

Electronic Supplementary Information

2 Haptens P2-3 and haptens P6-9 were synthesized via the same procedures as those of P1 and
P5, respectively. The data of nuclear magnetic resonance (NMR) spectra and electrospray
4 mass spectra of these haptens are described as follows.

Hapten P2

6 Yield: 58%. ^1H NMR (300 MHz, DMSO) δ (ppm): 15.84 (1H, s, ArOH), 13.06 (1H, s,
COOH), 8.64 (1H, d, $J = 8.6$ Hz, Ar), 8.44 (1H, s, Ar), 8.11 (2H, t, $J = 9.1$ Hz, Ar), 7.90 (2H,
8 d, $J = 7.0$ Hz, Ar), 7.58 (2H, t, $J = 7.8$ Hz, Ar), 7.44 (1H, t, $J = 7.3$ Hz, Ar), 7.03 (1H, d, $J =$
9.4 Hz, Ar). ^{13}C NMR (300 MHz, DMSO) δ (ppm): 168.60 (COOH), 167.23, 145.33,
10 140.04, 135.81, 130.97, 129.97, 129.97, 129.02, 128.85, 128.85, 127.86, 127.38, 124.67,
121.63, 119.57, 119.57 (Ar). Electrospray ionization - mass spectrometry (ESI-MS): m/z
12 290.8 [M - H] $^-$.

Hapten P3

14 Yield: 46%. ^1H NMR (300 MHz, DMSO) δ (ppm): 16.31(1H, s, ArOH), 13.04 (1H, s,
COOH), 8.59 (1H, d, $J = 8.7$ Hz, Ar), 8.41(1H, s, Ar), 8.09-8.05(2H, m, Ar), 7.80(1H, d, $J =$
16 8.1 Hz, Ar), 7.19 (2H, d, $J = 8.4$ Hz, Ar), 7.03 (1H, d, $J = 9.3$ Hz, Ar), 2.44(3H, s, ArCH₃),
2.33(3H, s, ArCH₃). ^{13}C NMR (300 MHz, DMSO) δ (ppm): 167.41, 167.26, 141.43, 139.29,
18 139.02, 135.57, 131.94, 130.88, 129.37, 128.61, 128.35, 127.16, 124.28, 121.38, 115.73(Ar),
20.95, 17.17. ESI-MS: m/z 318.9 [M - H] $^-$.

Hapten P6

Yield: 44%. ^1H NMR (300 MHz, DMSO) δ (ppm): 12.03 (1H, s, COOH), 8.30 (1H, d, $J =$
22 8.5 Hz, Ar), 8.04 (1H, d, $J = 9.1$ Hz, Ar), 7.97-7.92 (3H, m, Ar), 7.66-7.46 (5H, m, Ar), 7.45
(1H, t, $J = 5.8$ Hz, Ar), 4.21 (2H, t, $J = 5.7$ Hz, OCH₂CH₂), 2.27 (2H, t, $J = 7.0$ Hz,

CH₂CH₂COOH), 1.70 (4H, m, $J = 5.1$ Hz, OCH₂CH₂CH₂CH₂). ¹³C NMR (300 MHz,

2 DMSO) δ (ppm): 174.50 (COOH), 153.18, 148.02, 135.58, 131.42, 131.32, 131.32, 129.59,
128.76, 128.20, 128.00, 127.94, 124.58, 122.62, 122.30, 122.30, 116.61 (Ar), 69.59 (OCH₂),
4 33.43 (CH₂COOH), 28.50 (OCH₂CH₂CH₂), 21.33 (OCH₂CH₂CH₂). ESI-MS: m/z 349.1 [M
+ H]⁺.

6 Hapten P7

Yield: 49%. ¹H NMR (300 MHz, DMSO) δ (ppm): 12.10 (1H, s, COOH), 8.30 (1H, d, $J =$

8 7.8 Hz, Ar), 8.02-7.93 (2H, m, Ar), 7.61-7.43 (4H, m, Ar), 7.22 (1H, s, Ar), 7.17 (1H, d, $J =$
8.2 Hz, Ar), 4.20 (2H, t, $J = 5.8$ Hz, OCH₂), 2.62(3H, s, ArCH₃), 2.37(3H, s, ArCH₃), 2.25

10 (2H, t, $J = 6.9$ Hz, CH₂COOH), 1.76-1.60 (4H, m, OCH₂CH₂CH₂). ¹³C NMR (300 MHz,
DMSO) δ (ppm): 174.39 (COOH), 149.61, 148.02, 141.21, 137.44, 135.97, 131.92, 130.94,
12 128.78, 128.15, 127.93, 127.85, 127.45, 124.47, 122.63, 116.59, 114.85 (Ar), 69.61(OCH₂),
33.40, 28.58, 21.27, 21.07, 17.29. ESI-MS: m/z 377.1 [M + H]⁺.

