

Supplementary Material

Exploring the Substrate Specificity of OxyB, a Phenol Coupling P450 Enzyme Involved in Vancomycin Biosynthesis**Katharina Woithe, Nina Geib, Odile Meyer, Tanja Wörtz, Katja Zerbe and John A. Robinson**

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Peptide synthesis

Peptides were synthesized as described. Relevant analytical data for each new compound are given below.

*Pentapeptide 5A*¹H-NMR Chemical shift assignments (600 MHz, at 300 K, DMSO-d₆).

Residue	NH	C(α)-H	C(β)-H	Others
Tyr ¹	8.05	4.39	2.60, 2.83	C(δ)H=7.01, C(ε)H=6.63, OH=9.15, NAc=1.73
Asn ²	8.32	4.60	2.32, 2.44	N(δ)H=6.90, 7.30
Hpg ³	8.03	5.51	-	C(γ)H=7.18, C(δ)H=6.67, OH=9.34
Hpg ⁴	8.72	5.44	-	C(γ)H=7.08, C(δ)H=6.65, OH=9.36
Tyr ⁵	8.33	4.26	2.65, 2.79	C(δ)H=6.74, C(ε)H=6.52, OH=9.17
COOH	-	-	-	12.73

ESI-MS (MeCN/H₂O 1:1 + HCOOH 0.1%): *m/z* = 799.4 ([M+H]⁺).*Tetrapeptide 6A*¹H-NMR Chemical shift assignments (600 MHz, at 300 K, DMSO-d₆).

Residue	NH	C(α)-H	C(β)-H	Others
Asn ¹	8.12	4.61	2.29, 2.46	N(δ)H=6.88, 7.29, NAc=1.83
Hpg ²	8.04	5.49	-	C(γ)H=7.16, C(δ)H=6.67, OH=9.35
Hpg ³	8.69	5.42	-	C(γ)H=7.07, C(δ)H=6.65, OH=9.36
Tyr ⁴	8.30	4.25	2.64, 2.80	C(δ)H=6.75, C(ε)H=6.52, OH=9.17

ESI-MS (MeCN/H₂O 1:1 + HCOOH 0.1%): $m/z = 636.2$ ($[M+H]^+$).

Tripeptide 7A

¹H-NMR Chemical shift assignments (600 MHz, at 300 K, DMSO-d₆).

Residue	NH	C(α)-H	C(β)-H	Others
Hpg ¹	8.33	5.52	-	C(γ)H=7.18, C(δ)H=6.68, OH=9.35, NAc=1.85
Hpg ²	8.56	5.52	-	C(γ)H=7.05, C(δ)H=6.64, OH=9.34
Tyr ³	8.34	4.25	2.65, 2.81	C(δ)H=6.76, C(ε)H=6.52, OH=9.17

ESI-MS (MeCN/H₂O 1:1 + HCOOH 0.1%): $m/z = 522.2$ ($[M+H]^+$).

Tetrapeptide 8A

¹H-NMR Chemical shift assignments (500 MHz, at 300 K, DMSO-d₆).

Residue	NH	C(α)-H	C(β)-H	Others
Ala ¹	8.36	5.54	1.14	NAc=1.83
Hpg ²	8.25	5.52	-	C(γ)H=7.17, C(δ)H=6.68, OH=9.33
Hpg ³	8.62	5.43	-	C(γ)H=7.07, C(δ)H=6.65, OH=9.33
Tyr ⁴	8.28	4.26	2.66, 2.80	C(δ)H=6.76, C(ε)H=6.53, OH=9.15
COOH	-	-	-	12.69

ESI-MS (MeCN/H₂O 1:1 + HCOOH 0.1%): $m/z = 593.3$ ($[M+H]^+$), 615.3 ($[M+Na]^+$), 631.3 ($[M+K]^+$).

Tetrapeptide 9A

¹H-NMR Chemical shift assignments (500 MHz, at 300 K, DMSO-d₆).

Residue	NH	C(α)-H	C(β)-H	Others
Hpg ¹	8.36	5.54	-	C(γ)H=7.18, C(δ)H=6.65, OH=9.34, NAc=1.87

Hpg ²	8.58	5.50	-	C(γ)H=7.05, C(δ)H=6.61, OH=9.31
Hpg ³	8.63	5.44	-	C(γ)H=7.08, C(δ)H=6.65, OH=9.31
Tyr ⁴	8.29	4.26	2.66, 2.81	C(δ)H=6.77, C(ϵ)H=6.53, OH=9.15
COOH	-	-	-	12.69

ESI-MS (MeCN/H₂O 1:1 + HCOOH 0.1%): $m/z = 671.4$ ([M+H]⁺).

Hexapeptide 10A

¹H-NMR Chemical shift assignments (600 MHz, at 300 K, DMSO-d₆).

