Supporting Information.

Title: The Importance of Micelle-Bound States for the Bioactivities of Bifunctional Peptide Derivatives for δ/μ Opioid Receptor Agonists and Neurokinin 1 Receptor Antagonists

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Table S1. Sequence and analytical data of bifunctional peptide ligands

no	Sequence	*m/z* [a] (M + H)$^+$		HPLC [b] log *k'*		TLC [e] (R_f)		
		Obs. (ESI)	Calc.	(A)[c]	(B)[d]	(I)	(II)	(III)
1	H-Tyr-*D*-Ala-Gly-Phe-Met-Pro-Leu-Trp-O-3,5-Bzl$(CF_3)_2$ (**TY005**)	1210.4810	1210.4871	19.21	11.14	0.14	0.73	0.79
2	H-Tyr-*D*-Ala-Gly-Phe-Met-Pro-Leu-Trp-NH-3,5-Bzl$(CF_3)_2$ (**TY027**)	1209.3055	1209.5031	17.29	7.94	0.09	0.67	0.58
3	H-Tyr-*D*-Ala-Gly-Phe-Met-Pro-Leu-Trp-NH-3,5-Bzl (**TY025**)	1073.3096	1073.5283	14.14	4.19	0.14	0.67	0.55

[a] High-resolution mass spectroscopy using electrospray ionization method. [b] HPLC log *k'* = log [(peptide retention time - solvent retention time)/solvent retention time]. All the obtained final peptides showed > 98% purity (see ChartS1-S3 in this Supporting Information). [c] 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). [d] 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). [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-*D*-Ala-Gly-Phe-Met-Pro-Leu-Trp-O-3,5-Bzl$(CF_3)_2$□ TFA (**1**), 3.8 mM, only for the major isomer, δ:

AA	NH	α	β	misc.
Tyr^1		4.26	3.09, 3.20	2,6H: 7.18, 3,5H: 6.93
D-Ala^2	8.69	4.20	1.22	
Gly^3	8.52	3.86, 3.96		
Phe^4	7.89	4.70	3.15, 3.20	2,6H: 7.32, 3,5H: 7.35, 4H^a
Met^5	8.17	4.69	1.96, 2.05	γ: 2.45, 2.51, CH_3: 2.08
Pro^6		4.45	1.59, 2.20	γ: 1.78, 1.90, δ: 3.61, 3.71
Leu^7	7.84	4.44	1.59	γ: 1.59, δ: 0.86
Trp^8	8.11	4.73	3.34, 3.47	Ind2: 7.42, Ind4: 7.52, Ind5: 7.13, Ind6: 7.00, Ind7: 7.48
3,5-Bn$(CF_3)_2$		5.02, 5.08		2.6H: 7.70, 4H: 7.76

H-Tyr-*D*-Ala-Gly-Phe-Met-Pro-Leu-Trp-NH-3,5-Bzl$(CF_3)_2$□ TFA (**2**), 3.5 mM, only for the major isomer, δ:

AA	NH	α	β	misc.
Tyr^1		4.28	3.11, 3.22	2,6H: 7.20, 3,5H: 6.93
D-Ala^2	8.71	4.24	1.22	
Gly^3	8.56	3.86, 3.96		
Phe^4	7.82	4.68	3.13, 3.19	2,6H: 7.27, 3,5H: 7.34, 4H^a
Met^5	8.07	4.55	1.91	γ: 2.40, CH_3: 2.01
Pro^6		4.36	1.18, 2.01	γ: 1.56, 1.69, δ: 3.44, 3.60
Leu^7	8.35	4.18	1.71	γ: 1.61, δ: 0.92, 0.98
Trp^8	7.40	4.67	3.31, 3.47	Ind2: 7.40, Ind4: 7.37, Ind5: 6.83, Ind6: 7.10, Ind7: 7.51
3,5-Bn$(CF_3)_2$	8.03	4.41, 4.52		2.6H: 7.85, 4H: 7.75

H-Tyr-*D*-Ala-Gly-Phe-Met-Pro-Leu-Trp-NH-Bzl□ TFA (**3**), 4.0 mM, only for the major isomer, δ:

AA	NH	α	β	misc.
Tyr^1		4.20	3.04, 3.13	2,6H: 7.11, 3,5H: 6.85
D-Ala^2	8.62	4.17	1.14	
Gly^3	8.47	3.78, 3.88		
Phe^4	7.73	4.60	3.04, 3.11	2,6H: 7.19, 3,5H: 7.26, 4H^a
Met^5	7.94	4.41	1.79	γ: 2.31, 2.37, CH_3: 2.04
Pro^6		4.23	0.78, 1.83	γ: 1.23, 1.50, δ: 2.97, 3.43
Leu^7	8.46	4.05	1.67	γ: 1.55, δ: 0.85, 0.93
Trp^8	7.14	4.61	3.23, 3.54	Ind2: 7.36, Ind4: 7.53, Ind5: 6.96, Ind6: 7.10, Ind7: 7.48
Bzl	7.42	4.17, 4.33		2.6H: 7.14, 3,5H: 7.09, 4H^a

^{a}: not observed. Ind# represents the corresponding resonances in indole ring of Trp.

Table S3. ^{1}H Resonance Assignments of bifunctional peptides in DMSO at 298K.

