Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry

SUPPLEMENTARY INFORMATION FILE

Structure-immunogenicity relationship of kresoxim-methyl regioisomeric haptens

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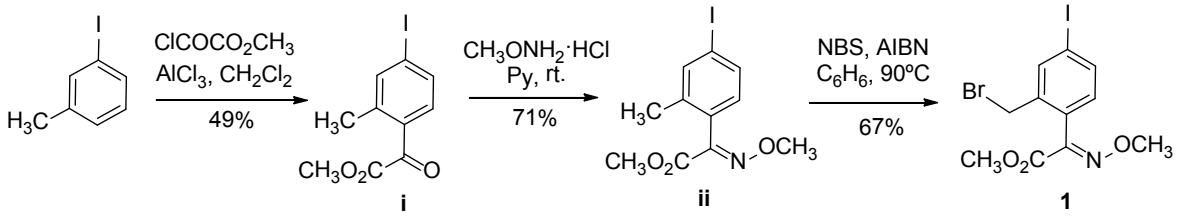
1. Preparation of bromide **1**



Methyl 2-(4-iodo-2-methylphenyl)-2-oxoacetate (**i**). Methyl 2-chloro-2-oxoacetate (323 μL, 3.510 mmol) was added to a solution of 3-iodotoluene (300 μL, 511.2 mg, 2.340 mmol) in anhydrous CH_2Cl_2 (5 mL). The mixture was stirred for 10 min and then cooled to −30ºC followed by the addition of $AlCl_3$ (1.2 g, 9.015 mmol) in small portions (in about 10 min). The mixture was stirred at the same temperature for 90 min, during which the color changes to dark red. The reaction mixture was poured into a mixture of crushed ice and conc. HCl and extruded with CH_2Cl_2. The combined organics layers were washed successively with a 10% aqueous solution of $Na_2S_2O_3$, a 0.1 M solution of NaOH and brine, dried over anhydrous Na_2SO_4 and concentrated to give a residue that was purified by chromatography, using hexane–Et_2O (9:1) as eluent, to afford, in order of elution, title compound **i** (348,6 mg, 49%), as a white solid, followed by methyl 2-oxo-2-(*m*-tolyl)acetate (92.4 mg, 13%), as a colorless oil, and regioisomeric methyl 2-(2-iodo-4-methylphenyl)-2-oxoacetate (248.5 mg, 35%) as a yellowish oil.

Physical and spectroscopic data of compound **i***:* Mp 86–88 ºC (crystallized from hexane); IR (NaCl) ν_{max} (cm^{-1}) 2954w, 2925w, 1734m, 1680s, 1585m, 1547m, 1429m, 1320m, 1203s, 922s, 912m, 735m; ^{1}H-NMR (300 MHz) δ (ppm) 7.22 (1H, br s, H-3), 7.68 (1H, dd, *J* = 8.2, 1.5 Hz, H-5), 7.38 (1H, d, *J* = 8.2 Hz, H-6), 3.95 (3H, s, CO_2CH_3), 2.53 (3H, s, CH_3); ^{13}C-NMR (75 MHz) δ (ppm) 187.65 (CO), 164.22 (CO_2CH_3), 142.78 (C-2), 141.30 (C-3), 135.21 (C-5), 133.04 (C-6), 130.67 (C-1), 102.23 (C-4), 52.90 (CO_2CH_3), 20.95 (CH_3); MS (EI) *m/z* (%) 304 (M^+, 7), 246 (9), 245 (100), 217 (12), 118 (5), 90 (17), 63 (5); HRMS *m/z* calcd. for $C_{10}H_9IO_3$ 303.9596, found 303.9595.

(*E*)-*Methyl 2-(4-iodo-2-methylphenyl)-2-(methoxyimino)acetate* (**ii**). A suspension of keto-ester **i** (202.4 mg, 0.666 mmol) and *N*-methylhydroxylamine hydrochloride (240 mg, 2.87 mmol) in anhydrous pyridine (3.5 mL) was stirred at room temperature under nitrogen overnight. Work up as usual followed by purification by column chromatography with hexane–Et_2O (from 8:2 to 6:4) as eluent afforded methoxyimino derivative **ii** (157.2 mg, 71%) as a white solid. Mp 50–52 ºC (crystallized from hexane); IR (KBr) ν_{max} (cm^{-1}) 2940w, 2886w, 1725s, 1597w, 1437m, 1323m, 1219s, 1066s, 1018s, 957m, 846m, 780m; ^{1}H-NMR (300 MHz) δ (ppm) 7.63 (1H, br s, H-3), 7.57 (1H, dd, *J* = 8.1, 1.4 Hz, H-5), 6.83 (1H, d, *J* = 8.1 Hz, H-6), 4.05 (3H, s, $NOCH_3$) 3.87 (3H, s, CO_2CH_3), 2.13 (3H, s, CH_3); ^{13}C-NMR (75 MHz) δ (ppm) 163.07 (CO_2CH_3), 149.69 (C=N), 138.83 (C-3), 138.33 (C-2), 134.61 (C-5), 129.64 (C-1), 129.50 (C-6), 95.64 (C-4), 63.85 ($NOCH_3$), 53.02 (CO_2CH_3), 19.14 (CH_3); MS (EI) *m/z* (%) 333 (M^+, 28), 302 (100), 270 (15), 259 (5), 243 (59), 242 (30), 175 (8), 143 (8), 116 (20), 89 (15), 63 (5), 59 (8); HRMS *m/z* calcd. for $C_{11}H_{12}INO_3$ 332.9862, found 332.9867.

(E)-Methyl 2-(2-(bromomethyl)-4-iodophenyl)-2-(methoxyimino)acetate (**1**). A mixture of intermediate **ii** (300 mg, 0.900 mmol), *N*-bromosuccinimide (NBS, 160 mg, 0.900 mmol), and azo-*bis*-isobutyronitrile (AIBN, 8.8 mg, 0.054 mmol) in dry benzene (4 mL) was degassed by nitrogen bubbling and ultrasound for 15 min. The reaction mixture was refluxed under nitrogen for 14 h and the solvent was removed in the rotary evaporator to leave an oil that was purified by chromatography, using hexane–Et_2O (9:1) as eluent, to give benzyl bromide **1** (248.4 mg, 67%) as a yellowish oil. IR (NaCl) ν_{max} (cm^{-1}) 3053w, 2941w, 1726s, 1595w, 1437m, 1388d, 1325m, 1302m, 1266m, 1223s, 1063s, 1017s, 957m, 785m, 736s; ^{1}H-NMR (300 MHz) δ (ppm) 7.84 (1H, d, *J* = 1.7 Hz, H-3), 7.70 (1H, dd, *J* = 8.1, 1.7 Hz, H-5), 6.88 (1H, d, *J* = 8.1 Hz, H-6), 4.23 (2H, s, CH_2Br), 4.07 (3H, s, $NOCH_3$), 3.88 (3H, s, CO_2CH_3); ^{13}C-NMR (75 MHz) δ (ppm) 162.71 (CO_2CH_3), 148.09 (C=N), 138.97 (C-3), 137.71 (C-2), 137.45 (C-5), 130.21 (C-6), 129.79 (C-1), 95.64 (C-4), 64.02 ($NOCH_3$), 53.20 (CO_2CH_3), 29.37 (CH_2Br); MS (EI) *m/z* (%) 414 (M^++1 for ^{81}Br, 12), 413 (M^+ for ^{81}Br, 97), 412 (M^+ +1 for ^{79}Br, 13), 411 (M^+ for ^{79}Br, 99), 380 (21), 378 (5), 332 (29), 301 (100), 272 (30), 269 (22), 258 (6), 242 (36), 193 (2), 145 (13), 114 (10); HRMS *m/z* calcd. for $C_{11}H_{11}{}^{79}BrINO_3$ 410.8967, found 410.8966.

