Supporting Information

Biological and Conformational Evaluation of Bifunctional Compounds for Opioid Receptor Agonists and Neurokinin 1 Receptor Antagonists Possessing Two Penicillamines

Takashi Yamamoto,[†] Padma Nair,[†] Neil E. Jacobsen,[†] Vinod Kulkarni,[†] Peg Davis,[‡] Shou-wu Ma,[‡] Edita Navratilova,[‡] Henry I. Yamamura,[‡] Todd W. Vanderah,[‡] Frank Porreca,[‡] Josephine Lai[‡] and Victor J. Hruby[†*]

The Chemistry and Biochemistry Department,[†] and Department of Pharmacology[‡], University of Arizona, Tucson, AZ, 85721, USA

contents

Characterization of Peptides.

The purified peptides were characterized by HRMS, TLC, analytical HPLC and ^{1}H-NMR. Sequential assignment of proton resonances was achieved by 2D-TOCSY NMR experiments.[1] High-resolution MS were taken in the positive ion mode using FAB methods at the University of Arizona Mass Spectrometry Facility. TLC was performed on aluminum sheets coated with a 0.2 mm layer of silica gel 60 F_{254} Merck using the following solvent systems: (1) $CHCl_3$: MeOH : AcOH = 90 : 10 : 3; (2) EtOAc : *n*-BuOH : water : AcOH = 5 : 3 : 1 : 1; and (3) *n*-BuOH : water : AcOH = 4 : 1 : 1. TLC chromatograms were visualized by UV light and by ninhydrin spray followed by heating (hot plate). Analytical HPLC was performed on a Hewlett Packard 1100 or Hewlett Packard 1090m with Waters NOVA-Pak C-18 column (3.9 x 150 mm, 5 □m, 60Å) or Vydac 218TP104 C-18 column (4.6 x 250 mm, 10 μm, 300 Å). ^{1}H-1D-NMR spectra were obtained on Bruker DRX-500 or DRX-600 spectrometer. 2D-TOCSY and 2D-NOESY NMR spectra were performed on a Bruker DRX-600 spectrometer equipped with a 5mm Nalorac triple-resonance single-axis gradient probe. The NMR experiments were conducted in DMSO-d_6 solution at 298K. Spectra were referenced to residual solvent protons as 2.49 ppm. The processing of NMR data was performed with the XwinNmr software (Bruker BioSpin, Fremont, CA). In the TOCSY experiments, the TPPI mode[2] with MLEV-17 Mixing Sequence[3] were used with a mixing time of 62.2 ms, at a spin-lock field of 8.33 kHz. The mixing time for the NOESY spectra was 450 ms for all cyclic peptide derivatives. All 2D spectra were acquired in the TPPI mode with 2k complex data points in t_2 and 750 real data points in t_1, and the spectral processing using shifted sine bell window functions in both dimensions.

Table S1. Sequence and analytical data of bifunctional peptide ligands

no	Sequence	*m/z* [a] (M + 2H) $^{2+}$		HPLC [b] log *k*'		TLC [e] (R_f)		
		Obs. (FAB)	Calc.	(A)[c]	(B)[d]	(I)	(II)	(III)
2	H-Tyr-c[*D*-Pen-Gly-Phe-Pen]-Pro-Leu-Trp-NH-3,5-Bzl(CF_3)$_2$ (**TY046**)	634.2522	634.2494	17.56	8.01	0.05	0.85	0.59
3	H-Tyr-c[*D*-Pen-Gly-Phe-*D*-Pen]-Pro-Leu-Trp-NH-3,5-Bzl(CF_3)$_2$ (**TY049**)	634.2522	634.2494	18.94	9.25	0.13	0.89	0.65
4	H-Tyr-c[*D*-Pen-Gly-Phe-Nle-Pro-Pen]-Trp-NH-3,5-Bzl(CF_3)$_2$ (**TY047**)	634.2522	634.2494	19.12	9.40	0.07	0.84	0.58
5	H-Tyr-c[*D*-Pen-Gly-Phe-Nle-Pro-*D*-Pen]- Trp-NH-3,5-Bzl(CF_3)$_2$ (**TY048**)	634.2518	634.2494	19.88	10.18	0.07	0.83	0.57

[a] High-resolution mass spectroscopy using FAB ionization method. [b] HPLC log *k'* = log [(peptide retention time - solvent retention time)/solvent retention time]. All the obtained final peptides showed > 98% purity, except for **4** (>95% purity). [c] 10-90% of acetonitrile containing 0.1% TFA within 40 min and up to 95% within additional 5 min, 1 mL/min, 230 nm, Waters NOVA-Pak C-18 column (3.9 x 150 mm, 5 μm, 60 Å). [d] 30-70% of acetonitrile containing 0.1% TFA within 40 min and up to 95% within an additional 5 min, 1 mL/min, 230 nm, Vydac 218TP104 C-18 column (4.6 x 250 mm, 10 μm, 300 Å). [e] (I) $CHCl_3$: MeOH : AcOH = 90 : 10 : 3; (II) EtOAc : *n*-BuOH : water : AcOH = 5 : 3 : 1 : 1; (III) *n*-BuOH : water : AcOH = 4 : 1 : 1.

Table S2. ^{1}H Resonance Assignments for Micelle-Bound Bifunctional peptides with 40-fold DPC in 90% H_2O/10% D_2O, 45mM CD_3CO_2Na, 1mM NaN_3 at 310 K

H-Tyr-c[*D*-Pen-Gly-Phe-Pen]-Pro-Leu-Trp-NH-3,5-Bzl(CF_3)$_2$·TFA (**2**), 4.2 mM, Only one isomer found; δ:				
AA	NH	α	β	misc.
Tyr[1]		4.63	2.96, 3.11	2,6H: 7.16, 3,5H: 6.82
*D*Pen[2]	8.8	4.36		γ: 1.15, 1.26
Gly[3]	8.89	3.56, 4.21		
Phe[4]	7.67	4.74	3.04, 3.17	2,6H: 7.20, 3,5H: 7.27, 4H[a]
Pen[5]	7.53	4.48		γ: 1.03, 1.24
Pro[6]		4.21	0.82, 1.85	γ: 1.44, 1.66, δ: 3.13, 3.66
Leu[7]	8.27	4.06	1.65	γ: 1.55, δ: 0.83, 0.90
Trp[8]	7.01	4.56	3.19, 3.43	Ind2: 7.29, Ind4: 7.34, Ind5: 6.74, Ind6: 7.01, Ind7: 7.42
Bzl	7.72	4.27, 4.32		2.6H: 7.65, 4H: 7.65

[a]: not observed.

