Viola "inconspicua" No More: An Analysis of Antibacterial Cyclotides

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Table S1: Putative cyclotide masses identified in *V. inconspicua* reduction/alkylation LC-MS mass shift analysis.

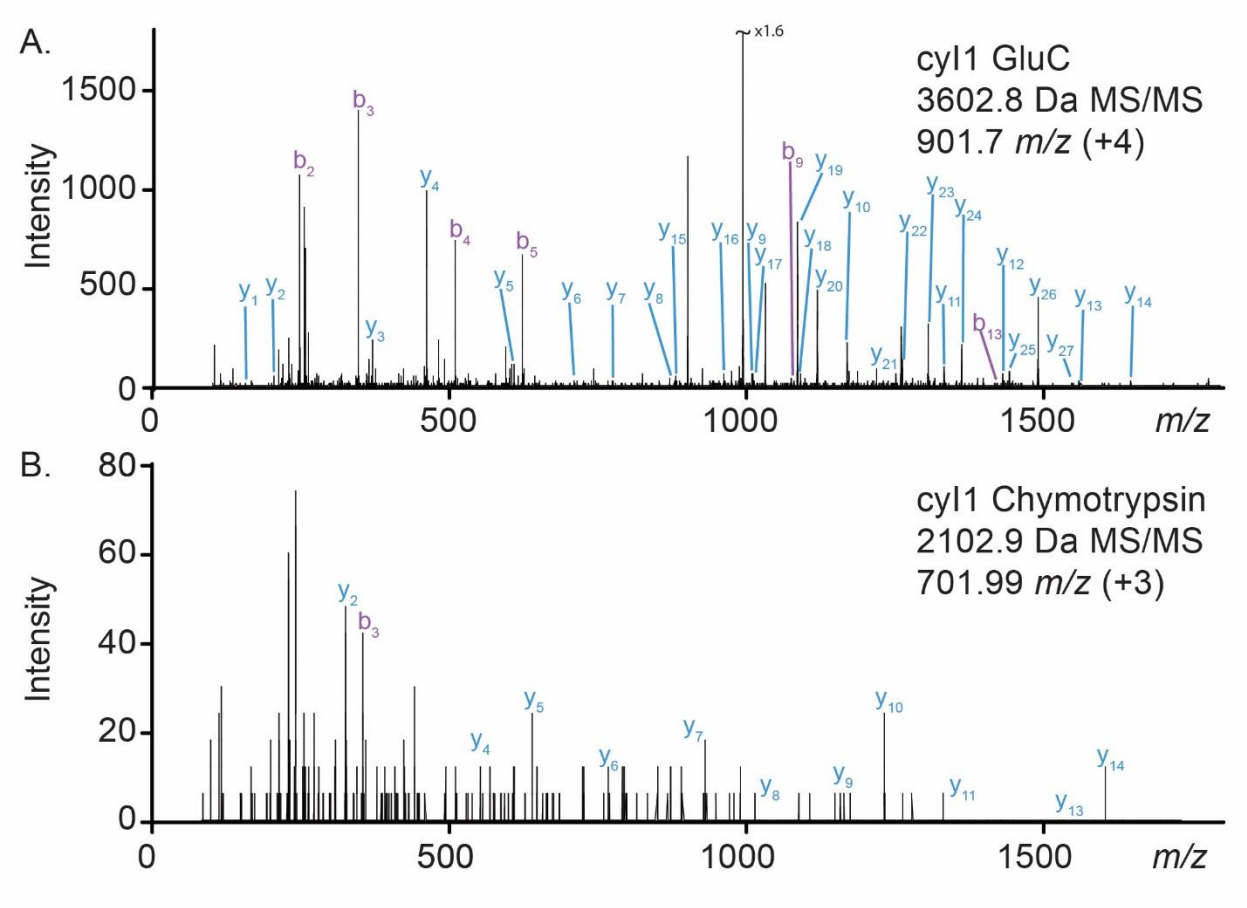

Figure S1. *V. inconspicua* cyclotide cyI1 (3236.4 Da) CID MS/MS spectra of (A) gluC linearized cyI1 (+4) and (B) cyI1 chymotrypsin fragment 2102.9 Da (+3). Spectra are annotated with b (purple) and y (blue) ion series.