Residue	NH	C(α)-H	C(β)-H	Others
Leu ¹	8.62	3.57	1.49, 1.38	C(γ)H=1.49, C(δ)H ₃ =0.78, 0.84, NMe=1.96
Tyr ²	8.81	4.76	2.96, 2.58	C(δ)H=7.04, C(ϵ)H=6.62, OH=9.17
Asn ³	8.45	4.68	2.47, 2.33	N(δ)H=7.41, 6.92
Hpg ⁴	8.08	5.52	-	C(γ)H=7.18, C(δ)H=6.67, OH=9.34
Hpg ⁵	8.77	5.46	-	C(γ)H=7.06, C(δ)H=6.61, OH=9.34
Trp ⁶	8.34	4.38	2.89, 3.02	C(δ)H=7.02, C(ϵ)H=7.30, C(ϕ)H=6.85

ESI-MS (MeCN/H₂O 1:1 + HCOOH 0.1%): $m/z = 907.6$ ([M+H]⁺).

Tripeptide 11A

¹H-NMR Chemical shift assignments (500 MHz, at 300 K, DMSO-d₆).

Residue	NH	C(α)-H	C(β)-H	Others
Tyr ¹	7.92	4.36	2.53, 2.80	C(δ)H=6.97, C(ϵ)H=6.61, OH=9.15, NAc=1.71
Tyr ²	7.86	4.43	2.65, 2.88	C(δ)H=6.99, C(ϵ)H=6.61, OH=9.15
Tyr ³	8.10	4.37	2.83, 2.93	C(δ)H=7.02, C(ϵ)H=6.66, OH=9.15
COOH	-	-	-	12.64

ESI-MS (MeCN/H₂O 1:1 + HCOOH 0.1%): $m/z = 550.2$ ([M+H]⁺).

*Tripeptide 12A*¹H-NMR Chemical shift assignments (500 MHz, at 300 K, DMSO-d₆).

Residue	NH	C(α)-H	C(β)-H	Others
Tyr ¹	7.91	4.35	2.53, 2.79	C(δ)H=6.96, C(ε)H=6.60, OH=9.10, NAc=1.72
Tyr ²	7.74	4.45	2.49, 2.69	C(δ)H=6.79, C(ε)H=6.56, OH=9.10
Tyr ³	8.26	4.33	2.71, 2.95	C(δ)H=7.02, C(ε)H=6.64, OH=9.16
COOH	-	-	-	12.70

ESI-MS (MeCN/H₂O 1:1 + HCOOH 0.1%): *m/z* = 550.2 ([M+H]⁺).**Synthesis of peptide-PCP-7S conjugates**

Peptide-PCP-7S conjugates were made as described. Thioester products were analyzed by MS and 1D/2D ¹H-NMR spectroscopy. NMR assignments were made by standard methods, using 2D DQF-COSY, TOCSY and NOESY spectra. Relevant analytical data for the thioester intermediates are given below.

*Pentapeptide-S-phenylthioester 5B*¹H-NMR Chemical shift assignments (600 MHz, at 300 K, DMSO-d₆).

Residue	NH	C(α)-H	C(β)-H	Others
Tyr ¹	8.03	4.39	2.57, 2.82	C(δ)H=6.99, C(ε)H=6.62, OH=9.14, NAc=1.71
Asn ²	8.31	4.61	2.32, 2.43	N(δ)H=6.92, 7.30
Hpg ³	8.00	5.56	-	C(γ)H=7.19, C(δ)H=6.59, OH=9.28
Hpg ⁴	8.91	5.53	-	C(γ)H=7.01, C(δ)H=6.64, OH=9.36
Tyr ⁵	9.00	4.44	2.73, 2.94	C(δ)H=6.91, C(ε)H=6.57, OH=9.23
COSPh	-	-	-	7.27, 7.47

ESI-MS (MeCN/H₂O 1:1 + HCOOH 0.1%): *m/z* = 891.3 ([M+H]⁺), 913.3 ([M+Na]⁺), 929.3 ([M+K]⁺).

*Tetrapeptide-S-phenylthioester 6B*¹H-NMR Chemical shift assignments (500 MHz, at 300 K, DMSO-d₆).

Residue	NH	C(α)-H	C(β)-H	Others
Asn ¹	8.11	4.62	2.30, 2.47	N(δ)H=6.86, 7.03, NAc=1.82
Hpg ²	8.02	5.53	-	C(γ)H=7.18, C(δ)H=6.61, OH=9.29
Hpg ³	8.88	5.52	-	C(γ)H=7.02, C(δ)H=6.64, OH=9.35
Tyr ⁴	8.98	4.45	2.74, 2.96	C(δ)H=6.92, C(ε)H=6.57, OH=9.21
COSPh	-	-	-	7.27, 7.47

ESI-MS (MeCN/H₂O 1:1 + HCOOH 0.1%): $m/z = 750.3$ ([M+Na]⁺).*Tripeptide-S-phenylthioester 7B*¹H-NMR Chemical shift assignments (600 MHz, at 300 K, DMSO-d₆).

