Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Supplementary Information

Design and Solid Phase Synthesis of New DOTA Conjugated (+)-Biotin Dimers Planned to Develop Molecular Weight-Tuned Avidin Oligomers

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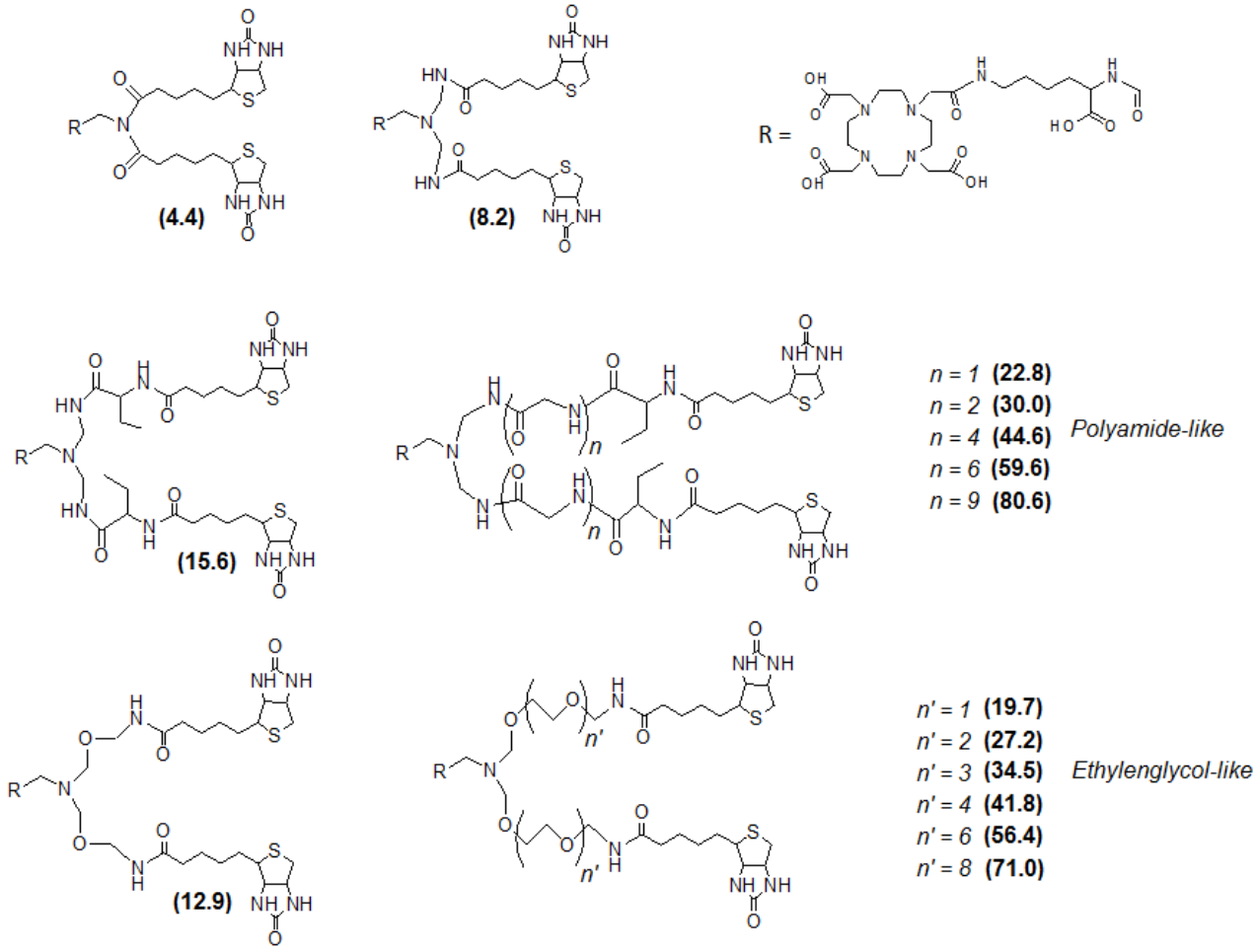


Figure 1S. Structures of bis-biotins designed for the molecular modeling study. In brackets is reported the linker arm length (Å) between the two amide C=O of the biotin residues, calculated on the fully extended planar conformation.

Table 1S. Potential energy data for type 1 complex.

	150 K		300 K		450 K	
Entry	**Ep** (Kcal/mol)	**sd**	**Ep** (Kcal/mol)	**sd**	**Ep** (Kcal/mol)	**sd**
bis-biotins with non-PEG spacers						
1	-6734	24	-5180	43	-3481	65
2	-6792	23	-5231	43	-3529	69
3	-6766	24	-5209	45	-3504	68
4	-6794	25	-5208	46	-3502	67
5	-6766	25	-5190	43	-3509	67
6	-6781	24	-5177	43	-3427	68
7	-6731	23	-5145	46	-3393	71
8	-6723	22	-5111	45	-3354	72
bis-biotins with PEG spacers						
1	-6730	24	-5180	41	-3506	64
2	-6775	29	-5215	41	-3549	72
9	-6795	23	-5229	45	-3538	69
10	-6773	24	-5224	41	-3528	67
11	-6810	22	-5244	45	-3521	77
12	-6795	26	-5211	41	-3519	68
13	-6772	24	-5185	47	-3468	63
14	-6744	23	-5160	44	-3439	67
15	-6744	25	-5141	44	-3422	73

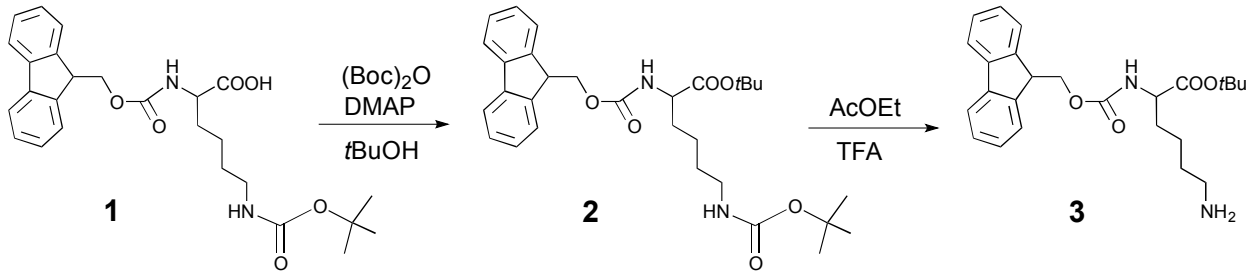
Type 1 Complex: potential energy average from 50 conformations recorded at a constant temperature (Ep) and standard deviation (sd) recorded during dynamic simulations. The structures 1 and 2 are the same in both series.