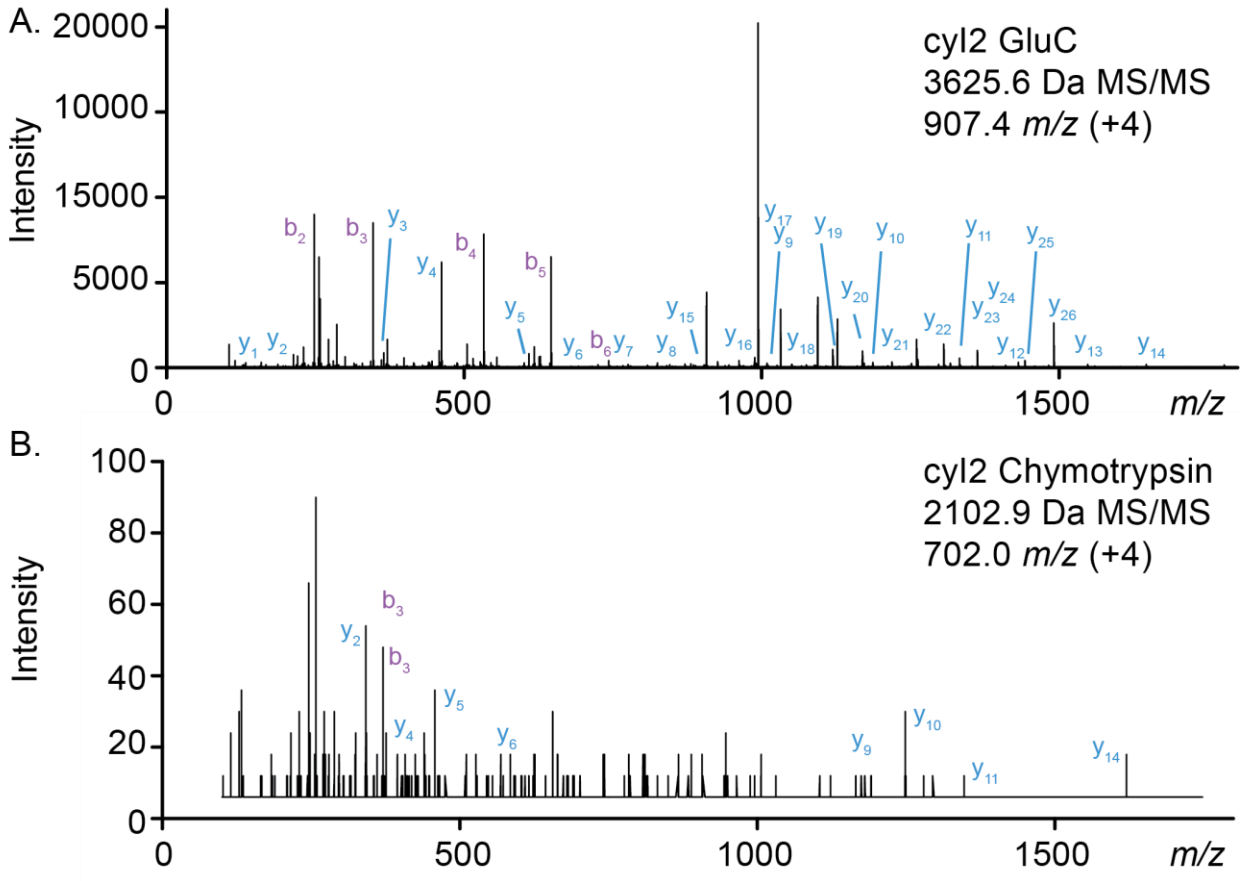

Figure S2. *V. inconspicua* cyclotide cyl2 (3259.4 Da) CID MS/MS spectra of (A) gluC linearized cyl2 (+4) and (B) cyl2 chymotrypsin fragment 2102.9 Da (+2). Spectra are annotated with b (purple) and y (blue) ion series.