Table S3. ^{1}H Resonance Assignments of bifunctional peptides in DMSO

H-Tyr-c[*D*-Pen-Gly-Phe-Pen]-Pro-Leu-Trp-NH-3,5-Bzl(CF_3)$_2$·TFA (**2**), Only one isomer found; δ:				
AA	NH	α	β	misc.
Tyr[1]	7.99(3H, bs)	4.38-4.48(1H, m)	2.70-2.78(1H, m), 2.92-3.00(1H, m)	6.69(2H, d, J=7.8Hz: PhH), 7.12(2H, d, J=8.4Hz: PhH), 9.32(1H, bs: PhOH)
*D*Pen[2]	8.58-8.63(1H, m)	4.36-4.46(1H, m)	0.94(3H, s), 1.62(3H, s)	-
Gly[3]	8.50(1H, t, J=3.6Hz)	3.17(1H, dd, J=3.6, 15.6), 4.20-4.30(1H, m)	-	-
Phe[4]	8.97(1H, d, J=7.2Hz)	4.47-4.53(1H, m)	2.83(1H, dd, J=10.8, 15.6Hz), 2.94-3.02(1H, m)	7.14-7.18(1H, m: PhH), 7.21-7.27(4H, m: PhH)
Pen[5]	7.69(1H, d, J=7.2Hz)	4.36-4.46(1H, m)	1.19(3H, s), 1.22(3H, s)	-
Pro[6]	-	4.25-4.31(1H, m)	1.59-1.65(1H, m), 1.88-1.95(1H, m)	1.69-1.78(1H, m: γCH_2), 1.80-1.88(1H, m: γCH_2), 3.45-3.51(1H, m: δCH_2), 3.70-3.77(1H, m: δCH_2)
Leu[7]	7.96-7.81(1H, m)	4.22-4.31(1H, m)	1.30-1.43(2H, m)	1.59-1.66(2H, m: γCH_2), 0.75(3H, d, J=6.0Hz: δCH_2), 0.81(3H, d, J=6.6Hz: δCH_2)
Trp[8]	7.92-7.95(1H, m)	4.47-4.53(1H, m)	2.94-3.02(1H, m), 3.11(1H, dd, J=5.4, 14.4Hz),	6.94(1H, t, J=7.8: Ind5), 7.04(1H, t, J=7.8Hz: Ind6), 7.07(1H, s: Ind2), 7.30(1H, d, J=7.8Hz: Ind7), 7.52(1H, d, J=7.8Hz: Ind4), 10.80(1H, bs, IndNH)
Bzl	8.59-8.62(1H, m)	2.92-3.00(1H, m), 4.36-4.46(1H, m)	-	7.88(2H, s: PhH), 7.94(1H, s: PhH)

[a] Can't be distinguished

H-Tyr-c[*D*-Pen-Gly-Phe-*D*-Pen]-Pro-Leu-Trp-NH-3,5-Bzl(CF_3)$_2$·TFA (**3**), 2 amide bond rotamers at the Pro^6 N, ca. 3 :1 ratio; δ:				
AA	NH	α	β	misc.
Tyr^1	8.04 (3H, bs)	4.25-4.35(1H, m)	2.70-2.80(1H, m), 2.89-2.99(1H, m)	6.70(2H, d, J=8.0Hz: PhH), 7.11(2H, m: PhH), 9.32(1H, bs: PhOH)
*D*Pen^2	8.61-8.66/8.53-8.58(1H, m)	4.30-4.38/4.44-4.51(1H, m)	1.01/1.03(3H, s), 1.28/1.27(3H, s)	-
Gly^3	8.45-8.52/8.53-8.57(1H, m)	3.10-3.20(2H, m)	-	-
Phe^4	8.84/8.64(1H, d, J=7.5/7.0Hz)	4.20-4.30/4.37-4.47(1H, m)	2.74-2.83(1H, m), 2.90-2.97/2.99-3.04(1H, m)	7.12-7.28(5H, m: PhH)
*D*Pen^5	8.54/8.57-8.66(1H, d, J=8.0Hz/m)	4.52-4.60/4.50-4.67(1H, m)	1.28/1.09(3H, s), 1.35/1.32(3H, s)	-
Pro^6	-	4.26-4.35/4.82(1H, m)	1.95-2.05/1.78-1.90(1H, m), 1.75-1.85/1.78-1.90(1H, m)	1.65-1.75(2H, m: γCH_2), 3.55-3.65/3.30-3.38(1H, m: δCH_2), 3.66-3.75/3.30-3.38(1H, m: δCH_2)
Leu^7	7.79/8.29(1H, d, J=7.5/8.0Hz)	4.16-4.24(1H, m)	1.45-1.55/1.32-1.48(2H, m)	1.45-1.55/1.32-1.48(1H, m: γCH_2), 1.55-1.63/1.32-1.48(1H, m: γCH_2), 0.80(3H, d, J=6.0Hz: δCH_2), 0.89/0.85(3H, d, J=6.5/6.5Hz: δCH_2)
Trp^8	7.79/7.85-7.90(1H, d, J=7.5Hz/m)	4.39-4.46/4.52-4.58(1H, m),	2.94-3.05(1H, m), 3.07-3.19(1H, m),	6.92/6.96(1H, t, J=7.5/7.0: Ind5), 7.00-7.08(1H, m: Ind6), 7.09(1H, s: Ind2), 7.30(1H, d, J=8.0Hz: Ind4), 7.45/7.53(1H, d, J=8.0/7.5Hz: Ind7), 10.79/10.82(1H, bs, IndNH)
Bzl	7.37/8.42-8.50(1H, d, J=5.5Hz/m)	4.35-4.45/420-4.30(1H, m), 4.50-4.58/4.32-4.42(1H, m)	-	7.86/7.90(2H, s: PhH), 7.91/7.97(1H, s: PhH)