2. Spectroscopic data of haptens and intermediates of their synthesis

2.1. Spectroscopic data of hapten KM*a* and intermediates of its synthesis

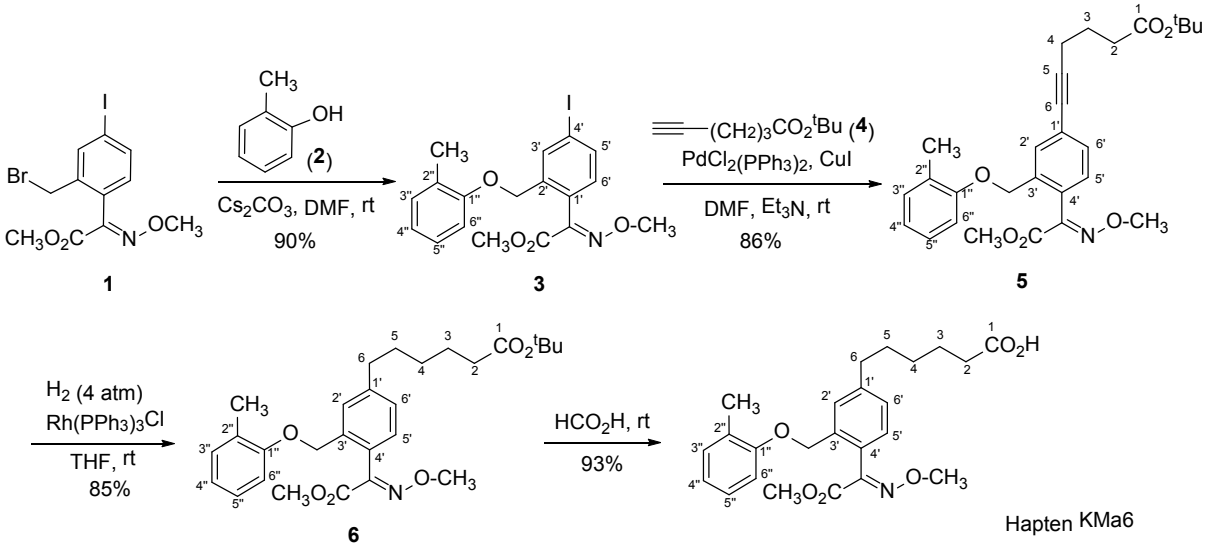


Figure S1. Synthetic sequence for the preparation of hapten KM*a*

(E)-Methyl 2-(4-iodo-2-((o-tolyloxy)methyl)phenyl)-2-(methoxyimino)acetate (**3**): IR (NaCl) ν_{max} (cm^{-1}) 2939w, 1725s, 1600m, 1588m, 1494s, 1454m, 1437m, 1323m, 1240s, 1222s, 1191w, 1121m, 1065s, 1018s, 956w, 787w, 752m; ^{1}H NMR (300 MHz) δ 7.95 (1H, d, *J* = 1.5 Hz, H-3), 7.72 (1H, dd, *J* = 8.1, 1.5 Hz, H-5), 7.13 (2H, m, H-3' and H-5'), 6.94 (1H, d, *J* = 8.1 Hz, H-6), 6.88 (1H, t, *J* = 7.3, 7.3 Hz, H-4'), 6.74 (1H, d, *J* = 8.1 Hz, H-6'), 4.89 (2H, s, OCH_2), 4.03 (3H, s, $NOCH_3$), 3.82 (3H, s, CO_2CH_3), 2.26 (3H, s, CH_3); ^{13}C NMR (75 MHz) δ 162.79 (CO_2CH_3), 156.28 (C-1'), 148.46 (CN), 137.90 (C-2), 136.65 (C-5), 136.55 (C-3), 130.83 (C-3'), 130.17 (C-6),128.42 (C-2'), 126.97 (C-1), 126.73 (C-5'), 120.90 (C-4'), 111.19 (C-6'), 95.82 (C-4), 67.38 (OCH_2), 63.84 ($NOCH_3$), 52.96 (CO_2CH_3), 16.16 (CH_3); MS (EI) *m/z* (%) 439 (M^+, 13), 408 (8), 332 (100), 302 (15), 272 (24), 268 (6), 257 (37), 243 (29), 242 (87), 161 (18), 146 (50), 131 (17), 115 (38), 114 (21), 107 (17), 91 (14), 89 (11), 77 (27), 59 (83); HRMS *m/z* calcd. for $C_{18}H_{18}INO_4$ 439.0281, found 439.0299.

(E)-tert-Butyl 6-(4-(2-methoxy-1-(methoxyimino)-2-oxoethyl)-3-((o-tolyloxy)methyl)phenyl)hex-5-ynoate (**5**): IR (NaCl) ν_{max} (cm^{-1}) 2977m, 2939m, 2258w, 1728s, 1603m, 1494s, 1458m, 1437m, 1312m, 1239s, 1149s, 1069s, 1020s, 952m, 912m, 835m, 785m, 733s; ^{1}H NMR (300 MHz) δ 7.59 (1H, d, *J* = 1.6 Hz, H-2'), 7.40 (1H, dd, *J* = 7.9, 1.6 Hz, H-6'), 7.14 (1H, m, H-3'' partially overlapped with the signals of H-5' and H-5''), 7.13 (1H, m, H-5' partially overlapped with the signals of H-3'' and H-5''), 7.10 (1H, m, H-5''partially overlapped with the signals of H-5' and H-3''), 6.86 (1H, ddd, *J* = 7.4, 7.3, 0.8 Hz, H-4''), 6.75 (1H, d, *J* = 8.1 Hz, H-6''), 4.91 (2H, s, OCH_2), 4.01 (3H, s, $NOCH_3$), 3.80 (3H, s, CO_2CH_3), 2.48 (2H, t, *J* = 7.0 Hz, H-4), 2.40 (2H, t, *J* = 7.5 Hz, H-2), 2.25 (3H,s, CH_3), 1.89 (2H, quint, *J* = 7.3 Hz, H-3), 1.46 (9H, s, $C(CH_3)_3$); ^{13}C NMR (75 MHz) δ 172.44 (CO_2*t*Bu), 163.01 (CO_2CH_3), 156.48 (C-1''), 148.93 (CN), 135.97 (C-3'), 130.69 (C-6'), 130.60 (C-2' and C-3''), 128.61 (C-5'), 128.16 (C-4'), 127.00 (C-2''), 126.67 (C-5''), 125.33 (C-1'), 120.71 (C-4''), 111.23 (C-6''), 90.58 (C-5),

80.75 (C-6), 80.28 (*C*$(CH_3)_3$), 67.93 (OCH_2), 63.78 ($NOCH_3$), 52.90 (CO_2*CH_3*), 34.36 (C-2), 28.08 (C*(*CH_3*)*$_3$), 24.01 (C-3), 18.84 (C-4), 16.19 (CH_3); MS (EI) *m/z* (%) 479 (M+, 20), 423 (4), 406 (16), 392 (4), 320 (100), 276 (27), 246 (17), 230 (19), 212 (12), 204 (4), 184 (5), 172 (17), 160 (5), 144 (18), 130 (9); HRMS *m/z* calcd. for $C_{28}H_{33}NO_6$ 479.2308, found 479.2304.

