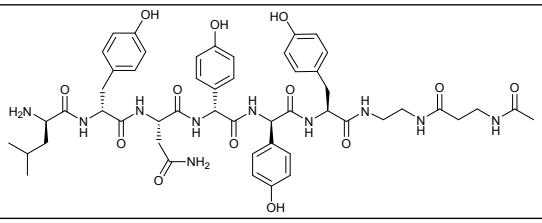
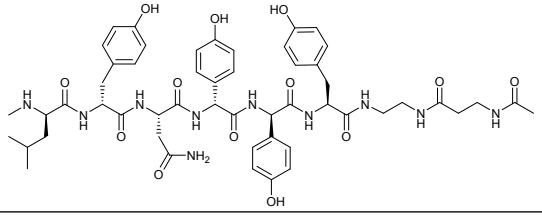
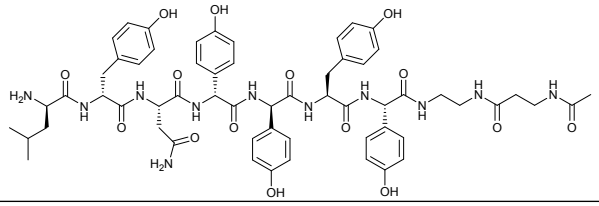
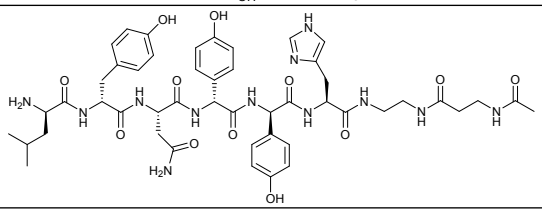
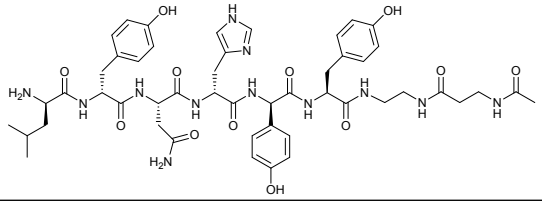
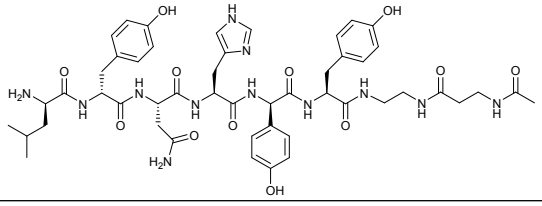
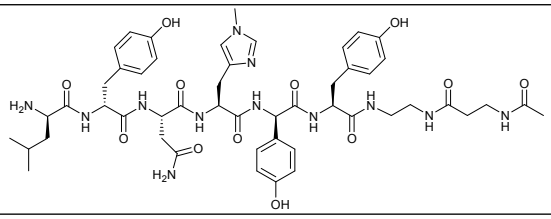
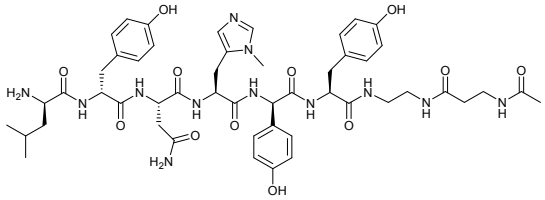
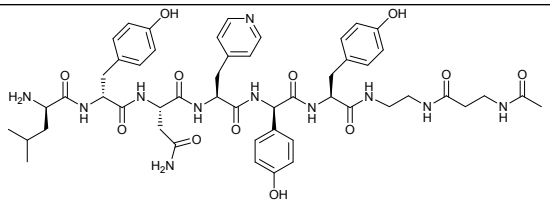
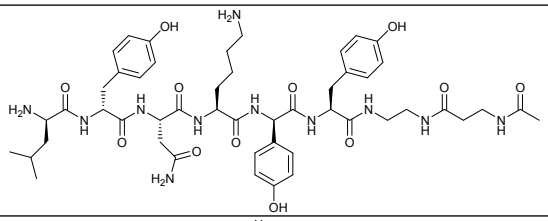
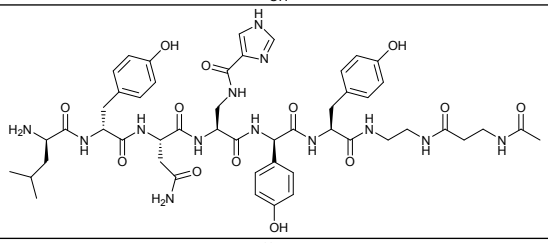
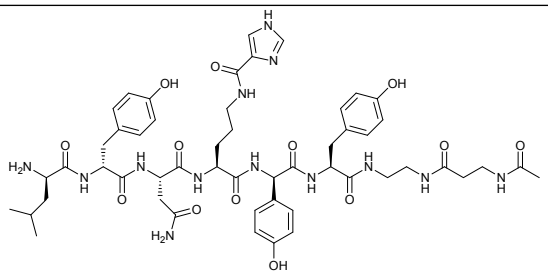
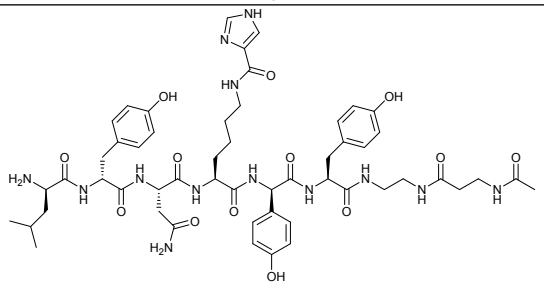
Supplementary Information for

Design and synthesis of peptide inhibitor conjugates as probes of the Cytochrome P450s from glycopeptide antibiotic biosynthesis