Residue	NH	C(α)-H	C(β)-H	Others
Hpg ¹	8.31	5.57	-	C(γ)H=7.21, C(δ)H=6.62, OH=9.31, NAc=1.85
Hpg ²	8.76	5.54	-	C(γ)H=7.00, C(δ)H=6.64, OH=9.37
Tyr ³	9.04	4.46	2.96, 2.75	C(δ)H=6.93, C(ε)H=6.58, OH=9.25
COSPh	-	-	-	7.47, 7.28

ESI-MS (MeCN/H₂O 1:1 + HCOOH 0.1%): $m/z = 636.3$ ([M+Na]⁺).*Tetrapeptide-S-phenylthioester 8B*¹H-NMR Chemical shift assignments (500 MHz, at 300 K, DMSO-d₆).

Residue	NH	C(α)-H	C(β)-H	Others
Ala ¹	8.01	4.38	1.14	NAc=1.82
Hpg ²	8.26	5.57	-	C(γ)H=7.19, C(δ)H=6.64, OH=9.30
Hpg ³	8.84	5.53	-	C(γ)H=7.02, C(δ)H=6.63, OH=9.36
Tyr ⁴	8.99	4.45	2.75, 2.95	C(δ)H=6.92, C(ε)H=6.58, OH=9.23
COSPh	-	-	-	7.27, 7.47

ESI-MS (MeCN/H₂O 1:1 + HCOOH 0.1%): $m/z = 685.4$ ([M+H]⁺).

Tetrapeptide-S-phenylthioester 9B

¹H-NMR Chemical shift assignments (500 MHz, at 300 K, DMSO-d₆).

Residue	NH	C(α)-H	C(β)-H	Others
Hp ^{g1}	8.35	5.56	-	C(γ)H=7.18, C(δ)H=6.65, OH=9.31, NAc=1.86
Hp ^{g2}	8.57	5.56	-	C(γ)H=7.06, C(δ)H=6.54, OH=9.26
Hp ^{g3}	8.83	5.54	-	C(γ)H=7.02, C(δ)H=6.64, OH=9.35
Tyr ⁴	8.99	4.46	2.75, 2.97	C(δ)H=6.93, C(ε)H=6.58, OH=9.21
COSPh	-	-	-	7.28, 7.47

ESI-MS (MeCN/H₂O 1:1 + HCOOH 0.1%): $m/z = 763.4$ ([M+H]⁺).

Hexapeptide-S-phenylthioester 10B

¹H-NMR Chemical shift assignments (600 MHz, at 300 K, DMSO-d₆).

Residue	NH	C(α)-H	C(β)-H	Others
Leu ¹	8.63	3.56	1.49, 1.37	C(γ)H=1.49, C(δ)H ₃ =0.77, 0.83, NMe=1.94
Tyr ²	8.78	4.77	2.97, 2.57	C(δ)H=7.04, C(ε)H=6.62, OH=9.17
Asn ³	8.46	4.69	2.47, 2.33	N(δ)H=7.30, 6.93
Hp ^{g4}	8.07	5.58	-	C(γ)H=7.20, C(δ)H=6.60, OH=9.30
Hp ^{g5}	8.95	5.55	-	C(γ)H=7.01, C(δ)H=6.60, OH=9.33
Trp ⁶	9.00	4.54	3.19, 3.02	C(δ)H=7.68, C(ε)H=7.33, 7.43, C(φ)H=6.95, 7.06, NH=9.37
COSPh	-	-	-	7.45, 7.24

ESI-MS (MeCN/H₂O 1:1 + HCOOH 0.1%): $m/z = 999.5$ ([M+H]⁺), 1021.5 ([M+Na]⁺).

Tripeptide-S-phenylthioester 11B

¹H-NMR Chemical shift assignments (600 MHz, at 300 K, DMSO-d₆).

Residue	NH	C(α)-H	C(β)-H	Others
Tyr ¹	7.94	4.38	2.53, 2.80	C(δ)H=6.96, C(ϵ)H=6.59, OH=9.13, NAc=1.72
Tyr ²	8.00	4.51	2.69, 2.97	C(δ)H=7.02, C(ϵ)H=6.62, OH=9.16
Tyr ³	8.75	4.59	2.91, 3.01	C(δ)H=7.05, C(ϵ)H=6.66, OH=9.23
COSPh	-	-	-	7.46, 7.32

ESI-MS (MeCN/H₂O 1:1 + HCOOH 0.1%): m/z = 642.4 ([M+H]⁺), 664.4 ([M+Na]⁺), 680.3 ([M+K]⁺).

