

Supplementary Tables

Supplementary Table 1. NMR data of **BA**, **BX** and **BY**.

←	BA		BX		BY	
	C	H (multiplicity, <i>J</i> in Hz)	C	H (multiplicity, <i>J</i> in Hz)	C	H (multiplicity, <i>J</i> in Hz)
1						
2	78.82		182.31		181.85	
3	202.23		61.41		62.11	
4	124.82	7.56 (d, 7.2)	125.97	7.23 (d, 7.2)	126.31	7.43 (d, 7.8)
5	137.79	7.45 (t, 7.8)	121.07	6.98 (dd, 7.2, 8.0)	120.85	6.99 (td, 7.8, 0.6)
6	119.35	6.81 (t, 7.8)	128.06	7.19 (dd, 7.2)	128.15	7.20 (td, 7.8, 0.6)
7	112.1	6.80 (d, 8.4)	109.18	6.82 (d, 7.2)	109.05	6.80 (d, 7.8)
8	121.11		142.32		142.42	
9	160.16		130.56		129.78	
10	37.43	2.78 (d 15.6) 2.33 (d 15.6)	33.12	2.19 (d, 13.6) 2.85 (d, 14.4)	33.63	2.12 (d, 15.6) 2.81 (d, 15.6)
11	67.82	-	65.63	-	67.17	
12	169.76	-	169.39	-	169.06	
14	44.06	3.46, m	43.39	3.40, m	43.27	3.26, m
15	25.1	2.04, m	24.33	1.79, m 1.99, m	24.47	1.78, m 1.98, m
16	28.96	2.79, m 1.86, m	28.99	2.50, overlap	28.45	1.79, m 2.47, m
17	69.52		68.07		69.1	
18	29.17	1.91, m 1.87, m	29.42	1.94 (dd, 10.4, 10.4) 1.76, m	27.97	1.93, m 1.64, m
19	55.7	2.4 (dd, 9.0, 7.8)	55.43	3.22 (dd, 8.8, 9.6)	50.04	3.16, overlapped
20	172.43		173.08		172.52	
22	48.44		45.10		46.86	
23	19.93	1.14, s	19.71	0.74, s	20.42	1.00, s
24	24.15	0.928, s	23.25	0.72, s	23.00	0.68, s

Supplementary Table 2. NMR data of compound 7.

	7			
	C	H (multiplicity, <i>J</i> in Hz)	HMBC	¹ H- ¹ H COSY
1				
2	189.29		10, 23, 24	
3	81.31		4,10	
4	122.32	7.48 (d, 7.8)	3, 5, 6	5
5	125.93	7.25 (t, 7.8)	4, 6	4, 6
6	129.21	7.37 (t, 7.8)	5, 7	5, 7
7	120.43	7.49 (d, 7.8)	5, 6	6
8	151.98		6, 7	
9	142.48		4, 10	
10	38.12	2.69 (d, 15.6) 1.70 (d, 15.6)	2, 3, 9, 11, 12, 19	
11	61.17	-	10, 19	
12	168.02	-	10, 14	
14	43.93	3.33, m 3.28, m	12, 15, 16, 17	15
15	23.92	1.95, m 1.82, m	14, 16, 17	14, 16
16	28.51	1.83, m 2.52, m	14, 15, 17, 18	15
17	66.64		14, 15, 16, 18	
18	31.40	1.94, m 2.13 m	16, 17, 19, 22	19
19	49.89	1.92 (dd, 27.6, 5.4)	18, 11, 22	18
20	172.22		16, 18,	
22	40.05		18, 19, 23, 24	
23	19.47	1.37, s	24, 22, 19, 2	
24	27.02	1.21, s	23, 22, 19, 2	

Supplementary Table 3. Crystal data and structure refinement for compounds **BY**, **BX** and **15**