H-Tyr-*D*-Ala-Gly-Phe-Pro-Met-Leu-Trp-O-3,5-Bzl(CF_3)$_2$ TFA (**1**); Only one isomer was found; ^{1}H-NMR (DMSO-d_6) δ:				
AA	NH	α	β	misc.
Tyr[1]	8.05(3H, bs)	3.95-4.02(1H, m)	2.80-2.91(2H, m)	6.71(2H, d, J=8.5Hz: PhH), 7.03(2H, d, J=9.0Hz: PhH), 9.33(1H, bs: PhOH)
D-Ala[2]	8.52(1H, d, J=6.5Hz)	4.29-4.35(1H, m)	1.06(3H, d, J=7.0Hz)	-
Gly[3]	8.19(1H, t, J=5.0Hz)	3.62(1H, dd, J=5.0, 17.0Hz), 3.68 (1H, dd, J=5.5, 17.0Hz)	-	-
Phe[4]	7.47(1H, d, J=7.5Hz)	4.52-4.60(1H, m)	2.68-2.75(1H, m), 2.88-2.98(1H, m)	7.13-7.28(5H, m: PhH)
Met[5]	8.41(1H, d, J=8.0Hz)	4.62(1H, d, J=7.5Hz)	1.75-1.82(1H, m), 1.89-1.98(1H, m)	2.42-2.52(2H, m: γCH_2), 2.02(3H, s: δCH_3)
Pro[5]	-	4.28-4.38(1H, m)	1.67-1.75(1H, m), 1.88-1.98(1H, m)	1.73-1.80(1H, m: γCH_2), 1.82-1.90(1H, m: γCH_2), 3.50-3.60(2H, m: δCH_2)
Leu[6]	7.89(1H, d, J=8.0Hz)	4.28-4.33(1H, m)	1.35(2H, dd, J=7.0, 7.0Hz)	1.53-1.61(1H, m: γCH_2), 0.76(3H, d, J=6.5Hz: δCH_2), 0.79(3H, d, J=6.5Hz: δCH_2)
Trp[7]	8.40(1H, d, J=7.5Hz)	4.52-4.60(1H, m)	3.13(1H, dd, J=6.5, 15.0Hz), 3.20(1H, dd, J=5.5, 14.5Hz)	6.96(1H, dd, J=7.0, 7.0Hz: Ind5), 7.05(1H, dd, J=7.0, 7.0Hz: Ind6), 7.17(1H, s: Ind2), 7.32(1H, d, J=8.0Hz: Ind4), 7.46(1H, d, J=7.5Hz: Ind7), 10.88(1H, bs, IndNH)
3,5-Bzl(CF_3)$_2$	-	5.11(1H, d, J=13.5Hz:), 5.21(1H, d, J=13.5Hz:)	-	7.89(2H, s: PhH), 8.04(1H, s: PhH)

H-Tyr-*D*-Ala-Gly-Phe-Pro-Met-Leu-Trp-NH-3,5-Bzl$(CF_3)_2$ · TFA (**2**); Only one isomer was found; ^{1}H-NMR (DMSO-d_6) δ:				
AA	NH	α	β	misc.
Tyr[1]	8.04(3H, bs)	3.93-4.00(1H, m)	2.80-2.92(2H, m)	6.69(2H, d, J=7.2Hz: PhH), 7.01(2H, d, J=7.2Hz: PhH), 9.31(1H, bs: PhOH)
D-Ala[2]	8.50(1H, d, J=7.8Hz)	4.28-4.33(1H, m)	1.04(3H, d, J=6.6Hz)	-
Gly[3]	8.17(1H, t, J=6.0Hz)	3.60(1H, dd, J=5.4, 16.8Hz), 3.69 (1H, dd, J=5.4, 16.2Hz)	-	-
Phe[4]	7.93-8.00(1H, m)	4.50-4.55(1H, m)	2.72(1H, dd, J=9.6, 13.8Hz), 2.89-2.95(1H, m)	7.13-7.28(5H, m: PhH)
Met[5]	8.38(1H, d, J=7.2Hz)	4.59(1H, dd, J=7.8, 14.4Hz)	1.70-1.80(1H, m), 1.86-1.94(1H, m)	2.42-2.47 (2H, m: γCH_2), 2.02(3H, s: δCH_3)
Pro[5]	-	4.28-4.33(1H, m)	1.63-1.68(1H, m), 1.85-1.92(1H, m)	1.66-1.84(2H, m: γCH_2), 3.50-3.60(2H, m: δCH_2)
Leu[6]	7.95-8.02(1H, m)	4.21(1H, dd, J=8.0, 15.0Hz)	1.38(2H, dd, J=7.2, 7.2Hz)	1.53-1.61(1H, m: γCH_2), 0.76(3H, d, J=6.6Hz: δCH_2), 0.82(3H, d, J=6.6Hz: δCH_2)
Trp[7]	7.92(1H, d, J=7.8Hz)	4.50-4.55(1H, m)	3.01(1H, dd, J=7.8, 14.4Hz), 3.13(1H, dd, J=5.4, 14.4Hz)	6.94(1H, dd, J=7.8, 7.8Hz: Ind5), 7.04(1H, dd, J=8.4, 8.4Hz: Ind6), 7.08(1H, s: Ind2), 7.30(1H, d, J=8.4Hz: Ind4), 7.51(1H, d, J=7.8Hz: Ind7), 10.88(1H, bs, IndNH)
3,5-Bzl$(CF_3)_2$	8.57(1H, t, J=6.0Hz)	4.32(1H, dd, J=5.4, 16.2Hz), 4.44(1H, d, J=6.6, 15.6Hz)	-	7.88(2H, s: PhH), 7.94(1H, s: PhH)