Tripeptide-S-phenylthioester 12B

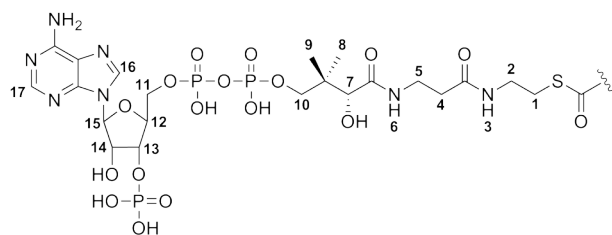
¹H-NMR Chemical shift assignments (600 MHz, at 300 K, DMSO-d₆).

Residue	NH	C(α)-H	C(β)-H	Others
Tyr ¹	7.93	4.40	2.53, 2.83	C(δ)H=6.97, C(ϵ)H=6.66, OH=9.12, NAc=1.72
Tyr ²	7.93	4.51	2.53, 2.67	C(δ)H=6.84, C(ϵ)H=6.59, OH=9.14
Tyr ³	8.84	4.57	2.79, 3.05	C(δ)H=7.08, C(ϵ)H=6.66, OH=9.22
COSPh	-	-	-	7.45, 7.34

ESI-MS (MeCN/H₂O 1:1 + HCOOH 0.1%): m/z = 642.3 ([M+H]⁺).

Pentapeptide-S-CoA thioester 5C

¹H-NMR Chemical shift assignments (700 MHz, at 300 K, DMSO-d₆) using the following nomenclature for the phosphopantetheinyl moiety.



Residue	NH	C(α)-H	C(β)-H	Others
Tyr ¹	8.03	4.39	2.58, 2.83	C(δ)H=7.00, C(ϵ)H=6.62, NAc=1.72

Asn ²	8.31	4.60	2.33, 2.44	N(δ)H=6.93, 7.30
Hpg ³	8.01	5.53	-	C(γ)H=7.17, C(δ)H=6.66
Hpg ⁴	8.81	5.48	-	C(γ)H=6.97, C(δ)H=6.62
Tyr ⁵	8.83	4.34	2.64, 2.90	C(δ)H=6.87, C(ϵ)H=6.55
COSCoA	-	-	-	1=2.80, 2.83, 2=3.11, 3=8.14, 4=2.28, 5=3.25, 3.33, 6=7.77, 7=3.76, 8=0.75, 9=0.95, 10=3.52, 3.91, 11=4.17, 12=4.39, 13=4.81, 14=4.72, 15=5.95, 16=8.58, 17=8.32

negative-ion ESI-MS: $m/z = 1546.3$ ($[M-H]^-$), 1568.3 ($[M+Na-2H]^-$), 1584.2 ($[M+K-2H]^-$).

Tetrapeptide-S-CoA thioester 6C

¹H-NMR Chemical shift assignments (500 MHz, at 300 K, DMSO-d₆).

Residue	NH	C(α)-H	C(β)-H	Others
Asn ¹	8.10	4.62	2.31, 2.47	N(δ)H=6.87, 7.29, NAc=1.83
Hpg ²	8.02	5.51		C(γ)H=7.16, C(δ)H=6.68
Hpg ³	8.77	5.47		C(γ)H=6.98, C(δ)H=6.63
Tyr ⁴	8.81	4.35	2.65, 2.90	C(δ)H=6.88, C(ϵ)H=6.56
COSCoA	-	-	-	1=2.82, 2=3.11, 3=8.13, 4=2.28, 5=3.27, 3.34, 6=7.76, 7=3.77, 8=0.75, 9=0.95, 10=3.54, 3.91, 11=4.17, 12=4.38, 13=4.83, 14=4.73, 15=5.98, 16=8.54, 17=8.29

negative-ion ESI-MS: $m/z = 1383.3$ ($[M-H]^-$), 1407.3 ($[M+Na-2H]^-$), 1421.2 ($[M+K-2H]^-$).

Tripeptide-S-CoA thioester 7C

¹H-NMR Chemical shift assignments (700 MHz, at 300 K, DMSO-d₆).

Residue	NH	C(α)-H	C(β)-H	Others
Hpg ¹	8.31	5.54	-	C(γ)H=7.18, C(δ)H=6.67, NAc=1.85
Hpg ²	8.64	5.49	-	C(γ)H=6.95, C(δ)H=6.62
Tyr ³	8.86	4.35	2.65, 2.92	C(δ)H=6.88, C(ϵ)H=6.55
COSCoA	-	-	-	1=2.28, 2.84, 2=3.12, 3=8.14, 4=2.28,

5=3.25, 3.33, 6=7.77, 7=3.76, 8=0.76,
9=0.95, 10=3.54, 3.91, 11=4.17, 12=4.40,
13=4.81, 14=4.71, 15=5.99, 16=8.62,
17=8.37

negative-ion ESI-MS: $m/z = 1269.1$ ($[M-H]^-$), 1291.0 ($[M+Na-2H]^-$), 1306.9 ($[M+K-2H]^-$).