14 Hapten P8

Yield: 53%. ¹H NMR (300 MHz, DMSO) δ (ppm): 12.06 (1H, s, COOH), 8.52 (1H, d, $J =$

16 8.6 Hz, Ar), 8.18-8.08 (5H, m, Ar), 7.99-7.96 (3H, m, Ar), 7.66-7.52 (5H, m, Ar), 7.49 (1H, t,
 $J = 1.1$ Hz, Ar), 4.26 (2H, t, $J = 5.5$ Hz, OCH₂), 2.31(2H, t, $J = 6.7$ Hz, CH₂COOH),

18 1.78-1.73(4H, m, OCH₂CH₂CH₂). ¹³C NMR (300 MHz, DMSO) δ (ppm): 174.53 (COOH),
154.43, 152.98, 152.22, 149.10, 135.16, 132.66, 132.06, 129.68, 129.68, 128.75, 128.43,
20 128.29, 128.15, 124.72, 123.96, 123.96, 123.44, 123.44, 122.90, 122.90, 122.87, 116.42(Ar),
69.59(OCH₂), 33.46, 28.51, 21.41. ESI-MS: m/z 453.1 [M + H]⁺.

22 Hapten P9

Yield: 61%. ¹H NMR (300 MHz, DMSO) δ (ppm): 12.04 (1H, s, COOH), 8.54 (1H, d, $J =$

9.0 Hz, Ar), 8.09(1H, d, $J = 9.0$ Hz, Ar), 7.97-7.84 (2H, m, Ar), 7.65-7.59 (3H, m, Ar),
2 7.51-7.45 (3H, m, Ar), 7.38-7.32 (1H, m, Ar), 4.26 (2H, t, $J = 5.9$ Hz, OCH₂), 2.80(3H, s,
ArCH₃), 2.74(3H, s, ArCH₃), 2.29(2H, t, $J = 6.9$ Hz, CH₂COOH), 1.82-1.67(4H, m,
4 OCH₂CH₂CH₂). ¹³C NMR (300 MHz, DMSO) δ (ppm): 174.43 (COOH), 153.26, 152.75,
150.30, 149.14, 138.60, 138.18, 135.38, 132.55, 131.84, 131.67, 128.73, 128.29, 128.23,
6 126.80, 125.87, 124.68, 122.86, 121.18, 116.32, 116.27, 115.24(Ar), 69.57(OCH₂), 33.43,
28.57, 21.35, 17.60, 17.30. ESI-MS: m/z 481.2 [M + H]⁺.

Table S1 Influence of different variables on ELISA performance.

Variables	IC ₅₀ ^a (ng mL ⁻¹)	A ₀ ^a (A. U.)
MeOH (%)		
5	2.5 ± 0.05	0.70 ± 0.06
10	2.4 ± 0.06	0.82 ± 0.05
15	2.7 ± 0.12	1.02 ± 0.06
20	5.6 ± 0.10	1.37 ± 0.08
30	-- ^b	1.52 ± 0.13
40	-- ^b	1.75 ± 0.16
pH		
4.9	3.2 ± 0.01	0.61 ± 0.01
5.9	2.4 ± 0.02	0.69 ± 0.03
6.9	2.6 ± 0.03	0.78 ± 0.05
7.4	2.2 ± 0.01	0.85 ± 0.08
8.0	2.5 ± 0.02	0.84 ± 0.05
9.0	2.6 ± 0.02	0.86 ± 0.06
10	3.9 ± 0.06	0.80 ± 0.02
NaCl (mol L⁻¹)		
0	2.5 ± 0.06	0.92 ± 0.04
0.068	2.8 ± 0.05	0.90 ± 0.06
0.137	2.4 ± 0.10	0.83 ± 0.02
0.274	2.3 ± 0.14	0.62 ± 0.02
0.547	1.9 ± 0.10	0.54 ± 0.02
1.094	2.2 ± 0.12	0.43 ± 0.03

^aMean value ± standard deviation (n = 3).

^bData are not available because of the unreasonable curve formed.