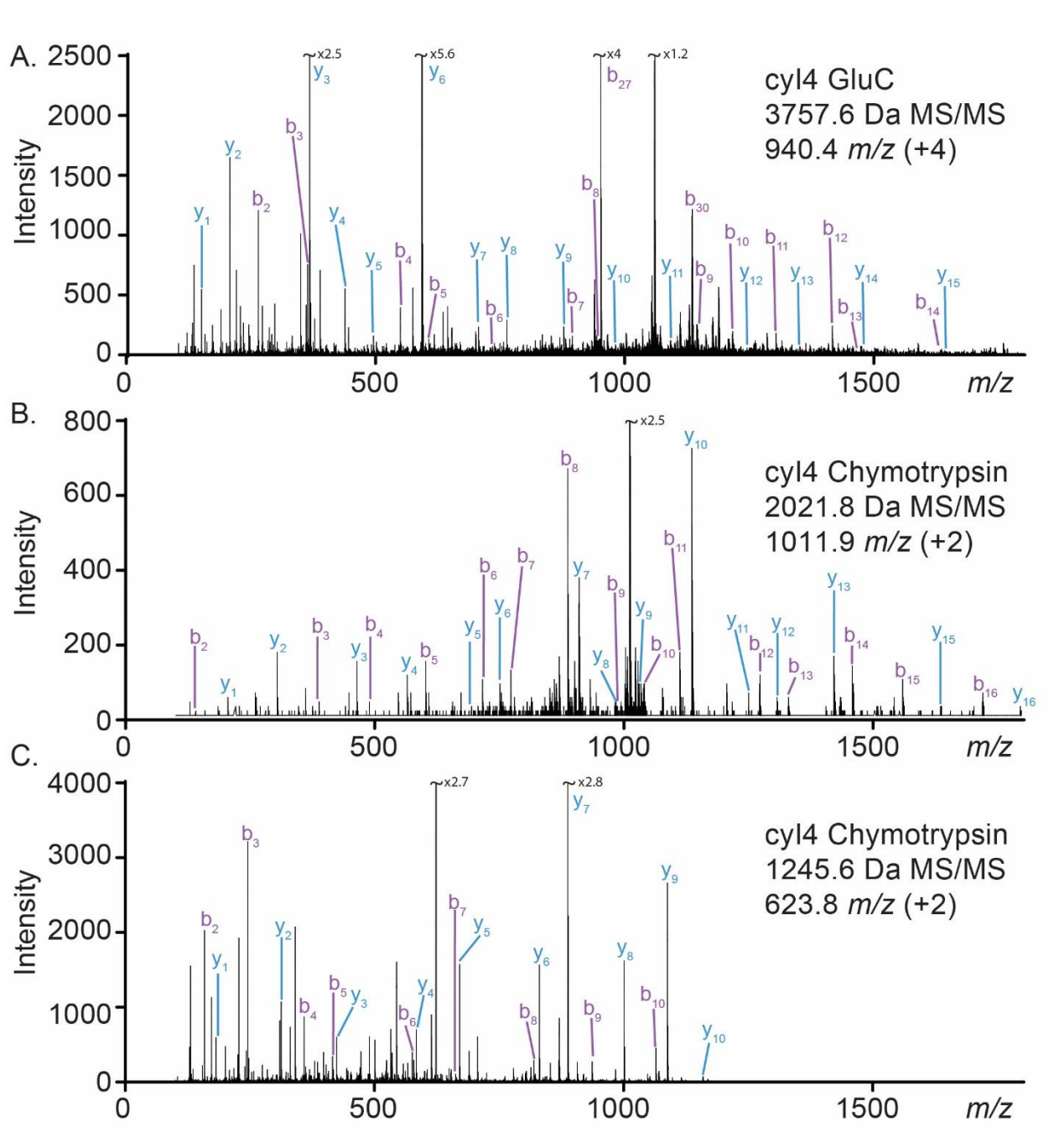

Figure S3. *V. inconspicua* cyclotide cyl4 (3391.4 Da) CID MS/MS spectra of (A) gluC linearized cyl4 (+4), (B) cyl3 chymotrypsin fragment 2021.8 Da (+2), and (C) cyl4 chymotrypsin fragment 1245.6 Da (+2). Spectra are annotated with b (purple) and y (blue) ion series.

