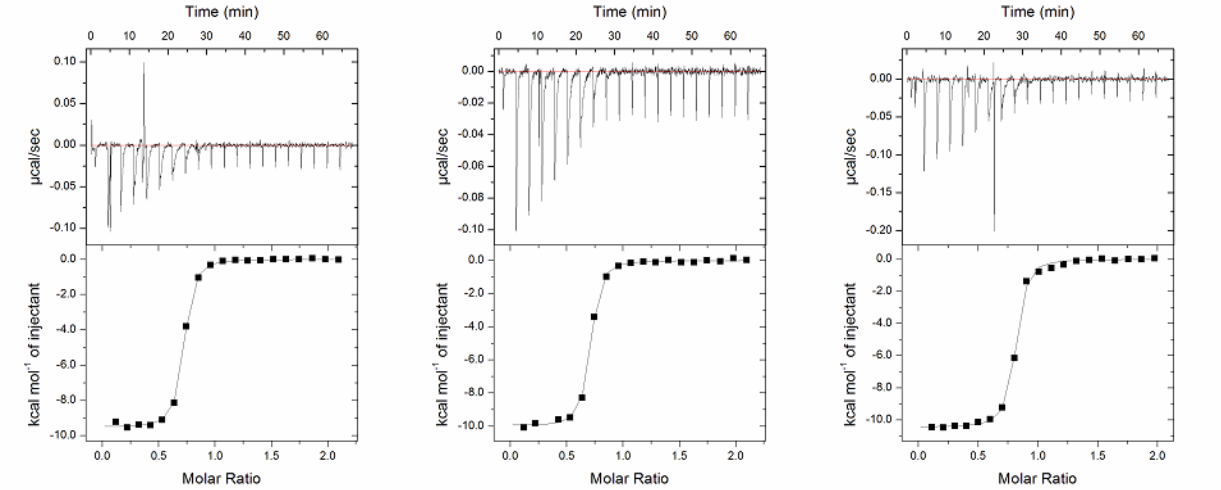


Figure S13: Raw isothermal titration calorimetry (ITC) data of Acyclo-Bio-AMS (**90**). A) Three independent direct binding experiments of **90** (100 µM) and *Mt*BPL (10 µM). B) Three independent competitive experiments with Biotin (100 µM), **90** (100 µM) and *Mt*BPL (10 µM).

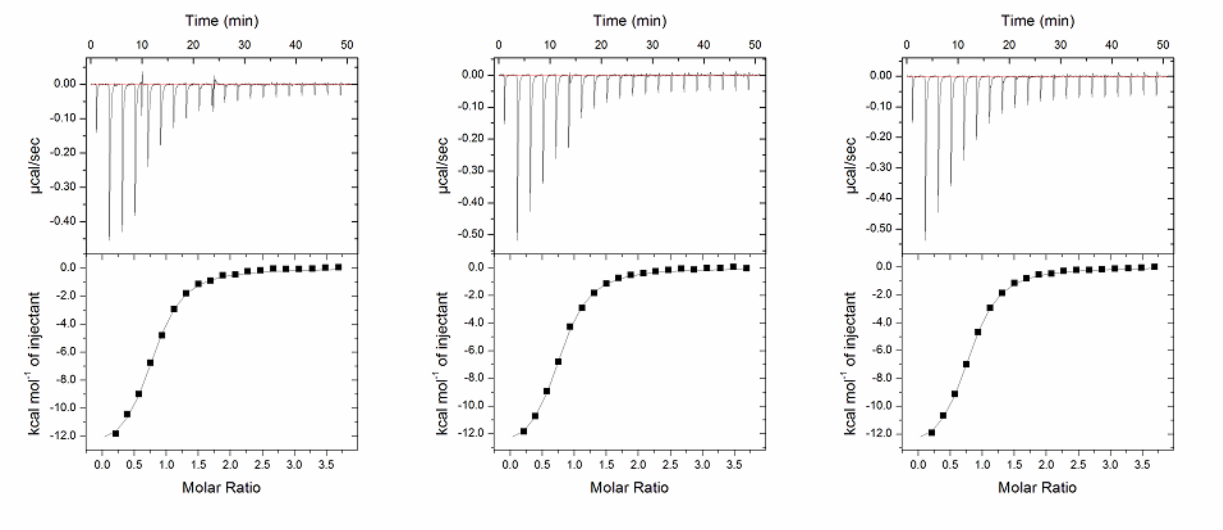


Figure S14: Raw isothermal titration calorimetry (ITC) data of biotin (**6**); three independent direct binding experiments of **6** (300 μM) and *Mt*BPL (10 μM).