	BY	BX	15
Empirical formula	C ₂₁ H ₂₃ N ₃ O ₃	C ₄₂ H ₄₈ N ₆ O ₇	C ₂₂ H ₂₇ N ₃ O ₃
Formula weight	365.42	748.86	381.46
Temperature	85(2) K	85(2) K	99.99(10) K
Wavelength	1.54184 Å	1.54184 Å	1.54184 Å
Crystal system, space group	Orthorhombic, P2 ₁ 2 ₁ 2 ₁	Monoclinic, P2 ₁	Monoclinic, P2 ₁
Unit cell dimensions	a = 8.64880(6) Å alpha = 90 deg.	a = 8.92445(7) Å alpha = 90 deg.	a = 9.95650(10) Å alpha = 90 deg.
	b = 14.08640(10) Å beta = 90 deg.	b = 16.05755(13) Å beta = 97.6976(7) deg.	b = 9.19010(10) Å beta = 109.0060(10) deg.
	c = 14.60695(10) Å gamma = 90 deg.	c = 13.03707(11) Å gamma = 90 deg.	c = 11.01750(10) Å gamma = 90 deg.
Volume	1779.57(2) Å ³	1851.44(3) Å ³	953.157(17) Å ³
Z, Calculated density	4, 1.364 Mg/m ³	2, 1.343 Mg/m ³	2, 1.329 Mg/m ³
Absorption coefficient	0.749 mM ⁻¹	0.753 mM ⁻¹	0.719 mM ⁻¹
F (000)	776	796	408
Crystal size	0.250 × 0.080 × 0.080 mm	0.110 × 0.100 × 0.070 mm	0.984 × 0.304 × 0.048 mm
Theta range for data collection	4.360 to 69.214 deg.	3.421 to 69.249 deg.	4.244 to 76.353 deg.
Limiting indices	-10 ≤ h ≤ 10, -17 ≤ k ≤ 16, -17 ≤ l ≤ 17	-10 ≤ h ≤ 10, -18 ≤ k ≤ 19, -15 ≤ l ≤ 15	-12 ≤ h ≤ 12, -11 ≤ k ≤ 11, -12 ≤ l ≤ 13
Reflections collected / unique	27067 / 3304 [R(int) = 0.0525]	28204 / 6550 [R(int) = 0.0504]	17458 / 3824 [R(int) = 0.0336]
Completeness to theta = 67.684	99.80%	99.90%	99.80%
Absorption correction	Semi-empirical from equivalents	Semi-empirical from equivalents	Semi-empirical from equivalents
Max. and min. transmission	1.00000 and 0.81488	1.00000 and 0.83164	1.00000 and 0.60142
Refinement method	Full-matrix least-squares on F ²	Full-matrix least-squares on F ²	Full-matrix least-squares on F ²
Data / restraints / parameters	3304 / 0 / 254	6550 / 1 / 524	3824 / 37 / 277
Goodness-of-fit on F ²	1.045	1.057	1.065
Final R indices	R1 = 0.0274, wR2 =	R1 = 0.0337, wR2 =	R1 = 0.0275, wR2 =

$[I > 2\sigma(I)]$	0.0715	0.0872	0.0687
R indices (all data)	R1 = 0.0277, wR2 = 0.0719	R1 = 0.0351, wR2 = 0.0900	R1 = 0.0302, wR2 = 0.0699
Absolute structure parameter	0.00(6)	0.05(10)	0.01(7)
Extinction coefficient	n/a	n/a	0.0111(10)
Largest diff. peak and hole	0.221 and -0.191 e.A ⁻³	0.655 and -0.175 e.A ⁻³	0.248 and -0.156 e.A ⁻³
Hooft parameters	0.00(5)	0.04(7)	0.00(6)

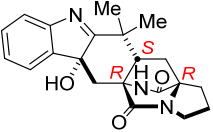
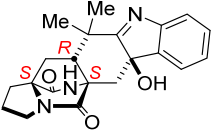
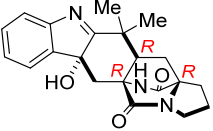
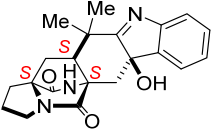
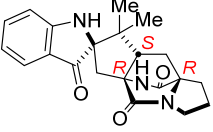
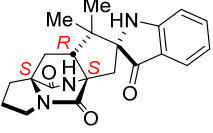
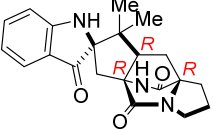
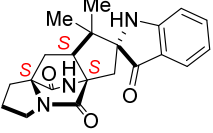
Supplementary Table 4. HPLC integrated peak areas and the calculated ratios of Brevianamide derivatives based on authentic standards.