Tetrapeptide-S-CoA thioester 8C

1H -NMR Chemical shift assignments (500 MHz, at 300 K, DMSO- d_6).

Residue	NH	C(α)-H	C(β)-H	Others
Ala ¹	8.01	4.38	1.14	NAc=1.82
Hpg ²	8.26	5.54	-	C(γ)H=7.17, C(δ)H=6.67
Hpg ³	8.74	5.47	-	C(γ)H=6.98, C(δ)H=6.62
Tyr ⁴	8.83	4.34	2.65, 2.91	C(δ)H=6.88, C(ϵ)H=6.56
COSCoA	-	-	-	1=2.82, 2=3.11, 3=8.15, 4=2.29, 5=3.25, 3.32, 6=7.77, 7=3.77, 8=0.75, 9=0.95, 10=3.50, 3.90, 11=4.15, 12=4.37, 13=4.82, 14=4.72, 15=5.97, 16=8.49, 17=8.25

negative-ion ESI-MS: $m/z = 1340.6$ ($[M-H]^-$), 1362.3 ($[M+Na-2H]^-$), 1378.2 ($[M+K-2H]^-$).

Tetrapeptide-S-CoA thioester 9C

1H -NMR Chemical shift assignments (500 MHz, at 300 K, DMSO- d_6).

Residue	NH	C(α)-H	C(β)-H	Others
Hpg ¹	8.36	5.55	-	C(γ)H=7.18, C(δ)H=6.64, NAc=1.87
Hpg ²	8.58	5.52	-	C(γ)H=7.04, C(δ)H=6.59
Hpg ³	8.75	5.49	-	C(γ)H=6.97, C(δ)H=6.62
Tyr ⁴	8.85	4.34	2.65, 2.91	C(δ)H=6.89, C(ϵ)H=6.56
COSCoA	-	-	-	1=2.83, 2=3.11, 3=8.15, 4=2.28, 5=3.26, 3.32, 6=7.77, 7=3.77, 8=0.75, 9=0.95, 10=3.49, 3.88, 11=4.15, 12=4.36, 13=4.82, 14=4.73, 15=5.96, 16=8.45, 17=8.21

negative-ion ESI-MS: $m/z = 1418.4$ ($[M-H]^-$), 1440.4 ($[M+Na-2H]^-$), 1456.3 ($[M+K-2H]^-$).

Hexapeptide-S-CoA thioester 10C

1H -NMR Chemical shift assignments (500 MHz, at 300 K, DMSO- d_6).

Residue	NH	C(α)-H	C(β)-H	Others
Leu ¹	8.63	3.58	1.49, 1.54	C(γ)H=1.49, C(δ)H ₃ =0.75, 0.83, NMe=1.99
Tyr ²	8.83	4.74	2.58, 2.95	C(δ)H=7.04, C(ϵ)H=6.62
Asn ³	8.51	4.64	2.35, 2.49	N(δ)H=6.89, 7.32
Hpg ⁴	7.97	5.51	-	C(γ)H=7.16, C(δ)H=6.66
Hpg ⁵	8.78	5.51	-	C(γ)H=6.96, C(δ)H=6.58
Trp ⁶	8.87	4.47	2.90, 3.14	C(δ)H=7.00, C(ϵ)H=7.33, 7.43, C(ϕ)H=6.92, 7.03, NH=9.23
COSCoA	-	-	-	1=2.77, 2=2.86, 2=3.11, 3=8.16, 4=2.29, 5=3.31, 6=7.77, 7=3.80, 8=0.74, 9=0.95, 10=3.59, 11=4.14, 12=4.36, 13=4.84, 14=4.76, 15=5.94, 16=8.44, 17=8.18

negative-ion ESI-MS: $m/z = 1655.5$ ($[M-H]^-$), 1677.6 ($[M+Na-2H]^-$), 1692.5 ($[M+K-2H]^-$).

Tripeptide-S-CoA thioester 11C

1H -NMR Chemical shift assignments (500 MHz, at 300 K, DMSO- d_6).