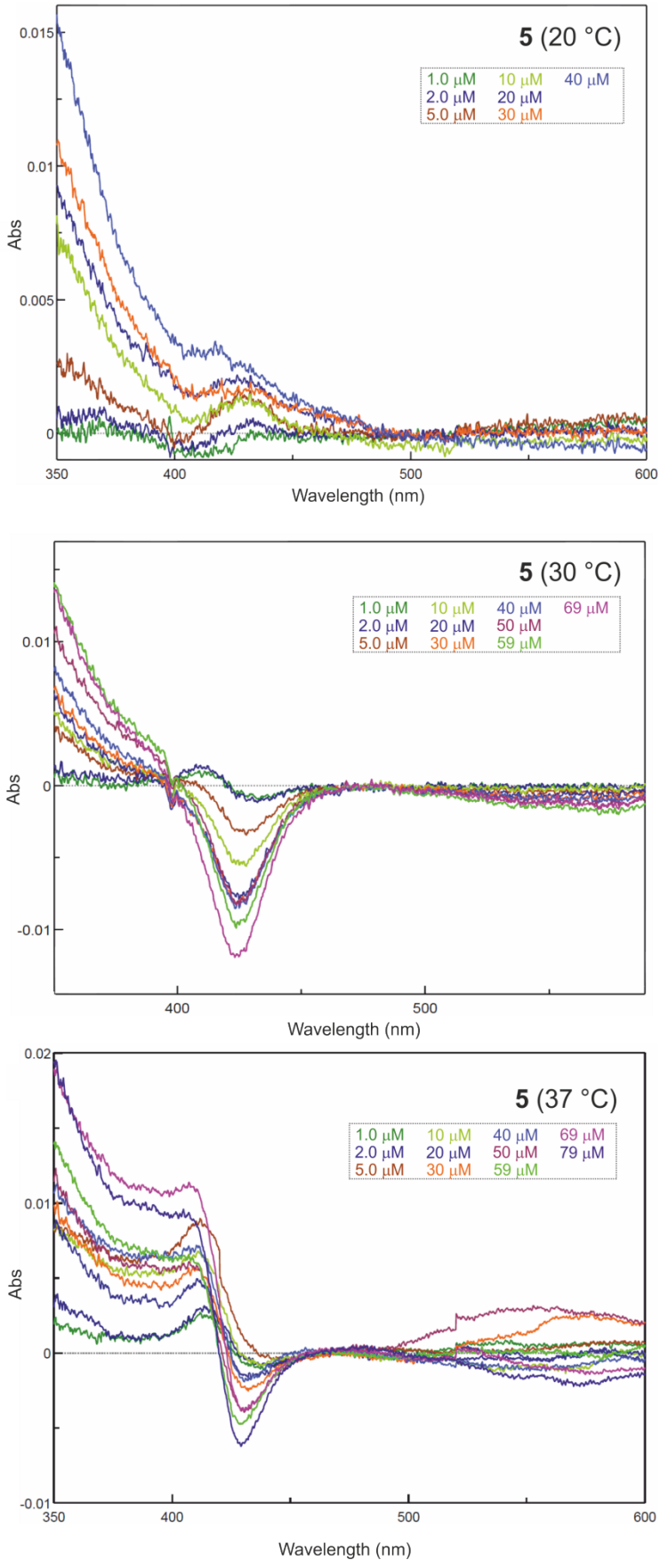
Clara Brieke,* Theresa Maier, Martin Schröter and Max J. Cryle*

Supplementary Table 1. Characterisation of linker peptides **5-17**.

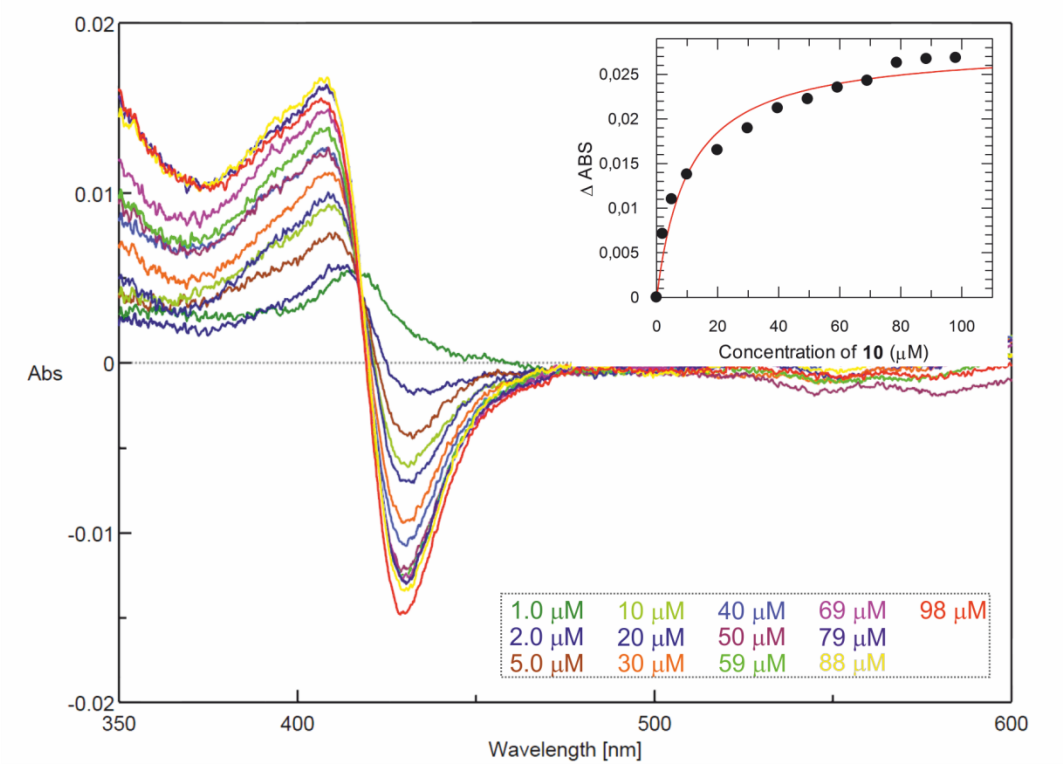
#	peptide structure	molecular data	mass found	R_t*
5		Chemical Formula: $C_{51}H_{64}N_{10}O_{13}$ Exact Mass: 1024,47 Molecular Weight: 1025,13	1025.55 $(M+H)^+$ 513.30 $(M+2H)^{2+}$	13.63 min
6		Chemical Formula: $C_{52}H_{66}N_{10}O_{13}$ Exact Mass: 1038,48 Molecular Weight: 1039,16	1039.65 $(M+H)^+$ 520.30 $(M+2H)^{2+}$	13.47 min
