Synthesis of Biotin-BPA affinity Probes

General methods

Preparative chromatography was performed using Sorbent technologies prepacked silica gel columns under medium pressure with ethyl acetate/hexanes (EtOAc/hex) or methanol/dichloromethane ($MeOH/CH_2Cl_2$) as eluent. Reverse phase chromatography was conducted using Isco 4.3 g C-18 columns eluted with acetonitrile/water (CH_3CN/H_2O). NMR spectra were acquired at ambient temperatures (18 ± 2 °C) unless otherwise noted. The 1H NMR spectra in CDCl3 were referenced to TMS unless otherwise noted. The 13C {1H} NMR spectra were recorded at 50 or 100 MHz and referenced relative to the 13C {1H} peaks of the solvent. Spectra are reported as (ppm), (multiplicity, coupling constants (Hz), and number of protons).

Synthesis of BPA-biotin affinity probe 1. (3a*S*,4*S*,6a*R*)-4-(5-(4-(2-(4-hydroxyphenyl)propan-2-yl)phenoxy)pentyl)tetrahydro-1*H*-thieno[3,4-*d*]imidazol-2(3*H*)-one.

Step a: Imidazole (0.381 g, 1.5 mmol) was added to a solution of triphenyl phosphine (0.395 g, 1.5 mmol) in CH_2Cl_2 (6 mL), followed by I_2 (0.102 g, 1.5 mmol) and stirred for 10 min. (3a*S*,4*S*,6a*R*)-4-(5-hydroxypentyl)tetrahydro-1*H*-thieno[3,4-*d*]imidazol-2(3*H*)-one was added, and the reaction stirred for 6 hrs. The mixture was diluted with CH_2Cl_2, (100 mL) then washed successively with 5% $Na_2S_2O_4$ and H_2O. The organic layer was dried over Na_2SO_4, concentrated *in vacuo*, and triturated with EtOAc to give the pure product (3a*S*,4*S*,6a*R*)-4-(5-iodopentyl)tetrahydro-1*H*-thieno[3,4-*d*]imidazol-2(3*H*)-one

(0.216 g, 64%) as a white solid. FT-IR (KBr) 3246, 3119, 2934, 2852, 2343, 1713, 1474 cm $^{-1}$. ^{1}H NMR ($CDCl_3$, 400MHz): δ 5.21 (s, 1H), 4.97 (s, 1H), 4.53 (m, 1H), 4.33 (dd, *J*= 7.7, 4.6 Hz, 1H), 3.20 (t, *J* = 7.0 Hz, 2H) 3.16 (m, 1H), 2.94 (dd, *J* = 12.8, 5.1 Hz, 1H), 2.75 (d, *J* = 12.8 Hz, 1H), 1.84 (m, 2H), 1.68(m, 2H) 1.45 (m, 4H). ^{13}C-NMR (DMSO, 100 MHz): δ 163.5, 61.7, 59.9, 56.1, 40.5, 33.4, 30.6, 28.8, 28.2, 9.7.

Step b: BPA (0.171 g, 0.75 mmol) was dissolved in DMF (2 mL) and cooled to 0°C. NaH (0.031 g, 0.78 mmol) was added, stirred 15 min at 0°C. A solution of the alkyl iodide (0.225 g, 0.75 mmol) in DMF (1 mL) was added slowly, and stirred 2 h at room temperature. The volatiles were removed *in vacuo* and the residue was purified by chromatography on silica gel (10% MeOH/CH_2Cl_2) to give the product **1** (0.221 g, 67%) as a white solid. FT-IR (KBr): 3256, 2929, 2857, 1701, 1673, 1510, 1470, 1249, 1178 cm^{-1}. ^{1}H NMR(400 MHz, CD_3OD,) δ 7.09 (d, *J*= 8.8 Hz, 2H), 7.01 (d, *J*= 8.8 Hz, 2H), 6.76 (d, *J*= 8.8 Hz , 2H), 6.65 (d, *J*= 8.8 Hz, 2H), 4.45 (dd, *J*= 7.9, 4.2 Hz, 1H), 4.26 (dd, *J*= 7.9, 4.5 Hz, 1H), 3.92 (t, *J* = 6.3 Hz, 2H), 3.15 (m, 1H), 2.89 (dd, *J*= 12.8, 4.9 Hz, 1H), 2.68 (d, *J*= 12.6 Hz, 1H), 1.74 (m, 3H), 1.56 (s, 6H), 1.70-1.45 (m, 5H). ^{13}C NMR (100 MHz, CD_3OD): δ 166.1, 158.3, 156.0, 144.5, 143.3, 128.7, 115.6, 114.9, 68.9, 63.4, 61.6, 42.5, 41.0, 31.6, 30.3, 30.1, 29.7, 27.2. HPLC-MS(ES^-) *m/z* : 439.25 (M^-, $C_{25}H_{31}N_2O_3S$ requires 439.21)