Table S1. Raw isothermal titration calorimetry (ITC) data including: n value (direct titration), association constant in the presence of 100 μM Biotin (K_A^{app}), association constant when corrected using equation 1 (K_A), dissociation constant (K_D), enthalpy (ΔH), Gibbs' free energy (ΔG), and entropy ($-T\Delta S$) for each analog indicating each separate trial.

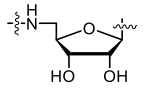
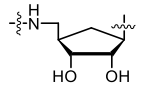
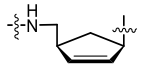
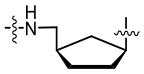
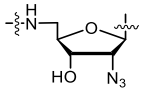
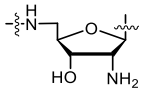
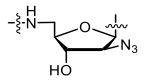
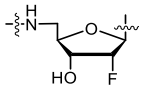
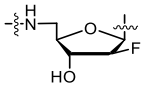
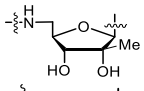
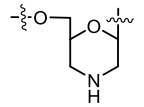
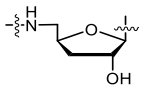
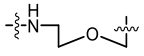
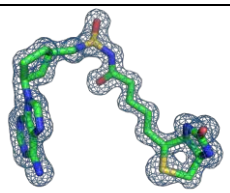
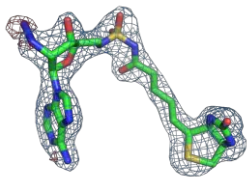
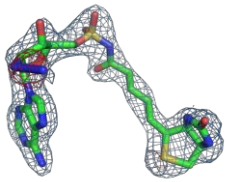
Compound	Trial	n	K_A^{app} (M^{-1})	K_A (M^{-1})	K_D (nM)	ΔH (kcal/mol)	ΔG (kcal/mol)	$-T\Delta S$ (kcal/mol)
	1	0.940	1.49E+07	1.10E+09	0.909	-19.06	-12.335	6.72
	2	0.962	1.63E+07	1.20E+09	0.831	-18.92	-12.388	6.53
	3	0.982	1.58E+07	1.17E+09	0.857	-18.85	-12.370	6.48
	1	0.877	1.80E+07	1.33E+09	0.752	-20.39	-12.447	7.94
	2	0.921	1.79E+07	1.32E+09	0.756	-20.17	-12.444	7.73
	3	0.796	1.80E+07	1.33E+09	0.752	-20.03	-12.447	7.58
	1	0.759	6.21E+06	4.59E+08	2.18	-21.92	-11.816	10.10
	2	0.748	6.16E+06	4.55E+08	2.20	-22.04	-11.812	10.23
	3	0.806	6.63E+06	4.90E+08	2.04	-22.38	-11.855	10.52
	1	0.785	2.50E+07	1.85E+09	0.542	-23.75	-12.641	11.11
	2	0.800	2.48E+07	1.83E+09	0.546	-23.72	-12.637	11.08
	3	0.853	2.54E+07	1.88E+09	0.533	-23.05	-12.651	10.40
	1	0.820	1.39E+07	1.03E+09	0.974	-23.47	-12.294	11.18
	2	0.873	1.53E+07	1.13E+09	0.885	-23.01	-12.351	10.66
	3	0.805	1.44E+07	1.06E+09	0.940	-23.11	-12.320	10.80
	1	0.737	2.20E+07	1.63E+09	0.615	-18.90	-12.566	6.65
	2	0.836	2.12E+07	1.57E+09	0.639	-18.82	-12.544	6.39
	3	0.827	-	-	-	-18.82	-	-
	1	0.645	4.89E+08	3.61E+10	0.0277	-29.21	-14.403	14.81
	2	0.704	4.97E+08	3.67E+10	0.0272	-29.35	-14.413	14.94
	3	0.687	-	-	-	-29.42	-	-
	1	0.768	2.21E+07	1.63E+09	0.613	-24.80	-12.569	12.23
	2	0.747	2.14E+07	1.58E+09	0.633	-24.39	-12.550	11.84
	3	0.817	2.27E+07	1.68E+09	0.596	-23.77	-12.585	11.17
	1	0.765	8.87E+07	6.55E+09	0.153	-28.43	-13.392	15.04
	2	0.802	8.87E+07	6.55E+09	0.153	-27.76	-13.392	14.37
	3	0.860	-	-	-	-29.05	-	-
	1	0.918	1.77E+07	1.31E+09	0.765	-20.13	-12.437	7.69
	2	0.940	1.75E+07	1.29E+09	0.774	-20.79	-12.430	8.36
	3	0.994	-	-	-	-20.08	-	-
	1	0.855	3.98E+07	2.94E+09	0.34	-18.90	-12.917	5.98
	2	0.885	4.06E+07	3.00E+09	0.333	-18.82	-12.929	5.89
	3	0.885	4.45E+07	3.35E+09	0.298	-18.82	-12.995	5.82
	1	0.810	2.26E+07	1.67E+09	0.599	-21.76	-12.581	9.17
	2	0.823	2.31E+07	1.71E+09	0.586	-20.87	-12.595	8.27
	3	0.790	2.27E+07	1.68E+09	0.596	-21.60	-12.584	9.01
	1	0.760	4.25E+07	3.14E+09	0.319	-25.22	-12.956	12.26
	2	0.784	4.32E+07	3.19E+09	0.313	-25.02	-12.965	12.05
	3	0.759	4.33E+07	3.20E+09	0.313	-26.37	-12.967	13.40