7		Chemical Formula: $C_{59}H_{71}N_{11}O_{15}$ Exact Mass: 1173,51 Molecular Weight: 1174,28	1174.65 $(M+H)^+$ 587.85 $(M+2H)^{2+}$	14.40 min
8		Chemical Formula: $C_{48}H_{62}N_{12}O_{12}$ Exact Mass: 998,46 Molecular Weight: 999,10	999.40 $(M+H)^+$ 500.25 $(M+2H)^{2+}$	11.15 min
9		Chemical Formula: $C_{49}H_{64}N_{12}O_{12}$ Exact Mass: 1012,48 Molecular Weight: 1013,12	1013.55 $(M+H)^+$ 507.25 $(M+2H)^{2+}$	11.72 min
10		Chemical Formula: $C_{49}H_{64}N_{12}O_{12}$ Exact Mass: 1012,48 Molecular Weight: 1013,12	1013.55 $(M+H)^+$ 507.30 $(M+2H)^{2+}$	11.95 min

#	peptide structure	molecular data	mass found	R_t*
11		Chemical Formula: $C_{50}H_{66}N_{12}O_{12}$ Exact Mass: 1026,49 Molecular Weight: 1027,15	1027.65 $(M+H)^+$ 514.30 $(M+2H)^{2+}$	12.23 min