Synthesis of BPA-biotin affinity probe 2. *N*-(4-(2-hydroxy-5-(2-(4-hydroxyphenyl)propan-2-yl)phenyl)but-3-ynyl)-6-(6-(5-((3a*S*,4*S*,6a*R*)-2-oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl)pentanamido)hexanamido)hexanamide

Step c, d, e: A mixture of *tert*-butyl but-3-ynylcarbamate (0.100 g, 0.59 mmol), bis-O-TBS-4-(2-(4-hydroxyphenyl)propan-2-yl)-2-iodophenol (0.313 g, 0.54 mmol), $Pd(OAc)_2$, (0.006 g, 0.027 mmol), PPh_3, (0.014 g, 0.054 mmol) and CuI (0.010 g, 0.054 mmol) in diethylamine (1.5 mL) was stirred at rt for 12 h. The mixture was diluted with EtOAc (15 mL), washed with satd. NH_4Cl (aq), dried over Na_2SO_4 and concentrated *in vacuo*. The resulting red residue was purified by chromatography on silica gel (5% EtOAc/Hexanes) to give bis-O-(TBS)-4-(2-hydroxy-5-(2-(4-hydroxyphenyl)propan-2-yl)phenyl)but-3-ynylcarbamate (0.291 g, 86%) as a colorless oil. ^{1}H NMR (400MHz, $CDCl_3$): δ 7.24 (d, *J*= 2.6 Hz, 1H), 7.04 (d, *J*= 8.6 Hz, 2H), 6.96 (dd, *J*= 8.6, 2.6 Hz, 1H), 6.71 (d, *J*= 8.6 Hz, 2H), 6.67 (d, *J*= 8.6 Hz, 1H), 3.34 (m, 2H), 2.60 (t, *J*= 5.8 Hz, 2H), 1.60 (s, 6H), 1.45 (s, 9H), 1.02 (s, 9H), 0.97 (s, 9H), 0.21 (s, 6H), 0.19 (s, 6H). ^{13}C NMR (100MHz, $CDCl_3$): δ 155.7, 154.2, 153.3, 143.6, 143.2, 131.4, 128.0, 127.6, 119.2, 118.9, 114.7, 90.0, 79.6, 79.3, 41.6, 39.4, 30.9, 28.4, 25.7, 21.2, 18.2, -4.3, -4.4.

A solution of TBAF in THF (1M, 0.2 mL) was added to the but-3-ynylcarbamate obtained from step c (0.220 g, 0.35 mmol) in anhydrous THF (1 mL) and stirred at rt for 3.5 h. The mixture was diluted with EtOAc (10 mL), washed with satd. NH_4Cl (aq), dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The crude product was purified by chromatography on silica gel (20% EtOAc/hexanes) to give *tert*-butyl 4-(2-hydroxy-5-(2-(4-hydroxyphenyl)propan-2-yl)phenyl)but-3-ynylcarbamate as a white solid (0.11 g,