Table 2S. Potential energy data for type 2 complex.

	150 K		300 K		450 K	
Entry	**Ep** (Kcal/mol)	**sd**	**Ep** (Kcal/mol)	**sd**	**Ep** (Kcal/mol)	**sd**
bis-biotins with non-PEG spacers						
1	-6719	21	-5162	43	-3455	76
2	-6751	26	-5189	45	-3502	67
3	-6754	24	-5178	44	-3473	68
4	-6743	24	-5173	46	-3494	68
5	-6728	25	-5140	44	-3406	69
6	-6692	24	-5105	49	-3373	67
7	-6711	23	-5103	43	-3363	77
8	-6663	28	-5070	44	-3322	72
bis-biotins with PEG spacers						
1	-6719	27	-5175	45	-3497	64
2	-6762	22	-5207	43	-3508	67
9	-6786	26	-5234	41	-3535	64
10	-6767	24	-5194	44	-3494	70
11	-6755	22	-5180	41	-3509	65
12	-6730	23	-5165	42	-3439	66
13	-6734	22	-5158	44	-3451	72
14	-6673	25	-5073	45	-3344	65
15	-6637	24	-5026	46	-3292	69

Type 2 complex: potential energy average from 50 conformations recorded at a constant temperature (Ep) and standard deviation (sd) recorded during dynamic simulations. The structures 1 and 2 are the same in both series

Scheme 1S. Synthetic route for compound **3**.

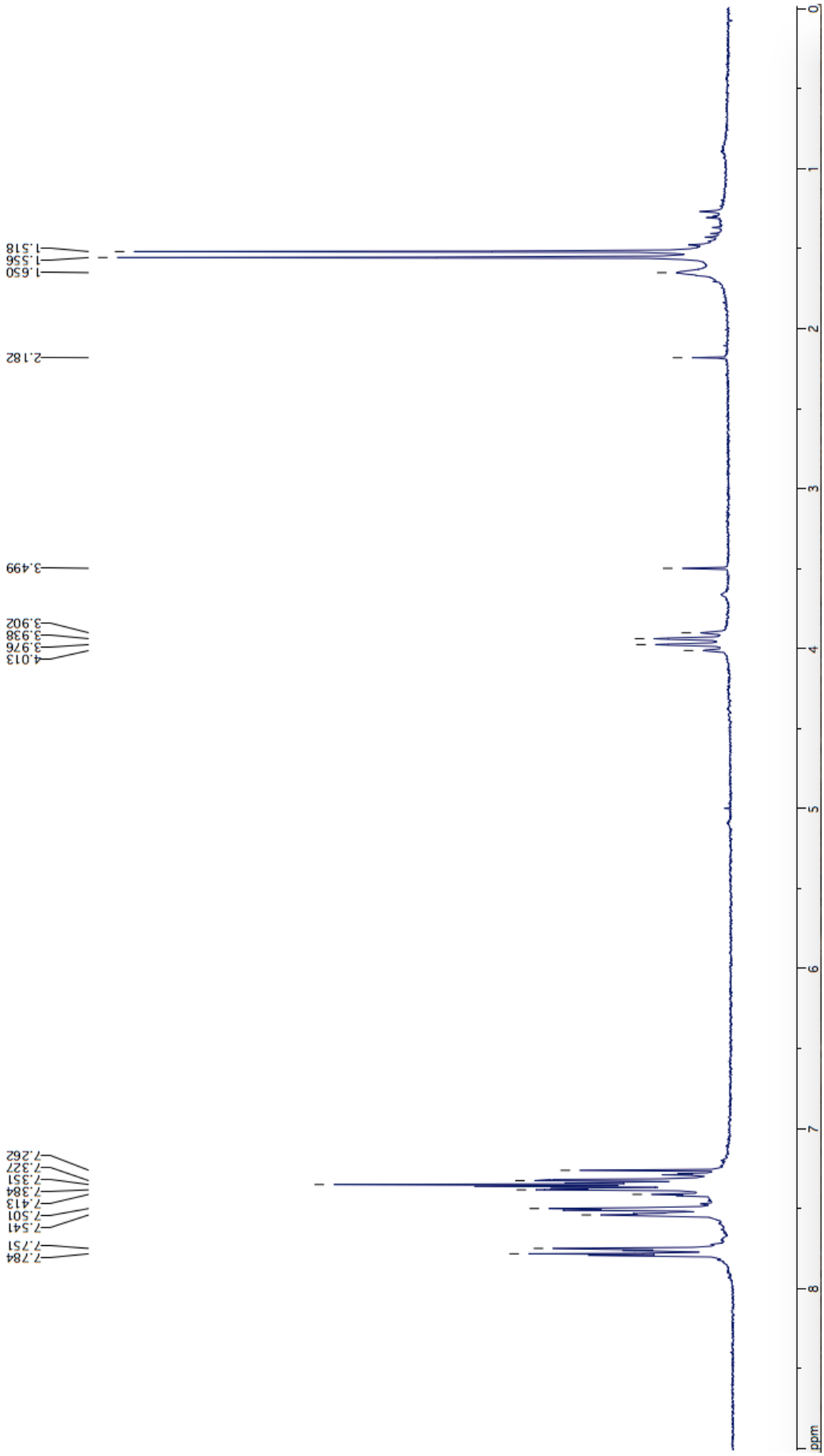


Synthesis of *tert*-butyl 2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-6-aminohexanoate (Fmoc-Lys-O*t*Bu, 3). 2.0 g of Fmoc-L-Lys(Boc)-OH (**1**) were added with 2.33 g of di-terbuthyl dicarbonate, Boc_2O, and 156.5 mg of 4-dimethylaminopiridine, DMAP, in 30 mL of anhydrous *tert*-buthanol. The reaction was stirred for 30 min at 35 °C and for 15h at room temperature. At the end of the reaction the product, **2**, was checked by TLC (AcOEt/exane 2:1), $R_f = 0.75$.