H-Tyr-*D*-Ala-Gly-Phe-Pro-Met-Leu-Trp-NH-Bzl□ TFA (□); Only one isomer was found; ^{1} H-NMR (DMSO-d_6) δ:				
AA	NH	α	β	misc.
Tyr[1]	8.03(3H, bs)	3.93-4.02(1H, m)	2.85(1H, dd, J=7.5, 14.0Hz), 2.89(1H, dd, J=7.0, 14.0Hz)	6.70(2H, d, J=7.0Hz: PhH), 7.02(2H, d, J=8.5Hz: PhH), 9.31(1H, bs: PhOH)
D-Ala[2]	8.55(1H, d, J=7.5Hz)	4.33(1H, dd, J=6.5, 6.5Hz)	1.06(3H, d, J=6.0Hz)	-
Gly[3]	8.19(1H, t, J=6.0Hz)	3.62(1H, dd, J=6.0, 17.0Hz), 3.70(1H, dd, J=6.0, 16.5Hz)	-	-
Phe[4]	7.97(1H, dd, J=7.5Hz)	4.56(1H, dd, J=7.0, 14.0Hz)	2.74(1H, dd, J=9.5, 13.5Hz), 2.95(1H, dd, J=5.5, 15.0Hz)	7.05-7.28(5H, m)
Met[5]	8.41(1H, d, J=7.5Hz)	4.61(1H, dd, J=7.0, 14.5Hz)	1.75-1.82(1H, m), 1.89-1.97(1H, m)	2.45-2.52(2H, m: γCH_2), 2.02(3H, s: δCH_3)
Pro[5]	-	4.33(1H, dd, J=6.5, 6.5Hz)	1.70-1.80(2H, m)	1.82-1.88(1H, m: γCH_2), 1.90-1.99(1H, m: γCH_2), 3.49-3.55(1H, m: δCH_2), 3.55-3.62(1H, m: δCH_2)
Leu[6]	8.01(1H, d, J=7.5Hz)	4.19-4.25(1H, m)	1.41(2H, dd, J=7.0, 7.0Hz)	1.55-1.64(1H, m: γCH_2), 0.80(3H, d, J=6.5Hz: δCH_2), 0.86(3H, d, J=6.5Hz: δCH_2)
Trp[7]	7.83(1H, dd, J=8.0Hz)	4.56(1H, dd, J=7.0, 14.0Hz)	3.02(1H, dd, J=7.5, 15.0Hz), 3.15(1H, dd, J=6.0, 14.5Hz)	6.97(1H, dd, J=7.5, 7.5Hz: Ind5), 7.03-7.08(1H, m: Ind6), 7.09(1H, s: Ind2), 7.34(1H, d, J=8.0Hz: Ind4), 7.55(1H, d, J=8.0Hz: Ind7), 10.88(1H, bs, IndNH)
Bzl	8.33(1H, t, J=6.0Hz)	4.18-4.30(2H, m)	-	7.05-7.28(5H, m)

