Discovery of novel μ-opioid receptor inverse agonist from a combinatorial library of tetrapeptides through structure-based virtual screening

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COMPOUNDS CHARACTERIZATION:

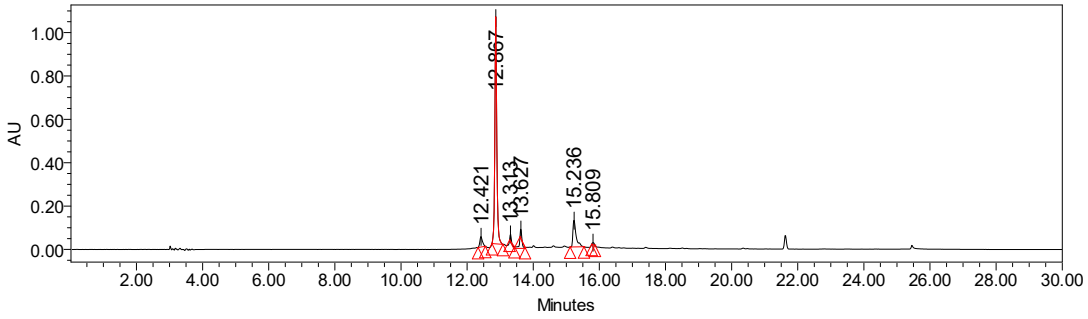
Peptide 1 (TFA·H-Tyr-Lys-Arg-Cys-OH): white solid powder, 36% yield; rt (analytical RP-HPLC): 12.86 (min). ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.63 (t, 1H, NH Arg side chain), 8.37 (d, 1H, NH Arg), 8.24 (t, 2H, NH_2 Lys), 8.17 (d, 1H, NH Lys), 7.99 (brs, 3H, NH_3^+ Tyr), 7.75-7.60 (m, 4H, NH guanidinium group), 7.03 (d, 3H, Tyr aromatics + NH Cys), 6.68 (dd, 2H, Tyr aromatics), 4.49-4.30 (m, 2H, CH^α Tyr, CH^α Arg), 3.96 (m, 1H, CH^α Lys), 3.18-2.87 (m, 4H, CH_2^β Tyr, CH_2^β Cys), 2.76-2.70 (m, 4H, CH_2^β Arg, CH_2^β Lys), 1.68-1.52 (m, 8H, CH_2^γ Arg, CH_2^δ Arg, CH_2^γ Lys, CH_2^δ Lys), 1.31 (m, 2H, CH_2^ε Lys). LRMS (ESI): m/z calc. for $C_{24}H_{40}N_8O_6S$ 568.2; found 568.5 [M].

Peptide 2 (TFA·H-Tyr-Trp-Trp-Trp-OH): white solid powder, 42% yield; rt (analytical RP-HPLC): 15.98 (min). ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.81, 10.75, 10.74 (m, 3H, NH indole), 8.64 (d, 1H, NH Trp) 8.21 (t, 2H, Trp aromatics), 7.85 (brs, 3H, NH_3^+ Tyr), 7.63-7.49 (td, 3H, 2H' indole), 7.31-7.26 (m, 3H, 1H Trp aromatic + 2 NH Trp), 7.12-6.91 (m, 12H, Trp aromatics + Tyr aromatics + NH Trp), 6.65 (dd, 2H, Tyr aromatics), 4.64-4.49 (m, 3H, CH^α Tyr + 2 CH^α Trp), 3.57 (m, 1H, CH^α Trp under water), 3.18-2.68 (m, 8H, CH_2^β Tyr + 3*CH_2^β Trp). LRMS (ESI): m/z calc. for $C_{42}H_{41}N_7O_6$ 739.3; found 740.3 $[M+H]^+$.

Peptide 3 (TFA·H-Tyr-Trp-Tyr-Trp-OH): white solid powder, 45% yield; rt (analytical RP-HPLC): 15.40 (min). ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.81, 10.76 (d, 2H, NH indole), 8.63 (d, 1H, NH Trp), 8.25 (d, 1H, NH Tyr), 8.13 (d, 1H, NH Trp), 7.86 (brs, 3H, NH_3^+ Tyr), 7.62 (d, 1H, Trp aromatic), 7.54 (d, 1H, Trp aromatic), 7.32-7.27 (m, 2H, Trp aromatics), 7.15-6.89 (m, 10H, 4H Tyr aromatics + 6H Trp aromatics), 6.66 (dd, 2H, Tyr aromatics), 6.55 (dd, 2H, Tyr aromatics) 4.61-4.48 (m, 3H, 2*CH^α Tyr + CH^α Trp), 3.69 (m, 1H, CH^α Trp under water), 3.14-2.67 (m, 8H, CH_2^β Trp + CH_2^β Tyr). LRMS (ESI): m/z calc. for $C_{40}H_{40}N_6O_7$ 716.2; found 717.3 $[M+H]^+$.