The solvent was removed under vacuum and the solid residue was dissolved 30 mL of ethyl acetate and 5 mL of water; the organic layer was washed with a saturated solution of $NaHCO_3$ (20 mL for 3 times), and with water (20 mL for 3 times). The organic phase was dried with anhydrous Na_2SO_4, the solvent was removed under vacuum and the crude product was purified by direct-phase LC on silica gel using AcOEt/exane 30:70.

1.70 g of the purified product **2** were dissolved in 56 mL of a solution containing 28% of TFA in AcOEt. The solution was stirred at 45 °C for 1h. After, another 10 mL of TFA were added. The reaction was stirred for 2h and at the end TFA was removed under nitrogen flow. The crude compound **3**, was solubilized in 10 mL of CH_3CN/H_2O 2:3 and purified by solid phase extraction on reverse-phase C18 silica gel, obtaining the pure compound Fmoc-Lys-O*t*Bu (**3**) (311 mg, 57% yield). ^1H NMR (298 K), δ (DMSO, 200 MHz): 7.78-7.75 (m, 2H), 7.54-7.50 (m, 2H) 7.41-7.26 (m, 4H), 4.01-3.90 (m, 2H), 1.65 (m, 2H), 1.56-1.52 (m, 15H). ESI-MS: calculated *m/z* 425.5 $[M+H]^+$; found 425.5.

Figure 2S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7.501
7.541
7.751
7.784
ppm

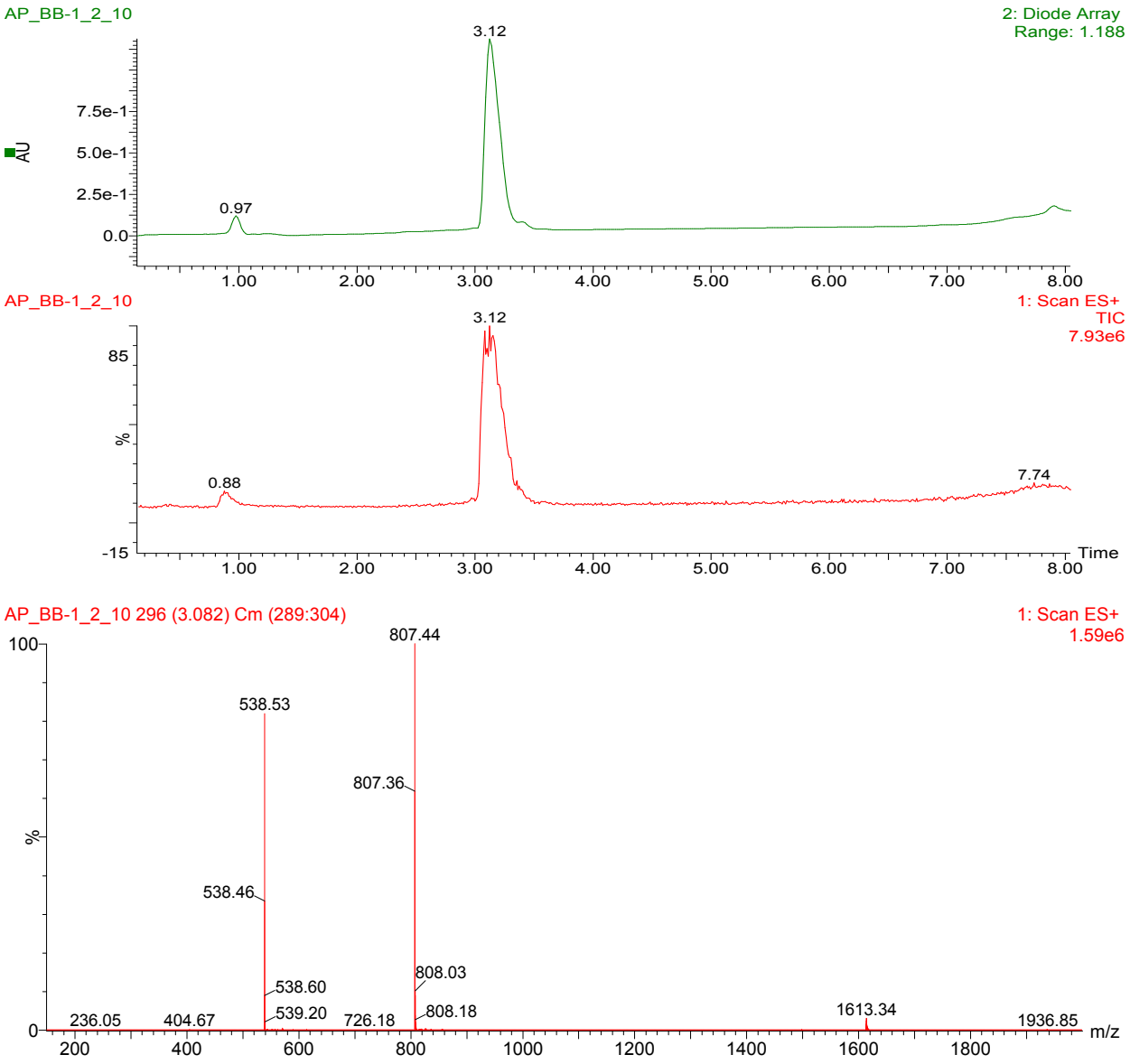

Figure 3S. HPLC-MS analysis of **6**.