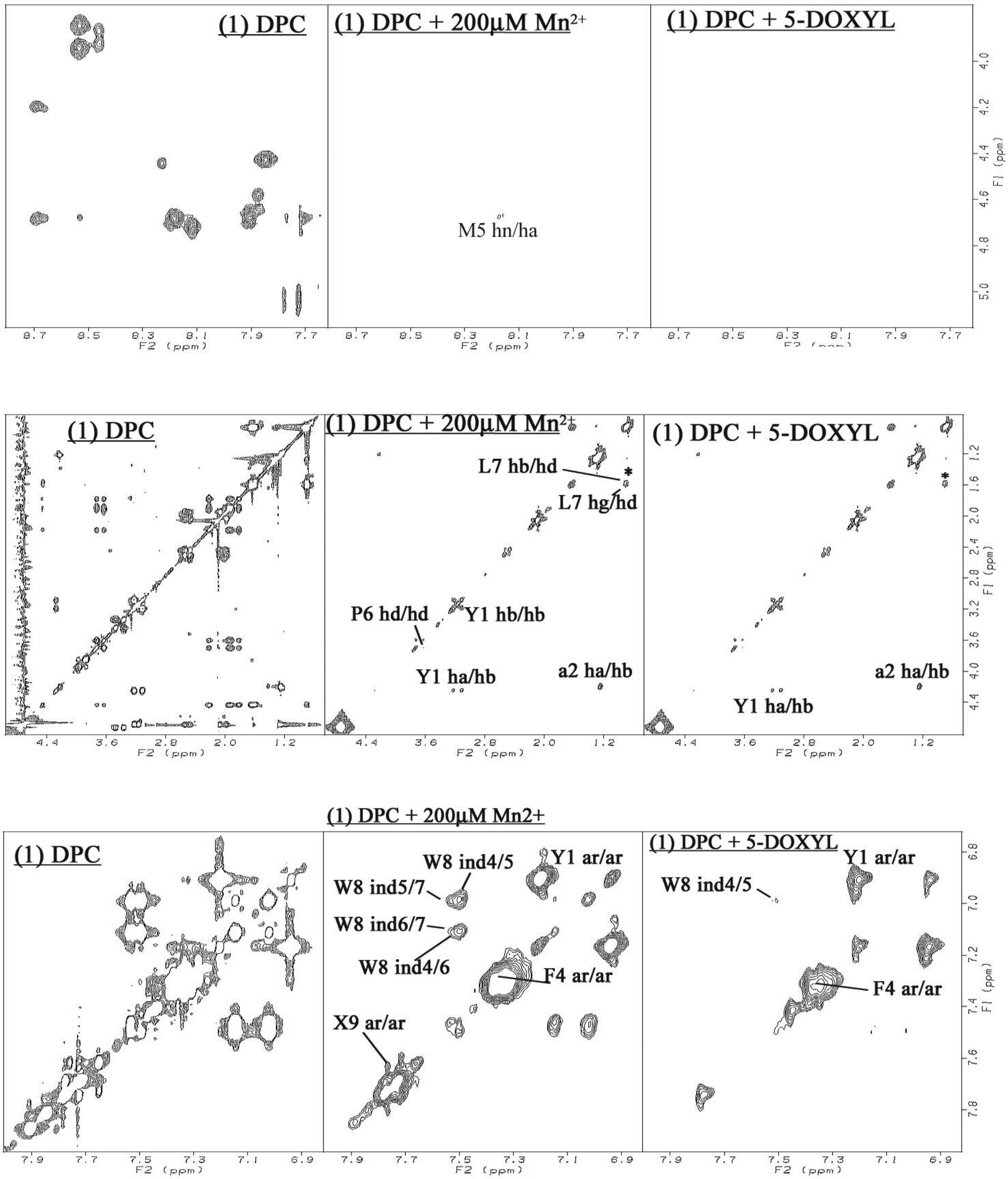

Figure S1. Effect of Radicals on TOCSY Spectra. **1** with DPC micelles (left column), with 200 μM Mn^{2+} (middle) and 5-DOXYL stearic acid (right). Preserved resonances (labeled) are in a phase not missed by the phase-specific radical probe (Mn^{2+} or DOXYL). X9 represents the cross-peaks derived from the corresponding aromatic protons of benzyl moiety. The resonances with asterisk (*) are DPC or 5-DOXYL derived ones. Spectra were compared from the same noise level.

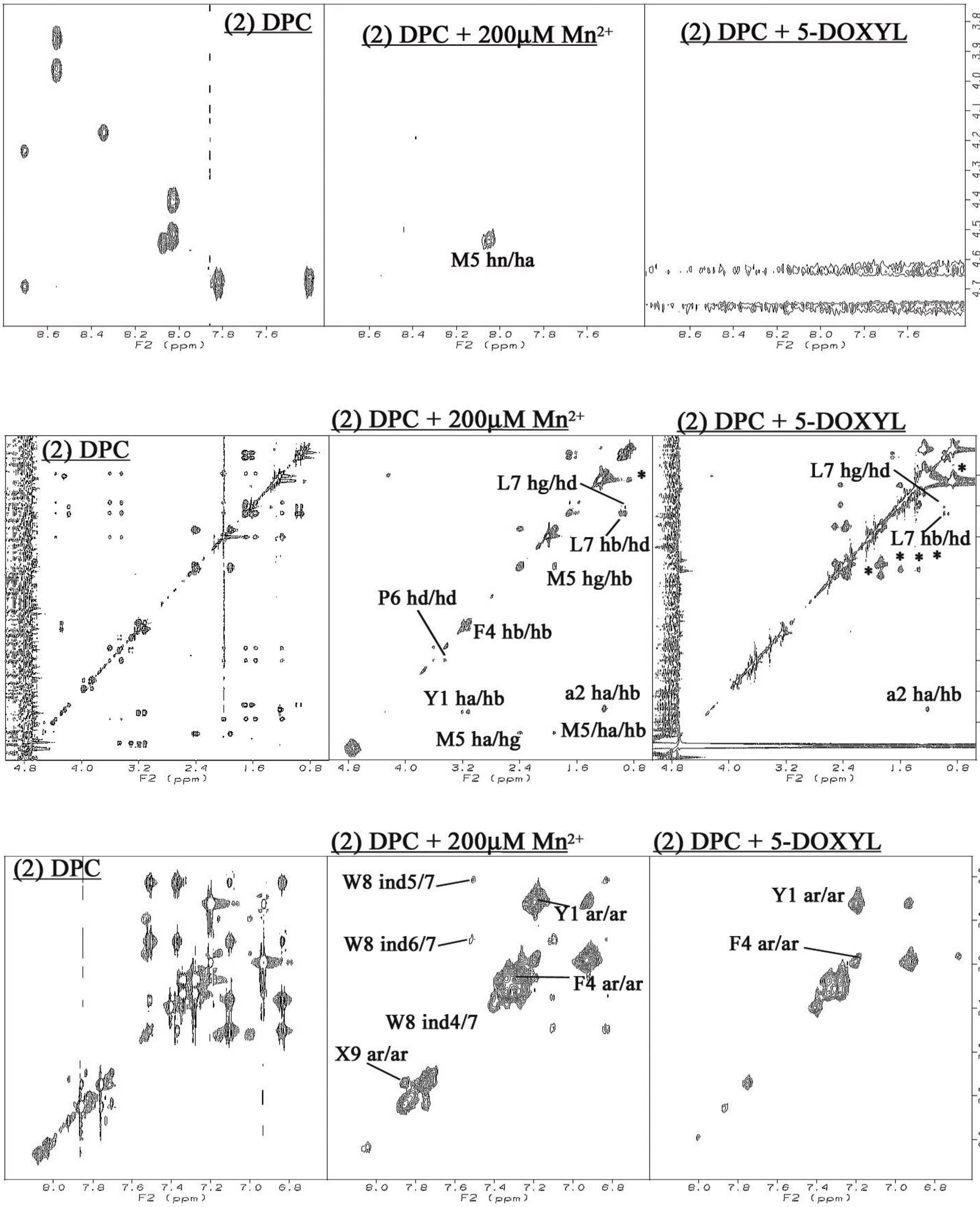

Figure S2. Effect of Radicals on TOCSY Spectra. **2** with DPC micelles (left column), with 200 □M Mn^{2+} (middle) and 5-DOXYL stearic acid (right). Preserved resonances (labeled) are in a phase not be missed by the phase-specific radical probe (Mn^{2+} or DOXYL). X9 represents the cross-peaks derived from the corresponding aromatic protons of benzyl moiety. The resonances with asterisk (*) are DPC or 5-DOXYL derived ones. Spectra were compared from the same noise level.