(E)-tert-Butyl 6-(4-(2-methoxy-1-(methoxyimino)-2-oxoethyl)-3-((o-tolyloxy)methyl)phenyl)hexanoate (**6**): IR (NaCl) ν_{max} (cm^{-1}) 2976w, 2936m, 2859m, 1727s, 1602w, 1495m, 1460m, 1438m, 1366m, 1311m, 1243s, 1214s, 1156s, 1067s, 1020m, 955w, 847w, 752m; ^{1}H NMR (300 MHz) δ 7.38 (1H, s, H-2'), 7.19 (1H, dd, *J* = 7.8, 1.6 Hz, H-6'), 7.14 (1H, m, H-5' partially overlapped with the signals of H-3'' and H-5''), 7.13 (1H, m, H-3'' partially overlapped with the signals of H-5' and H-5''), 7.10 (1H, m, H-5'' partially overlapped with the signals of H-5' and H-3''), 6.85 (1H, ddd, *J* = 7.4, 7.4, 0.8 Hz, H-4''), 6.78 (1H, d, *J* = 8.1 Hz, H-6''), 4.93 (2H, s, OCH_2), 4.02 (3H, s, $NOCH_3$), 3.81 (3H, s, CO_2CH_3), 2.65 (2H, t, *J* = 7.6 Hz, H-6), 2.25 (3H, s, CH_3), 2.21 (2H, t, *J* = 7.5 Hz, H-2), 1.68-1.60 (4H, m, H-5 and H-3), 1.45 (9H, s, $C(CH_3)_3$), 1.40 (2H, m, H-4); ^{13}C NMR (75 MHz) δ 173.09 (CO_2^{t}Bu), 163.37 (CO_2CH_3), 156.60 (C-1''), 149.42 (CN), 144.12 (C-1'), 135.59 (C-3'), 130.02 (C-3''), 128.53 (C-5'), 127.65 (C-2'), 127.53 (C-6'), 126.94 (C-2''), 126.64 (C-5''), 126.27 (C-4'), 120.56 (C-4''), 111.23 (C-6''), 79.98 (*C*$(CH_3)_3$), 68.31 (OCH_2), 63.65 ($NOCH_3$), 52.82 (CO_2*CH_3*), 35.58 (C-6), 35.41 (C-2), 30.62 (C-5), 28.69 (C-4), 28.07 (C*(*CH_3*)*$_3$), 24.88 (C-3), 16.17 (CH_3); MS (EI) *m/z* (%) 483 (M+, 4), 452 (4), 396 (7), 320 (100), 276 (23), 246 (15), 230 (18), 212 (9), 204 (5), 184 (10), 172 (15), 160 (8), 144 (18), 130 (7); HRMS *m/z* calcd. for $C_{28}H_{37}NO_6$ 483.2621, found 483.2631.

(E)-6-(4-(2-Methoxy-1-(methoxyimino)-2-oxoethyl)-3-((o-tolyloxy)methyl)phenyl)hexanoic acid (Hapten **KMa**): IR (NaCl) ν_{max} (cm^{-1}) 3293w (br), 2937m, 2858w, 1725s, 1708s, 1601w, 1494m, 1460w, 1438m, 1310m, 1242s, 1190w, 1122m, 1067s, 1019s, 953w, 782w, 752m; ^{1}H NMR (300 MHz) δ 7.38 (1H, s, H-2'), 7.20 (1H, dd, *J* = 7.9, 1.5 Hz, H-6'), 7.14 (1H, m, H-3'' partially overlapped with the signals of H-5' and H-5''), 7.13 (1H, m, H-5' partially overlapped with the signals of H-3'' and H-5''), 7.10 (1H, m, H-5'' partially overlapped with the signals of H-5' and H-3''), 6.86 (1H, ddd, *J* = 7.3, 7.3, 0.7 Hz, H-4''), 6.77 (1H, d, *J* = 8.1 Hz, H-6''), 4.94 (2H, s, OCH_2), 4.02 (3H, s, $NOCH_3$), 3.81 (3H, s, CO_2CH_3), 2.66 (2H, t, *J* = 7.7 Hz, H-6), 2.35 (2H, t, *J* = 7.5 Hz, H-2), 2.25 (3H, s, CH_3), 1.68-1.60 (4H, m, H-5 and H-3), 1.41 (2H, m, H-4); ^{13}C NMR (75 MHz) δ 179.65 (CO_2H), 163.40 (CO_2CH_3), 156.59 (C-1''), 149.39 (CN), 143.98 (C-1'), 135.64 (C-3'), 130.61 (C-3''), 128.56 (C-5'), 127.65 (C-2'), 127.55 (C-6'), 126.94 (C-2''), 126.64 (C-5''), 126.31 (C-4'), 120.59 (C-4''), 111.26 (C-6''), 68.29 (OCH_2), 63.68 ($NOCH_3$), 52.87 (CO_2*CH_3*), 35.52 (C-6), 33.84 (C-2), 30.54 (C-5), 28.63 (C-4), 24.44 (C-3), 16.18 (CH_3); MS (EI) *m/z* (%) 427 (M^+, 6), 396 (6), 320 (100), 276 (21), 246 (14), 230 (19), 212 (8), 204 (3), 184 (5), 172 (15), 160 (8), 144 (18), 130 (8); HRMS *m/z* calcd. for $C_{24}H_{29}NO_6$ 427.1995, found 427.2007.

