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Supporting Information

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Sandwich Immunoassay as a New High-Throughput Screening Method for Coupling reactions.

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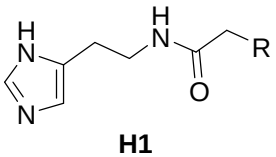
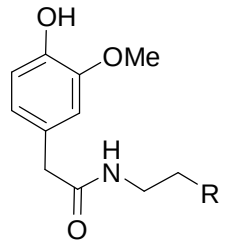
1. Hapten identity and antibody properties.

The antibodies used in the screening method were raised against hapten **H1** for anti-tag 1 mAbs and against hapten **H2** for anti-tag 2 mAbs (Scheme S1).



H1



H2

Scheme S1. Structures of Haptens **H1** and **H2**. R = linker for carrier protein attachment (R = SH for **H1** and R = NH_2 for **H2**).

The monoclonal antibodies raised against hapten **H1** display good affinities (10^{-7} M > Kd > 10^{-9} M) for a broad range of molecules bearing the **H1** moiety (cross-reactivity > 10% for R = alkyl, aryl, heteroaryl…) but are highly specific toward the imidazole part of the hapten (H. Volland, PhD, **1999**, Paris VI University).

The monoclonal antibodies raised against hapten **H2** display moderate to good affinities (10^{-7} M > Kd > 10^{-5} M) for a broad range of molecules bearing the **H2** moiety (cross-reactivity > 10% for R = alkyl, aryl, heteroaryl…) but are highly specific toward the guaiacol part of the hapten.

2. Development of the sandwich immunoassay.

Several combinations of mAbs were tested to find the most accurate immunological sandwich for the detection of product **3**. While the immobilisation of **3** *via* anti-tag 2 mAbs and detection by addition of anti-tag 1 mAbs gave poor results (Figure A), the reverse strategy highlighted several efficient sandwich immunoassays Figure B). Among them, the combination of mAb 203 for the capture of **3** and mAb 46 for detection was found to be the most interesting.

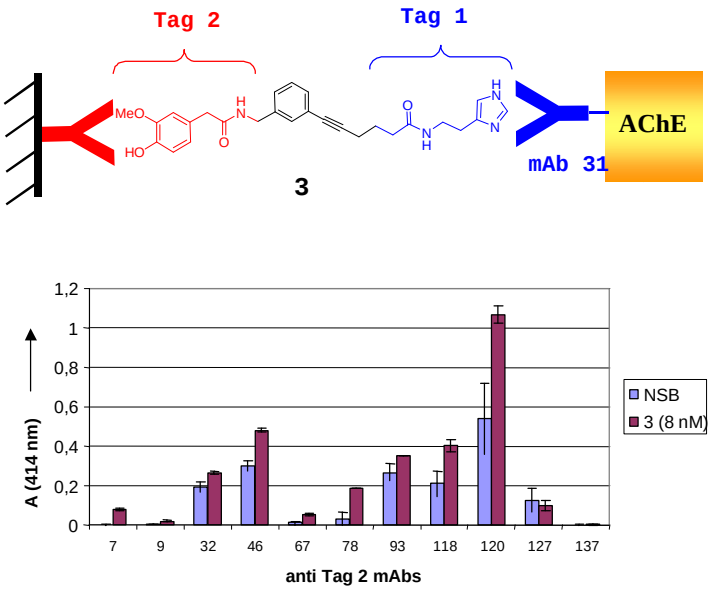


Figure A. Sandwich immunoassay of compound **3** assayed at 8 nM. Conditions = capture: incubation of 100 µL of **3** (8 nM) in a plate previously coated with anti-Tag 2 mAbs for 14 hours at 4°C; staining: after washing, 100 µL of the enzymatic tracer anti-Tag 1 mAb 31-AChE conjugate were added to each well of the plate, and absorbance was measured (after addition of the Ellman reagent) after 15 min at 414 nm. NSB = non-specific binding.