Table S2. Summary of Crystallographic Data

Inhibitor	**36**	**46***	**57***
Final 1.5σ $2F_o$-F_c density (blue) -3σ F_o-F_c density (red)			
PDB ID code	4xtv	4xtw	4xtx
Source	APS 17 ID	IMCA 17-ID	IMCA 17-ID
Resolution (Å)	1.45	2.3	2.3
Space group	P 2_1 2_1 2_1	P 2_1 2_1 2_1	P 2_1 2_1 2_1
Cell axis length (Å)	63.896 68.937 115.713	63.076 68.939 115.094	63.367 68.841 114.452
Data Processing	Auto-Proc	Auto-Proc	Auto-Proc
Resolution range (Å) (high shell)	115.71-1.45 (1.455-1.450)	115.09-2.300 (2.308-2.300)	114.45-2.30 (2.308-2.300)
Observ. measured (high shell)	585195 (5893)	148467 (1477)	146421 (1444)
Unique reflections (high shell)	91151 (889)	22970 (219)	22929 (218)
Average multiplicity (high shell)	6.4 (6.6)	6.5 (6.7)	6.4 (6.6)
Completeness (%) (high shell)	100.0 (99.9)	100.0 100.0	99.0 (99.5)
R_{merge} (high shell)	0.040 (0.347)	0.080 (0.400)	0.076 (0.298)
Mean $<I/\sigma_I>$ (high shell)	26.8 (5.4)	17.5 (5.0)	19.0 (6.8)
Refinement statistics			
Resolution range (Å) (high shell)	36.415-1.450 (1.466-1.450)	55.314-2.30 (2.404-2.30)	58.99-2.30 (2.405-2.300)
Working reflections (high shell)	91060 (2843)	22913 (2683)	22873 (2659)
R_{free} reflections (high shell)	4562 (164)	1177 (126)	1169 (146)
R (%) (high shell)	0.1862 (0.2350)	0.1848 (0.2335)	0.1895 (0.2350)
R_{free} (%) (high shell)	0.2056 (0.2745)	0.2161 (0.3111)	0.2497 (0.2972)
Non-hydrogen atoms	4753	4120	4039
Solvent waters	637	126	117
Mean B-factors (Å^2)	19.84	30.13	35.62
Protein atoms	4040	3911	3939
RMS deviations From Ideal Geometry			
Bond lengths (Å)	0.003	0.003	0.002
Bond angles (°)	0.976	0.834	0.842
Ramachandran plot outliers (%)	0.2	0.2	0.4
MolProbity score	1.21	1.33	1.32