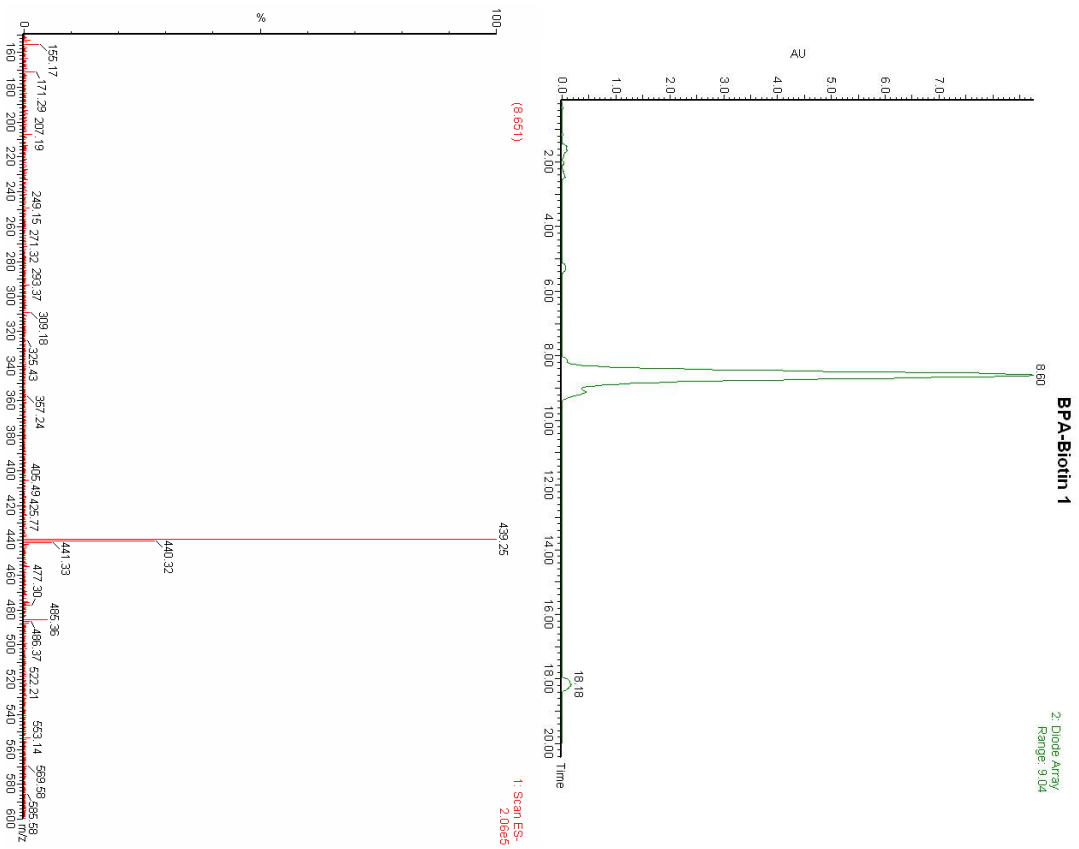
80%). ^{1}H NMR (400MHz, $CDCl_3$): δ 7.16(d, *J*= 2.5Hz, 1H), 7.06(d, *J*= 8.6 Hz, 2H), 7.03(dd, *J*= 8.6, 2.5 Hz, 1H), 6.81(d, *J*= 8.6 Hz, 1H), 6.73(d, *J*= 8.6 Hz, 2H). ^{13}C NMR (100MHz, $CDCl_3$): δ 156.50, 154.93, 153.66, 142.62, 142.45, 129.54, 128.73, 127.78, 114.78, 114.43, 108.84, 93.56, 80.07, 41.54, 39.47, 30.90, 28.35, 21.60.