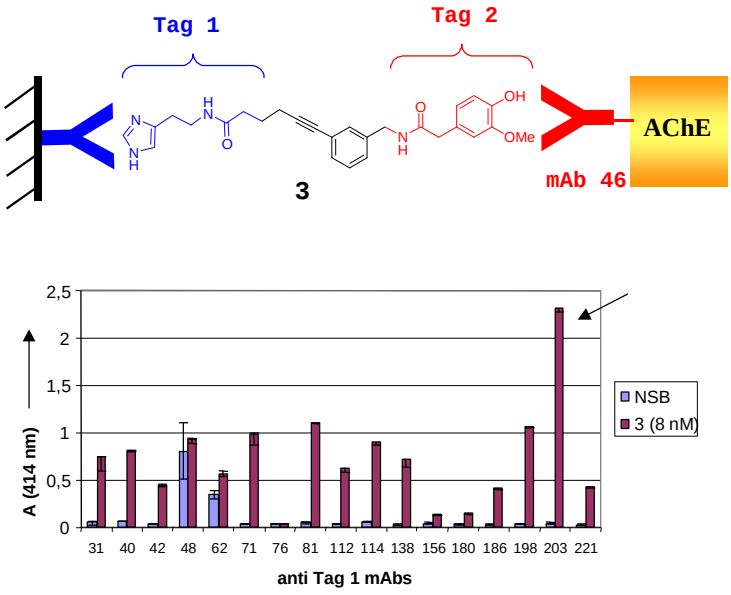
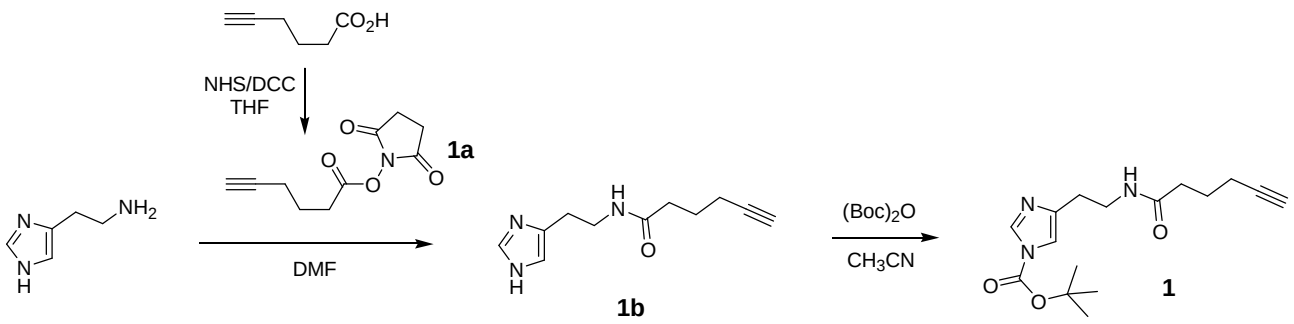


Figure B. Sandwich immunoassay of compound **3** assayed at 8 nM. Conditions = capture: incubation of 100 µL of **3** (8 nM) in a plate previously coated with anti-Tag 1 mAbs for 14 hours at 4°C; staining: after washing, 100 µL of the enzymatic tracer anti-Tag 2 mAb 46-AChE conjugate were added to each well of the plate, and absorbance was measured (after addition of the Ellman reagent) after 15 min at 414 nm. NSB = non-specific binding.

3. Substrate and product synthesis

Substrate **1**:



