Supporting Information

Zhang et al. 10.1073/pnas.1113863108

SI Materials and Methods

Peptide and β-Amino Acid Analog Purification and Characterization. The peptide analogs were purified on a Waters C_{18} Sep Pak cartridge and a Delta-Pak C_{18} RP column (Waters) (8 × 100 mm, 15-μm particle size, 100-Å pore size) on a Waters 510 HPLC system controlled with a Millennium 2010 chromatography manager system (Waters), with detection at 214 nm at ambient temperature. Solvent A was 0.1% aqueous TFA, and solvent B was 80% (vol/vol) aqueous acetonitrile containing 0.1% TFA. Conditions were as follows: The initial solvent consisting of 20% (vol/vol) solvent B was followed by the Waters linear program to 100% (vol/vol) solvent B over 40 min, with a flow rate of 2 mL/min. Delta-Pak C_{18} retention times (t_Rs) were as follows: 1935 (2Abf-Suc-A[dK]PRLa), 13.5 min; 1477 (2Abf-Suc-AGPRLa), 15.5 min; 1894 (2Abf-Suc-FSPRLa), 18.0 min; 1895 (2Abf-Suc-FGPRLa), 18.0 min; 1896 (2Abf-Suc-FTPRIa), 18.0 min; DH-2Abf-K (2Abf-Suc-FKPRLa), 15.0 min; 1903 (2Abf-Suc-FVPRLa), 18.2 min; 1868 (2Abf-Suc-[β^{3}F]TPRLa), 5.0 min; 1631 (2Abf-Suc-FT[β^{3}P]RLa), 5.0 min; 1823 (Hex-Suc-FTPRLa), 14.1 min; 17113 (2Abf-Suc-FT[Oic]RLa), 24.3 min; 1790A (2Abf-Suc-LWA[dF]PRLa), 23.5 min; 1608-2 (2Abf-Suc-F[dF][β^{3}P]RLa), 11.6 min; 1373 (2Abf-Suc-A[dF]PRLa), 24.0 min; 1478 (2Abf-Suc-AGPRAa), 16.5 min; 1525 (2Abf-Suc-AAAA[dF]PRLa), 20.9 min; 1604 (2Abf-Suc-F[dF]PRAa), 21.8 min; 1374 (2Abf-Suc-F[dF]PRLa), 25.5 min; 1605 (Hex-Suc-A[dF]PRLa), 13.5 min; and 1607 (2Abf-Suc-[β^{3}F][dF]PRLa), 31.1 min. The peptides were further purified on a Waters Protein Pak I125 column (7.8 × 300 mm; Milligen Corp.). Conditions were as follows: 2.0 mL/min flow rate, solvent A was 95% (vol/vol) acetonitrile made to 0.01% TFA, solvent B was 50% (vol/vol) aqueous acetonitrile made to 0.01% TFA, 100% (vol/vol) solvent A isocratic for 4 min and then a linear program to 100% (vol/vol) solvent B over 80 min. Waters t_Rs (in min) were as follows: 1935 (2Abf-Suc-A[dK]PRLa), 6.0 min; 1477 (2Abf-Suc-AGPRLa), 6.0 min; 1894 (2Abf-Suc-FSPRLa), 6.0 min; 1895 (2Abf-Suc-FGPRLa), 5.0 min; 1896 (2Abf-Suc-FTPRIa), 6.0 min; DH-2Abf-K (2Abf-Suc-FKPRLa), 6.0 min; 1903 (2Abf-Suc-FVPRLa), 4.5 min; 1868 (2Abf-Suc-[β^{3}F]TPRLa), 5.0 min; 1631 (2Abf-Suc-FT[β^{3}P]RLa), 5.0 min; 1823 (Hex-Suc-FTPRLa), 6.0 min; 17113 (2Abf-Suc-FT[Oic]RLa), 5.5 min; 1790A (2Abf-Suc-LWA[dF]PRLa), 4.5 min; 1608-2 (2Abf-Suc-F[dF][β^{3}P]RLa), 6.0 min; 1478 (2Abf-Suc-AGPRAa), 7.5 min; 1525 (2Abf-Suc-AAAA[dF]PRLa), 6.0 min; 1604 (2Abf-Suc-F[dF]PRAa), 5.0 min; 1605 (Hex-Suc-A[dF]PRLa), 6.0 min; and 1607 (2Abf-Suc-[β^{3}F][dF]PRLa), 6.0 min.