ANALYTICAL RP-HPLC TRACES OF PEPTIDES 1-3

Peptide 1



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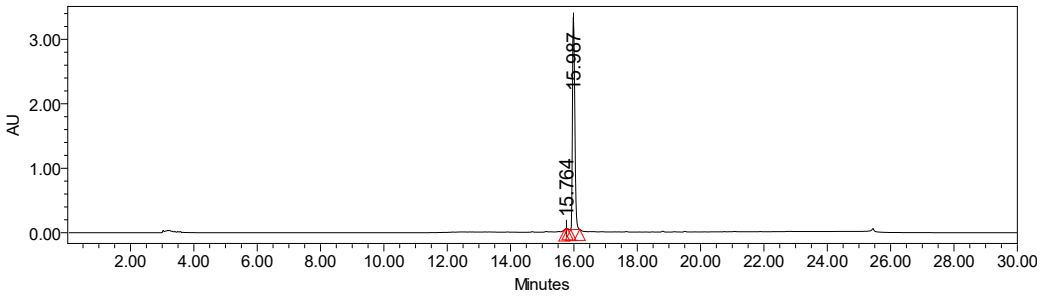

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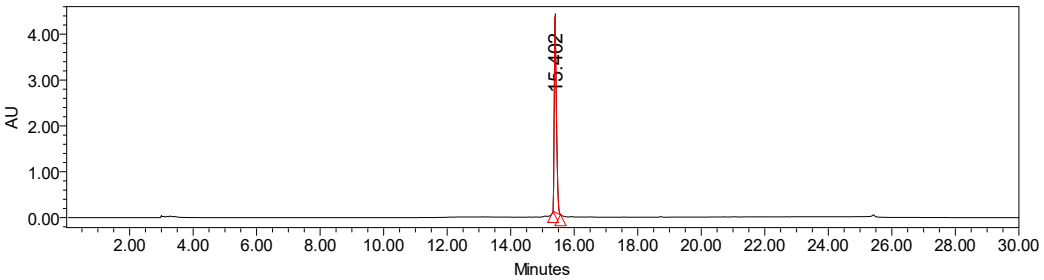
Peptide 3