Hex-5-ynoic acid 2,5-dioxo-pyrrolidin-1-yl ester 1a
At room temperature, with stirring and under argon, *N*-hydroxysuccinimide (2.25 g, 18.96 mmols) and *N,N'*-dicyclohexylcarbodiimide (1 eq, 3.91 g) were added to a solution of hex-5-ynoic acid (2.24 g, 1 eq) in dry THF. After 10 hours, the crude reaction mixture was filtered, evaporated to dryness and purified by flash chromatography (AcOEt/hexane 50/50, R*f* 0.4) to yield the target compound as a white solid (2.89 g, 73%).
^{1}H NMR (300 MHz, CD_3COCD_3): δppm 2.90 (s, 4H), 2.80 (t, J=7.3 Hz, 2H), 2.46 (t, J=2.5 Hz, 1H), 2.28 (dt, J=2.5, 7.3 Hz, 2H), 1.94 (quint, J=7.3 Hz, 2H); ^{13}C NMR (100 MHz, CD_3COCD_3): δppm 169.5 (2C), 168.3, 82.5, 70.0, 25.3 (2C), 25.0, 23.6, 16.9; MS (ESI) *m/z*: 232 (M+23); IR (NaCl): 3505, 3285, 2947, 2116, 1814, 1783, 1737, 1431 cm^{-1}.

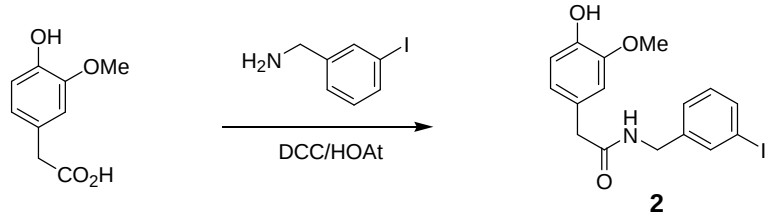
Hex-5-ynoic acid [2-(1H-imidazol-4-yl)-ethyl]-amide 1b
At room temperature, with stirring and under argon, the activated ester **1a** (2.79 g, 1 eq) was added to a solution of histamine in dry DMF (1.53 g, 13.35 mmols, 1 eq). After 20 hours, the reaction mixture was evaporated to yield an orange oil. The target compound was isolated as a white solid (2.65 g, 97%) after flash chromatography (CH_2Cl_2/MeOH 96/4, R*f* 0.2).
^{1}H NMR (400 MHz, CD_3OD): δppm 7.58 (s, 1H), 6.83 (s, 1H), 3.42 (t, J=7.2 Hz, 2H), 2.77 (t, J=7.2 Hz, 2H), 2.28 (t, J=7.2 Hz, 2H), 2.24 (d, J=2.4 Hz, 1H), 2.17 (dt, J=7.2, 2.4 Hz, 2H), 1.78 (quint, J=7.2Hz, 2H); ^{13}C NMR (CD_3OD, 100 MHz): δppm 174.0, 134.5, 134.2, 116.5, 82.7, 68.7, 38.8, 34.3, 26.2, 24.8, 24.5;MS (ESI) *m/z*: 411 (2M+1), 228 (M+23), 206 (M+1); IR: 3247, 3223, 3140, 2943, 2303, 2242, 1782, 1702, 1680, 1647, 1553 cm^{-1}.

4-(2-Hex-5-ynoylamino-ethyl)-imidazole-1-carboxylic acid tert-butyl ester 1
Compound **1b** (1.82 g, 8.89 mmols, 1 eq) was dissolved in dry acetonitrile under argon, at room temperature. DMAP (110 mg, 0.1 eq), triethylamine (1.25 mL, 1e q) and di-*tert*-butyl dicarbonate (2.35 g, 1.2 eq) were added. After 18 hours, the crude was evaporated and purified by flash chromatography (CH_2Cl_2/MeOH 98/2, R*f* 0.11) to yield the target compound as a colourless oil (1.60 g, 55%).
^{1}H NMR (300 MHz, CD_3COCD_3): δppm 8.02 (s, 1H), 7.26 (s, 1H), 7.16 (bs, 1H), 4.47 (dd, J=7.3, 12.8 Hz, 2H), 2.70 (t, J=7.3 Hz, 2H), 2.35 (m, 1H), 2.23 (m, 4H), 1.78 (quint, J=7.3 Hz, 2H), 1.63 (s, 9H); ^{13}C NMR (75 MHz, CD_3COCD_3): δppm 171.3, 169.9, 147.0, 141.4, 136.5, 84.8, 83.5, 69.2, 38.3, 34.4, 28.0, 27.0

(3C), 24.5, 17.4; MS ESI *m/z*: 633 (2M+23), 328 (M+23), 306 (M+1), 250, 206; IR (NaCl): 3293, 2979, 2117, 1754, 164, 1550 cm^{-1}.