Amino acid analysis was carried out under previously reported conditions (1) and used to quantify the peptide analogs and to confirm identity, leading to the following analyses: 1935 (2Abf-Suc-A[dK]PRLa): A[1.0], K[1.1], L[1.0], P[0.9], R[1.0]; 1477 (2Abf-Suc-AGPRLa): A[0.9], G[0.7], L[1.0], P[0.7], R[0.7]; 1894 (2Abf-Suc-FSPRLa): F[1.0], L[1.0], P[1.0], R[1.0], S[1.0]; 1895 (2Abf-Suc-FGPRLa): F[1.0], G[0.7], L[0.9], P[0.9], R[1.1]; 1896 (2Abf-Suc-FTPRIa): F[1.0], I[0.9], P[0.9], R[1.0], T[1.0]; DH-2Abf-K (2Abf-Suc-FKPRLa): F[1.0], K[0.9], L[1.0], P[0.9], R[1.0]; 1903 (2Abf-Suc-FVPRLa): F[1.0], L[1.1], P[01.0], R[1.1], V[1.0]; 1868 (2Abf-Suc-[β^{3}F]TPRLa): L[1.0], P[1.0], R[1.0], T[1.0]; 1631 (2Abf-Suc-FT[β^{3}P]RLa): F[1.0], L[1.0], R[1.0], T[1.0]; 1823 (Hex-Suc-FTPRLa): F[1.0], L[1.0], P[1.0], R[1.0], T[0.9]; 17113 (2Abf-Suc-FT[Oic]RLa): F[1.0], L[1.0], R[1.0], T[0.9]; 1790A (2Abf-Suc-LWA[dF]PRLa): F[2.0], L[1.0], R[1.1]; 1608-2 (2Abf-Suc-F[dF][β^{3}P]RLa): F[1.0], L[0.9], R[0.9], T[0.9]; 1373 (2Abf-Suc-A[dF]PRLa): A[1.1], F[0.0], L[1.0], P[1.3], R[1.0]; 1478 (2Abf-Suc-AGPRAa): A[2.0], G[1.0], P[1.1], R[1.0]; 1525 (2Abf-Suc-AAAA[dF]PRLa): A[4.0], F[1.0], L[1.0], P[1.0], R[1.0]; 1604 (2Abf-Suc-F[dF]PRAa): A[0.9], F[2.0], P[1.1], R[1.0]; 1374 (2Abf-Suc-F[dF]PRLa): F[2.0], L [1.0], P[1.0], R[1.0]; 1605 (Hex-Suc-A[dF]PRLa): A[1.0], F[1.0], L[1.0], P[0.9], R[1.0]; and 1607 (2Abf-Suc-[β^{3}F][dF]PRLa): F[1.0], L[1.0], P[1.0], R[1.0]. Identities of the peptide analogs were confirmed via MALDI-TOF-MS on a Kratos Kompact Probe MALDI-TOF-MS machine (Kratos Analytical, Ltd.) with the presence of the following molecular ions [MH$^+$]: 1935 (2Abf-Suc-A[dK]PRLa), 927.2 Da [calculated (calc) 925.9 Da]; 1477 (2Abf-Suc-AGPRLa), 855.7 (calc 855.0); 1894 (2Abf-Suc-FSPRLa), 965.6 (calc 964.9); 1895 (2Abf-Suc-FGPRLa), 930.9 (calc 930.9); 1896 (2Abf-Suc-FTPRIa), 974.4 (calc 974.9); DH-2Abf (2Abf-Suc-FKPRLa), 1001.6 (calc 1001.0); 1903 (2Abf-Suc-FVPRLa), 972.7 (calc 972.0); 1868 (2Abf-Suc-[β^{3}F]TPRLa), 988.9 (calc, 988.0); 1631 (2Abf-Suc-FT[β^{3}P]RLa), 988.9 (calc 988.0); 1823 (Hex-Suc-FTPRLa), 815.8 (calc 816.0); 17113 (2Abf-Suc-FT[Oic]RLa), 1030.0 (calc 1030.6); 1790A (2Abf-Suc-LWA[dF]PRLa), 1245.5 (calc 1244.0); 1608-2 (2Abf-Suc-F[dF][β^{3}P]RLa), 1034.0 (calc 1034.0); 1373 (2Abf-Suc-A[dF]PRLa), 944.9 (calc 945.0); 1478 (2Abf-Suc-AGPRAa), 813.8 (calc 813.0); 1525 (2Abf-Suc-AAAA[dF]PRLa), 1159.3 (calc 1158); 1604 (2Abf-Suc-F[dF]PRAa), 977.9 (calc 977.0); 1374 (2Abf-Suc-F[dF]PRLa), 1022.3 (calc 1022.0); 1605 (Hex-Suc-A[dF]PRLa), 785.5 (calc 785.0); and 1607 (2Abf-Suc-[β^{3}F][dF]PRLa), 1033.4 (calc 1034.0).