Residue	NH	C(α)-H	C(β)-H	Others
Tyr ¹	7.91	4.35	2.51, 2.77	C(δ)H=6.99, C(ϵ)H=6.61, NAc=1.71
Tyr ²	7.91	4.46	2.63, 2.93	C(δ)H=6.95, C(ϵ)H=6.60
Tyr ³	8.62	4.48	2.82, 2.95	C(δ)H=7.02, C(ϵ)H=6.65
COSCoA	-	-	-	1=2.84, 2=3.12, 3=8.12, 4=2.26, 5=3.24, 3.32, 6=7.74, 7=3.78, 8=0.72, 9=0.95, 10=3.46, 3.90, 11=4.13, 12=4.36, 13=4.83, 14=4.73, 15=5.94, 16=8.43, 17=8.17

negative-ion ESI-MS: $m/z = 1298.1$ ($[M-H]^-$), 1320.4 ($[M+Na-2H]^-$).

Tripeptide-S-CoA thioester 12C

¹H-NMR Chemical shift assignments (500 MHz, at 300 K, DMSO-d₆).

Residue	NH	C(α)-H	C(β)-H	Others
Tyr ¹	7.92	4.36	2.53, 2.78	C(δ)H=6.96, C(ε)H=6.61, NAc=1.72
Tyr ²	7.80	4.45	2.48, 2.65	C(δ)H=6.79, C(ε)H=6.57
Tyr ³	8.69	4.44	2.71, 3.00	C(δ)H=7.06, C(ε)H=6.65
COSSCoA	-	-	-	1=2.88, 2=3.16, 3=8.13, 4=2.27, 5=3.24, 3.32, 6=7.75, 7=3.77, 8=0.75, 9=0.94, 10=3.55, 3.91, 11=4.19, 12=4.40, 13=4.81, 14=4.72, 15=5.99, 16=8.63, 17=8.38

negative-ion ESI-MS: $m/z = 1298.0$ ([M-H]⁻).

The identity of each peptide-PCP-7S conjugates was confirmed by MALDI-MS:

5D, $m/z 11479 \pm 2$ [M+H]⁺, calcd mass 11478;

6D, $m/z 11313 \pm 2$ [M+H]⁺, calcd mass 11315;

7D, $m/z 11201 \pm 2$ [M+H]⁺, calcd mass 11201;

8D, $m/z 11273 \pm 2$ [M+H]⁺, calcd mass 11273;

9D, $m/z 11351 \pm 2$ [M+H]⁺, calcd mass 11351;

10D, $m/z 11588 \pm 2$ [M+H]⁺, calcd mass 11586;

11D, $m/z 11227 \pm 2$ [M+H]⁺, calcd mass 11229;

12D, $m/z 11229 \pm 2$ [M+H]⁺, calcd mass 11229.

OxyB activity assays

The OxyB assay was as described. The peptide hydrazide products were purified by analytical HPLC and analyzed by MS and 1D and 2D ¹H-NMR spectroscopy. NMR assignments were made by standard methods, using 2D DQF-COSY, TOCSY and NOESY spectra, and are given below.

*Monocyclic pentapeptide hydrazide 13*¹H-NMR Chemical shift assignments (600 MHz, at 300 K, DMSO-d₆).

Residue	NH	C(α)-H	C(β)-H	Others
Tyr ¹	8.02	4.42	2.57, 2.82	C(δ)H=7.00, C(ε)H=6.62, OH=9.13, NAc=1.72
Asn ²	8.34	4.66	2.35, 2.50	N(δ)H=6.96, 7.33
Hpg ³	7.97	5.27	-	C(γ1)H=5.90, C(γ2)H=6.64, C(δ)H=6.77, OH=9.41,
Hpg ⁴	8.88	5.23	-	C(γ)H=7.08, C(δ)H=6.68, OH=9.40
Tyr ⁵	7.39	4.65	2.92, 3.27	C(δ1)H=7.22, C(δ2)H=7.28, C(ε1)H=6.94, C(ε2)H=7.09
CONHNH ₂	-	-	-	7.15, 7.07, 7.01

ESI-MS/MS (MeCN/H₂O 1:1 + HCOOH 0.1%): *m/z* 320.0 (b₂), 779.3 (b₅), 492.1 (y₃), 606.3 (y₄), 475.1 (z₃), 589.2 (z₄).

*Linear tetrapeptide-hydrazide 6E*¹H-NMR Chemical shift assignments (600 MHz, at 300 K, DMSO-d₆).

Residue	NH	C(α)-H	C(β)-H	Others
Asn ¹	8.12	4.61	2.30, 2.46	N(δ)H=6.89, 7.29, NAc=1.83
Hpg ²	8.06	5.46	-	C(δ)H=7.17, C(ε)H=6.67, OH=9.36
Hpg ³	8.64	5.38	-	C(γ)H=7.01, C(δ)H=6.63, OH=9.34
Tyr ⁴	8.31	4.26	2.56, 2.70	C(γ)H=6.81, C(δ)H=6.52, OH=9.14
CONHNH ₂	-	-	-	7.16, 7.07, 6.99

ESI-MS/MS (MeCN/H₂O 1:1 + HCOOH 0.1%): *m/z* 427.1 (a₃), 306.1 (b₂), 455.1 (b₃), 618.2 (b₄), 328.1 (z₂).