[a] Can't be distinguished

H-Tyr-c[*D*-Pen-Gly-Phe-Nle-Pro-Pen]-Trp-NH-3,5-Bzl(CF_3)$_2$·TFA (**4**), 2 amide bond rotamers at the Pro^6 N, ca. 3 : 1 ratio; δ:				
AA	NH	α	β	misc.
Tyr^1	7.92 (3H, bs)	4.06-4.14/4.13-4.20(1H, m)	2.65-2.75/2.75-2.80(1H, m), 3.02-3.12/2.94-3.00(1H, m)	6.70(2H, d, J=7.2Hz: PhH), 7.13/7.11(2H, d, J=8.4: PhH), 9.34(1H, bs: PhOH)
*D*Pen^2	8.80/8.85(1H, d, J=9.6/9.0Hz)	4.60/4.55-4.60(1H, d, J=9.6Hz/m)	1.17/1.02(3H, s), 1.30/1.19(3H, s)	-
Gly^3	7.90-8.00/8.78-8.86(1H, m)	2.75-2.83/3.08-3.14(1H, m), 3.32-3.45/3.38-3.35(1H, m)	-	-
Phe^4	8.35-8.43/7.37(1H, m/d, J=9.0Hz)	4.05-4.15/4.54-4.64(1H, m)	2.77-2.82/2.52-2.60(1H, m), 3.11-3.20/2.86-2.94(1H, m)	7.12-7.28(5H, m: PhH)
Nle^5	-[a]/8.24-8.30(1H, m)	4.05-4.15/4.55-4.65(1H, m)	1.25-1.36/1.12-1.28(2H, m)	1.60-1.68/1.44-1.51(1H, m: γCH_2), 1.60-1.68/1.58-1.66(1H, m: γCH_2), 1.25-1.36/1.12-1.28(2H, m: δCH_2), 0.68-0.78(3H, m: εCH_3)
Pro^6	-	4.44-4.52/4.77(1H, m/d, J=7.2Hz)	1.55-1.62/1.76-1.83(1H, m), 1.88-2.00/1.90-1.96(1H, m)	1.68-1.75/1.55-1.60(1H, m: γCH_2), 1.74-1.83/1.60-1.70(1H, m: γCH_2), 3.30-3.38/3.38-3.43(1H, m: δCH_2), 3.56-3.64/3.38-3.43(1H, m: δCH_2)
Pen^7	7.85-7.95(1H, m)	4.18-4.28/4.28-4.35(1H, m)	1.10/1.20(3H, s), 1.30/1.38(3H, s)	-
Trp^8	8.02-8.09(1H, m)	4.48-4.58(1H, m),	3.00-3.10(1H, m), 3.11-3.18(1H, m),	6.93/6.91(1H, t, J=7.8/8.4Hz: Ind5), 7.03/7.02(1H, t, J=7.2/7.2Hz: Ind6), 7.08(1H, s: Ind2), 7.30/7.28(1H, d, J=8.4/9.6HzHz: Ind7), 7.49(1H, d, J=7.8Hz: Ind4), 10.78(1H, bs, IndNH)
Bzl	8.49-8.57(1H, m)	4.27-4.35/4.22-4.32(1H, m), 4.35-4.47(1H, m)	-	7.88(2H, s: PhH), 7.86(1H, s: PhH)

[a] Can't be distinguished

H-Tyr-c[*D*-Pen-Gly-Phe-Nle-Pro-*D*-Pen]-Trp-NH-3,5-Bzl(CF_3)$_2$·TFA (**5**), 2 amide bond rotamers at the Pro^6 N, ca. 2 : 1 ratio; δ:				
AA	NH	α	β	misc.
Tyr^1	7.96/8.02(3H, bs)	4.08-4.22(1H, m)	2.68-2.78/2.73-2.82(1H, m), 2.97-3.052.95-3.02(1H, m)	6.71/6.73(2H, d, J=7.2/7.2Hz: PhH), 7.10-7.20(2H, m: PhH), 9.33/9.35(1H, bs: PhOH)
*D*Pen^2	8.68-8.78/8.82-8.88(1H, m)	4.53-4.65/4.43-4.50(1H, m)	1.12/1.09(3H, s), 1.16(3H, s)	-
Gly^3	-[a]	2.90-3.00/3.90-4.03(1H, m), 3.18-3.26/3.90-4.03(1H, m)	-	-
Phe^4	8.03-8.39/7.70-7.78(1H, m)	4.30-4.40/4.55-4.62(1H, m)	2.80-2.88/2.58-2.65(1H, m), 3.03-3.10/2.88-2.96(1H, m)	7.12-7.30(5H, m: PhH)
Nle^5	8.29-8.35/8.64-8.73(1H, m)	4.10-4.18/4.36-4.45(1H, m)	1.18-1.31/1.22-1.36(2H, m)	1.45-1.64/1.65-1.86(2H, m: γCH_2), 1.18-1.31/1.22-1.36(2H, m: δCH_2), 0.75-0.90(3H, m: εCH_3)
Pro^6	-	4.40-4.49/4.35-4.44(1H, m)	1.70-1.79/1.65-1.74(1H, m), 1.80-1.90/1.75-1.86(1H, m)	1.55-1.62/1.40-1.50(2H, m: γCH_2), 3.32-3.41/3.23-3.32(1H, m: δCH_2), 3.55-3.63/3.33-3.44(1H, m: δCH_2)
*D*Pen^7	8.60-8.75(1H, m)	4.42-4.52(1H, m)	0.97(3H, s), 1.07(3H, s)	-
Trp^8	8.45-8.54/8.54-8.61(1H, m)	4.48-4.57 (1H, m),	2.88-3.01/2.94-3.04(1H, m), 3.16-3.27/3.27-3.35(1H, m),	6.91-6.97(1H, m: Ind5), 7.01(1H, t, J=7.8Hz: Ind6), 7.13/7.17 (1H, s: Ind2), 7.26-7.34(1H, m: Ind7), 7.57/7.56(1H, d, J=8.4/8.4Hz: Ind4), 10.73/10.74(1H, bs, IndNH)
Bzl	8.60-8.76(1H, m)	4.42-4.53(2H, m)	-	7.91(2H, s: PhH), 7.96(1H, s: PhH)