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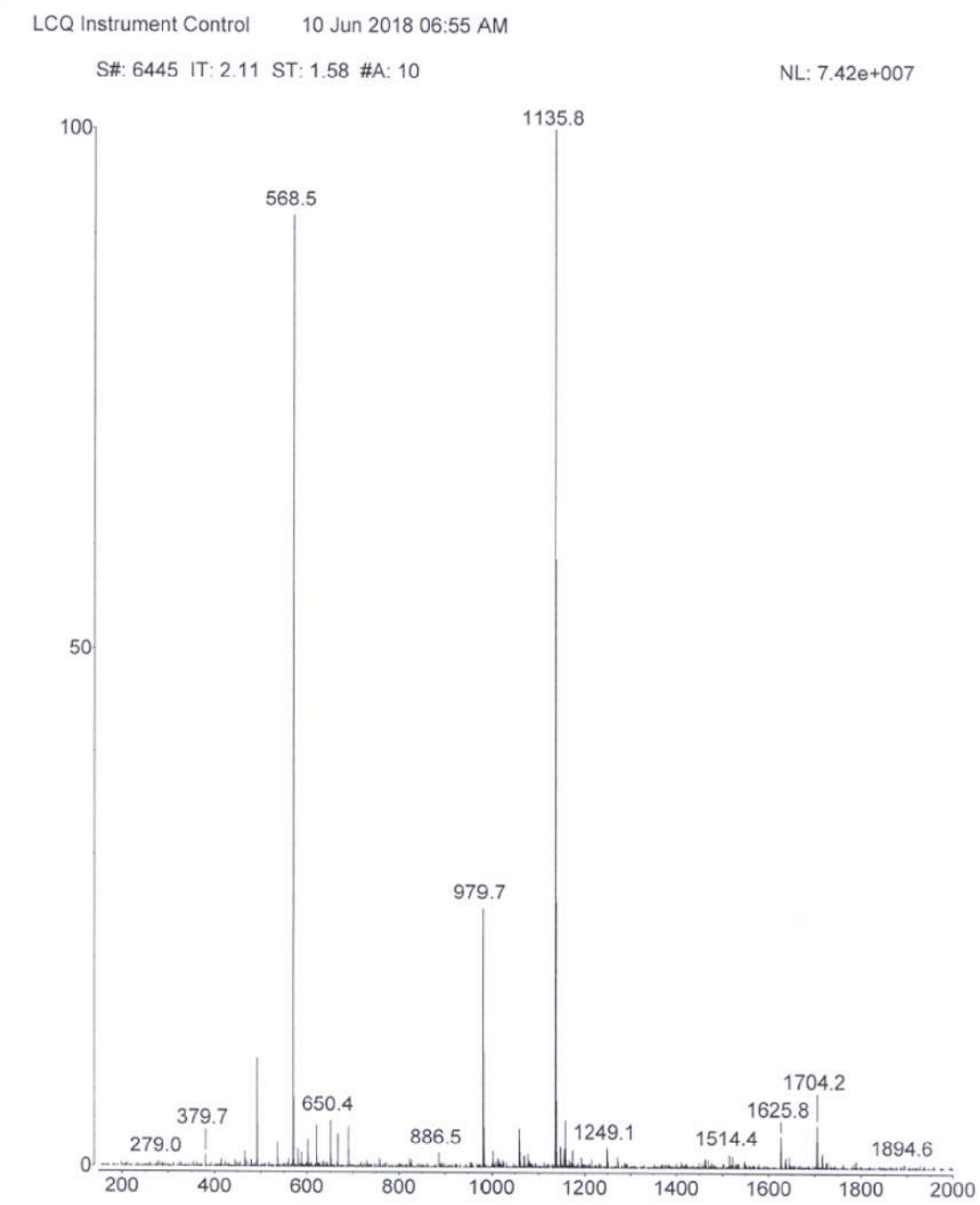
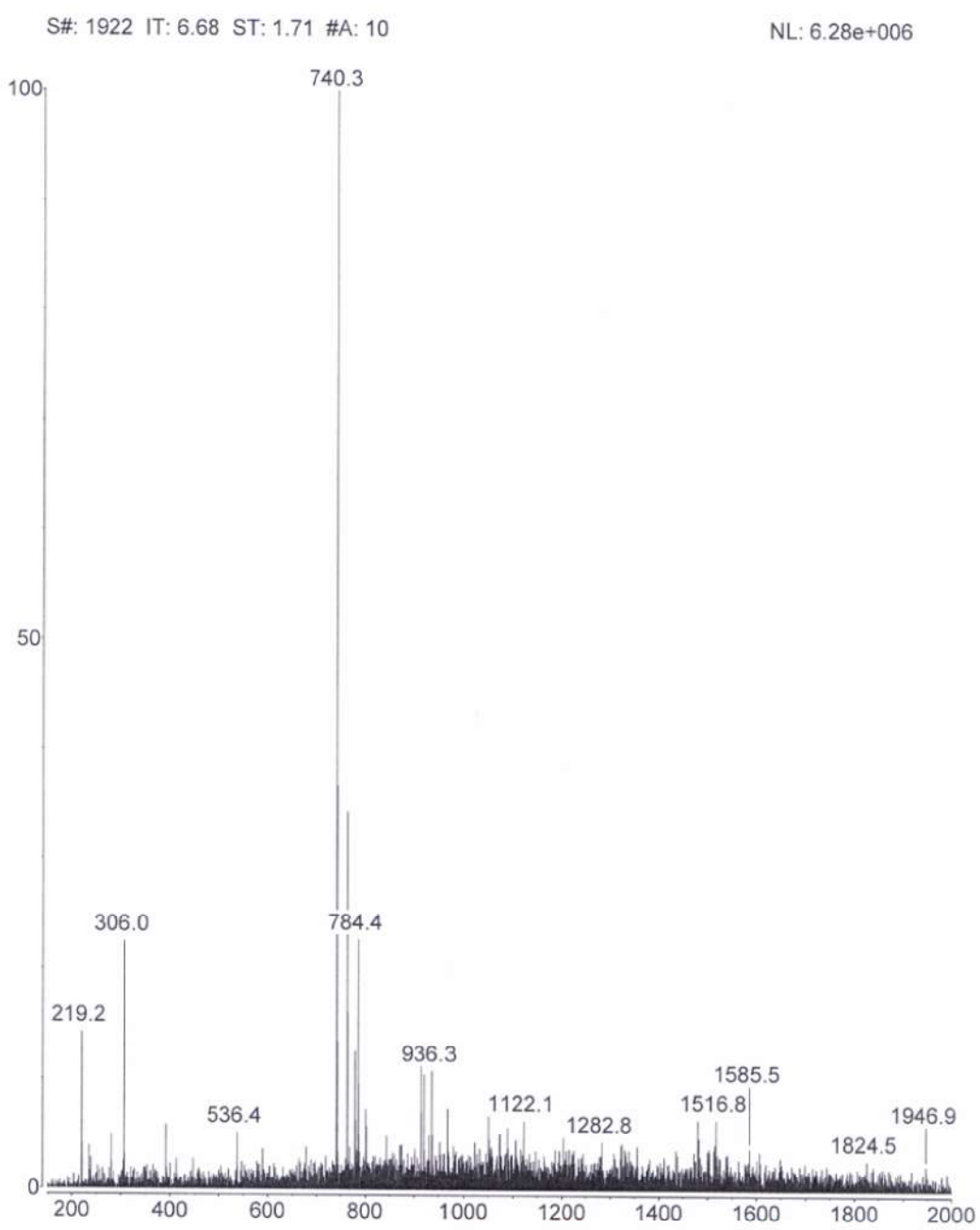
LRMS for peptides 1-3