*Monocyclic tripeptide hydrazide 14*¹H-NMR Chemical shift assignments (600 MHz, at 300 K, DMSO-d₆).

Residue	NH	C(α)-H	C(β)-H	Others
Hpg ¹	8.44	5.27	-	C(γ 1)H=5.84, C(γ 2)H=6.69 C(δ)H=6.80, OH=9.39, NAc=1.78
Hpg ²	8.88	5.21	-	C(γ)H=7.08, C(δ)H=6.67, OH=9.42
Tyr ³	7.38	4.69	2.89, 3.27	C(δ 1)H=7.21, C(δ 2)H=7.28, C(ϵ 1)H=6.95, C(ϵ 2)H=7.10
CONHNH ₂	-	-	-	7.16, 7.07, 6.99

ESI-MS/MS (MeCN/H₂O 1:1 + HCOOH 0.1%): m/z 502.1 (b₃), 517.2 (x₃), 492.0 (y₃), 475.1 (z₃).

Linear and monocyclic tetrapeptide hydrazide 8E + 15

The mixture of linear (**8E**) and monocyclic peptide hydrazide (**15**) was isolated and used to derive assignments for each molecule by ¹H-NMR (600MHz, at 300K, DMSO-d₆).

Peptide 8E:

Residue	NH	C(α)-H	C(β)-H	Others
Ala ¹	8.03	4.38	1.14	NAc=1.82
Hpg ²	8.31	5.48	-	C(γ)H=7.18, C(δ)H=6.70, OH=9.37
Hpg ³	8.62	5.31	-	C(γ)H=7.06, C(δ)H=6.67, OH=9.42
Tyr ⁴	7.96	4.29	2.62, 2.80	C(δ)H=6.89, C(ϵ)H=6.61, OH=9.18
CONHNH ₂	-	-	-	7.00, 7.08, 7.16

ESI-MS/MS (MeCN/H₂O 1:1 + HCOOH 0.1%): m/z 384.2 (a₃), 412.3 (b₃), 575.3 (b₄).

Peptide 15:

Residue	NH	C(α)-H	C(β)-H	Others
Ala ¹	8.02	4.44	1.20	NAc=1.81
Hpg ²	8.33	5.27	-	C(γ 1)H=5.85, C(γ 2)H=6.63, C(δ)H=6.79, OH=9.41
Hpg ³	8.88	5.23	-	C(γ)H=7.07, C(δ)H=6.65, OH=9.38

Tyr ⁴	7.38	4.67	2.90, 3.27	C(δ1)H=7.22, C(δ2)H=7.28, C(ε1)H=6.94, C(ε2)H=7.09
CONHNH ₂	-	-	-	7.00, 7.08, 7.16

ESI-MS/MS (MeCN/H₂O 1:1 + HCOOH 0.1%): *m/z* 573.1 (b₄), 492.3 (y₃), 563.0 (y₄), 475.2 (z₃).

Linear and monocyclic tetrapeptide hydrazide 9E + 16

The mixture of linear (**9E**) and monocyclic peptide hydrazide (**16**) was isolated and used to derive assignments for each molecule by ¹H-NMR (600MHz, at 300K, DMSO-d₆).

Peptide 9E:

Residue	NH	C(α)-H	C(β)-H	Others
Hpg ¹	8.39	5.55	-	C(γ)H=7.18, C(δ)H=6.65, OH=9.33, NAc=1.86
Hpg ²	8.64	5.46	-	C(γ)H=7.05, C(δ)H=6.63, OH=9.34
Hpg ³	8.64	5.32	-	C(γ)H=7.06, C(δ)H=6.66, OH=9.382
Tyr ⁴	7.93	4.29	2.60, 2.80	C(δ)H=6.89, C(ε)H=6.60, OH=9.18
CONHNH ₂	-	-	-	6.99, 7.07, 7.16

ESI-MS/MS (MeCN/H₂O 1:1 + HCOOH 0.1%): *m/z* 462.2 (a₃), 490.2 (b₃), 653,4 (b₄).

Peptide 16:

Residue	NH	C(α)-H	C(β)-H	Others
Hpg ¹	8.38	5.59	-	C(γ)H=7.26, C(δ)H=6.69, OH=9.377, NAc=1.86
Hpg ²	8.65	5.23	-	C(γ1)H=5.78, C(γ2)H=6.21, C(δ)H=6.57, OH=9.36
Hpg ³	8.90	5.24	-	C(γ)H=7.08, C(δ)H=6.67, OH=9.43
Tyr ⁴	7.39	4.66	2.90, 3.26	C(δ1)H=7.20, C(δ2)H=7.26, C(ε1)H=6.92, C(ε2)H=7.05
CONHNH ₂	-	-	-	6.99, 7.07, 7.16

ESI-MS/MS (MeCN/H₂O 1:1 + HCOOH 0.1%): *m/z* 651.2 (b₄), 492.3 (y₃), 475.2 (z₃), 624.6 (z₄).