[a] Can't be distinguished

Table S4. The Statistical Analysis of the Obtained NMR Conformations of **2**.

Compound	**2**	
	final 10 structs	most stable
rms deviation from NOE dist restraints (Å)[b]	0.0267 ± 0.0008	0.0260
rms deviation from backbone φ angle restraints (deg)[c]	0.000 ± 0.000	0.000
NOE dist restraints violations		
> 0.01 Å	21.0 ± 0.9	21
> 0.1 Å	4.4 ± 0.7	4
max dist violations (Å)	0.15 ± 0.00	0.14
dihedral backbone angle violations		
> 0.1°	0.0 ± 0.0	0
> 1°	0.0 ± 0.0	0
max dihdral violations (deg)	0.0 ± 0.0	0
AMBER energies (kcal mol^{-1})		
restraint[i]	4.10 ± 0.22	3.91
bond stretching	3.41 ± 0.05	3.40
bond angles	20.47 ± 0.20	20.30
dihedral angles	18.80 ± 0.60	18.40
planarity	2.27 ± 0.07	2.23
van der Waals[j]	9.86 ± 0.15	9.80
electrostatic[k]	−10.21 ± 0.13	−10.13
total	43.86 ± 0.75	43.26

[a] reference.[4] [b] The total number of NOE restraints were 184. [c] 3 backbone φ angle restraints were applied. [d] no restraints used. [e] Derived from the rMD calculations using the AMBER force field in DISCOVER. [f] The number of bond length was 167. [g] The number of bond valence angles was 300. [h] The number of out-of-plane angles was 37. [i] Calculated with force constants of 25 kcal mol^{-1} $Å^{-2}$ and 100 kcal mol^{-1} rad^{-2} for the NOE distance and dihedral angle restraints, respectively. [j] Calculated with the Lennard-Jones potential using the AMBER force field and a 12 Å cutoff. [k] Calculated with a distance-dependent dielectric constant ($\varepsilon = 4r$).

Table S5. Atomic rmsd values (Å) for the final 9 conformers compared to the most stable conformer of bifunctional peptide derivatives **1**.[4]

Aligned residues	Whole molecule	1-4 resdues	5-8 residues and *C*-terminus
backbone toms (N, C^{α}, C')	1.03 ± 0.57	0.97 ± 0.75	0.36 ± 0.40
all non-hydrogen atoms	1.85 ± 0.81	1.83 ± 1.13	0.99 ± 0.25

Table S6. Number of β-turn structural elements and the distance between alpha carbons of *i* th and (*i* + 3)rd residues of compound **1**.[4]

Residues[b]	number of β-turns[a]	distance (Å)
$C^{2}\alpha$-$C^{5}\alpha$	10	5.04 ± 0.65
$C^{6}\alpha$-Bzl	10	6.22 ± 0.39

[a] Out of the best 10 calculated structures. The distance is the mean distance between two alpha carbons ± standard deviation (SD). The sequences with less than 7 Å distance between alpha carbons of *i* th and (*i* + 3) rd residues without helical structure were considered as a *β*-turn.[5] Bzl stands for the benzyl moiety at the *C*-terminus. [b] Only residues possessing *β*-turn structural element were displayed.

Table S7. φ and ψ angle values of the ligand **1** in the observed NMR structure.[a]

angle	Tyr[1]	*D*-Ala[2]	Gly[3]	Phe[4]
φ		−82.3 ± 87.2	−67.0 ± 71.2	−83.0 ± 19.3
ψ	−113.4 ± 89.6	20.0 ± 66.7	−23.9 ± 31.7	−34.6 ± 4.4
angle	Met[5]	Pro[6]	Leu[7]	Trp[8]
φ	71.5 ± 2.2	−47.8 ± 8.3	−48.5 ± 60.5	−65.9 ± 4.6
ψ	135.0 ± 6.3	103. ± 67.3	−5.3 ± 9.6	−27.7 ± 4.4

[a] Average ± SD values out of the best 10 calculated structures were listed. The *C*-terminus was considered as residue 9.

Table S2. Observed hydrogen bonds in the NMR structures of ligands **1** and **2**.[a]

Molecule	No.[b]	Donor	Acceptor	Distance (Å)[c]	Angle (deg)[d]
TY027 (1)	6	Bzl^{9} H^{Ne}	Pro^{6} O	2.17 ± 0.12	159.4 ± 1.5
	4	Gly^{3} H^{N}	Tyr^{1} O	2.00 ± 0.07	143.0 ± 0.36
2	No hydrogen bond observed				

[a] The hydrogen bonds which were observed in more than five structures were listed. [b] The number of structures of the final 10 for which the listed hydrogen bond is observed. [c] The distance is the mean proton-acceptor atom distance (± SD) in the structures for which a hydrogen bond is observed. [d] The angle is the mean angle (± SD) in the structures for which a hydrogen bond is observed. [e] Amide proton of *C*-terminal benzyl moiety. [g] Fluorine atom at *C*-terminal benzyl moiety.