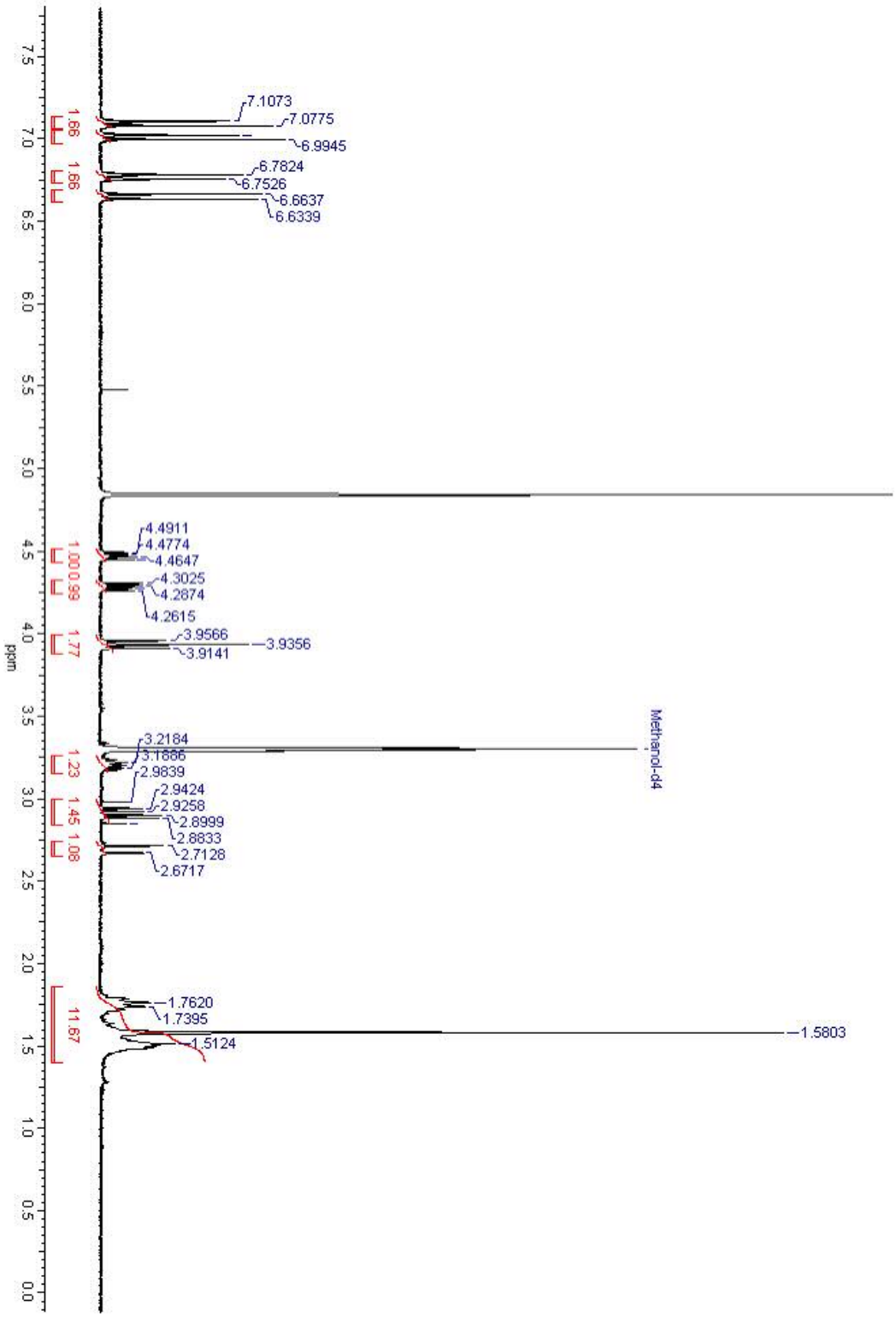
Trifluoroacetic acid (0.2 mL) was added dropwise over 1 min to a cooled solution of *tert*-butyl 4-(2-hydroxy-5-(2-(4-hydroxyphenyl)propan-2-yl)phenyl)but-3-ynylcarbamate (0.056 g, 0.14 mmol) in CH_2Cl_2 (0.8 mL) and stirred at 0 °C for 1h. The mixture was concentrated *in vacuo*, and the residue was purified by chromatography on silica gel (10% MeOH/CH_2Cl_2) to give 2-(4-aminobut-1-ynyl)-4-(2-(4-hydroxyphenyl)propan-2-yl)phenol as white solid (0.042 g, 100%). ^{1}H NMR (400 MHz, CD_3OD): δ 7.37 (d, *J*= 1.0 Hz, 1H), 7.22 (d, *J*= 8.6 Hz, 2H), 7.02 (d, *J*= 8.8 Hz, 2H), 6.65 (d, *J*= 8.8 Hz, 2H), 6.44 (d, *J*= 1.0 Hz, 1H), 2.98 (t, *J*= 6.5Hz, 2H), 2.89 (t, *J*= 6.5 Hz, 2H), 1.64(s, 6H). ^{13}C NMR (100 MHz, CD_3OD): δ 154.3, 153.5, 141.1, 140.1, 129.6, 126.6, 126.0, 113.0, 112.9, 108.0, 86.7, 78.0, 39.7, 37.5, 28.8, 17.9.

Step f: A mixture of Biotin-L_2-NHS (0.015 g, 0.026 mmol), 2-(4-aminobut-1-ynyl)-4-(2-(4-hydroxyphenyl)propan-2-yl)phenol (0.011 g, 0.026 mmol), and triethylamine (30 μL) in dry DMF (1 mL) was allowed to stir at 0 °C, then warm to rt for 12 h. The volatiles were removed *in vacuo*, and the residue was purified by reverse phase column chromatography (20% CH_3CN/H_2O) to give **2** as a colorless solid (0.015 g, 82%). ^{1}H NMR (400 MHz, CD_3OD): δ 7.09 (d, *J*= 2.4 Hz, 1H), 7.00 (d, *J*= 8.8 Hz, 2H), 6.98 (dd, *J*= 8.8, 2.4 Hz, 1H), 6.69 (d, *J*= 8.8 Hz, 1H), 6.65 (d, *J*= 8.8 Hz, 2H), 4.47-4.43 (m, 1H), 4.28-4.24 (m, 1H), 3.38 (t, *J*= 6.8 Hz, 2H), 3.20-3.07 (m, 6H), 2.92-2.86 (m, 2H, 2.70-