2.2. Spectroscopic data of hapten KM*b* and intermediates of its synthesis

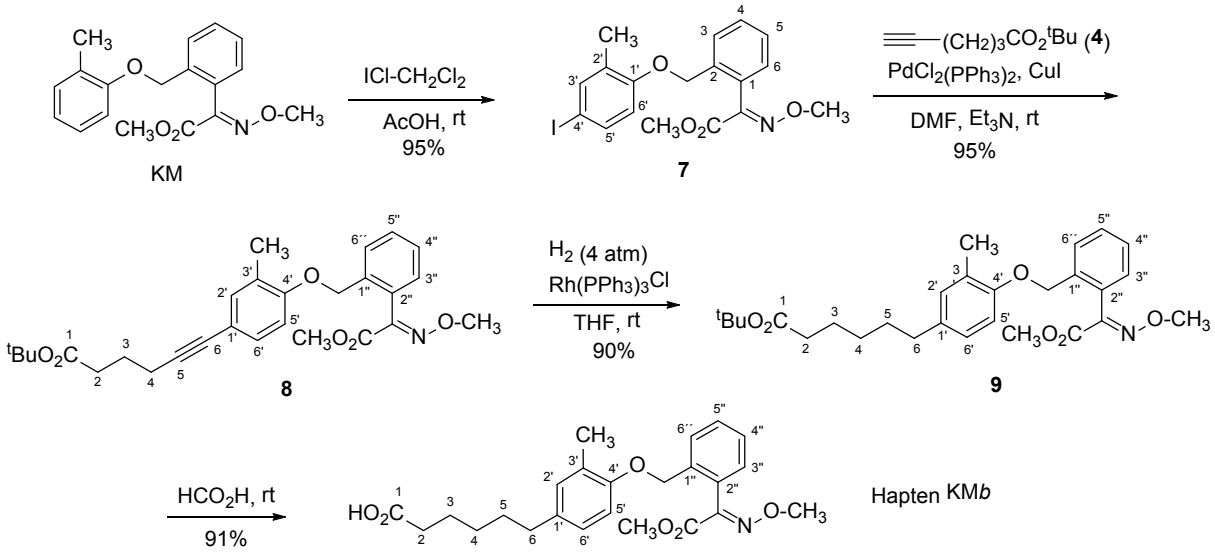


Figure S2. Synthetic sequence for the preparation of hapten KM*b*

(E)-Methyl 2-(2-((4-iodo-2-methylphenoxy)methyl)phenyl)-2-(methoxyimino)acetate (**7**): IR (KBr) ν_{max} (cm^{-1}) 2985w, 2969w, 2938w, 1738s, 1585m, 1487s, 1464s, 1436s, 1384m, 1296s, 1241s, 1188s, 1132s, 1002s, 978s, 887m, 801s, 786s, 766s, 644m; ^{1}H NMR (300 MHz) δ 7.52 (1H, dd, *J* = 7.3, 1.4 Hz, H-6), 7.49-7.33 (4H, m, H-3', H-5, H-5', H-4), 7.20 (1H, dd, *J* = 7.3, 1.3 Hz, H-3), 6.52 (1H, d, *J* = 8.6 Hz, H-6'), 4.93 (2H, s, OCH_2), 4.02 (3H, s, $NOCH_3$), 3.83 (3H, s, CO_2CH_3), 2.19 (3H, s, CH_3); ^{13}C NMR (75 MHz) δ 163.18 (CO_2CH_3), 156.40 (C-1'), 149.19 (CN), 139.08 (C-3'), 135.40 (C-5'), 135.15 (C-2), 129.78 (C-3), 129.65 (C-5),128.95 (C-2'), 128.61 (C-1), 127.72 (C-4), 127.48 (C-6), 113.41 (C-6'), 82.97 (C-4'), 68.21 (OCH_2), 63.84 ($NOCH_3$), 53.02 (CO_2CH_3), 15.88 (CH_3); MS (EI) *m/z* (%) 439 (M^+, 9), 232 (7), 206 (48), 146 (7), 131 (44), 117 (16), 116 (100), 106 (5), 89 (21), 78 (27), 59 (67); HRMS *m/z* calcd. for $C_{18}H_{18}INO_4$ 439.0280, found 439.0281.

(E)-tert-Butyl 6-(4-((2-(2-methoxy-1-(methoxyimino)-2-oxoethyl)benzyl)oxy)-3-methylphenyl)hex-5-ynoate (**8**): IR (NaCl) ν_{max} (cm^{-1}) 2977w, 2939w; 1727s, 1604w, 1500m, 1461w, 1437w, 1367w, 1313w, 1262w, 1233m, 1147m, 1132m, 1069m, 1019m, 958w, 758w, 738w; ^{1}H NMR (300 MHz) δ 7.54 (1H, dd, *J* = 7.3, 1.5 Hz, H-3''), 7.44 (1H, ddd, *J* = 7.4, 7.3, 1.3 Hz, H-4''), 7.38 (1H, ddd, *J* = 7.4, 7.4, 1.5 Hz, H-5''), 7.20 (1H, dd, *J* = 7.4, 1.5 Hz, H-6''), 7.19 (1H, s, H-2'), 7.14 (1H, dd, *J* = 8.4, 2.0 Hz, H-6'), 6.66 (1H, d, *J* = 8.4 Hz, H-5'), 4.94 (2H, s, OCH_2), 4.02 (3H, s, $NOCH_3$), 3.82 (3H, s, CO_2CH_3), 2.43 (2H, t, *J* = 7.0 Hz, H-4), 2.39 (2H, t, *J* = 7.5 Hz, H-2), 2.19 (3H, s, CH_3), 1.86 (2H, quint, *J* = 7.2 Hz, H-3), 1.45 (9H, s, $C(CH_3)_3$); ^{13}C NMR (75 MHz) δ 172.62 (CO_2*t*Bu), 163.19 (CO_2CH_3), 156.15 (C-4'), 149.22 (CN), 135.34 (C-1''), 133.86 (C-2'), 130.18 (C-6'), 129.63 (C-4''), 128.93 (C-2''), 128.57 (C-6''), 127.64 (C-5''), 127.52 (C-3''), 126.95 (C-3'), 115.76 (C-1'), 110.92 (C-5'), 87.25 (C-5), 81.03 (C-6), 80.23 ($C(CH_3)_3$), 68.13 (OCH_2), 63.83 ($NOCH_3$), 52.99 (CO_2CH_3), 34.46 (C-2), 28.10 ($C(CH_3)_3$), 24.21 (C-3), 18.83 (C-4), 16.03 (CH_3); MS (EI) *m/z* (%) 479 (M^+, 26), 423 (3), 406 (16), 392 (4), 217 (6), 206

(100), 176 (8), 162 (11), 159 (2), 146 (9), 132 (26), 131 (58), 116 (95), 105 (5), 59 (25), 57 (21); HRMS *m/z* calcd. for $C_{28}H_{33}NO_6$ 479.2308, found 479.2302.

(E)-tert-Butyl 6-(4-((2-(2-methoxy-1-(methoxyimino)-2-oxoethyl)benzyl)oxy)-3-methylphenyl)hexanoate (**9**): IR (NaCl) ν_{max} (cm^{-1}) 2976m, 2933m, 2857w, 1728s, 1609w, 1592w, 1503s, 1456w, 1437m, 1367s, 1219s, 1155s, 1133s, 1069s, 1046w, 1020s, 1046w, 1020s, 957w, 759w; ^{1}H NMR (300 MHz) 7.58 (1H, d, *J* = 7.4 Hz, H-3''), 7.44 (1H, ddd, *J* = 7.6, 7.4, 1.5 Hz, H-4''), 7.38 (1H, ddd, *J* = 7.6, 7.4, 1.5 Hz, H-5''), 7.19 (1H, dd, *J* = 7.4, 1.5 Hz, H-6''), 6.94 (1H, s, H-2'), 6.88 (1H, dd, *J* = 8.2, 1.8 Hz, H-6'), 6.67 (1H, d, *J* = 8.2 Hz, H-5'), 4.92 (2H, s, OCH_2), 4.03 (3H, s, $NOCH_3$), 3.82 (3H, s, CO_2CH_3), 2.49 (2H, t, *J* = 7.6 Hz, H-6), 2.22 (5H, m, CH_3 and H-2), 1.67-1.51 (4H, m, H-5 and H-3), 1.43 (9H, s, $C(CH_3)_3$), 1.33 (2H, m, H-4); δ ^{13}C NMR (75 MHz) δ 172.25 (CO_2^{t}Bu), 163.22 (CO_2CH_3), 156.64 (C-4'), 149.32 (CN), 135.66 (C-1''), 134.66 (C-1'), 130.82 (C-6'), 129.58 (C-4''), 128.88 (C-2''), 128.41 (C-6''), 127.50 (C-5''), 127.46 (C-3''), 126.64 (C-3'), 126.20 (C-2'), 111.05 (C-5'), 79.93 ($C(CH_3)_3$), 68.10 (OCH_2), 63.81 ($NOCH_3$), 52.97 (CO_2CH_3), 35.50 (C-2), 34.82 (C-6), 31.41 (C-5), 28.68 (C-4). 28.08 ($C(CH_3)_3$), 24.91 (C-3), 16.25 (CH_3); MS (EI) *m/z* (%) 483 (M^+, 4), 452 (4), 396 (7), 206 (94), 176 (8), 162 (12), 146 (9), 132 (28), 131 (57), 121 (19), 116 (100), 105 (6), 91 (10), 57 (40); HRMS *m/z* calcd. for $C_{28}H_{37}NO_6$ 483.2621, found 483.2632.