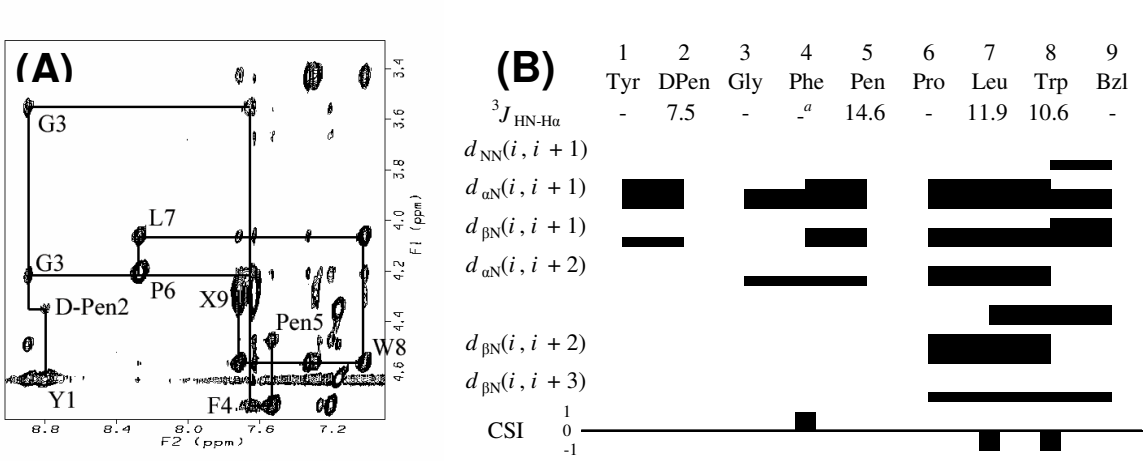

Figure S1. (A) Fingerprint (H^N-H^α) region of the NOESY spectrum of **2** in DPC micelles. Intraresidue H^N-H^α NOE cross-peaks are labeled with residue numbers, and arrows indicate the connectivity path from *N*-terminal to *C*-terminal. X9 represents the cross-peaks derived from the corresponding *C*-terminal H^N and benzyl protons. (B) Diagram of H^N-H^α coupling constants, NOE connectivities, and H^α chemical shift index (CSI) for **2**. The H^α CSI was calculated using the random-coil values reported by Andersen et al.[4,5]

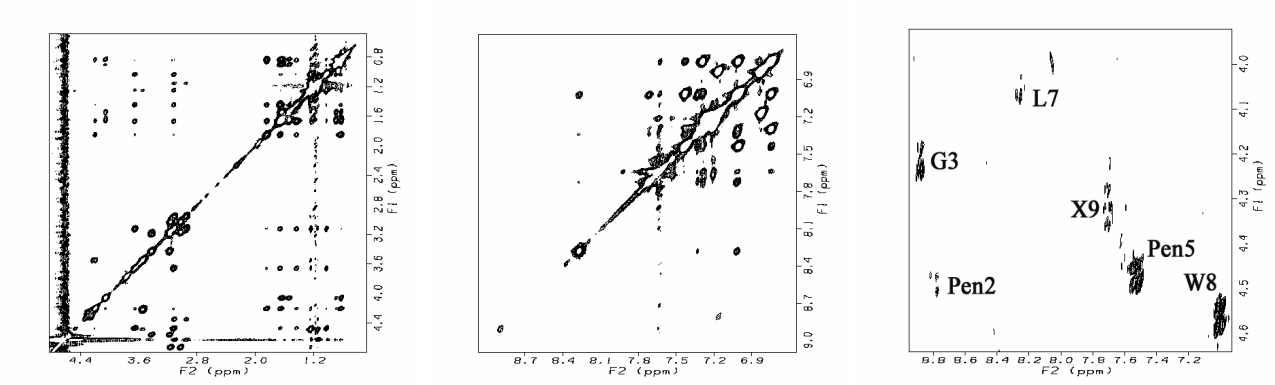

Figure S2. Side-chain (left) and aromatic regions (middle) of the NOESY spectrum of **2** in DPC micelles. Fingerprint (H^N-H^α) region of the DQF-COSY spectrum of **2** in DPC micelles (right) was also shown. Intraresidue H^N-H^α cross-peaks in the DQF-COSY spectrum are labeled with residue number. X9 represents the cross-peaks derived from the corresponding *C*-terminal H^N and benzyl protons. The Phe[4] derived H^N-H^α cross-peak was not found in the DQF-COSY spectrum.