***** No density for azide. 3σ (-) diff density envelops current modeled position

Table S2. Summary of Crystallographic Data. Part 2

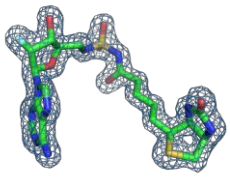
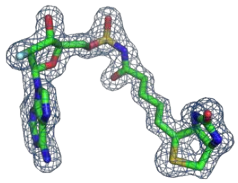
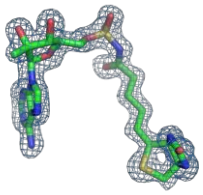
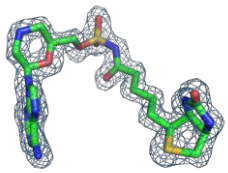
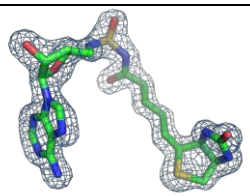
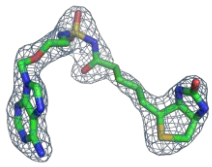
Inhibitor	**63**	**69**	**73**
Final 1.5σ $2F_o$-F_c density (blue) -3σ F_o-F_c density (red)			
PDB ID code	4xty	4xtz	4xu0
Source	IMCA 17-ID	IMCA 17-ID	APS 17 ID
Resolution (Å)	1.8	1.9	1.6
Space group	P 2_1 2_1 2_1	P 2_1 2_1 2_1	P 2_1 2_1 2_1
Cell axis length (Å)	63.408 68.731 114.488	63.449 68.875 115.581	63.550 68.907 115.333
Data Processing	Auto-Proc	Auto-Proc	Auto-Proc
Resolution range (Å) (high shell)	114.5-1.80 (1.806-1.800)	115.58-1.900 (1.906-1.900)	115.33-1.600 (1.605-1.600)
Observ. measured (high shell)	304872 (3088)	263045 (2962)	440593 (4290)
Unique reflections (high shell)	47014 (484)	40735 (433)	67262 (664)
Average multiplicity (high shell)	6.5 (6.4)	6.5 (6.8)	6.6 (6.5)
Completeness (%) (high shell)	99.8 (98.6)	100.0 (100.0)	99.6 (99.8)
R_{merge} (high shell)	0.055 (0.273)	0.073 (0.388)	0.052 (0.388)
Mean $<I/\sigma_I>$ (high shell)	21.6 (6.0)	19.6 (5.4)	22.3 (5.0)
Refinement statistics			
Resolution range (Å) (high shell)	36.14-1.80 (1.8368-1.8000)	42.73-1.90 (1.94-1.90)	43.32-1.600 (1.623-1.600)
Working reflections (high shell)	46947 (2557)	40669 (2569)	67183 (2601)
R_{free} reflections (high shell)	2376 (138)	2037 (119)	3405 (131)
R (%) (high shell)	0.1770 (0.1898)	0.1832 (0.2059)	0.1952 (0.2307)
R_{free} (%) (high shell)	0.2116 (0.2563)	0.2172 (0.2434)	0.2201 (0.2407)
Non-hydrogen atoms	4340	4347	4642
Solvent waters	332	328	617
Mean B-factors (Å^2)	21.94	21.19	21.15
Protein atoms	4008	3944	3947
RMS deviations From Ideal Geometry			
Bond lengths (Å)	0.008	0.003	0.003
Bond angles (°)	1.295	0.955	0.941
Ramachandran plot outliers (%)	0	0.2	0
MolProbity score	1.13	1.26	1.25

Table S2. Summary of Crystallographic Data. Part 3

Inhibitor	**82**	**87**	**90**
Final 1.5σ $2F_o$-F_c density (blue) -3σ F_o-F_c density (red)			
PDB ID code	4xu1	4xu2	4xu3
Source	GMCAT-23-ID-B	IMCA 17-ID	ALS 4.2.2
Resolution (Å)	1.7	1.85	2.25
Space group	P 2_1 2_1 2_1	P 2_1 2_1 2_1	P 2_1 2_1 2_1
Cell axis length (Å)	63.219 71.38 113.702	63.753 68.875 115.609	63.326 68.730 114.093
Data Processing	HKL2000	Auto-Proc	HKL2000
Resolution range (Å) (high shell)	50.00-1.70 (1.73-1.70)	115.61-1.85 (1.856-1.850)	50.0-2.25 (2.29-2.25)
Observ. measured (high shell)	419551	282718 (2943)	71806
Unique reflections (high shell)	57349 (2803)	44260 (459)	23132 (1023)
Average multiplicity (high shell)	7.3 (7.1)	6.4 (6.4)	3.1 (2.5)
Completeness (%) (high shell)	99.94 (99)	100.0 (99.8)	94.4 (84.7)
R_{merge} (high shell)	0.080 (0.410)	0.055 (0.334)	0.043 (0.257)
Mean $<I/\sigma_I>$ (high shell)	24.7 (5.3)	21.9 (5.2)	12.3 (1.47)
Refinement statistics			
Resolution range (Å) (high shell)	30.45-1.70 (1.728-1.700)	36.37-1.85 (1.890-1.850)	31.66-2.24 (2.335-2.250)
Working reflections (high shell)	54370 (2538)	44191 (2607)	23108 (2165)
R_{free} reflections (high shell)	2905 (113)	2227 (122)	1198 (120)
R (%) (high shell)	0.1709 (0.2022)	0.1919 (0.2192)	0.1854 (0.2379)
R_{free} (%) (high shell)	0.2028 (0.2531)	0.2219 (0.2439)	0.2324 (0.2819)
Non-hydrogen atoms	4678	4486	4171
Solvent waters	654	511	179
Mean B-factors (Å^2)	20.31	24.51	38.02
Protein atoms	4024	3901	3923
RMS deviations From Ideal Geometry			
Bond lengths (Å)	0.007	0.002	0.002
Bond angles (°)	1.309	0.797	0.767
Ramachandran plot outliers (%)	0.2	0.2	0.2
MolProbity score	1.24	1.25	1.27