DH-Jo, Backbone Cyclic, and DH Cyclic Analog Purification and Characterization. The peptide and/or pseudopeptide analogs were purified on a C_{18} Sep Pak cartridge and a Delta-Pak C_{18} RP column (8 × 100 mm, 15-μm particle size, 100-Å pore size) on a 510 HPLC system controlled with a Millennium 2010 chromatography manager system with detection at 214 nm at ambient temperature. Solvent A was 0.1% aqueous TFA, and solvent B was 80% (vol/vol) aqueous acetonitrile containing 0.1% TFA. Conditions were as follows: The initial solvent consisting of 20% solvent B was followed by the Waters linear program to 100% solvent B over 40 min, with a flow rate of 2 mL/min. Delta-Pak C_{18} t_Rs were as follows: DH-Jo (Ac-GLWA[Jo]RLa), 12.0 min; 1798A (*cyclo*[Suc-RWF[dF][a4G]]RLa), 16.4 min; and 35191 (*cyclo*[GLWFGPRL]), 16.5 min. The peptide and/or pseudopeptide analogs were further purified on a Protein Pak I125 column (7.8 × 300 mm). Conditions were as follows: 2.0 mL/min flow rate, solvent A was 95% acetonitrile made to 0.01% TFA, solvent B was 50% aqueous acetonitrile made to 0.01% TFA, 100% solvent A isocratic for 4 min and then a linear program to 100% solvent B over 80 min. Protein Pak I125 t_Rs were as follows: DH-Jo (Ac-GLWA[Jo]RLa), 7.0 min; 1798A (*cyclo*[Suc-RWF[dF][a4G]]RLa), 6.0 min; and 35191 (*cyclo*[GLWFGPRL]), 5.5 min.

Amino acid analysis was carried out under previously reported conditions (1) and used to quantify the peptide and/or pseudopeptide analogs and to confirm identity, leading to the following analyses: DH-Jo (Ac-GLWA[Jo]RLa): A[1.3], G[0.9], L[2.0], R[1.1]; 1798A (*cyclo*[Suc-RWF[dF][a4G]]RLa): F[2.0], G[1.9], L[1.3], R[1.0]; and 35191 (*cyclo*[GLWFGPRL]): F[1.0], G[1.9], L[2.0], P[1.0], R[1.0]. Identities of the peptide and/or pseudopeptide analogs were confirmed via MALDI-TOF-MS on a Kratos Kompact Probe MALDI-TOF-MS machine, with the presence of the following molecular ions [MH$^+$]: DH-Jo (Ac-GLWA[Jo]RLa), 896.6 Da (calc 896.0 Da); 1798A (*cyclo*[Suc-RWF[dF][a4G]]RLa), 1133.0 (calc 1133.0); and 35191 (*cyclo*[GLWFGPRL]), 927.8 (calc 928.1).

1. Nachman RJ, et al. (2009) An amphiphilic, PK/PBAN analog is a selective pheromonotropic antagonist that penetrates the cuticle of a heliothine insect. *Peptides* 30:616–621.

Table S1. Sequences and reference numbers of putative DH analogs and antagonists evaluated in the three diapause assays described in this study

	Reference no.	Sequence
For diapause termination	Pk-2Abf	2Abf-Suc-FTPRLa
	DH-2Abf-K	2Abf-Suc-FKPRLa
	1823	Hex-Suc-FTPRLa
	17113	2Abf-Suc-FT[Oic]Rla
For diapause prevention	Pk-2Abf	2Abf-Suc-FTPRLa
	DH-2Abf-K	2Abf-Suc-FKPRLa
	858C-1	2Abf-Suc-AARAAa
	1477	2Abf-Suc-AGPRLa
	1894	2Abf-Suc-FSPRLa
	1895	2Abf-Suc-FGPRLa
	1896	2Abf-Suc-FTPRIa
	1903	2Abf-Suc-FVPRLa
	1868	2Abf-Suc-[β^3F]TPRLa
	1631	2Abf-Suc- FT[β^3P]RLa
For preventing diapause termination	1373	2Abf-Suc-A[dF]PRLa
	1374	2Abf-Suc-F[dF]PRLa
	1478	2Abf-Suc-AGPRAa
	1525	2Abf-Suc-AAAA[dF]PRLa
	1604	2Abf-Suc-F[dF]PRAa
	1605	Hex-Suc-A[dF]PRLa
	1607	2Abf-Suc-[β^3F][dF]PRLa
	1608-2	2Abf-Suc- F[dF] [β^3P]PRLa
	1790A	2Abf-Suc- LWA[dF]PRLa
	1798A	*Cyclo*[Suc-RWF[dF][a4G]]RLa
	DH-Jo	Ac-GLWA[JoA]RLa
	1935	2Abf-Suc-A[dK]PRLa
	35191	*Cyclo*[GLWFGPRL]