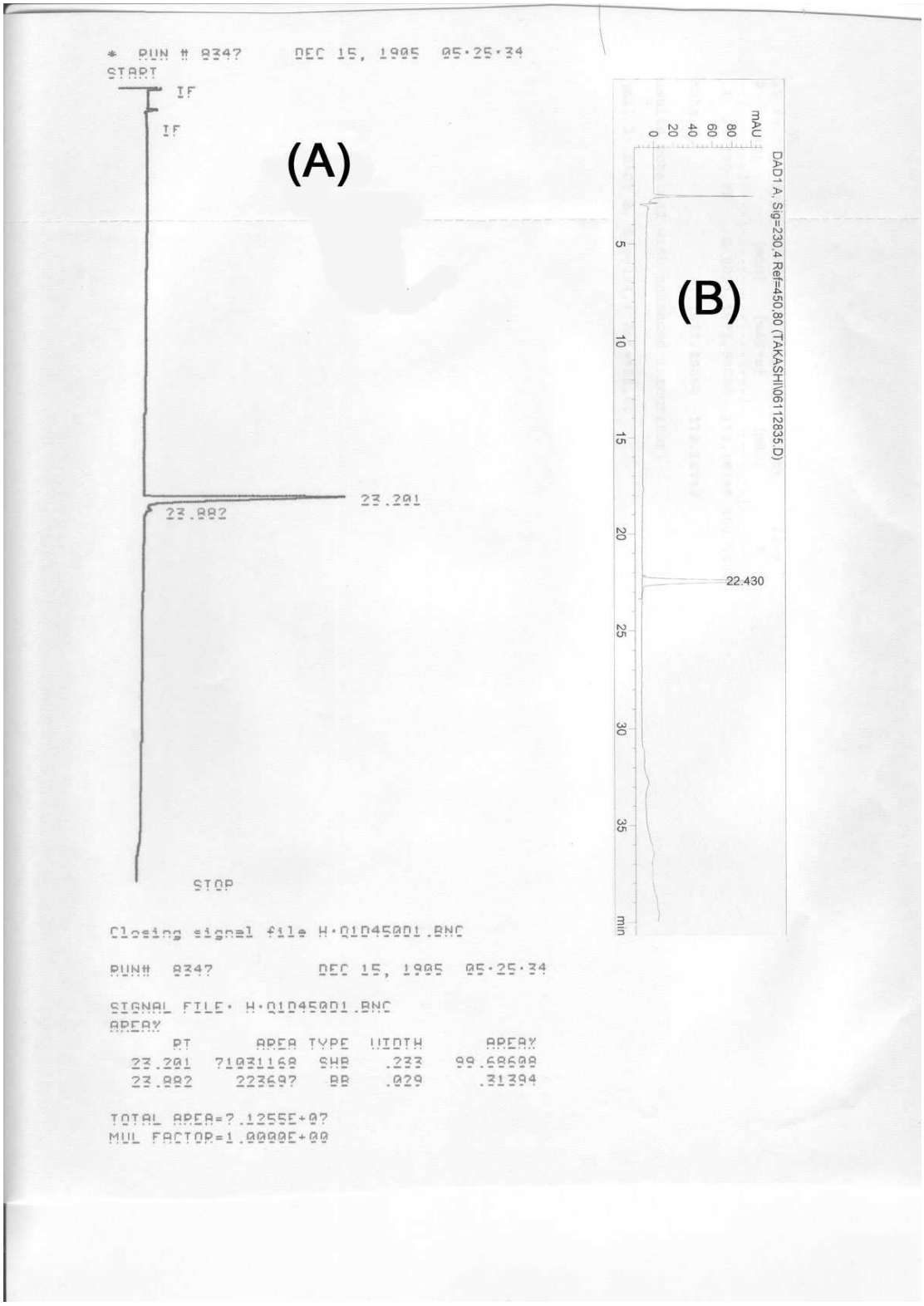

Chart S1. HPLC trace of **2**: (A) 10-90% of acetonitrile containing 0.1% TFA within 40 min and up to 95% within an additional 5 min, 1 mL/min, 230 nm, Waters NOVA-Pak C-18 column (3.9 x 150 mm, 5 µm, 60 Å). (B) 30-70% acetonitrile containing 0.1% TFA within 40 min and up to 95% within an additional 5 min, 1 mL/min, 230 nm, Vydac 218TP104 C-18 column (4.6 x 250 mm, 10 µm, 300 Å).