Substrate **2**:

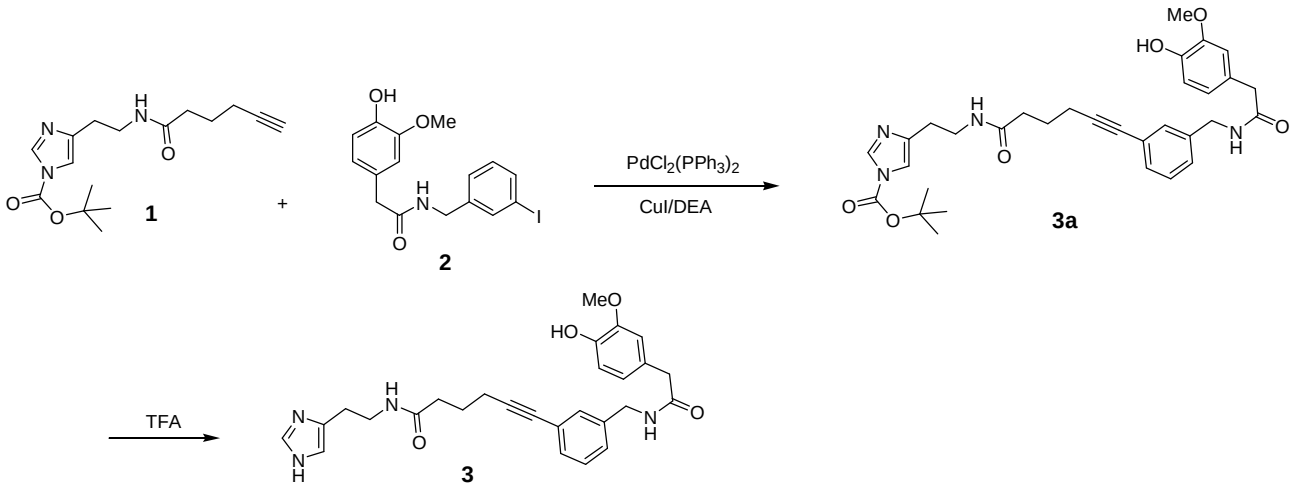
2-(4-Hydroxy-3-methoxy-phenyl)-N-(3-iodo-benzyl)-acetamide 2



2

Under argon, at room temperature, iodobenzylamine hydrochloride (3.87 g, 14.36 mmol, 1 eq) was dissolved in dry THF. *N,N'*-Dicyclohexylcarbodiimide (2.93 g, 1 eq) and 1-hydroxy-7-aza-benzotriazole (195.5 mg, 0.1 eq), triethylamine (6 mL, 3 eq) were added in sequence to give a yellow solution. Homovanillic acid (2.67 g, 1 eq) dissolved in dry THF was then added drop by drop and the mixture was stirred at room temperature overnight. After evaporation of the solvent, substrate **2** was obtained as a white solid (5.02 g, 88%) after flash chromatography (AcOEt/hexane 60/40, R*f*= 0.3).
^{1}H NMR (300 MHz, CD_3COCD_3): δppm 7.61 (m, 3H), 7.29 (d, J=7.3 Hz, 1H), 7.11 (t, J=7.3 Hz, 1H), 6.94 (s, 1H), 6.77 (s, 2H), 4.36 (s, 2H), 3.83 (s, 3H), 3.48 (s, 2H); ^{13}C NMR (100 MHz, CD_3COCD_3): δppm 171.3, 148.0, 146.1, 143.3, 136.9, 136.36, 130.9, 127.9, 127.4, 122.3, 115.5, 113.2, 94.3, 56.0, 43.2, 42.5; MS (ESI) *m/z*: 817 (2M+23), 436 (M+39), 420 (M+23); IR (NaCl): 3507, 3288, 3077, 1647, 1514 cm^{-1}.