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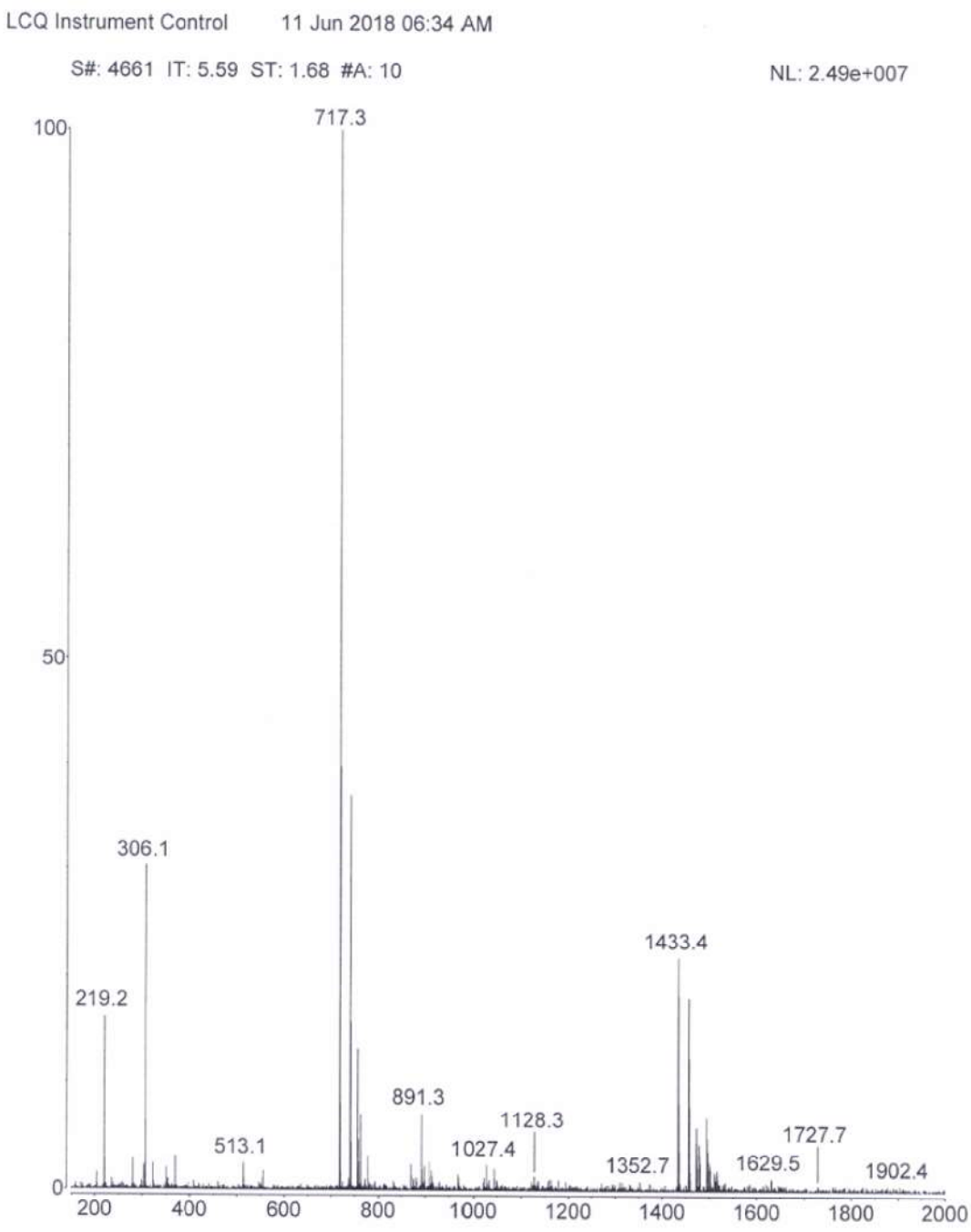