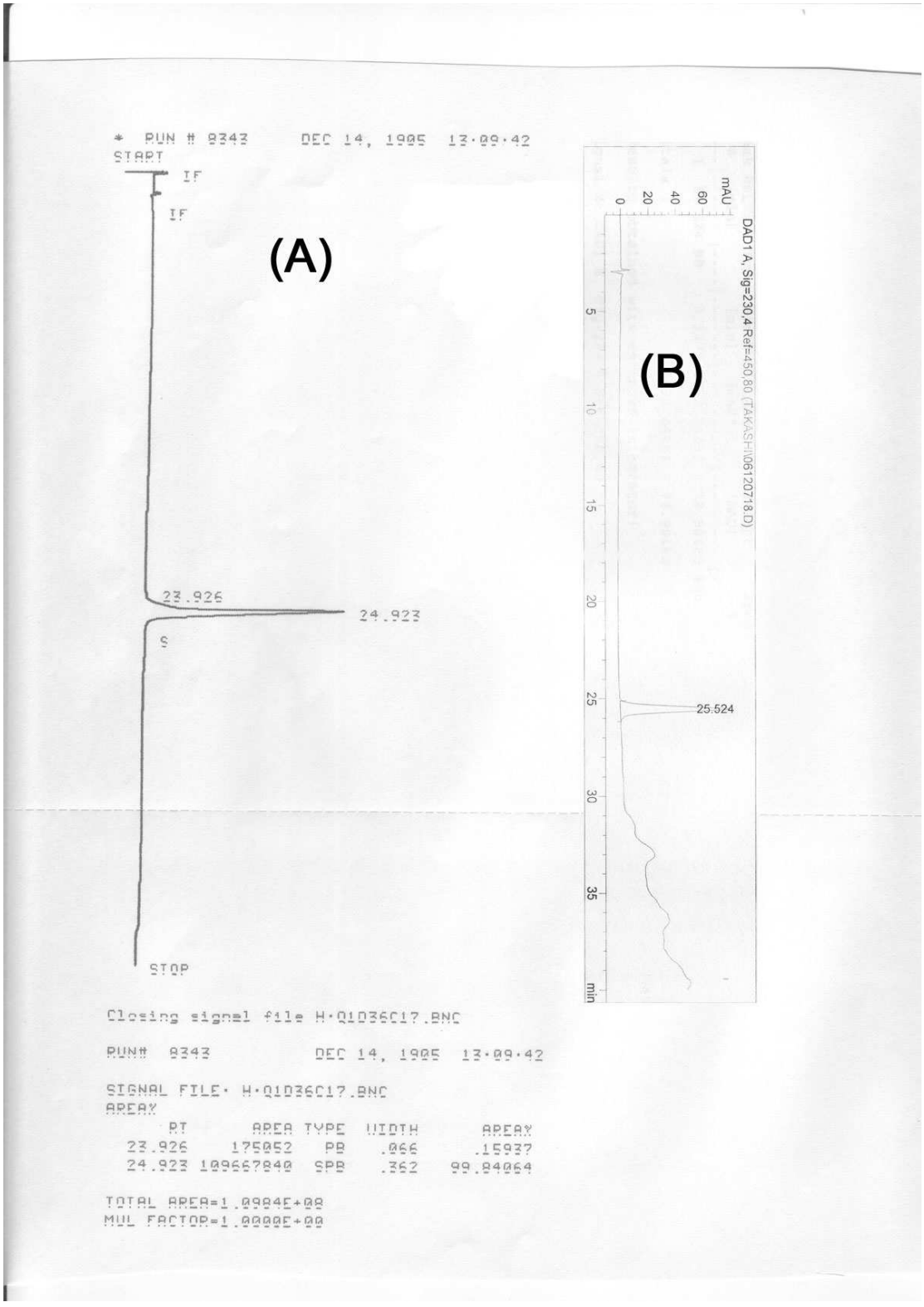

Chart S2. HPLC trace of **3**: (A) 10-90% of acetonitrile containing 0.1% TFA within 40 min and up to 95% within an additional 5 min, 1 mL/min, 230 nm, Waters NOVA-Pak C-18 column (3.9 x 150 mm, 5 µm, 60 Å). (B) 30-70% acetonitrile containing 0.1% TFA within 40 min and up to 95% within an additional 5 min, 1 mL/min, 230 nm, Vydac 218TP104 C-18 column (4.6 x 250 mm, 10 µm, 300 Å).

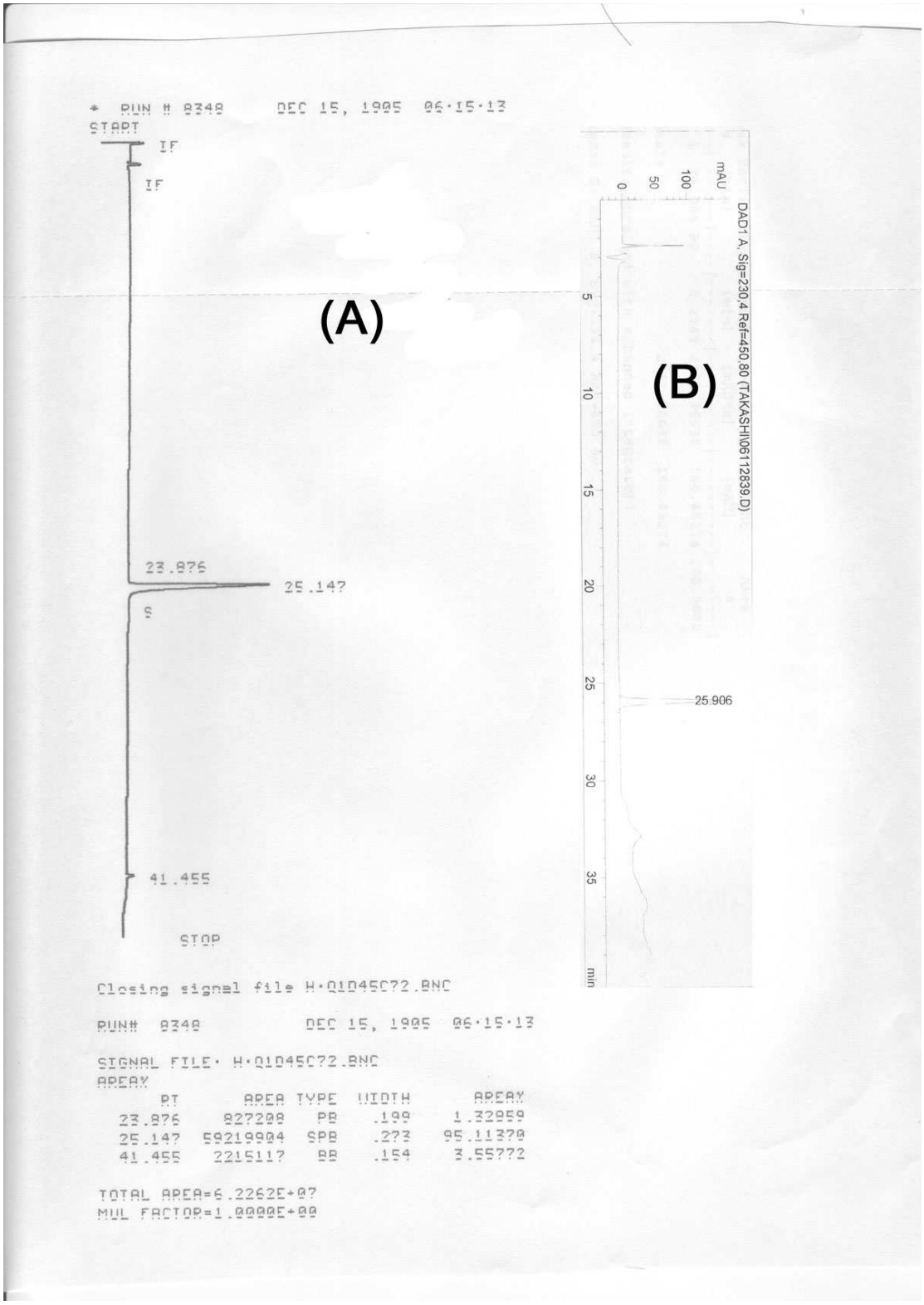

Chart S3. HPLC trace of **4**: (A) 10-90% of acetonitrile containing 0.1% TFA within 40 min and up to 95% within an additional 5 min, 1 mL/min, 230 nm, Waters NOVA-Pak C-18 column (3.9 x 150 mm, 5 µm, 60 Å). (B) 30-70% acetonitrile containing 0.1% TFA within 40 min and up to 95% within an additional 5 min, 1 mL/min, 230 nm, Vydac 218TP104 C-18 column (4.6 x 250 mm, 10 µm, 300 Å).