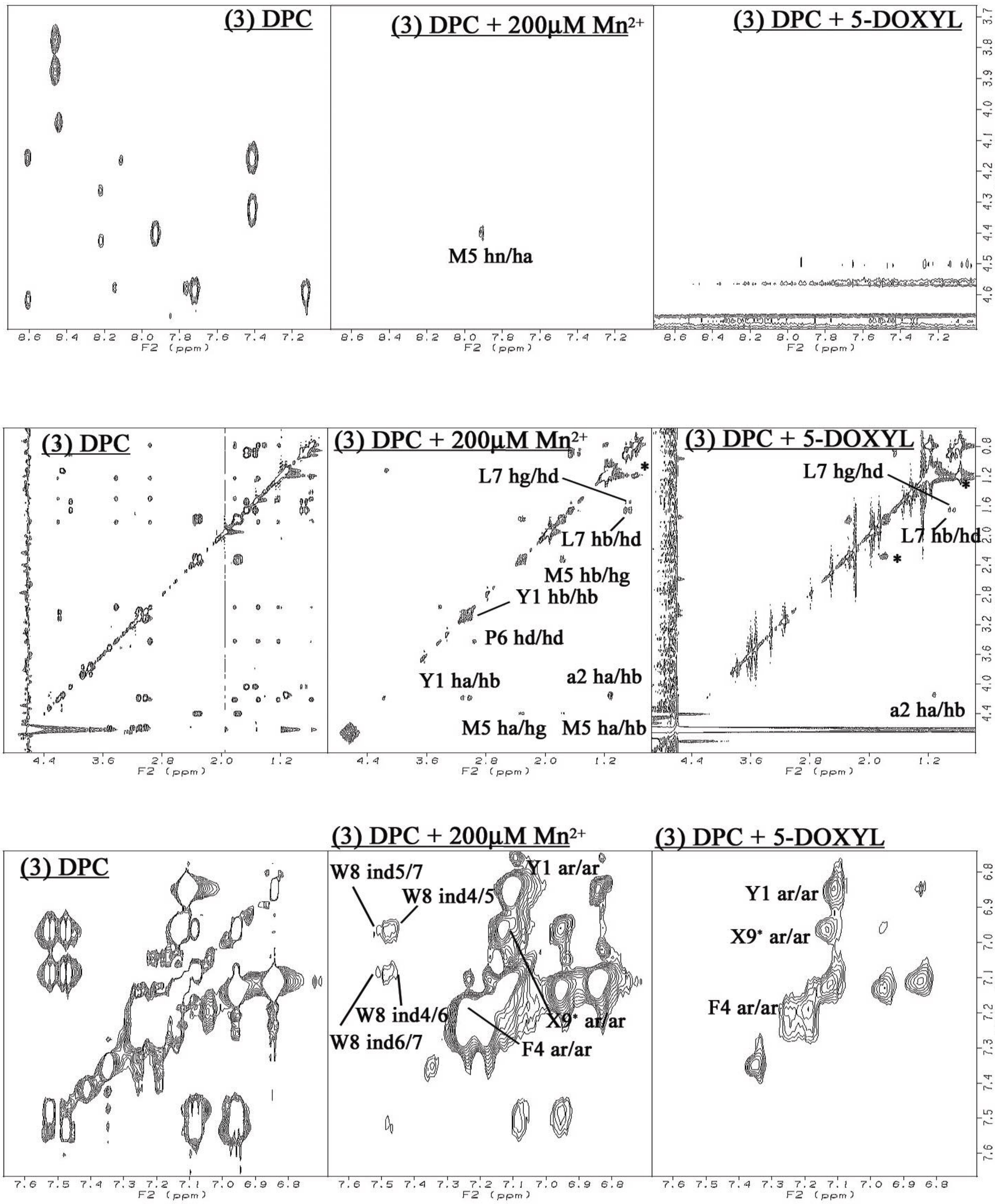

Figure S3. Effect of Radicals on TOCSY Spectra. **3** with DPC micelles (left column), with 200 □ M Mn^{2+} (middle) and 5-DOXYL stearic acid (right). Preserved resonances (labeled) are in a phase not be affected by the phase-specific radical probe (Mn^{2+} or DOXYL). X9 represents the cross-peaks derived from the corresponding aromatic protons of benzyl moiety. The resonances with asterisk (*) are DPC or 5-DOXYL derived ones. Spectra were compared from the same noise level.