Product **3**:



5-{2-[6-(3-{[2-(4-Hydroxy-3-methoxy-phenyl)-acetylamino]-methyl}-phenyl)-hex-5-ynoylamino]-ethyl}-imidazole-1-carboxylic acid tert-butyl ester 3a
Under argon, a round-bottom flask was charged with **2** (556.8 mg, 51,40 mmols, 1 eq) and $PdCl_2(PPh_3)_2$ (30 mg, 0.03 eq). A 0.0168 M solution of CuI (3.4 mL, 0.04 eq) in Et_2NH was added to the flask giving a brown solution. Alkyne **1** (1.14 eq, 803.6 mg) dissolved in Et_2NH was then added and the resulting mixture was stirred for 20 h at room temperature. The crude solution was evaporated to dryness and subjected to flash chromatography (AcOEt/acetone

60/40, *Rf* = 0.24), yielding the target compound as a brown oil (27.2 mg, 28%).
^{1}H NMR (300 MHz, CD_3COCD_3): δppm 8.03 (s, 1H), 7.68 (bs, 1H), 7.54 (bs, 1H), 7.24 (m, 5H), 6.96 (s, 1H), 6.78 (s, 2H), 4.38 (s, 2H), 3.82 (s, 3H), 3.50 (t, J=6.7 Hz, 2H), 3.48 (s, 2H), 2.73 (t, J=6.7 Hz, 2H), 2.45 (t, J=7.3 Hz, 2H), 2.33 (t, J=7.3 Hz, 2H), 1.88 (quint, J=7.3 Hz, 2H), 1.63 (s, 9H); ^{13}C NMR (100 MHz, CD_3COCD_3): δppm 171.9, 171.0, 147.4, 147.0, 145.4, 141.29, 140.0, 136.6, 130.2, 129.7, 128.2, 127.3, 126.7, 123.9, 121.6, 115.0, 113.5, 112.6, 89.4, 84.9, 80.7, 55.3, 42.6, 42.2, 38.4, 34.6, 27.9, 27.0 (3C), 24.7, 18.4; MS (ESI) *m/z*: 597 (M+23), 575 (M+1), 475; IR (NaCl): 3291, 2939, 2830, 2230, 1759, 1651, 1602, 1515 cm^{-1}.

6-(3-{[2-(4-Hydroxy-3-methoxy-phenyl)-acetylamino]-methyl}-phenyl)-hex-5-ynoic acid [2-(3H-imidazol-4-yl)-ethyl]-amide 3
Compound **3a** (0.18 mmol, 101.3 mg, 1 eq) was dissolved at room temperature in CH_2Cl_2. CF_3COOH (1,5 mL, 108 eq) was added and the solution was stirred for 1 h at room temperature. The crude solution was then evaporated to dryness, yielding the target compound as a brown oil in quantitative yield.
^{1}H NMR (400 MHz, CD_3COCD_3): δppm 8.74 (s, 1H), 7.97 (bs, 1H), 7.75 (bs, 1H), 7.33 (s, 1H), 7.25 (s, 1H), 7.20 (s, 3H), 6.91 (s, 1H), 6.74 (s,2H), 4.37 (d, J=6.0 Hz, 2H), 3.76 (s, 3H), 3.54 (t, J=6.2 Hz, 2H); 3.52 (s, 2H), 2.94 (t, J=6.2 Hz, 2H), 2.39 (t, J=7.2 Hz, 2H), 2.33 (t, J=7.2 Hz, 2H), 1.82 (quint, J=7.2 Hz, 2H); ^{13}C NMR (100 MHz, CD_3COCD_3): δppm 172.8, 171.9, 147.4, 145.5, 139.7, 133.5, 131.8, 130.2, 129.7, 128.3, 127.0, 126.7, 124.9, 121.7, 116.3, 115.0, 112.6, 89.3, 80.8, 55.2, 42.4, 37.8, 34.4, 29.6, 24.6, 24.5, 18.3; MS (ESI) *m/z*: 497 (M+23), 475 (M+1); IR (NaCl): 3291, 2232, 1956, 1784, 1677, 1552, 1519 cm^{-1}.