Method: 15% to 50% of eluent B in 5 min; flow 0.6 mL/min. R*t*= 3.12 min

HPLC purity >98.8%

ESI-MS analysis - *m/z:* $[M+H]^+$ 1613.34; $[M+2H]^{2+}$ 807.44; $[M+3H]^{3+}$ 583.53

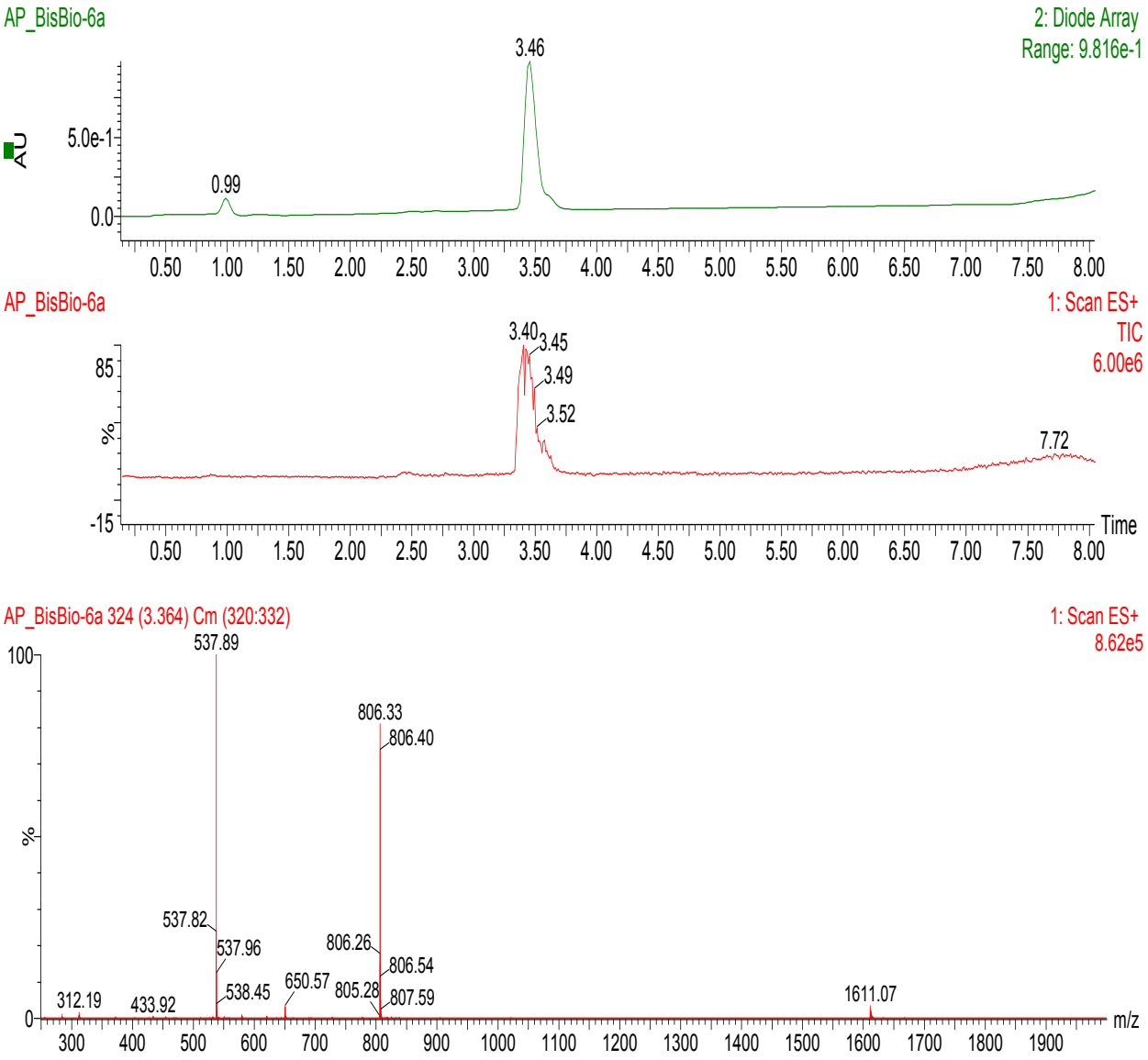

Figure 4S. HPLC-MS analysis of **9**.

Method: 15% to 50% of eluent B in 5 min; flow 0.6 mL/min. R*t*= 3.46 min

HPLC purity >98.5%

ESI-MS analysis - *m/z:* $[M+H]^+$ 1611.07; $[M+2H]^{2+}$ 806.33; $[M+3H]^{3+}$ 537.89