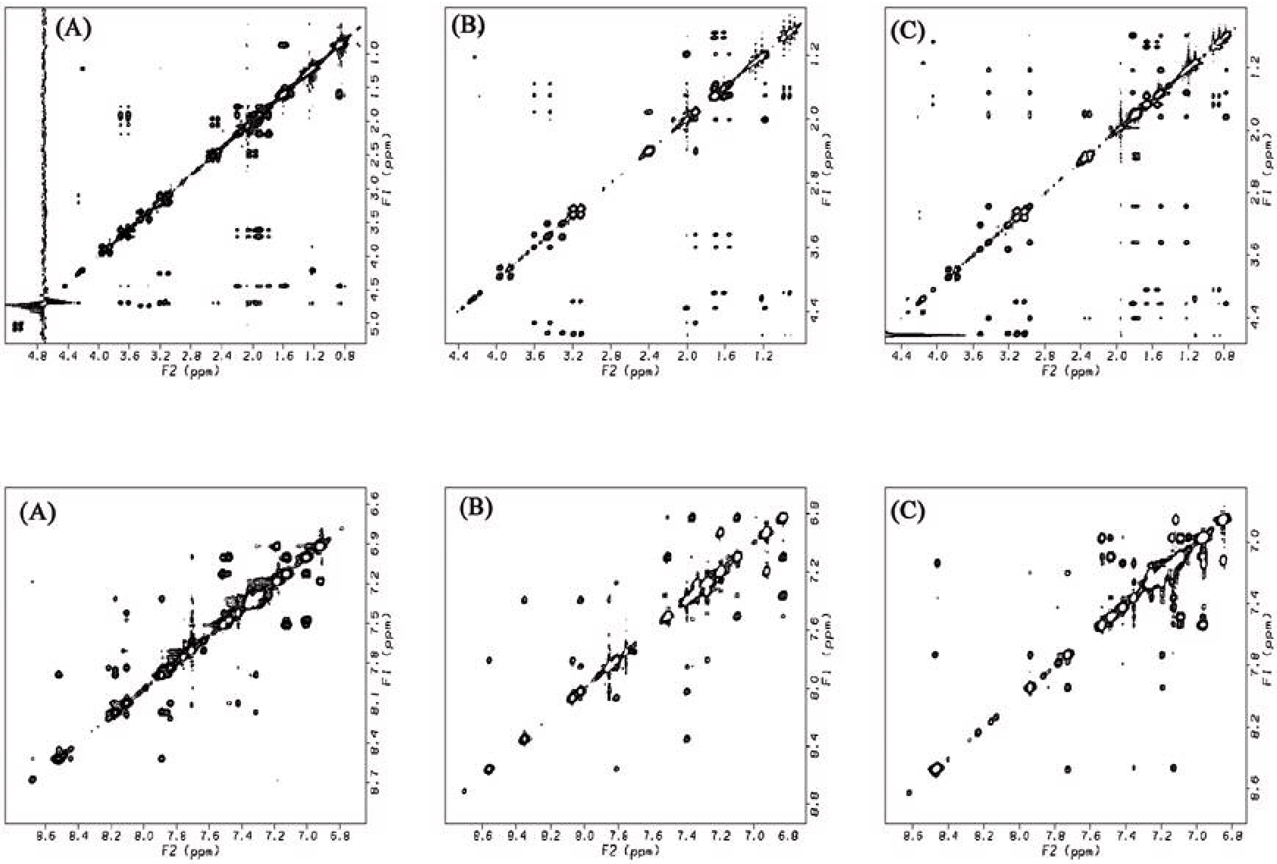

Figure S4. Side-chain region (upper row) and H^N-H^N reagion (bottom) of the NOESY spectrum of (A) **1**, (B) **2** and (C) **3** in DPC micelles.

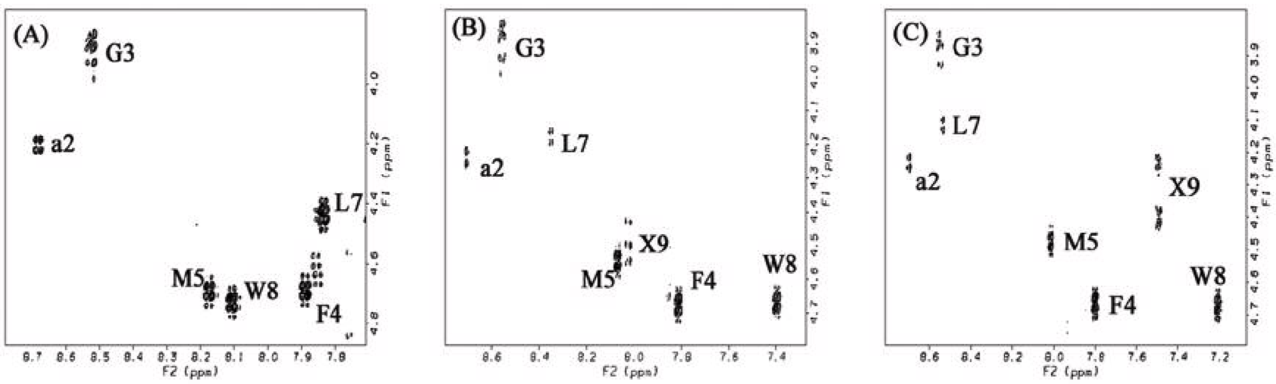

Figure S5. Fingerprint (H^N-H^α) region of the DQF-COSY spectrum of (A) **1**, (B) **2** and (C) **3** in DPC micelles. Intraresidue H^N-H^α cross-peaks are labeled with residue number. X9 represents the cross-peaks derived from the corresponding *C*-terminal H^N and benzyl protons.