4. HPLC separation.

HPLC separations were carried out using a Waters apparatus on a xterra C18 column. Gradient elution at a flow-rate of 1 mL/min was achieved with acetonitrile-water containing 0.1% of formic acid as eluting solvent (from 5/95 to 0/100 V/V in 8 min).

5. Scope of the heterogeneous catalytic system highlighted by the screening.

To demonstrate the usefulness of the heterogeneous catalytic system highlighted by the screening method, a variety of alkynes and aryl halides were tested (Table 1). Typical procedure use 1 mg of Pd/C for 1 mL of solvent.
The reactions were carried out in a round-bottom flask on a 0.5 to 5 mmol scale under the following conditions: aryl halide 1 eq; alkyne 1.2 to 1.5 eq; Pd/C 30% w/w (3% w/w of Pd); CuBr-Me_2S 10% w/w; tetramethylguanidine 1.5 to 4 eq; PPh_3 0.15 eq; CH_3CN/H_2O 9/1.

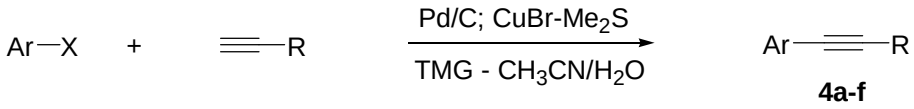
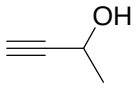
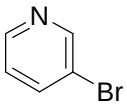
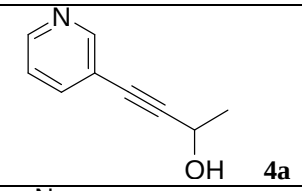
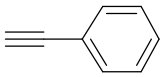
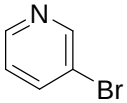
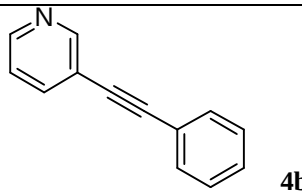
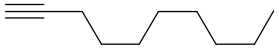
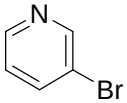
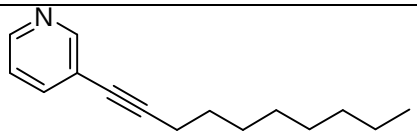
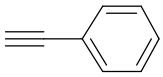
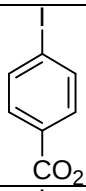
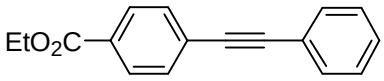
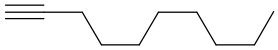
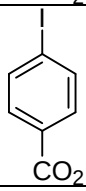
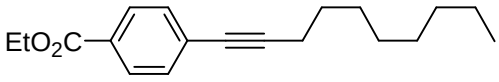
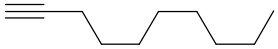
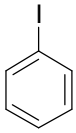
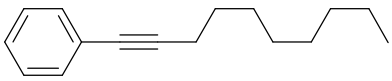


Table 1.

Alkyne	Aryl Halide	Product	Yields
		 **4a**	80%
		 **4b**	85%
		 **4c**	68%
		 **4d**	95%
		 **4e**	72%
		 **4f**	94%

4-Pyridin-3-yl-but-3-yn-2-ol 4a

^{1}H NMR (400 MHz, CD_3OD): δppm 8.55 (s, 1H), 8.46 (d, J=4.6Hz, 1H), 7.81 (d, J=7.4 Hz, 1H), 7.37 (dd, J=4.6, 7.4 Hz, 1H), 4.71 (q, J=6.4 Hz, 1H), 1.49 (d, J=6.4 Hz, 3H); ^{13}C NMR (100 MHz, CD_3OD): δppm 151.0, 147.8, 139.1, 123.5, 120.6, 95.2, 78.8, 57.4, 23.1; MS (ESI) *m/z*:148 (M+1); IR (NaCl): 3253, 2982, 2233, 1736, 1589, 1566, 1478, 1509 cm^{-1}.