Standard	BA	7	BX	BY
Peak area / 1 mM	22950830	12386848	6650753	6820322
Peak area (230 nm)				
Product	<i>Pb</i> -WT	<i>Pb</i> - <i>bvnE</i> -KO	<i>Ao</i> - <i>bvnBCD</i>	<i>Ao</i> - <i>bvnBCDE</i>
BA	6070509	1645891	89398	1135720
BB	385483	1512301	62211	145886
7	0	5656029	170993	0
BX	0	1740695	44157	0
BY	0	4145174	106020	0
Peak area (404 nm)				
Product	<i>Pb</i> -WT	<i>Pb</i> - <i>bvnE</i> -KO	<i>Ao</i> - <i>bvnBCD</i>	<i>Ao</i> - <i>bvnBCDE</i>
BA	422582	32811	2784	62595
BB	37730	38626	2608	7441
ratio	11:1	1:1	1:1	8:1
BA:BB:7:BX:BY				
ratio	<i>Pb</i> -WT	<i>Pb</i> - <i>bvnE</i> -KO	<i>Ao</i> - <i>bvnBCD</i>	<i>Ao</i> - <i>bvnBCDE</i>
	BA:BB:7:BX:BY			
	11:1:0:0:0	1:1:7:5:9	1:1:5:2:6	8:1:0:0:0

Supplementary Table 5. NMR data of compounds **15**, **16** and **17**.

	15		16		17	
	C	H (multiplicity, <i>J</i> in Hz)	C	H (multiplicity, <i>J</i> in Hz)	C	H (multiplicity, <i>J</i> in Hz)
1						
2	191.57		70.22		178.77	
3	86.32		201.52		54.57	
4	123.25	7.45 (d, 7.8)	123.2	7.22 (d, 7.8)	128.73	7.05 (d, 7.8)
5	125.3	7.14 (dd, 7.8, 1.2)	116.78	6.56, m	119.52	6.84 (t, 7.2)
6	129.12	7.31 (dd, 7.8, 1.2)	136.4	7.31 (ddd, 8.4, 7.2, 1.2)	127.55	7.11 (t, 7.2)
7	120.2	7.41 (d, 7.8)	111.18	6.82 (d, 8.4)	108.66	6.71 (d, 7.8)
8	141.69		120.13		143.31	
9	151.64		161.05		128.73	
10	34.32	3.12 (dd, 6.6, 15.6) 1.8 (dd, 3.0, 15.6)	32.69	2.47 (dd, 4.8, 15.6) 2.67 (dd, 3, 15.0)	30.91	2.939, m 2.55 (dd, 6, 16.2)
11	56.6	4.72	59.46	3.9	59.99	3.97
12	165.92		163.57		162.56	
14	45.35	3.41, m	44.79	3.23 (td, 9.6, 3.6) 3.73, m	43.7	3.19 (dt, 11.4, 8.4) 2.94, m
15	22.01	1.85, m	20.74	1.67, m	20.18	1.51, m 1.4, m
16	28.21	2.18, m 1.87, m	28.15	1.68, m 0.47, m	28.11	1.72, m 0.45 m
17	58.29	4.3 (t, 4.3)	58.04	3.77 (ddd, 1.8, 5.4, 11.4)	57.95	3.69 (dd, 6.0, 12.0)
18	168.09		165.95		165.8	
19-NMe	30.11	2.36, s	32.69	2.6, s	32.81	2.76, s
20	42.8		44.79		42.72	
21	145.12	6.31 (dd, 17.4, 10.2)	142.11	6.02 (dd, 10.8, 16.8)	143.06	6.03, m
22	111.93	5.15 (dd, 0.6, 10.2) 5.05 (dd, 0.6, 10.2)	113.5	5.1 (d, 17.4) 5.0 (d, 12.0)	113.52	5.03 (d, 10.8) 4.91 (d, 17.4)
23	26.01	1.52, s	21.16	0.92, s	20.87	0.86, s
24	27.53	1.47, s	21.16	0.95, s	21.75	0.96, s

Supplementary Table 6. Selectivity calculation results of different Brevianamide derivatives.

Compound type	Product	Stereochemistry	Relative G of different TS (kcal/mol)	Probability	Selectivity
3-hydroxy indolenines	7		0.00	0.521	7: 52.1%
	8 (converted to BY)		0.19 1.92	0.375 0.020	(BY) 39.5%
	9 (converted to BX)		1.62 2.47	0.034 0.008	(BX) 4.2%
	22		1.49	0.042	4.2%
3-spiro-ψ-indoxyls	BA		0.00 1.52 0.72	0.706 0.055 0.210	97.1%
	BB		2.07 3.51 2.82	0.022 0.002 0.006	2.9%
	23		8.67 9.14 7.91	0.000 0.000 0.000	0.0%
	24		8.38 15.71 10.51 9.27	0.000 0.000 0.000 0.000	0.0%

Supplementary Table 7. Data collection and refinement statistics for ligand-free BvnE.