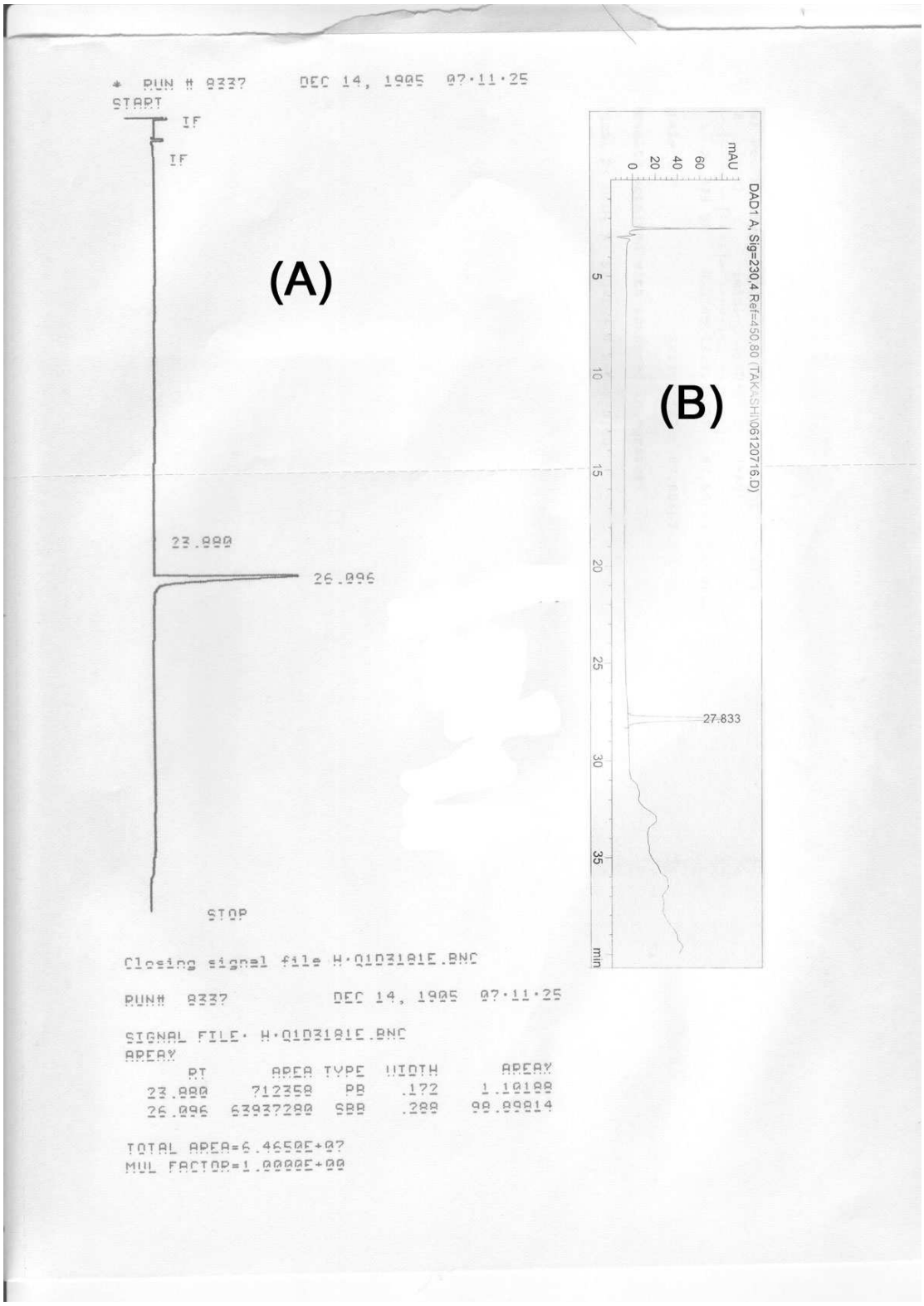

Chart S4. HPLC trace of **5**: (A) 10-90% of acetonitrile containing 0.1% TFA within 40 min and up to 95% within an additional 5 min, 1 mL/min, 230 nm, Waters NOVA-Pak C-18 column (3.9 x 150 mm, 5 µm, 60 Å). (B) 30-70% acetonitrile containing 0.1% TFA within 40 min and up to 95% within an additional 5 min, 1 mL/min, 230 nm, Vydac 218TP104 C-18 column (4.6 x 250 mm, 10 µm, 300 Å).

1. Davis, D. G.; Bax, A. Assignment of complex proton NMR spectra via two-dimensional homonuclear Hartmann-Hahn spectroscopy *J. Am Chem. Soc.* **1985,** 107, 2820-2821.

2. Marion, D. W., K. Application of phase sensitive two-dimensional correlated spectroscopy (COSY) for measurements of 1H-1H spin-spin coupling constants in proteins. *Biochem. Biophys. Res. Commun.* **1983,** 113, 967-974.

3. Braunschweiler, L.; Ernst, R. R. Coherence Transfer by Isotropic Mixing: Application of Proton Correlation Spectroscopy. *J. Magn. Reson.* **1983,** 53, 521-528.

4. Yamamoto, T.; Nair, P.; Jacobsen, N. E.; Davis, P.; Ma, S. W.; Navratilova, E.; Lai, J.; Yamamura, H. I.; Vanderah, T. W.; Porreca, F.; Hruby, V. J. The Importance of Micelle-Bound States for the Bioactivities of Bifunctional Peptide Derivatives for δ/μ Opioid Receptor Agonists and Neurokinin 1 Receptor Antagonists. *J. Med. Chem.* **2008,** 51, 6334-6347.

5. Wagner, G.; Neuhaus, D.; Worgotter, E.; Vasak, M.; Kagi, J. H.; Wuthrich, K. Nuclear magnetic resonance identification of "half-turn" and 3(10)-helix secondary structure in rabbit liver metallothionein-2. *J. Mol. Biol.* **1986,** 187, 131-135.