12		Chemical Formula: $C_{50}H_{66}N_{12}O_{12}$ Exact Mass: 1026,49 Molecular Weight: 1027,15	1027.65 $(M+H)^+$ 514.30 $(M+2H)^{2+}$	12.21 min
13		Chemical Formula: $C_{51}H_{65}N_{11}O_{12}$ Exact Mass: 1023,48 Molecular Weight: 1024,15	1024.60 $(M+H)^+$ 512.80 $(M+2H)^{2+}$	12.13 min
14		Chemical Formula: $C_{49}H_{69}N_{11}O_{12}$ Exact Mass: 1003,51 Molecular Weight: 1004,16	1004.60 $(M+H)^+$ 502.80 $(M+2H)^{2+}$	12.18 min
15		Chemical Formula: $C_{50}H_{65}N_{13}O_{13}$ Exact Mass: 1055,48 Molecular Weight: 1056,13	1056.65 $(M+H)^+$ 528.80 $(M+2H)^{2+}$	12.50 min
16		Chemical Formula: $C_{52}H_{69}N_{13}O_{13}$ Exact Mass: 1083,51 Molecular Weight: 1084,20	1084.65 $(M+H)^+$ 542.85 $(M+2H)^{2+}$	12.50
17		Chemical Formula: $C_{53}H_{71}N_{13}O_{13}$ Exact Mass: 1097,53 Molecular Weight: 1098,23	1098.70 $(M+H)^+$ 549.80 $(M+2H)^{2+}$	12.69 min

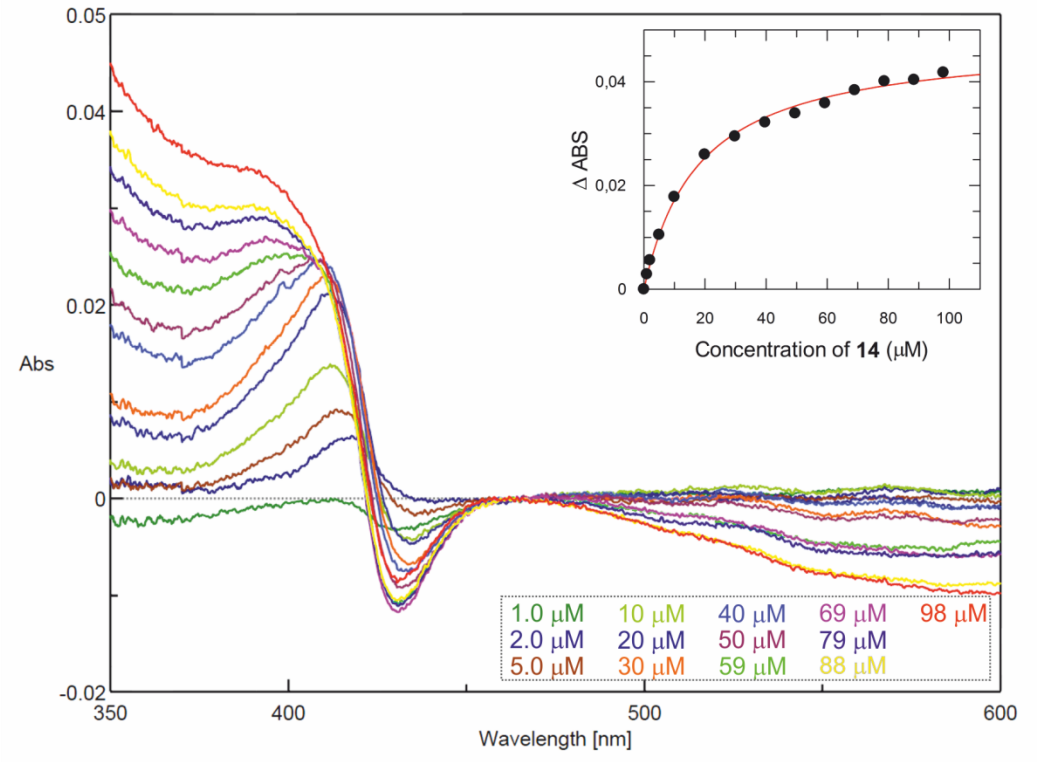
* Gradient used: 0 – 4 min 95 % solvent A; 4 – 25 min up to 55 % solvent B, then column wash with 100% B and re-equilibration to 95% A; Waters XBridge BEH300 C_{18} column 5 µm, 4.6 x 250 mm, flow rate 1 mL/min. Solvents used: water + 0.1% formic acid (solvent A) and HPLC-grade MeCN + 0.1% formic acid (solvent B).