Linear hexapeptide-hydrazide 10E

¹H-NMR Chemical shift assignments (600 MHz, at 300 K, DMSO-d₆).

Residue	NH	C(α)-H	C(β)-H	Others
Leu ¹	8.66	3.59	1.40, 1.50	C(γ)H=1.50, C(δ)H ₃ =0.79, 0.85, NMe=1.95
Tyr ²	8.82	4.77	2.57, 2.97	C(δ)H=7.05, C(ε)H=6.63, OH=9.18
Asn ³	8.46	4.67	2.33, 2.46	N(δ)H=6.95, 7.33
Hpg ⁴	8.10	5.51	-	C(γ)H=7.19, C(δ)H=6.67, OH=9.37
Hpg ⁵	8.73	5.41	-	C(γ)H=6.99, C(δ)H=6.58, OH=9.31
Trp ⁶	8.36	4.42	2.80, 2.95	C(δ)H=6.92, C(ε)H=7.30, 7.51, C(φ)H=6.94, 7.03, NH=9.37
CONHNH ₂				7.02, 7.08, 7.17

ESI-MS/MS (MeCN/H₂O 1:1 + HCOOH 0.1%): *m/z* 526.4 (a₄), 405.3 (b₃), 554.4 (b₄), 703.5 (b₅), 889.5 (b₆), 571.4 (c₄).

A minor product (≈20%) isolated from the assay with peptide **10D** was assigned as an epimer of **10E**, with the following chemical shift assignments (600 MHz, at 300 K, DMSO-d₆):

Residue	NH	C(α)-H	C(β)-H	Others
Leu ¹	8.66	3.59	1.40, 1.50	C(γ)H=1.50, C(δ)H ₃ =0.79, 0.85, NMe=1.95
Tyr ²	8.82	4.77	2.97, 2.57	C(δ)H=7.05, C(ε)H=6.63, OH=9.18
Asn ³	8.46	4.67	2.46, 2.33	N(δ)H=7.33, 6.95
Hpg ⁴	8.10	5.51	-	C(γ)H=7.19, C(δ)H=6.69, OH=9.37
Hpg ⁵	8.76	5.35	-	C(γ)H=7.08, C(δ)H=6.64, OH=9.38
Trp ⁶	8.03	4.41	2.84, 3.03	C(δ)H=6.92, C(ε)H=7.31, 7.48 C(φ)H=6.94, 7.03, NH=9.40
CONHNH ₂				6.99, 7.08, 7.17

ESI-MS (MeCN/H₂O 1:1 + HCOOH 0.1%): *m/z* = 921.6 ([M+H]⁺).

*Linear tripeptide-hydrazide 11E*¹H-NMR Chemical shift assignments (600 MHz, at 300 K, DMSO-d₆).

Residue	NH	C(α)-H	C(β)-H	Others
Tyr ¹	7.96	4.35	2.53, 2.80	C(γ)H=6.97, C(δ)H=6.60, OH=9.13, NAc=1.72
Tyr ²	7.89	4.37	2.63, 2.84	C(γ)H=6.96, C(δ)H=6.61, OH=9.14
Tyr ³	8.02	4.36	2.72, 2.82	C(γ)H=6.99, C(δ)H=6.64, OH=9.18
CONHNH ₂	-	-	-	7.16, 7.08, 6.99

ESI-MS/MS (MeCN/H₂O 1:1 + HCOOH 0.1%): *m/z* 178.0 (a₁), 504.3 (a₃), 369.2 (b₂), 532.4 (b₃), 547.5 (x₃).*Linear tripeptide-hydrazide 12E*¹H-NMR Chemical shift assignments (600 MHz, at 300 K, DMSO-d₆).

Residue	NH	C(α)-H	C(β)-H	Others
Tyr ¹	7.99	4.30	2.51, 2.77	C(γ)H=6.95, C(δ)H=6.60, OH=9.14, NAc=1.73
Tyr ²	7.68	4.42	2.45, 2.70	C(γ)H=6.74, C(δ)H=6.55, OH=9.11
Tyr ³	8.34	4.38	2.66, 2.85	C(γ)H=7.06, C(δ)H=6.64, OH=9.17
CONHNH ₂	-	-	-	7.16, 7.08, 6.99

ESI-MS/MS (MeCN/H₂O 1:1 + HCOOH 0.1%): *m/z* 504.3 (a₃), 369.3 (b₂), 532.3 (b₃), 196.0 (y₁), 359.3 (y₂).