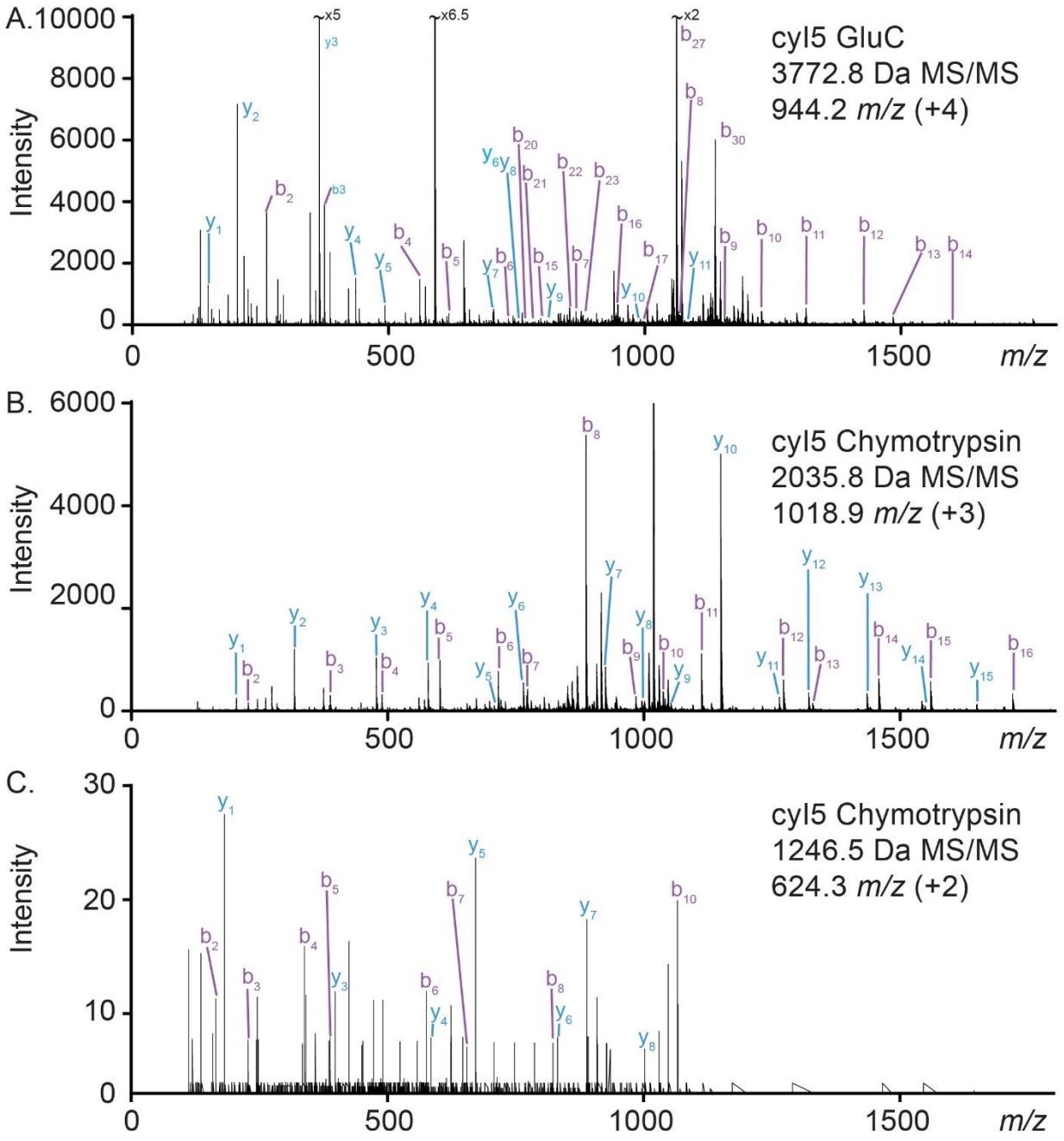

Figure S4. *V. inconspicua* cyclotide cyl5 (3406.5 Da) CID MS/MS spectra of (A) gluC linearized cyl5 (+4), (B) cyl5 chymotrypsin fragment 2035.8 Da (+3), and (C) cyl5 chymotrypsin fragment 1246.5 Da (+2). Spectra are annotated with b (purple) and y (blue) ion series.