Parameter	
Wavelength (Å)	1.033
Resolution range	36.72-1.78 (1.84-1.78)
Space group	<i>P</i> 3 ₂ 2 1
Unit cell (Å)	105.6×105.6×61.6
Total reflections	761908 (69001)
Unique reflections	38383 (3783)
Multiplicity	19.9 (18.2)
Completeness (%)	99.9 (99.5)
Mean I/sigma(I)	20.71 (2.60)
Wilson B-factor	22.37
R _{merge}	0.129 (0.950)
R _{meas}	0.132 (0.978)
CC _{1/2}	0.999 (0.886)
Reflections used in refinement	38359 (3780)
Reflections used for R _{free}	2000 (196)
R _{work}	0.182 (0.267)
R _{free}	0.203 (0.316)
Number of non-hydrogen atoms	2604
macromolecules	2290
Ligands	25
Solvent	289
RMS(bonds)	0.006
RMS(angles)	0.82
Ramachandran favored (%)	99.3
Ramachandran allowed (%)	0.7
Ramachandran outliers (%)	0.0
Average B-factor	26.15
macromolecules	25.07
Ligands	37.70
Solvent	33.70

Supplementary Table 8. Strains and vectors used in this study.

Stains/vectors	Genotype	Description
<i>Penicillium brevicompactum</i> NRRL 864 (<i>Pb</i>)	Wild type	
<i>Pb-bvnB</i> -KO		The <i>bvnB</i> knockout mutant of <i>Pb</i>
<i>Pb-bvnC</i> -KO		The <i>bvnC</i> knockout mutant of <i>Pb</i>
<i>Pb-bvnD</i> -KO		The <i>bvnD</i> knockout mutant of <i>Pb</i>
<i>Pb-bvnE</i> -KO		The <i>bvnE</i> knockout mutant of <i>Pb</i>
<i>Aspergillus oryzae</i> NSAR1 (<i>Ao</i>)	<i>niad</i> ⁻ , <i>sC</i> ⁻ , Δ <i>argB</i> , <i>adeA</i> ⁻	Heterologous gene expression host
<i>Ao-bvnA</i>		Heterologous expression of <i>bvnA</i> in <i>Ao</i>
<i>Ao-bvnC</i>		Heterologous expression of <i>bvnC</i> in <i>Ao</i>
<i>Ao-bvnCDE</i>		Heterologous expression of <i>bvnCDE</i> in <i>Ao</i>
<i>Ao-bvnBC</i>		Heterologous expression of <i>bvnBC</i> in <i>Ao</i>
<i>Ao-bvnBCE</i>		Heterologous expression of <i>bvnBCE</i> in <i>Ao</i>
<i>Ao-bvnBCD</i>		Heterologous expression of <i>bvnBCD</i> in <i>Ao</i>
<i>Ao-bvnBCDE</i>		Heterologous expression of <i>bvnBCDE</i> in <i>Ao</i>
<i>Ao-bvnD</i>		Heterologous expression of <i>bvnD</i> in <i>Ao</i>
<i>Ao-bvnDE</i>		Heterologous expression of <i>bvnDE</i> in <i>Ao</i>
<i>E. coli</i> DH5 α		For general molecular cloning
<i>E. coli</i> BL21(DE3)		For protein overexpression
<i>E. coli</i> BL21(DE3) pRARE2		For BvnE expression for crystallization
pRSF-hyg		Backbone vector for gene knockout
pTAex3- <i>bvnA</i>		For heterologous expression of <i>bvnA</i> in <i>Ao</i>
pUARA2- <i>bvnB</i>		For heterologous expression of <i>bvnB</i> in <i>Ao</i>
pAdeA2- <i>bvnBD</i>		For heterologous expression of <i>bvnBD</i> in <i>Ao</i>
pAdeA2- <i>bvnC</i>		For heterologous expression of <i>bvnC</i> in <i>Ao</i>
pUSA2- <i>bvnE</i>		For heterologous expression of <i>bvnE</i> in <i>Ao</i>
pET28b- <i>bvnB</i>		For expression of BvnB in <i>E. coli</i>
pET28b- <i>bvnC</i>		For expression of BvnC in <i>E. coli</i>
pET28b- <i>bvnE</i>		For expression of BvnE in <i>E. coli</i>

Supplementary Table 9. Primers used in this study.