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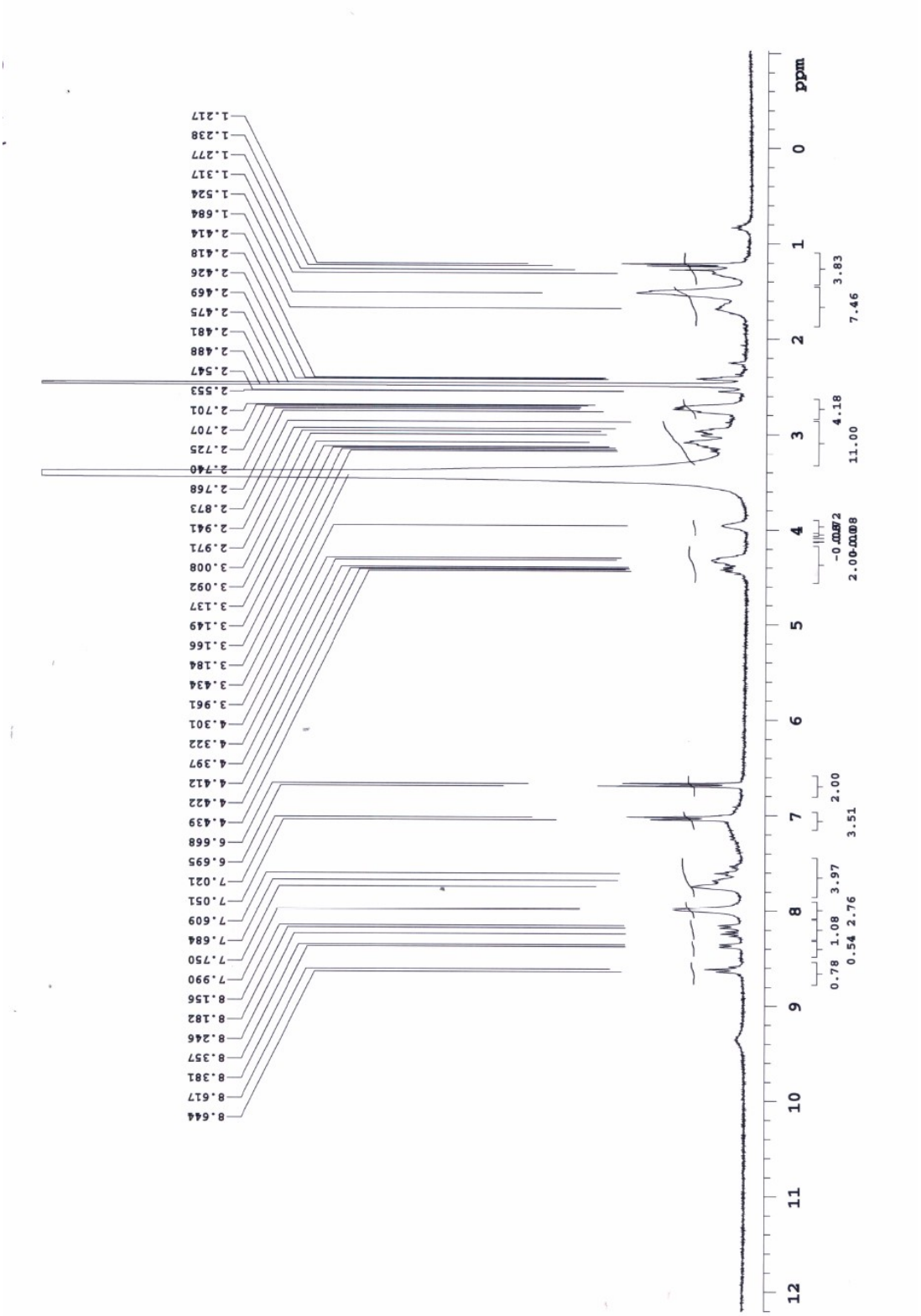
^{1}H-NMR in DMSO-d_6 for peptide **1**