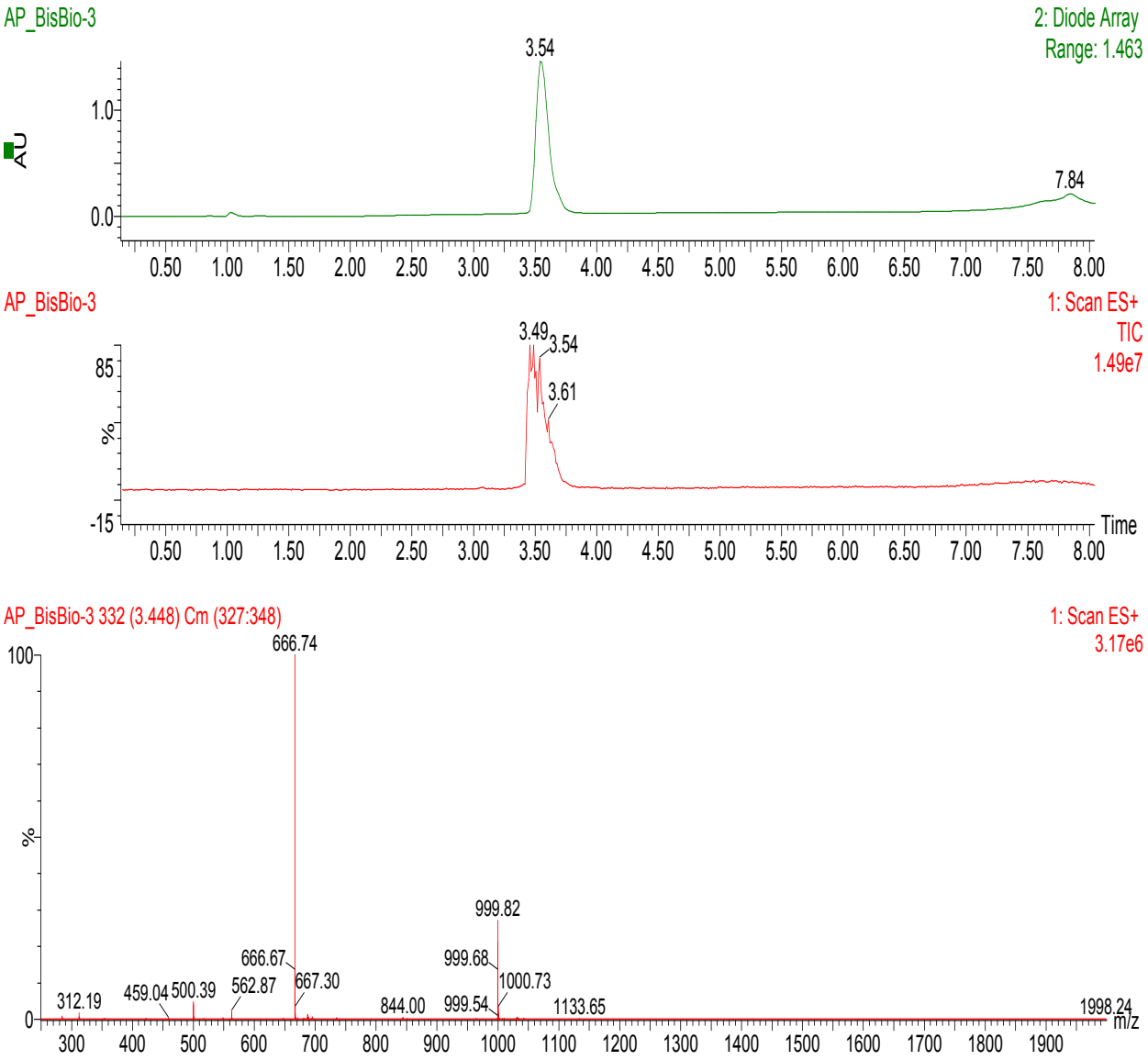

Figure 5S. HPLC-MS analysis of **11**.

method: 20% to 60% of eluent B in 5 min; flow 0.6 mL/min. R*t* = 3.54 min

HPLC purity >99%

ESI-MS analysis - *m/z:* $[M+H]^+$ 1998.24; $[M+2H]^{2+}$ 999.82; $[M+3H]^{3+}$ 666.74

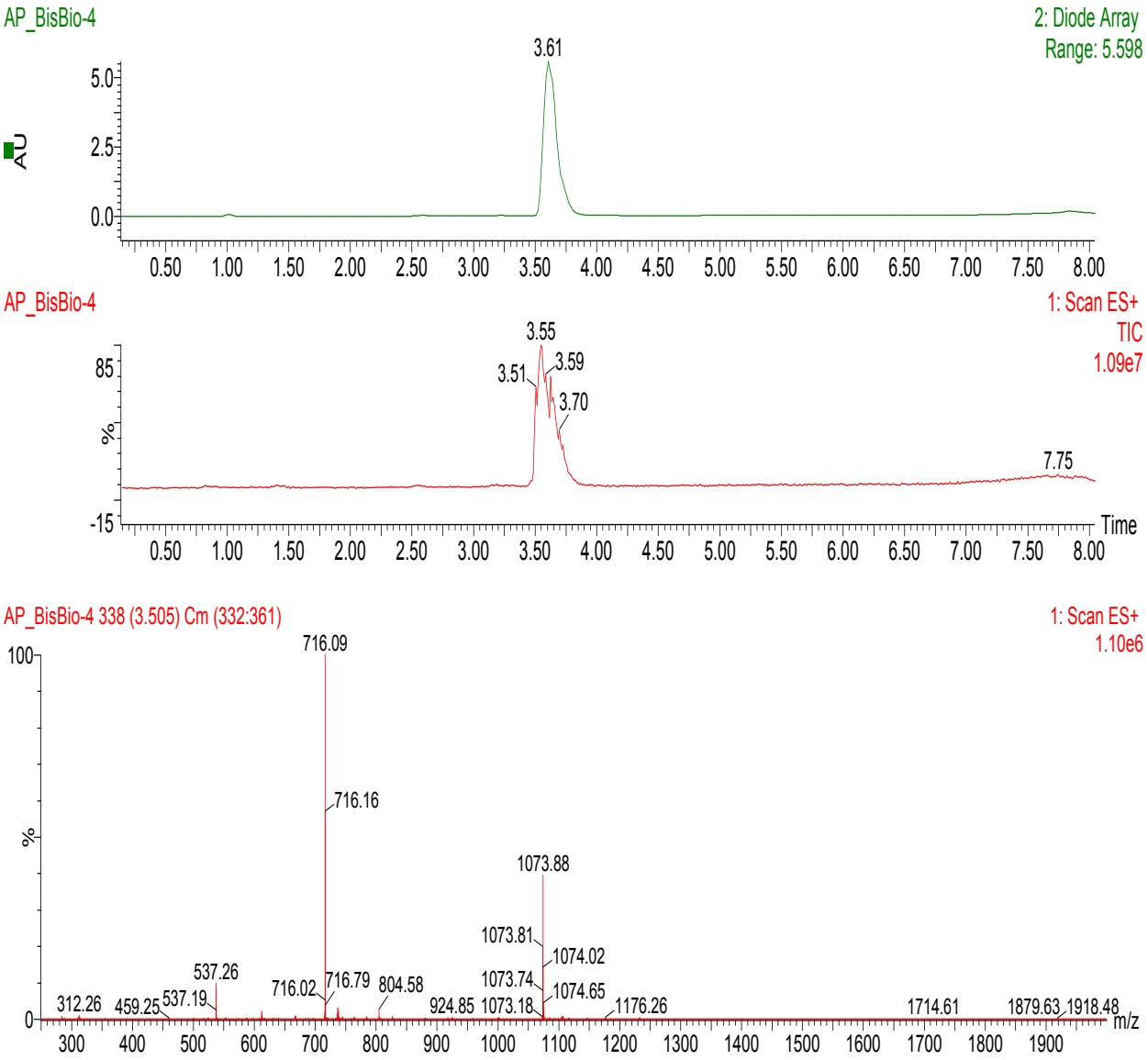

Figure 6S. HPLC-MS analysis of **14**.