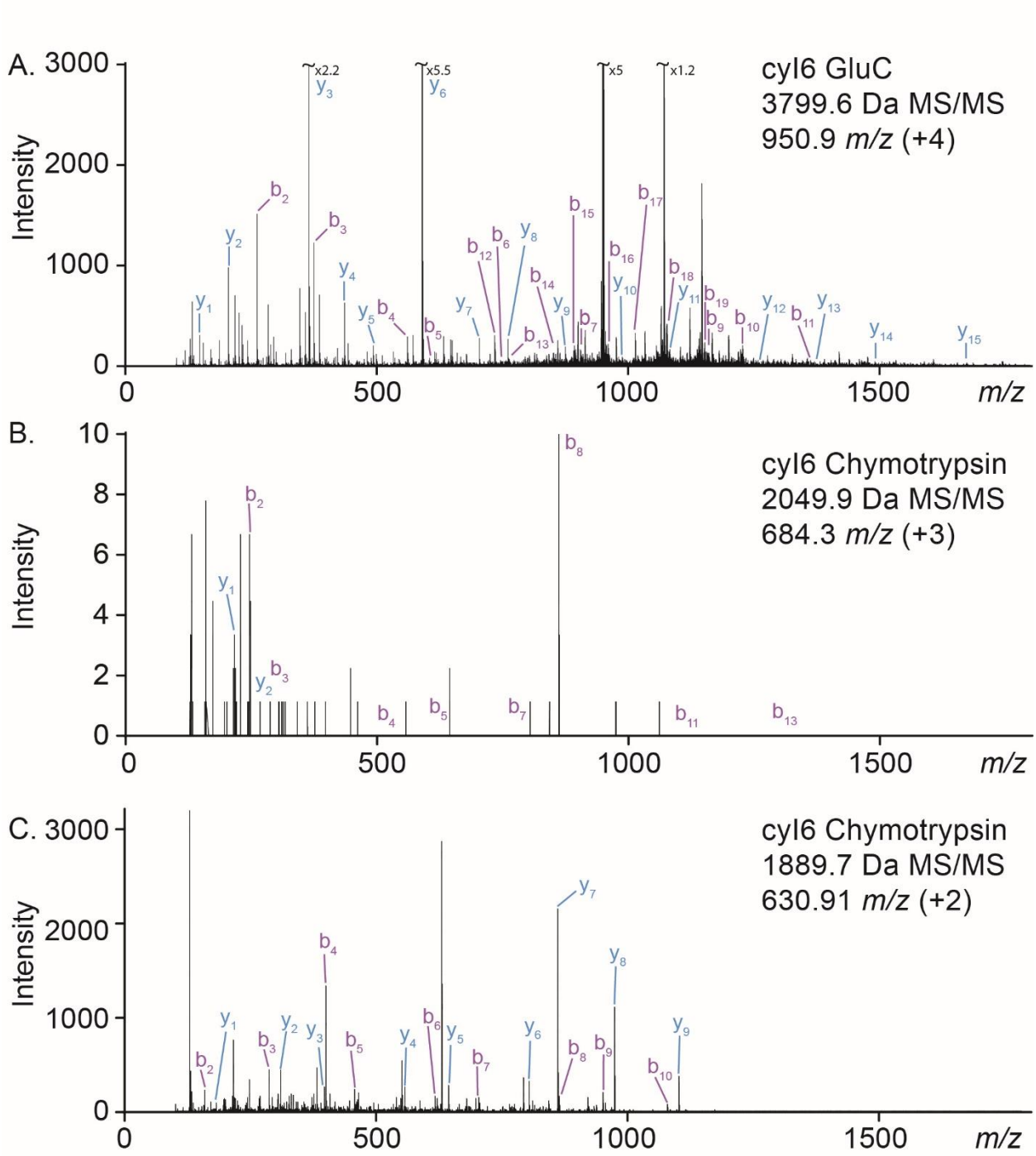

Figure S5. *V. inconspicua* cyclotide cyl6 (3433.5 Da) CID MS/MS spectra of (A) gluC linearized cyl6 (+4), (B) cyl6 chymotrypsin fragment 2049.9 Da (+3), and (C) cyl6 chymotrypsin fragment 1889.7 Da (+2). Spectra are annotated with b (purple) and y (blue) ion series.

Table S1. Putative cyclotide masses identified in *V. inconspicua* reduction/alkylation LC-MS mass shift analysis exhibiting 348.16 Da mass shift. Asterisks indicate known cyclotide sequences previously identified in different botanical species. Novel cyclotide sequences described herein are denoted with cyI1-6 nomenclature. Mass species are denoted by Type I, Type II, or Mixed as per CID MS/MS fingerprint ion identifications. Mixed species were not resolved from other cyclotidyl species in the MS1 and thus conflicting fingerprint fragment ions confounded motif type identification.

	Mass (Da)	Identity			Mass (Da)	Identity
1	3099.3			24	3277.5	
2	3108.4	viba 11*		25	3291.4	
3	3116.3			26	3303.5	
4	3138.4	cyO9*		27	3317.4	**Type I**
5	3140.4			28	3319.4	
6	3142.4			29	3332.4	
7	3156.4			30	3347.4	
8	3170.4			31	3373.5	**Mixed**
9	3208.4	**Type I**		32	3378.5	cyI3
10	3225.4	cyO8*		33	3382.5	
11	3229.5			34	3391.5	cyI4
12	3236.4	cyI1		35	3394.5	
13	3242.5			36	3396.5	**Mixed**
14	3243.4			37	3406.5	cyI5
15	3252.4			38	3408.4	
16	3253.4			39	3410.5	
17	3254.4			40	3423.5	
18	3255.5			41	3433.5	cyI6
19	3259.4	cyI2		42	3437.5	**Type II**
20	3263.5			43	3451.5	
21	3271.4			44	3465.5	
22	3273.5			45	3526.5	
23	3275.4					