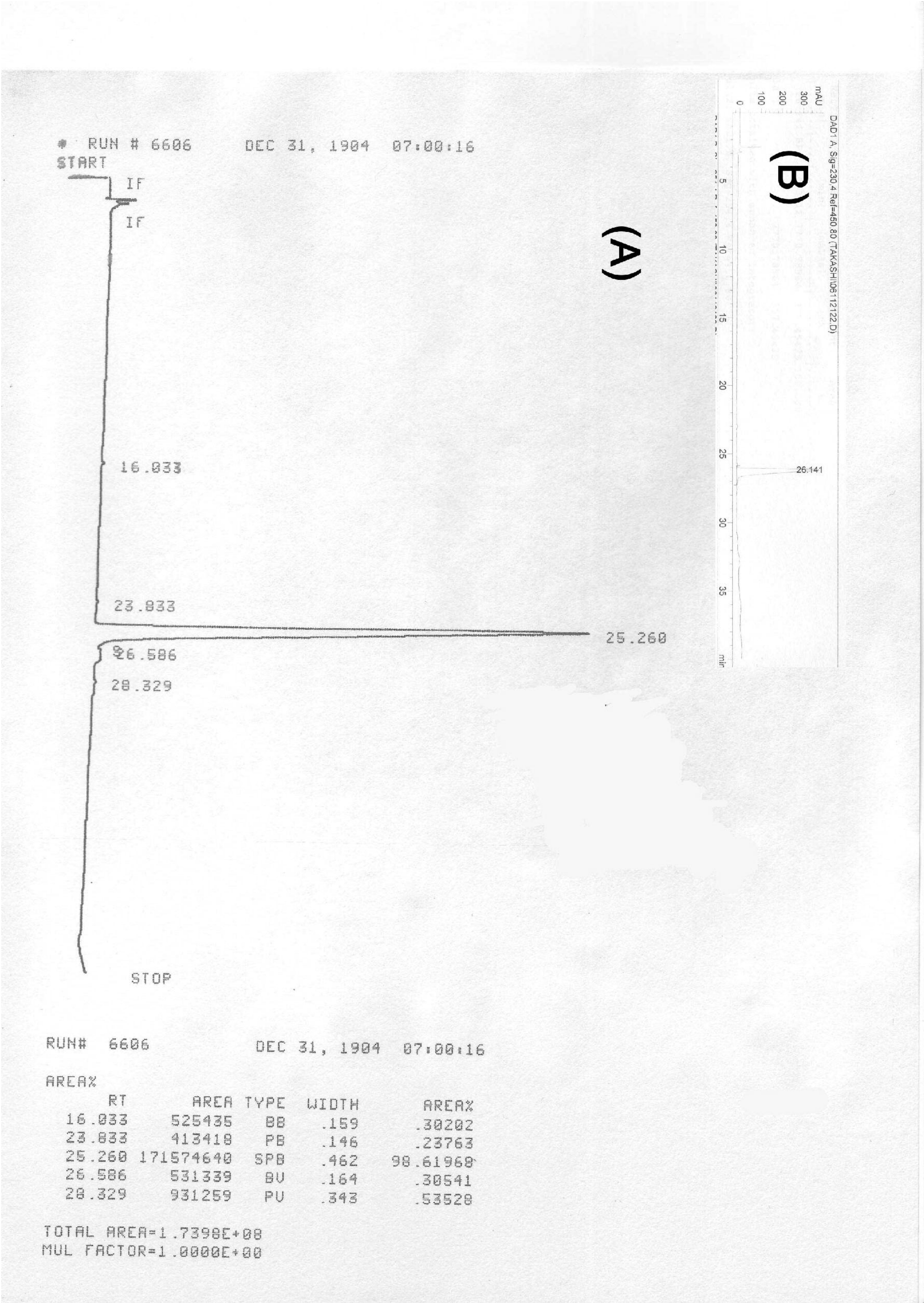

Chart S1. HPLC trace of **1**: (A) 10-90% of acetonitrile containing 0.1% TFA within 40 min and up to 95% within an additional 5 min, 1 mL/min, 230 nm, Waters NOVA-Pak C-18 column (3.9 x 150 mm, 5 µm, 60 Å). (B) 30-70% acetonitrile containing 0.1% TFA within 40 min and up to 95% within an additional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