method: 20% to 60% of eluent B in 5 min; flow 0.6 mL/min. R*t* = 3.61 min

HPLC purity >99%

ESI-MS analysis - *m/z:* $[M+2H]^{2+}$ 1073.88; $[M+3H]^{3+}$ 716.09

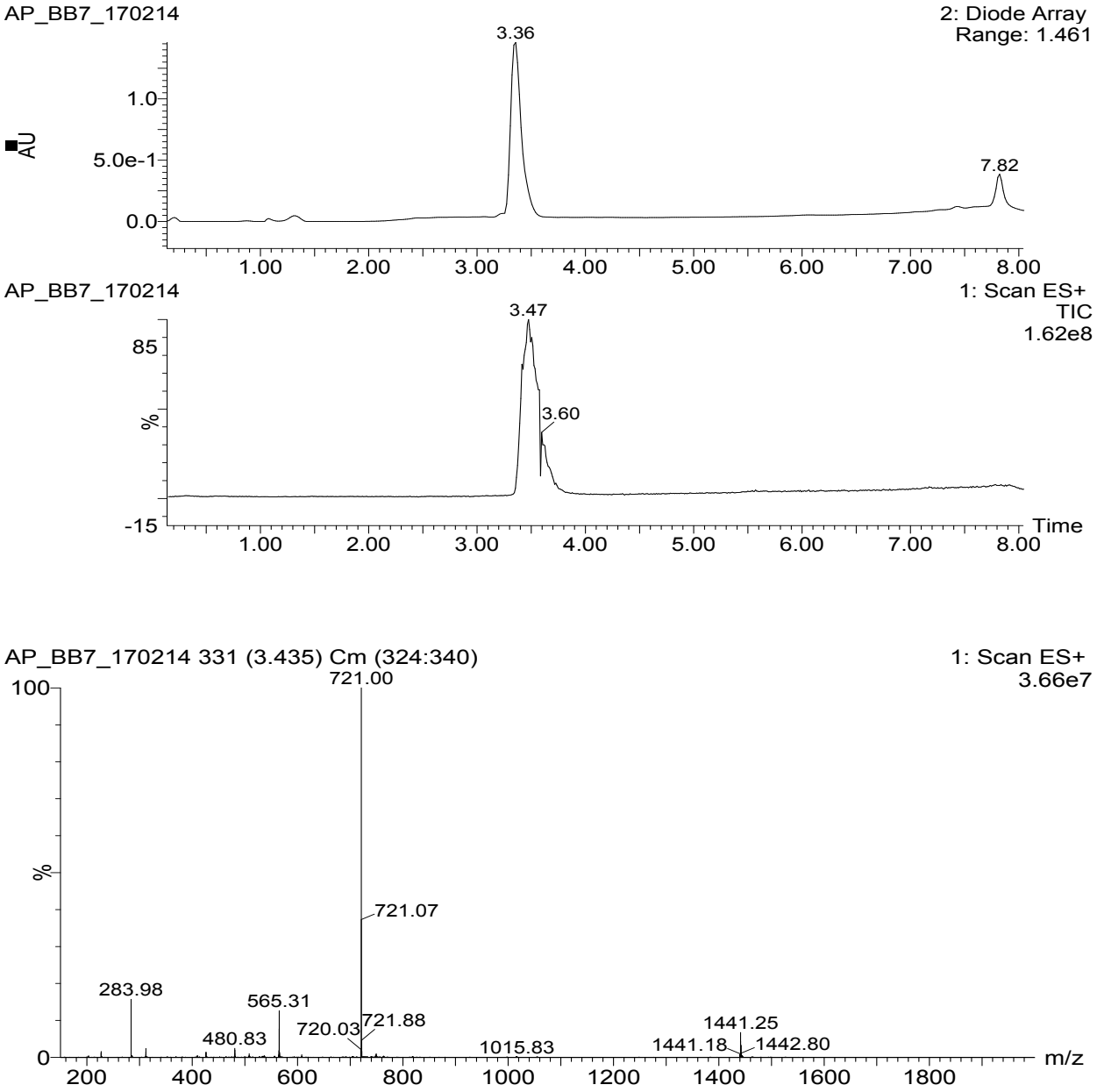

Figure 7S. HPLC-MS analysis of **16**.