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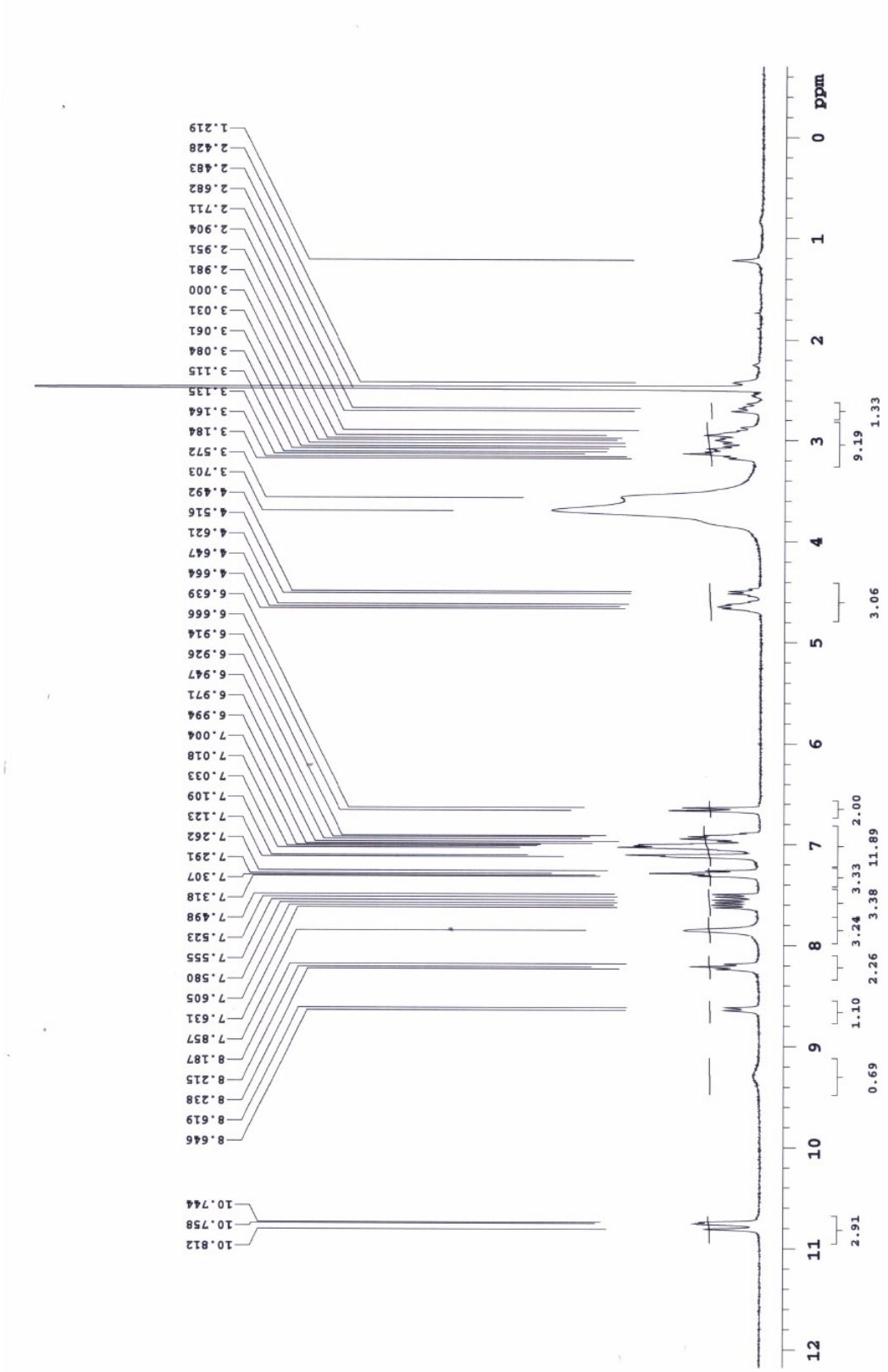

^{1}H-NMR in DMSO-d_6 for peptide **3**