Vector/primers	Sequence	Description
Hyg-F	CTGCTCCATACAAGCCAACC	For amplifying knockout split fragments
Hyg-R	GATGTAGGAGGGCGTGGATAT GTCCT	For amplifying knockout split fragments
bvnB-up-F	GCCTGCAGGTCGACAAGCTTCC CTATCCCTAGAAGTGCCAGTAT	For amplifying up arm of <i>bvnB</i> .
bvnB-up-R	AAACTACCGCATTAAAGCTTGT TAATCATCTAGTCAACATCCTTC	For amplifying up arm of <i>bvnB</i> .
bvnB-down-F	GTGGAGCGGCGTCGAGATCTGG TTCAGCACAGTCTGATGATACG T	For amplifying down arm of <i>bvnB</i>
bvnB-down-R	CCGATATCCAATTGAGATCTGCA GCGGATACAACGAATACACTT	For amplifying down arm of <i>bvnB</i>
bvnB-anchor-F	CACCGTTGTCCACCGAGATT	For PCR verification of <i>bvnB</i> knockout mutants
bvnB-anchor-R	CGTGAAGATGGGAACCCTGAT	For PCR verification of <i>bvnB</i> knockout mutants
bvnB-in-F	GTAGGTATGCCGATGGACT	For PCR verification of <i>bvnB</i> knockout mutants
bvnB-in-R	ATCCTGTGCCCTTTAGTGGT	For PCR verification of <i>bvnB</i> knockout mutants
bvnC-up-F	GCCTGCAGGTCGACAAGCTTTG CCCATCATAACCATAACATCCT	For amplifying up arm of <i>bvnC</i>
bvnC-up-R	AAACTACCGCATTAAAGCTTAG TAGAAGCATTGCGAATTTGAG	For amplifying up arm of <i>bvnC</i>
bvnC-down-F	GTGGAGCGGCGTCGAGATCTGA TGTTGACTGTCCCAGTAAGAT A	For amplifying down arm of <i>bvnC</i>
bvnC-down-R	CCGATATCCAATTGAGATCTCGT CTGATCCTTCAGTCGGTCTAG	For amplifying down arm of <i>bvnC</i>
bvnC-anchor-F	TGAGACACCCAGGTCCGTAG	For PCR verification of <i>bvnC</i> knockout mutants
bvnC-anchor-R	TGCAAAGCCGCCACTCGTAA	For PCR verification of <i>bvnC</i> knockout mutants
bvnC-in-F	ACTTGGGCACGGGCCAGGAA	For PCR verification of <i>bvnC</i> knockout mutants
bvnC-in-R	GGCCCAGTGGTGGCAAGATA	For PCR verification of <i>bvnC</i> knockout mutants
bvnD-up-F	GCCTGCAGGTCGACAAGCTTTA GTTCCAGATGATGGGCGAGGC	For amplifying up arm of <i>bvnD</i>
bvnD-up-R	AAACTACCGCATTAAAGCTTATT	For amplifying up arm of <i>bvnD</i>