method: 20% to 50% of eluent B in 5 min; flow 0.6 mL/min. R*t* = 3.36 min

HPLC purity 99%

ESI-MS analysis - *m/z:* $[M+H]^+$ 1441.25; $[M+2H]^+$ 721.00

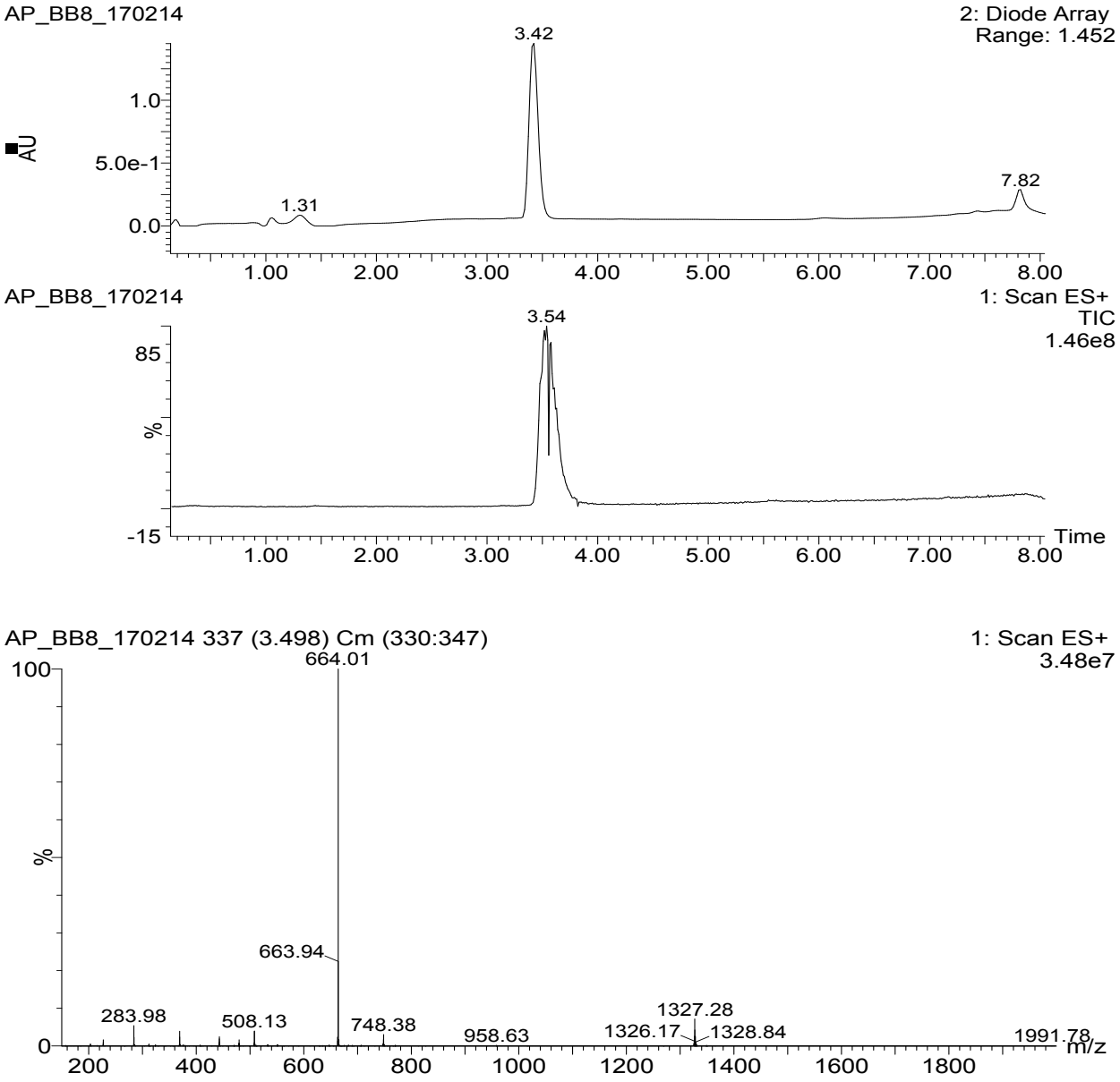

Figure 8S. HPLC-MS analysis of **18**.

method: 20% to 50% of eluent B in 5 min; flow 0.6 mL/min. R*t* = 3.42 min

HPLC purity 98%

ESI-MS analysis - *m/z:* $[M+H]^+$ 1327.28; $[M+2H]^+$ 664.01

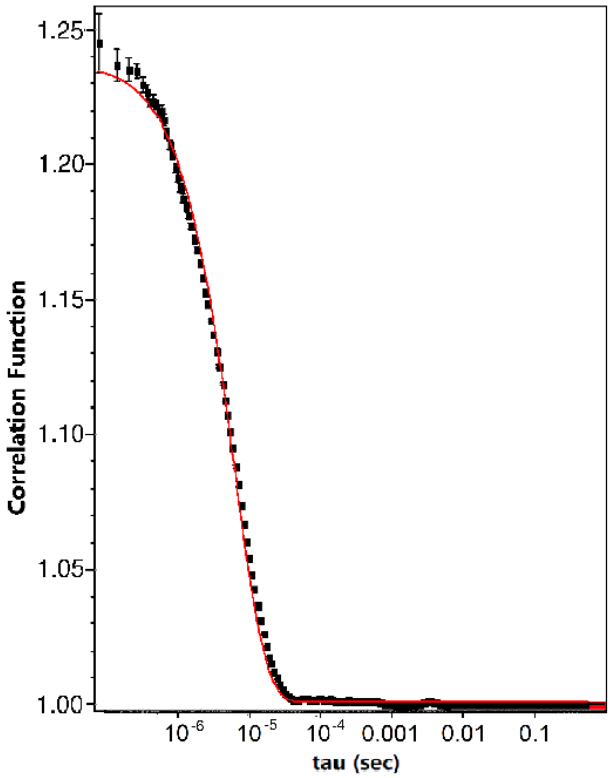

Figure 9S. Correlation function of native avidin, experimental data are showed as black dots, while the result of interpolation is showed as red line.