(E)-6-(4-((2-(2-Methoxy-1-(methoxyimino)-2-oxoethyl)benzyl)oxy)-3-methylphenyl)hexanoic acid (Hapten **KM*b***). IR (NaCl) ν_{max} (cm^{-1}) 3325w (broad), 2937s, 2856m, 2660m, 1727s, 1708s, 1609w, 1503s, 1437m, 1380w, 1317m, 1251s, 1220s, 1132m, 1070s, 1046w, 1019s, 938m, 788w; ^{1}H NMR (300 MHz) 7.59 (1H, d, *J* = 7.4 Hz, H-3''), 7.45 (1H, ddd, *J* = 7.6, 7.4, 1.4 Hz, H-4''), 7.39 (1H, ddd, *J* = 7.6, 7.4, 1.5 Hz, H-5''), 7.21 (1H, dd, *J* = 7.4, 1.4 Hz, H-6''), 6.96 (1H, d, *J* =1.8 Hz, H-2'), 6.90 (1H, dd, *J* = 8.2, 1.8 Hz, H-6'), 6.69 (1H, d, *J* = 8.2 Hz, H-5'), 4.94 (2H, s, OCH_2), 4.04 (3H, s, $NOCH_3$), 3.83 (3H, s, CO_2CH_3), 2.52 (2H, t, *J* = 7.5 Hz, H-6), 2.36 (2H, t, J = 7.5 z, H-2), 2.24 (3H, s, CH_3), 1.74-1.54 (4H, m, H-5, H-3), 1.38 (2H, m, H-4); δ ^{13}C NMR (75 MHz) δ180.04 (CO_2H), 163.20 (CO_2CH_3), 154.64 (C-4'), 149.28 (CN), 135.82 (C-1''), 134.46 (C-1'), 130.78 (C-6'), 129.54 (C-4''), 128.85 (C-2''), 128.39 (C-6''), 127.48 (C-5''), 127.43 (C-3''), 126.63 (C-3'), 126.16 (C-2'), 111.03 (C-5'), 68.08 (OCH_2), 63.75 ($NOCH_3$), 52.92 (CO_2CH_3), 34.71 (C-6), 33.92 (C-2), 31.29 (C-5), 28.59 (C-4), 24.45 (C-3), 16.20 (CH_3); MS (EI) *m/z* (%) 427 (M^+, 25), 396 (4), 337 (3), 206 (61), 176 (4), 162 (8), 146 (9), 135 (10), 132 (23), 131 (51), 121 (42), 116 (100), 105 (8), 91 (15), 59 (44); HRMS *m/z* calcd. for $C_{24}H_{29}NO_6$ 427.1995, found 427.2004.

2.3. Spectroscopic data of hapten KM*c* and intermediates of its synthesis

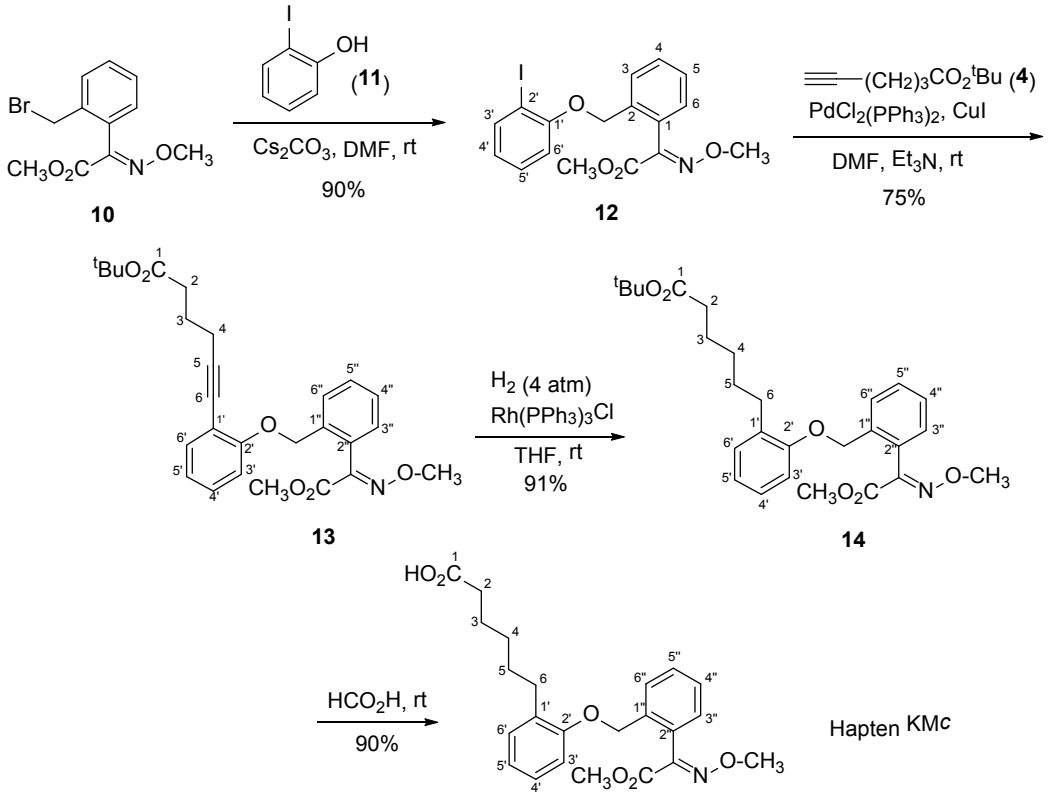


Figure S3. Synthetic sequence for the preparation of hapten KM*c*

(E)-Methyl 2-(2-((2-iodophenoxy)methyl)phenyl)-2-(methoxyimino)acetate (**12**): IR (KBr) ν_{max} (cm^{-1}) 3000w, 2945w, 1715s, 1579w, 1470m, 1446m, 1318m, 1222m, 1069s, 1012s, 948m, 745s; ^{1}H NMR (300 MHz) δ 7.78 (1H, dd, *J* = 7.8, 1.6 Hz, H-3'), 7.71 (1H, dd, *J* = 7.7, 1.2 Hz, H-6), 7.46 (1H, ddd, *J* = 7.7, 7.6, 1.4 Hz, H-5), 7.38 (1H, ddd, *J* = 7.6, 7.4, 1.2 Hz, H-4), 7.24-7.16 (2H, m, H-3 and H-5'), 6.77-6.68 (2H, m, H-4' and H-6'), 5.01 (2H, s, OCH_2), 4.05 (3H, s, $NOCH_3$), 3.87 (3H, s, CO_2CH_3); ^{13}C NMR (75 MHz) δ 163.71 (CO_2CH_3), 157.41 (C-1'), 149.21 (CN), 139.56 (C-3'), 132.20 (C-2), 129.80 (C-5), 129.40 (C-3), 128.27 (C-5'), 127.63 (C-4), 127.43 (C-6), 125.21 (C-1), 122.87 (C-4'), 112.59 (C-6'), 85.90 (C-2'), 68.76 (OCH_2), 63.91 ($NOCH_3$), 53.14 (CO_2CH_3); ESI-HRMS *m/z* calcd. for $C_{17}H_{16}INNaO_4$ $[M+Na]^+$ 448.0022, found 448.0019.