	GTAACGGATTACTGGAAGGAAT	
bvnD-down-F	GTGGAGCGGCGTCGAGATCTAA TTTACTCGATGTAGCGCAACTG A	For amplifying down arm of <i>bvnD</i>
bvnD-down-R	CCGATATCCAATTGAGATCTTCA GCAAACCTTGACGCAGTAATCG	For amplifying down arm of <i>bvnD</i>
bvnD-anchor-F	CGTCAAGTCAGCCATACATAGC	For PCR verification of <i>bvnD</i> knockout mutants
bvnD-anchor-R	GAAGACATAGGATAAGCGGAGT	For PCR verification of <i>bvnD</i> knockout mutants
bvnD-in-F	TGTCAATCGGTATCGGTGGG	For PCR verification of <i>bvnD</i> knockout mutants
bvnD-in-R	GAATTTGTCAGGCTCAGGGT	For PCR verification of <i>bvnD</i> knockout mutants
bvnE-up-F	CGCCTGCAGGTCGACAGGCTGG TGACCGTGCGAAAT	For amplifying up arm of <i>bvnE</i>
bvnE-up-R	CGTTAGCAATTTAACTGTGATAA ACTACCGCATTAACGAGGACTG TAGGAGTAGGC	For amplifying up arm of <i>bvnE</i>
bvnE-down-F	GGGCCTTGACATGTGCAGCCGG TGGAGCGGCGTCGATAGCGATT ACTGCGTCAAG	For amplifying down arm of <i>bvnE</i>
bvnE-down-R	GCCGATATCCAATTGACTACGG CTGAGAAATACCAT	For amplifying down arm of <i>bvnE</i>
bvnE-anchor-F	ACCTGGTGGACGGTAGCAAT	For PCR verification of <i>bvnE</i> knockout mutants
bvnE-anchor-R	GCCATTTATTCAGCACCCAC	For PCR verification of <i>bvnE</i> knockout mutants
bvnE-in-F	CAATCAAACGACAGGTCCCA	For PCR verification of <i>bvnE</i> knockout mutants
bvnE-in-R	ACATCCCGTAGCAAAGTCCC	For PCR verification of <i>bvnE</i> knockout mutants
pUARA2-BvnB-F	ATCGATTTGAGCTAGATGACTA GGAACAATACCAT	For amplifying <i>bvnB</i> for pUARA2 insertion by infusion
pUARA2-BvnB-R	TAGTGCGGCCGCTAGCTACTCC TGACGATATTTCC	For amplifying <i>bvnB</i> for pUARA2 insertion by infusion
pAdeA2-BvnD-F	AAGCTCCGGAATTCGAGCTCGG TACCATGACCAAGGCCACCACC CC	For amplifying <i>bvnD</i> for pAdeA2 insertion by HiFi assembly
pAdeA2-BvnD-R	GATCCCCGGTACCCTATATTTT GACCTCCTCTTGTCGCCG	For amplifying <i>bvnD</i> for pAdeA2 insertion by HiFi assembly
pAdeA2-linker-F	GGTCAAATATAGGGTACCGG GGATCTGTAGTAG	For amplifying linker from pAdeA2 for pAdeA2- <i>bvnBD</i> by

		HiFi assembly
pAdeA2-linker-R	TTGACTTGGTCATGCTAGCTCA AATCGATTCTGAATTC	For amplifying linker from pAdeA2 for pAdeA2- <i>bvnBD</i> by HiFi assembly
pAdeA2-BvnC-F	GATTTGAGCTAGCATGACCAAG TCAAACGAAGTC	For amplifying <i>bvnC</i> for pAdeA2 insertion by HiFi assembly
pAdeA2-BvnC-R	ACTACCCGGGTCAGTAGTGC GG CCGCTAGCTCAGTTCTCCACG GATAGG	For amplifying <i>bvnC</i> for pAdeA2 insertion by HiFi assembly
pUSA2-BvnE-F	AAGCTCCGGAATTCGAGCTCGG TACCATGACGCTCAATCAAACG AC	For amplifying <i>bvnE</i> for pUSA2 insertion by HiFi assembly
pUSA2-BvnE-R	ATCCCCGGGTACCCTATATGGA TGTCGATTCTGC	For amplifying <i>bvnE</i> for pUSA2 insertion by HiFi assembly
BvnE Y109F	GGCGGCATGTGGCCTTTTTGAG AATGAGTACATGGTCGTA CTTACC	BvnE site specific mutagenesis
BvnE R38A	CAGACGCGATGGTCTCACACGC CACCGAGGAATGTGTACGC	BvnE site specific mutagenesis
BvnE R49A	ACGCGTCCC GCGCCGGCCTCGC TTGGCTTTGCACCGCTAA	BvnE site specific mutagenesis
BvnE Y113F	GTGGCCTTTATGAGAATGAGTT CATGGTCGTA CTTACCTTTAATG	BvnE site specific mutagenesis
BvnE E131A	GACTTTGCTACGGGATGTTATC GCGTTTGCAGATAGCGATTACT GC	BvnE site specific mutagenesis
BvnE E131Q	GACTTTGCTACGGGATGTTATC CAGTTTGCAGATAGCGATTACT GC	BvnE site specific mutagenesis
BvnE R38Q	CAGACGCGATGGTCTCACACCA GACCGAGGAATGTGTACGC	BvnE site specific mutagenesis