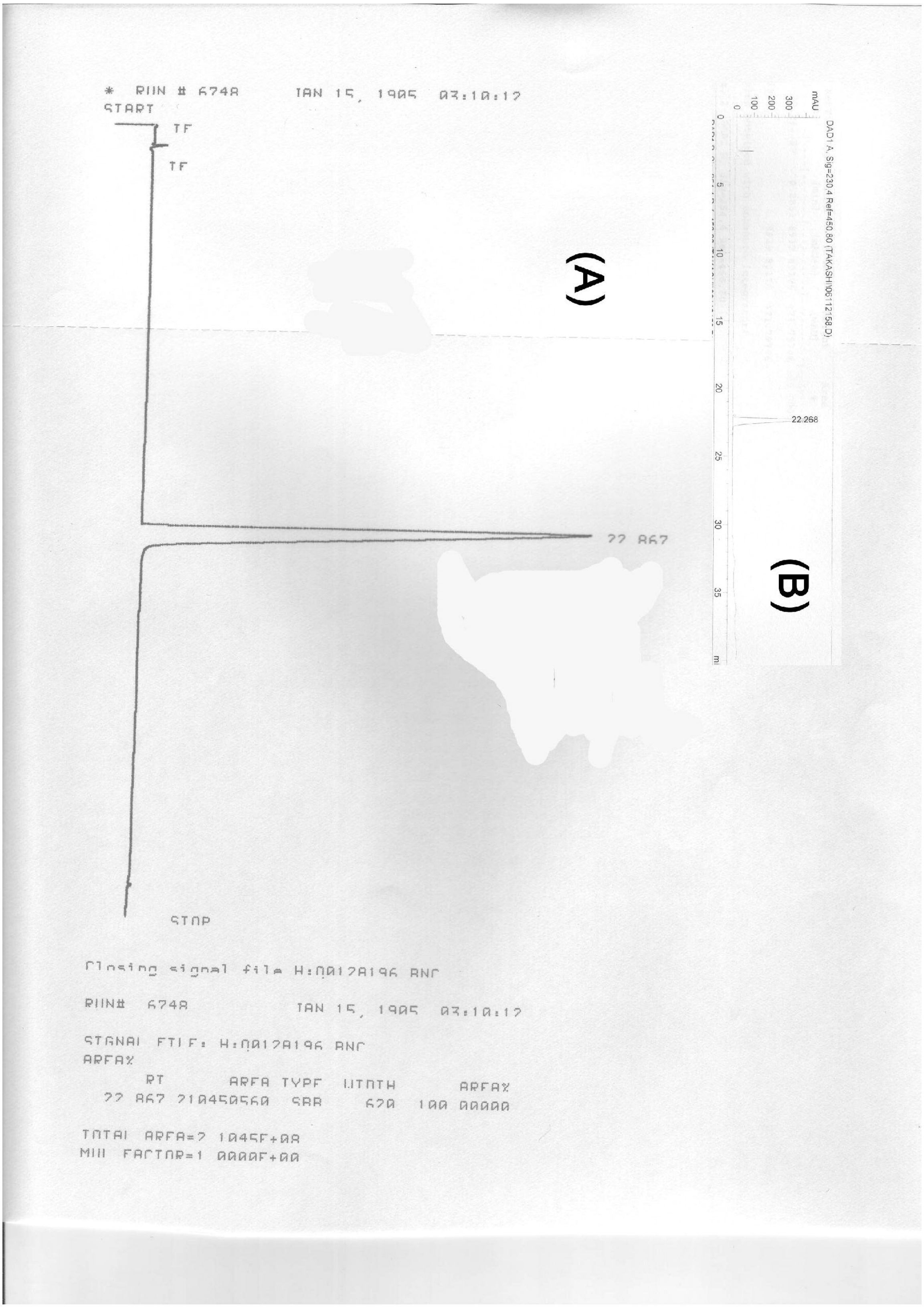

Chart S2. HPLC trace of **2**: (A) 10-90% of acetonitrile containing 0.1% TFA within 40 min and up to 95% within an additional 5 min, 1 mL/min, 230 nm, Waters NOVA-Pak C-18 column (3.9 x 150 mm, 5 µm, 60 Å). (B) 30-70% acetonitrile containing 0.1% TFA within 40 min and up to 95% within an additional 5 min, 1 mL/min, 230 nm, Vydac 218TP104 C-18 column (4.6 x 250 mm, 10 µm, 300 Å).

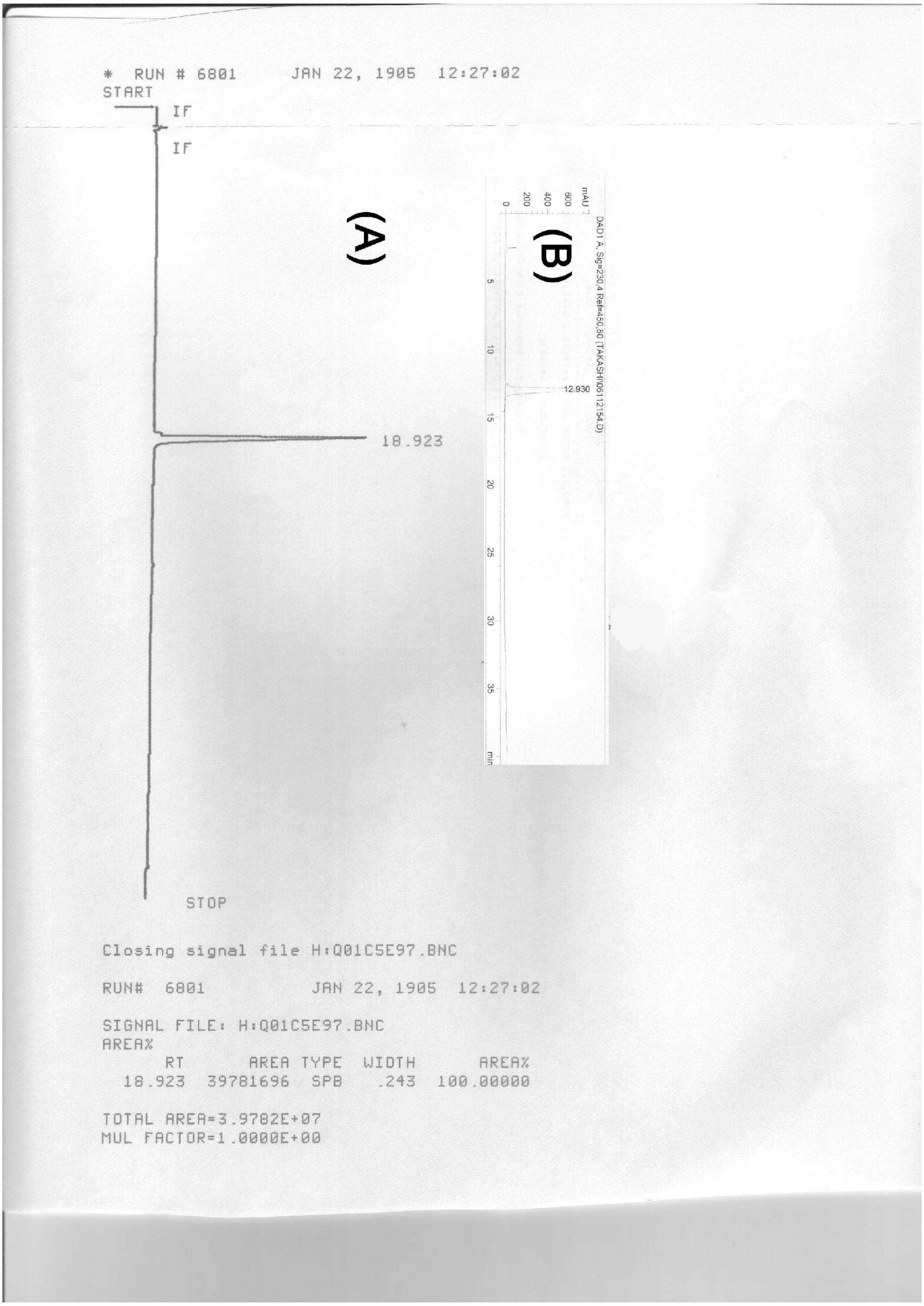

Chart S3. HPLC trace of **3**: (A) 10-90% of acetonitrile containing 0.1% TFA within 40 min and up to 95% within an additional 5 min, 1 mL/min, 230 nm, Waters NOVA-Pak C-18 column (3.9 x 150 mm, 5 µm, 60 Å). (B) 30-70% acetonitrile containing 0.1% TFA within 40 min and up to 95% within an additional 5 min, 1 mL/min, 230 nm, Vydac 218TP104 C-18 column (4.6 x 250 mm, 10 µm, 300 Å).