2.58 (m, 2H), 2.22-2.16 (m, 6H), 1.71-1.27 (m, 25H). HPLC-MS(ES^-) *m/z* : 746.43 (M^-, $C_{41}H_{56}N_5O_6S$ requires 746.40).

BPA-Biotin 1 was eluted from a Waters Symmetry® C_{18} 5µm 3.0 X 150mm column with 47:53 CH_3CN/H_2O containing 0.01% formic acid, RT= 8.60 min. UV-Vis at RT = 6.80 min. λ_{max} 229 and 278 nm. ESI-MS *m/z* (ES-) calcd for $C_{25}H_{31}N_2O_3S$ $(M-H)^-$ 439.21, found 439.25.

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Methanol-d4
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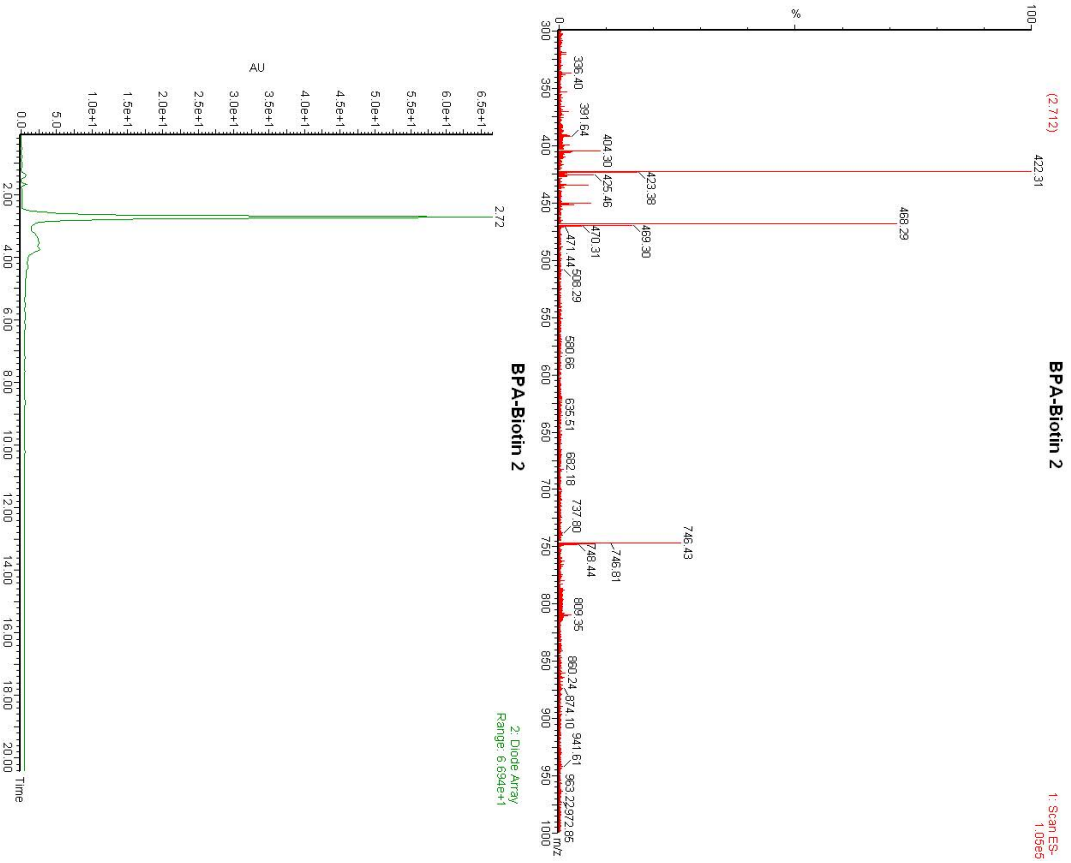