(E)-tert-Butyl 6-(2-((2-(2-methoxy-1-(methoxyimino)-2-oxoethyl)benzyl)oxy)phenyl)hex-5-ynoate (**13**): IR (NaCl) ν_{max} (cm^{-1}) 2975w, 2934w, 1735s, 1488w, 1441w, 1369w, 1307w, 1214m, 1137s, 1064m, 1013m, 961w, 754m; ^{1}H NMR (300 MHz) δ 7.69 (1H, dd, *J* = 7.7, 0.7 Hz, H-3''), 7.44 (1H, ddd, *J* = 7.7, 7.6, 1.4 Hz, H-4''), 7.39-7.32 (2H, m, H-3' and H-5''), 7.19-7.11 (2H, m, H-6'' and H-5'), 6.87 (1H, ddd, *J* = 7.5, 7.5, 0.7 Hz, H-4'), 6.76 (1H, d, *J* = 8.3 Hz, H-6'), 5.01 (2H, s, OCH_2), 4.05 (3H, s, $NOCH_3$), 3.86 (3H, s, CO_2CH_3), 2.53 (2H, t, *J* = 7.2 Hz, H-4), 2.42 (2H, t, *J* = 7.2 Hz, H-2), 1.91 (2H, quint, *J* = 7.2 Hz, H-3), 1.44 (9H, s, $C(CH_3)_3$); ^{13}C NMR (75 MHz) δ 172.64 (CO_2*t*Bu), 158.81 (CO_2CH_3), 157.01 (C-1'), 149.18 (CN), 135.42 (C-1''), 133.47 (C-3'), 129.60 (C-4''), 128.82 (C-5'), 128.02 (C-6''), 127.39 (C-5''), 127.08 (C-3''), 120.84 (C-4'), 119.74 (C-2''), 113.67

(C-2'), 112.57 (C-6'), 110.55 (C-5), 93.51 (C-6), 80.19 ($C(CH_3)_3$), 68.04 (OCH_2), 63.85 ($NOCH_3$), 53.06 (CO_2CH_3), 34.47 (C-2), 28.11 ($C(CH_3)_3$), 24.21 (C-3), 19.22 (C-4); ESI-HRMS *m/z* calcd. for $C_{27}H_{31}NNaO_6$ $[M+Na]^+$ 488.2049, found 488.2047.

(E)-tert-Butyl 6-(2-((2-(2-methoxy-1-(methoxyimino)-2-oxoethyl)benzyl)oxy)phenyl)hexanoate (**14**): IR (NaCl) ν_{max} (cm^{-1}) 2967w, 2936m, 2862w, 1727s, 1594w, 1492w, 1450m, 1366w, 1318m, 1224s, 1156s, 1068s, 1019s, 955w, 752m; ^{1}H NMR (300 MHz) δ 7.57 (1H, dd, *J* = 7.6, 1.4 Hz, H-3''), 7.45 (1H, ddd, *J* = 7.6, 7.6, 1.5 Hz, H-4''), 7.38 (1H, ddd, *J* = 7.6, 7.5, 1.4 Hz, H-5''), 7.19 (1H, dd, *J* = 7.5, 1.5 Hz, H-6''), 7.15-7.05 (2H, m, H-6' and H-4'), 6.87 (1H, ddd, *J* = 7.4, 7.4, 1.0 Hz, H-5'), 6.76 (1H, d, *J* = 8.1 Hz, H-3'), 4.94 (2H, s, OCH_2), 4.04 (3H, s, $NOCH_3$), 3.84 (3H, s, CO_2CH_3), 2.65 (2H, t, *J* = 7.5 Hz, H-6), 2.19 (2H, t, *J* = 7.5 Hz, H-2), 1.68-1.55 (4H, m, H-5 and H-3), 1.41-1.32 (11H, m, ($C(CH_3)_3$ and H-4); ^{13}C NMR (75 MHz) δ 173.27 ($CO_2{}^t$Bu), 163.23 (CO_2CH_3), 156.23 (C-2'), 149.21 (CN), 135.83 (C-1''), 131.27 (C-1'), 129.82 (C-6'), 129.65 (C-4''), 128.59 (C-2''), 128.29 (C-6''), 127.39 (C-5''), 127.16 (C-3''), 126.77 (C-4'), 120.71 (C-5'), 111.61 (C-3'), 79.88 ($C(CH_3)_3$), 67.74 (OCH_2), 63.84 ($NOCH_3$), 52.97 (CO_2CH_3), 35.57 (C-2), 29.96 (C-6), 29.95 (C-5), 28.44 (C-4), 28.11 ($C(CH_3)_3$), 25.30 (C-3); ESI-HRMS *m/z* calcd. for $C_{27}H_{35}NNaO_6$ $[M+Na]^+$ 492.2362, found 492.2357.

(E)-6-(2-((2-(2-Methoxy-1-(methoxyimino)-2-oxoethyl)benzyl)oxy)phenyl)hexanoic acid (Hapten **KMc**): IR (KBr) ν_{max} (cm^{-1}) 3031-2935s (broad), 2770w, 1718s, 1596w, 1493m, 1455m, 1287m, 1233s, 1203s, 1071s, 1049m, 1020s, 761m, 743m; ^{1}H NMR (300 MHz) δ 9.90 (1H, s, OH), 7.58 (1H, dd, *J* = 7.6, 1.4 Hz, H-3''), 7.45 (1H, ddd, *J* = 7.6, 7.5, 1.5 Hz, H-4''), 7.38 (1H, ddd, *J* = 7.6, 7.5, 1.4 Hz, H-5''), 7.20 (1H, dd, *J* = 7.6, 1.5, Hz, H-6''), 7.15-7.06 (2H, m, H-4' and H-6'), 6.88 (1H, ddd, *J* = 7.4, 7.4, 1.0 Hz, H-5'), 6.77 (1H, d, *J* = 8.1 Hz, H-3'), 4.95 (2H, s, OCH_2), 4.04 (3H, s, $NOCH_3$), 3.84 (3H, s, CO_2CH_3), 2.66 (2H, t, *J* = 7.5 Hz, H-6), 2.38 (2H, t, *J* =7.5 Hz, H-2), 1.72-1.56 (4H, m, H-5 and H-3), 1.45-1.33 (2H, m, H-4); ^{13}C NMR (75 MHz) δ 179.40 (CO_2H), 163.26 (CO_2CH_3), 156.24 (C-2'), 149.19 (CN), 135.78 (C-1''), 131.13 (C-1'), 129.83 (C-6'), 129.66 (C-4''), 128.62 (C-2''), 128.31 (C-6''), 127.44 (C-5''), 127.16 (C-3''), 126.83 (C-4'), 120.73 (C-5'), 111.62 (C-3'), 67.82 (OCH_2), 63.82 ($NOCH_3$), 52.97 (CO_2CH_3), 33.89 (C-2), 29.93 (C-6), 29.37 (C-5), 28.87 (C-4), 24.52 (C-3); ESI-HRMS *m/z* calcd. for $C_{23}H_{27}NNaO_6$ $[M+Na]^+$ 436.1736, found 436.1736.