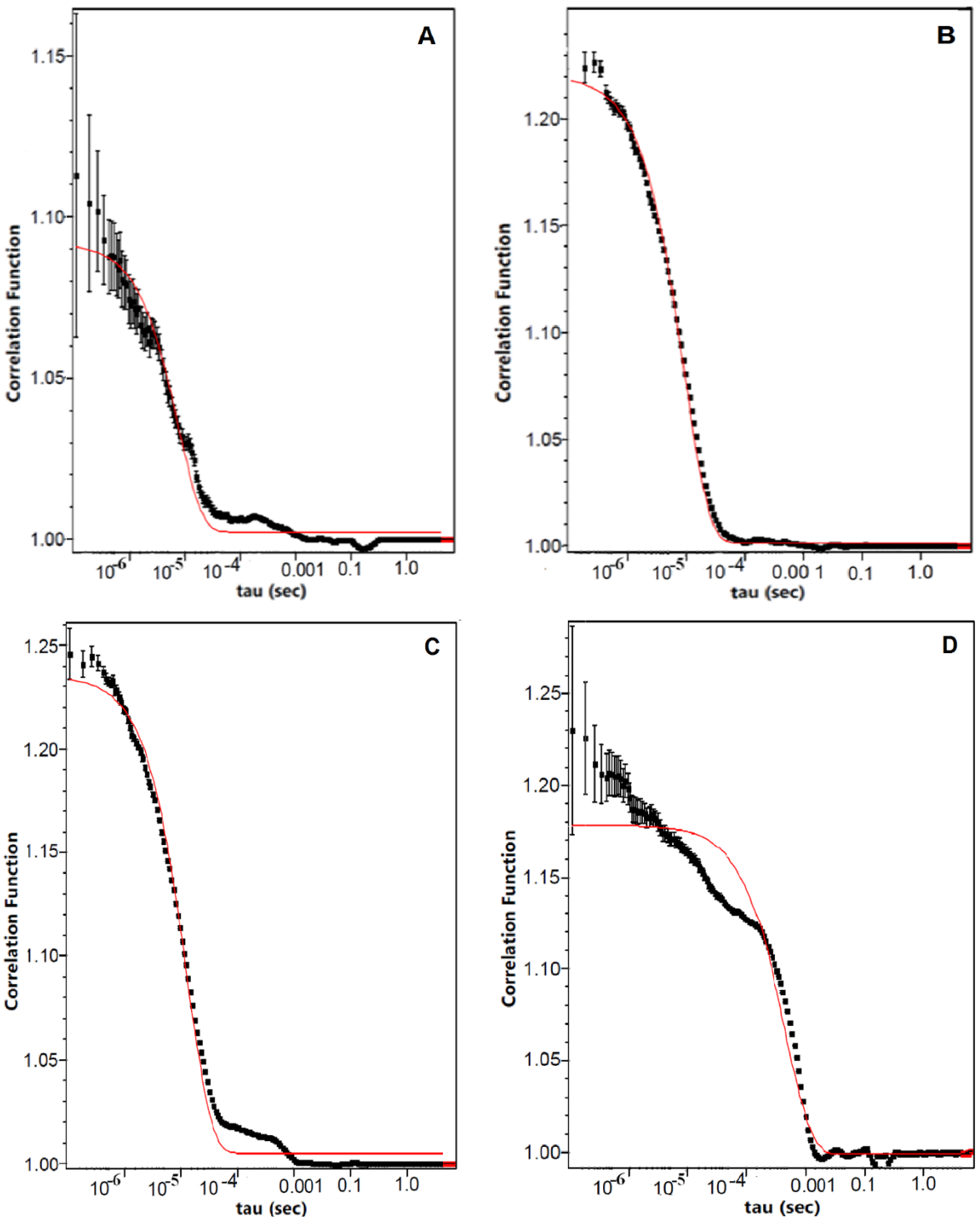

Figure 10S. Correlation function for Av/**16** complexes, experimental data are showed as black dots, while the result of interpolation is showed as red line. A) ^{t}R 10.76 min; B) ^{t}R 9.44 min; C) ^{t}R 8.54 min; D) ^{t}R 8.11 min.

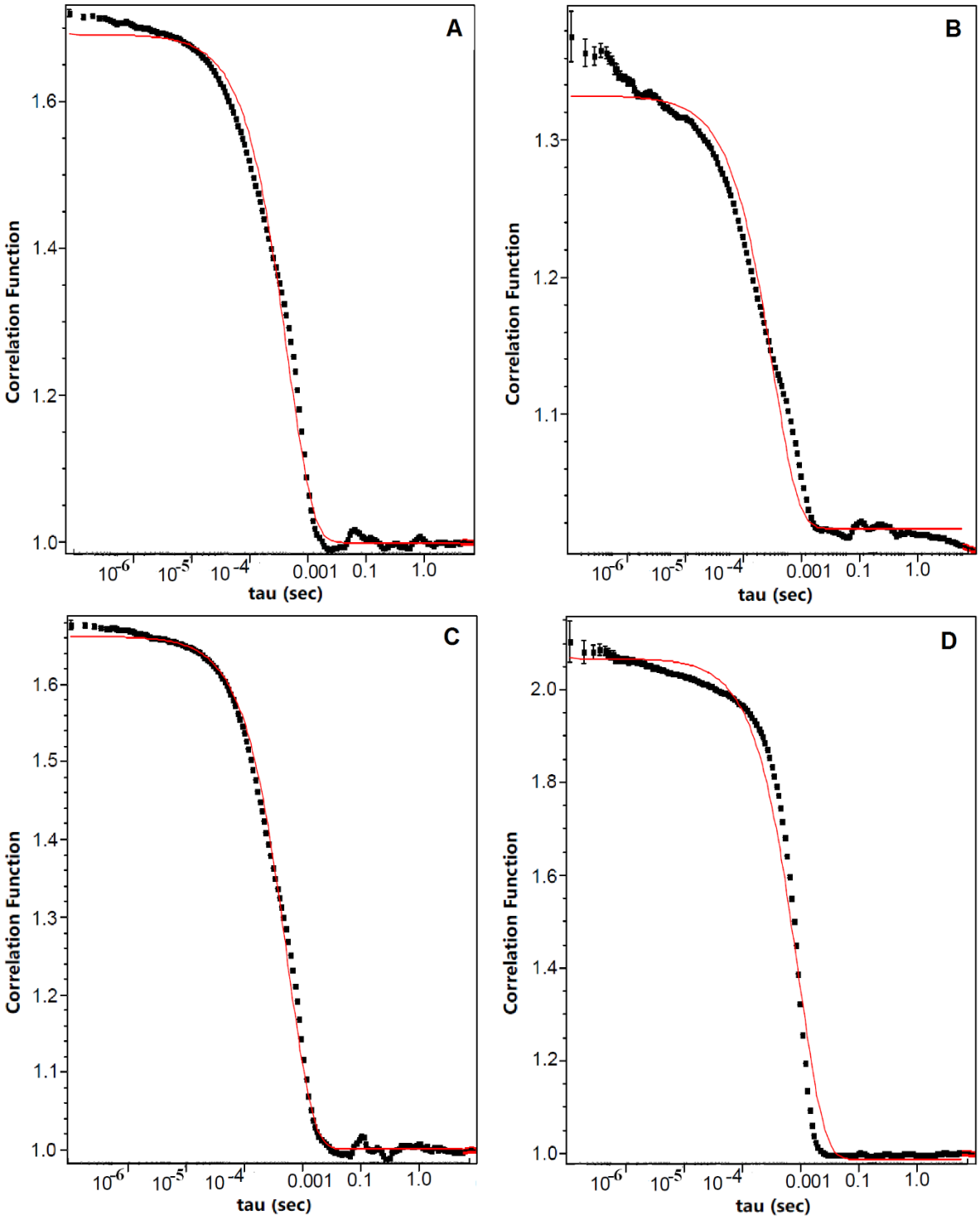

Figure 11S. Correlation functions of Av/**18** polymer at different incubation time: A) 0 min.; B) 45 min; C) 150 min.; D) 360 min.