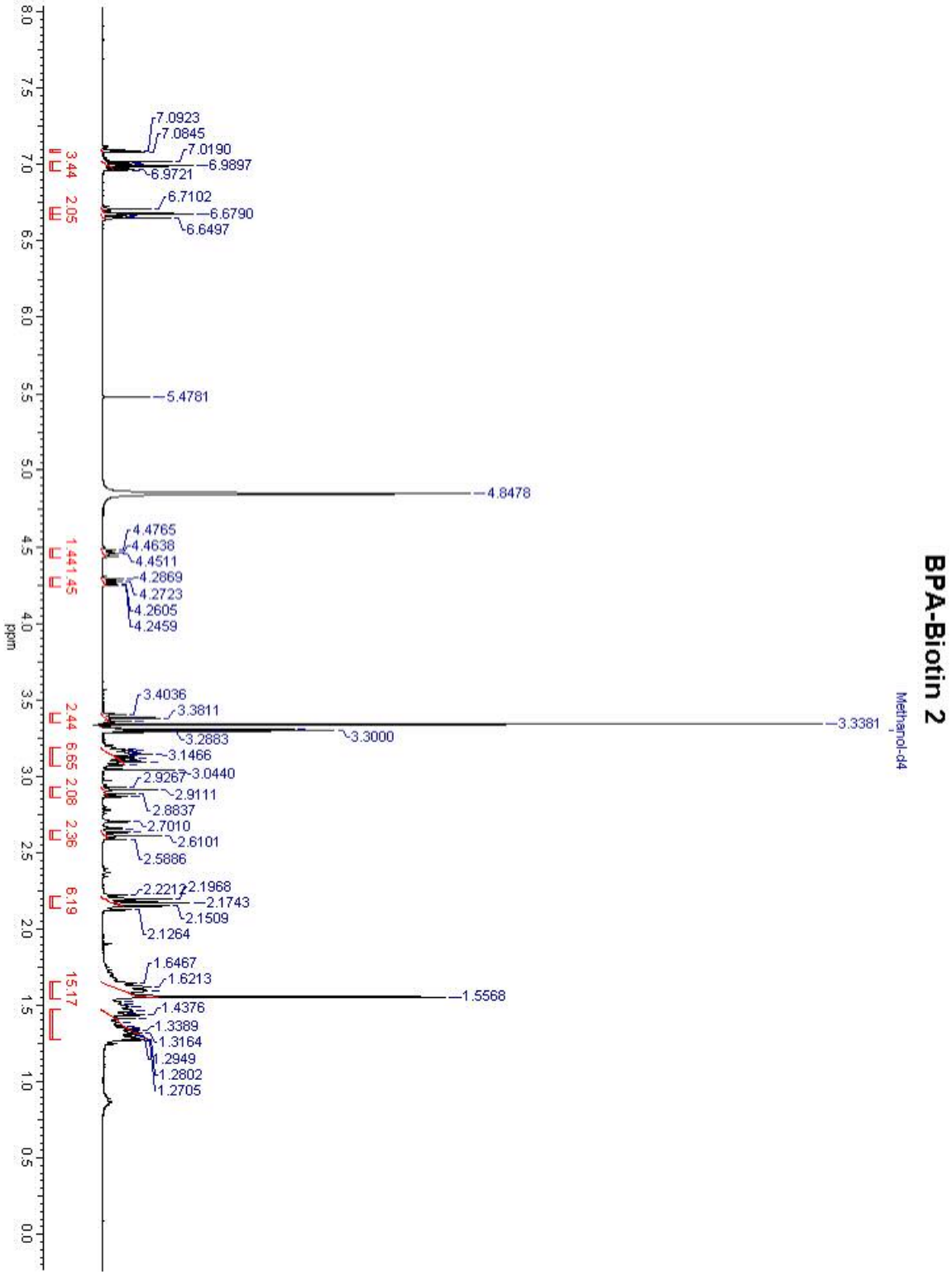
1.66
1.00
0.99
1.77
1.23
1.45
1.08
11.67

BPA-Biotin 2 was eluted from a Waters Symmetry® C_{18} 5µm 3.0 X 150mm column with 37:63 CH_3CN/H_2O containing 0.01% formic acid, RT= 2.72 min. UV-Vis at RT = 2.72 min. λ_{max} 228 and 298 nm. ESI-MS *m/z* (ES-) calcd for $C_{41}H_{56}N_5O_6S$ $(M-H)^-$ 746.40, found 746.43.

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4.4511
4.2869
4.2723
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4.2459
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