2.4. Spectroscopic data of hapten KMe and intermediates of its synthesis

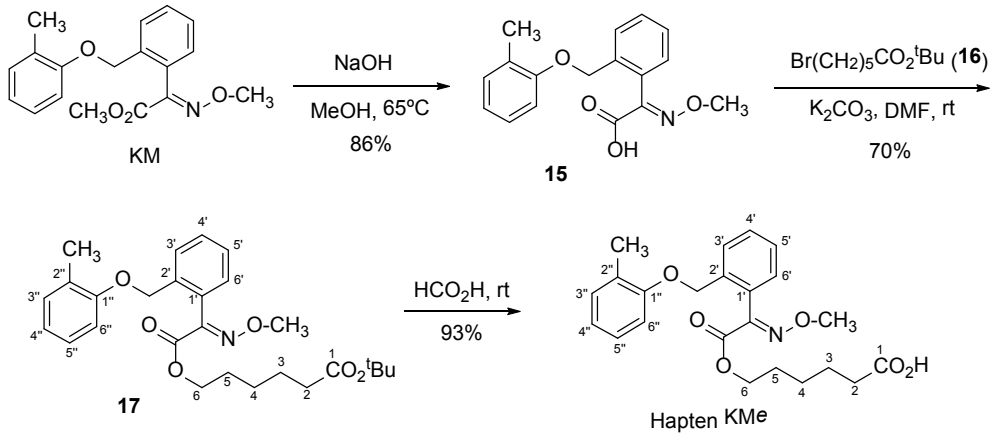


Figure S4. Synthetic sequence for the preparation of hapten KMe

(E)-tert-Butyl 6-(2-(methoxyimino)-2-(2-((o-tolyloxy)methyl)phenyl)acetoxy)hexanoate (**17**): IR (NaCl) ν_{max} (cm^{-1}) 2969m, 2938m, 2868w, 1726s, 1602w, 1586w, 1494m, 1459m, 1367m, 1313m, 1214s, 1194m, 1151s, 1123m, 1068s, 1013m, 953w, 847w, 752m; ^{1}H NMR (300 MHz) δ 7.57 (1H, d, *J* = 7.5 Hz, H-6'), 7.44 (1H, ddd, *J* = 7.5, 7.5, 1.5 Hz, H-5'), 7.37 (1H, ddd, *J* = 7.5, 7.5, 1.4 Hz, H-4'), 7.20 (1H, dd, *J* = 7.4, 1.4 Hz, H-3'), 7.12 (1H, m, H-3'' partially overlapped with the signal of H-5''), 7.10 (1H, m, H-5'' partially overlapped with the signal of H-3''), 6.85 (1H, dd, *J* = 7.5, 7.3, 0.7 HZ, H-4''), 6.76 (1H, d, *J* = 8.0 Hz, H-6''), 4.95 (2H, s, OCH_2), 4.20 (2H, t, *J* = 6.8 Hz, H-6), 4.02 (3H, s, $NOCH_3$), 2.25 (3H, s, CH_3), 2.15 (2H, t, *J* = 7.5 Hz, H-2), 1.68-1.50 (4H, m, H-5 and H-3), 1.43 (9H, s $C(CH_3)_3$), 1.29 (2H, m ,H-4); ^{13}C NMR (75 MHz) δ 172.88 (CO_2*t*Bu), 162.81 (CO_2), 156.53 (C-1''), 149.48 (CN), 135.05 (C-2'), 130.67 (C-3''), 129.49 (C-5'), 129.08 (C-1'), 128.45 (C-3'), 127.44 (C-4'), 127.42 (C-6'), 126.96 (C-2''), 126.67 (C-5''), 120.58 (C-4''), 111.14 (C-6''), 80.06 ($C(CH_3)_3$), 67.99 (OCH_2), 65.88 (C-6), 63.54 ($NOCH_3$), 35.29 (C-2), 28.11 (C-5), 28.09 ($C(CH_3)_3$), 25.22 (C-4), 24.58 (C-3), 16.30 (CH_3); MS (EI) *m/z* (%) 469 (M^+, 2), 382 (6), 362 (5), 306 (11), 282 (2), 275 (3), 223 (3), 205 (15), 192 (100), 116 (51), 97 (7), 89 (3), 73 (2), 69 (12), 57 (38); HRMS *m/z* calcd. for $C_{27}H_{35}NO_6$ 469.2464, found 469.2499.

(E)-6-(2-(Methoxyimino)-2-(2-((o-tolyloxy)methyl)phenyl)acetoxy)hexanoic acid (Hapten **KMe**): IR (NaCl) ν_{max} (cm^{-1}) 3329m (broad), 2940m, 2804w, 1709s, 1601w, 1592w, 1494m, 1462m, 1388w, 1312m, 1240s, 1195m, 1123m, 1069s, 1012m, 953w, 753m; ^{1}H NMR (300 MHz) δ 7.57 (1H, d, *J* = 7.5 Hz, H-6'), 7.44 (1H, ddd, *J* = 7.5, 7.5, 1.6 Hz, H-5'), 7.38 (1H, ddd, *J* = 7.5, 7.5, 1.4 Hz, H-4'), 7.20 (1H, dd, *J* = 7.5, 1.6 Hz, H-3'), 7.13 (1H, m, H-3'' partially overlapped with the signal of H-5''), 7.10 (1H, m, H-5'' partially overlapped with the signal of H-3''), 6.85 (1H, ddd, *J* = 7.5, 7.4, 0.8 Hz, H-4''), 6.76 (1H, d, *J* = 8.1 Hz, H-6''), 4.96 (2H, s, OCH_2), 4.21 (2H, t, *J* = 6.7 Hz, H-6), 4.03 (3H, s, $NOCH_3$), 2.29 (2H, t, *J* = 7.5 Hz, H-2), 2.25 (3H, s, CH_3), 1.72-1.52 (4H, m, H-5 and H-3), 1.33 (2H, m ,H-4); ^{13}C NMR (75 MHz) δ 179.35 (CO_2H), 162.79 (CO_2CH_2), 156.52 (C-1''), 149.47 (CN), 135.62 (C-2'), 130.67 (C-3''), 129.49 (C-5'), 129.09 (C-1'), 128.46 (C-3'), 127.46 (C-4' and C-6'), 126.95 (C-2''), 126.66 (C-5''), 120.61 (C-4''), 111.13 (C-6''), 68.03 (OCH_2), 65.73 (C-6), 63.73 ($NOCH_3$), 33.66 (C-2), 28.01 (C-5),

25.20 (C-4), 24.09 (C-3), 16.28 (CH_3); MS (EI) *m/z* (%) 413 (M^+, 1), 306 (9), 192 (53), 162 (1), 144 (1), 116 (100), 108 (8), 97 (7), 89 (7), 78 (2), 69 (26), 55 (13); HRMS *m/z* calcd. for $C_{23}H_{27}NO_6$ 413.1838, found 413.1830.