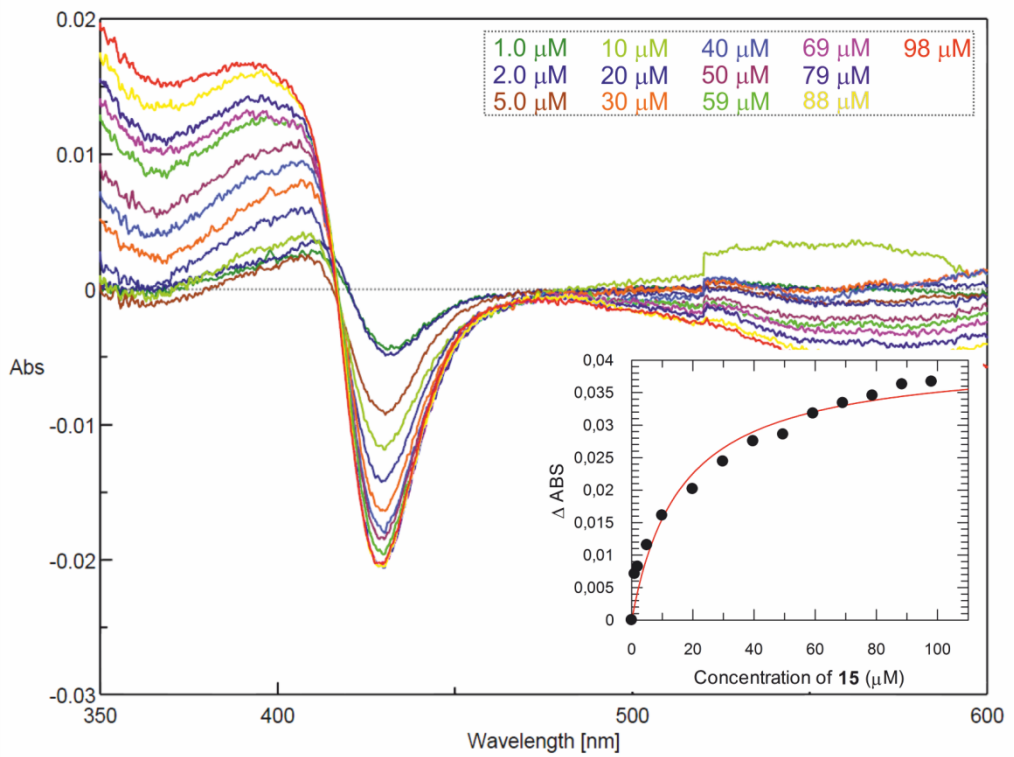
Supplementary Figure 1. The difference of temperature on the binding signal produced by the titration of $OxyB_{van}$ with **5**.



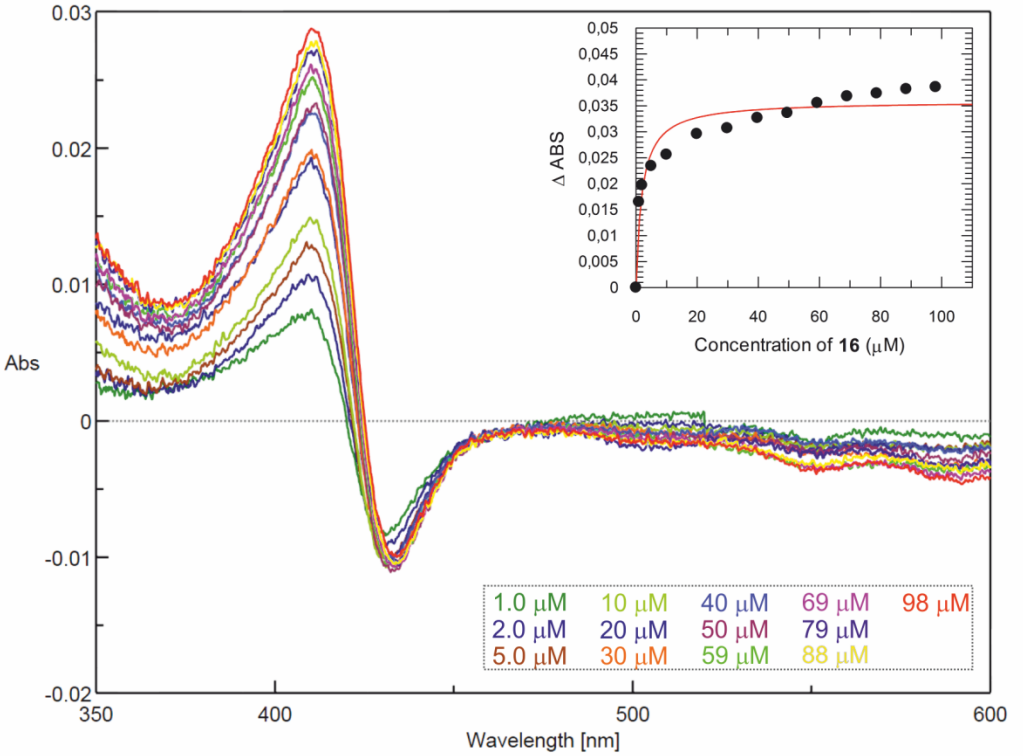
Supplementary Figure 2. Difference spectra of the binding of **10** to $OxyB_{van}$ with the difference in absorbance plotted against the concentration of **10** and fitted to a single site binding model (insert).



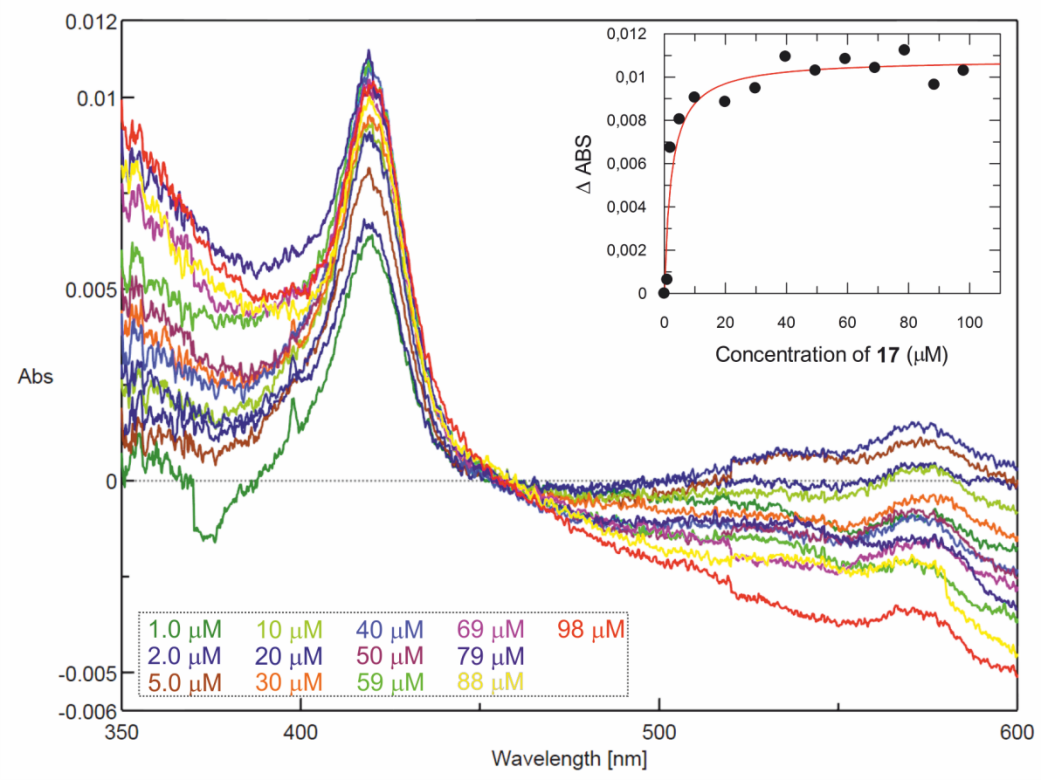
Supplementary Figure 3. Difference spectra of the binding of **14** to $OxyB_{van}$ with the difference in absorbance plotted against the concentration of **14** and fitted to a single site binding model (insert).



Supplementary Figure 4. Difference spectra of the binding of **15** to $OxyB_{van}$ with the difference in absorbance plotted against the concentration of **15** and fitted to a single site binding model (insert).



Supplementary Figure 5. Difference spectra of the binding of **16** to $OxyB_{van}$ with the difference in absorbance plotted against the concentration of **16** and fitted to a single site binding model (insert).



Supplementary Figure 6. Difference spectra of the binding of **17** to $OxyB_{van}$ with the difference in absorbance plotted against the concentration of **17** and fitted to a single site binding model (insert).