2.5. Spectroscopic data of hapten KM*o* and intermediates of its synthesis

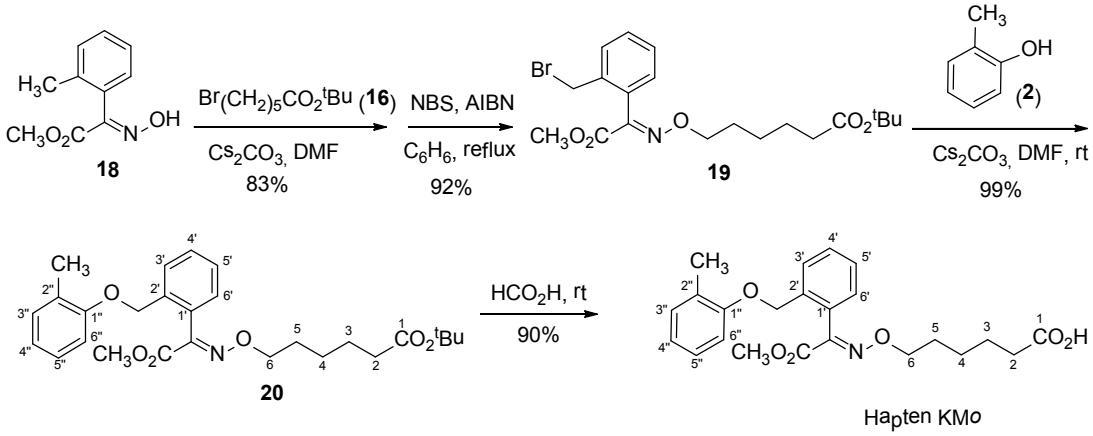


Figure S5. Synthetic sequence for the preparation of hapten KM*o*

(E)-tert-Butyl 6-(((2-methoxy-2-oxo-1-(2-((o-tolyloxy)methyl)phenyl)ethylidene)amino)oxy)hexanoate (**19**): IR (NaCl) ν_{max} (cm^{-1}) 2974w, 2935w, 1727s, 1602w, 1494w, 1460w, 1437w, 1367w, 1321w, 1241m, 1219m, 1152m, 1123s, 1068m, 1001m, 914w, 753m, 732m; ^{1}H NMR (300 MHz) δ 7.57 (1H, d, *J* = 7.4 Hz, H-6'), 7.44 (1H, ddd, *J* = 7.4, 7.4, 1.4 Hz, H-5'), 7.37 (1H, ddd, *J* = 7.5, 7.4, 1.4 Hz, H-4'), 7.19 (1H, dd, *J* = 7.5, 1.4 Hz, H-3'), 7.13 (1H, m, H-3'' partially overlapped with the signal of H-5''), 7.09 (1H, m, H-5'' partially overlapped with the signal of H-3''), 6.85 (1H, ddd, *J* = 7.3, 7.2, 0.8 HZ, H-4''), 6.75 (1H, d, *J* = 8.1 Hz, H-6''), 4.95 (2H, s, OCH_2), 4.23 (2H, t, *J* = 6.8 Hz, H-6), 3.81 (3H, s, CO_2CH_3), 2.25 (3H,s, CH_3), 2.14 (2H, t, *J* = 7.5 Hz, H-2), 1.66 (2H, quint, *J* = 7.0 Hz, H-5), 1.53 (2H, quint, *J* = 7.3 Hz, H-3), 1.42 (9H, s, $C(CH_3)_3$), 1.27 (2H, m, H-4); ^{13}C NMR (75 MHz) δ 172.88 (CO_2*t*Bu), 163.39 (CO_2CH_3), 156.51 (C-1''), 148.96 (CN), 135.65 (C-2'), 130.65 (C-3''), 129.44 (C-5'), 129.10 (C-1'), 128.45 (C-3'), 127.44 (C-4'), 127.38 (C-6'), 126.88 (C-2''), 126.65 (C-5''), 120.56 (C-4''), 111.08 (C-6''), 79.99 ($C(CH_3)_3$), 76.21 (C-6), 67.99 (OCH_2), 52.87 (CO_2CH_3), 35.28 (C-2), 28.69 (C-5), 28.06 ($C(CH_3)_3$), 25.11 (C-4), 24.69 (C-3), 16.24 (CH_3); MS (EI) *m/z* (%) 469 (M^+, 5), 382 (10), 360 (5), 306 (12), 282 (2), 275 (5), 223 (3), 205 (15), 192 (100), 116 (51), 97 (8), 89 (3), 73 (2), 69 (12), 57 (39); HRMS *m/z* calcd. for $C_{27}H_{35}NO_6$ 469.2464, found 469.2499.

(E)-6-(((2-Methoxy-2-oxo-1-(2-((o-tolyloxy)methyl)phenyl)ethylidene)amino)oxy)hexanoic acid (Hapten **KMo**): IR (NaCl) ν_{max} (cm^{-1}) 3424m (broad), 3026m, 2948m, 2853m, 1733s, 1708s, 1602w, 1590w, 1495s, 1437m, 1310m, 1241s, 1215s, 1123m, 1069s, 1001s, 781d,754s; ^{1}H NMR (300 MHz) δ 7.57 (1H, d, *J* = 7.5 Hz, H-6'), 7.44 (1H, ddd, *J* = 7.5, 7.5, 1.5 Hz, H-5'), 7.37 (1H, ddd, *J* = 7.5, 7.5, 1.4 Hz, H-4'), 7.19 (1H, dd, *J* = 7.5, 1.5 Hz, H-3'), 7.12 (1H, m, H-3'' partially overlapped with the signal of H-5''), 7.10 (1H, m, H-5'' partially overlapped with the signal of H-3''), 6.84 (1H, ddd, *J* = 7.3, 7.2, 0.8 Hz, H-4''), 6.76 (1H, d, *J* = 8.1 Hz, H-6''), 4.95 (2H, s, OCH_2),

4.24 (2H, t, *J* = 6.8 Hz, H-6), 3.81 (3H, s, CO_2CH_3), 2.27 (2H, m, H-2 partially overlapped with the signal of CH_3), 2.25 (3H, s, CH_3 partially overlapped with the signal of H-2), 1.66 (2H, quint, *J* = 7.3 Hz, H-5), 1.57 (2H, quint, *J* = 7.4 Hz, H-3), 1.30 (2H, m ,H-4); ^{13}C NMR (75 MHz) δ 179.35 (CO_2H), 163.39 (CO_2CH_3), 156.51 (C-1''), 149.07 (CN), 135.64 (C-2'), 130.08 (C-3''), 129.46 (C-5'), 129.08 (C-1'), 128.45 (C-3'), 127.46 (C-4'), 127.42 (C-6'), 126.90 (C-2''), 126.65 (C-5''), 120.60 (C-4''), 111.08 (C-6''), 76.06 (C-6), 68.02 (OCH_2), 52.90 (CO_2CH_3), 33.66 (C-2), 28.59 (C-5), 25.11 (C-4), 24.21 (C-3), 16.24 (CH_3); MS (EI) *m/z* (%) 413 (M^+, 2), 306 (32), 223 (2), 192 (20), 184 (1), 175 (3), 160 (10), 148 (27), 143 (2), 132 (6), 116 (100), 107 (4), 97 (6), 89 (7), 77 (7), 69 (16), 58 (13); HRMS *m/z* calcd. for $C_{23}H_{27}NO_6$ 413.1838, found 413.1820.

Table S1

IC_{50} values (nM) for kresoxim-methyl obtained by indirect assay using monoclonal antibodies.[a]

	OVA conjugate[b]				
mAb	KM*a*	KM*b*	KM*c*	KM*e*	KM*o*
KM*a*#26	41.08	–[c]	–	–	–
KM*a*#29	95.34	–	–	–	–
KM*b*#19	–	17.42	–	–	–
KM*b*#32	1.15	0.80	1.35	1.90	2.38
KM*b*#44	–	3.78	2.07	–	2.25
KM*b*#322	1.57	1.52	0.60	2.66	1.77
KM*c*#35	–	–	27.08	–	–
KM*e*#14	–	–	–	3.24	5.80
KM*e*#18	–	–	–	5.51	3.91
KM*e*#26	–	–	–	2.78	2.19
KM*e*#211	–	–	–	3.11	2.15
KM*o*#111	–	–	–	4.34	3.02
KM*o*#114	3.29	6.20	–	–	6.18
KM*o*#117	–	–	–	1.76	1.61

[a] Values are the mean of three independent determinations and correspond to an inhibition curve with an A_{max} value between 0.8 and 1.5. [b] OVA conjugate concentration was either 100 or 1000 ng mL^{-1}. [c] A_{max} values were lower than 0.8 at 300 ng mL^{-1} mAb concentration and the highest coating concentration.

Assay protocol

Microplates were coated by overnight incubation with 100 µL per well of OVA conjugate solution in CB. All incubation steps were performed at room temperature with sealed plates, and after each step, microwells were washed four times with washing solution. The competitive step was carried out during 1 h with 50 µL per well of analyte solution in PBS plus 50 µL per well of antibody dilution in PBST. Retained antibodies were amplified by incubation during 1 h with 100 µL per well of rabbit anti-mouse immunoglobulins polyclonal antibody–peroxidase conjugate diluted 1/2000 in PBST. Signal was produced with 100 µL per well of freshly prepared enzyme substrate solution. After 10 min at room temperature, enzyme activity was stopped upon adding 100 µL per well of 2.5 M sulfuric acid. Absorbances were immediately read at 492 nm with a reference wavelength at 650 nm.

4. Copies of ^{1}H NMR spectra of activated haptens