3-Phenylethynyl-pyridine 4b

^{1}H NMR (400MHz, $CDCl_3$): δppm 8.78 (s, 1H), 8.55 (d, J=3.2 Hz, 1H), 7.81 (dt, J=1.6, 3.2 Hz, 1H), 7.55 (m, 2H), 7.37 (m, 3H), 7.28 (dd, J=4.8, 7.6 Hz, 1H); ^{13}C NMR (100MHz, $CDCl_3$): δppm 152.2, 148.5, 138.3, 131.6 (2C), 128.8, 128.4 (2C), 123.0, 122.5, 120.4, 92.6, 85.9; MS (ESI) *m/z*: 180 (M+1); IR (KBr): 2217, 1560, 1487, 1411 cm^{-1}.

4-Phenylethynyl-benzoic acid ethyl ester 4c

^{1}H NMR (400 MHz, $CDCl_3$): δppm 8.60 (s, 1H), 8.45 (d, J = 4.5 Hz, 1H), 7.63 (d, J=7.6 Hz, 1H), 7.18 (dd, J=7.6, 4.5 Hz, 1H), 2.39 (t, J= 7.2 Hz, 2H), 1.59 (quint, J= 7.2 Hz, 2H), 1.42 (bs, 2H), 1.27 (bs, 8H), 0.86 (t, J= 6.4 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δppm 152.3, 147.8, 138.3, 122.8, 121.1, 94.0, 77.3, 31.7, 29.1, 29.0, 28.8, 28.5, 22.5, 19.3, 14.0; IR (NaCl): 2954, 2928, 2856, 2236, 1560, 1475 cm^{-1}; MS (ESI) *m/z*: 216 (M+1).

4-Phenylethynyl-benzoic acid ethyl ester 4d

^{1}H NMR (400 MHz, $CDCl_3$): δppm 8.03 (d, J=8.4 Hz, 2H), 7.59 (d, J=8.4 Hz, 2H), 7.55 (m, 2H), 7.37 (m, 3H), 4.39 (q, J=7.2 Hz, 2H), 1.41 (t, J=7.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δppm 166.0, 131.7 (2C), 131.4 (2C), 129.8, 129.4 (2C), 128.7, 128.4 (2C), 127.8, 122.7, 92.2, 88.6, 61.1,14.1; IR (KBr): 2213, 1703, 1604, 1276 cm^{-1}; MS (EI 70eV) *m/z*:250 (M), 222, 205,176.

4-Dec-1-ynyl-benzoic acid ethyl ester 4e

^{1}H NMR (400 MHz, $CDCl_3$): δppm 7.95 (d, J=7.6 Hz, 2H), 7.43 (d, J=7.6 Hz, 2H), 4.36 (q, J=7.2 Hz, 2H), 2.42 (t, J=7.2 Hz, 2H), 1.61 (quintet, J=7.2 Hz, 2H), 1.45 (m, 2H), 1.39 (t, J=7.2 Hz, 3H), 1.29 (m, 8H), 0.89 (t, J=6.4 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δppm 166.1, 131.3 (2C), 129.3 (2C), 129.1, 128.8, 93.8, 80.1, 60.9, 31.8, 29.1, 29.0, 28.9, 28.5, 22.6, 19.4, 14.2, 14.0; IR (NaCl): 2956, 2928, 2856, 2234, 1721, 1607 $cm^{-1;}$ MS (ESI) *m/z*: 287 (M+1).

Dec-1-ynyl-benzene 4f

^{1}H NMR (400 MHz, $CDCl_3$): δppm 7.29 (m, 2H), 7.15 (m, 3H), 2.28 (t, J=7.2, 2H), 1.49 (quintet, J=7.2 Hz, 2H), 1.34 (m, 2H), 1.19 (m, 8H), 0.78 (t, J=7.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δppm 131.3 (2C), 128.0 (2C), 127.2, 124.0, 90.3, 80.4, 31.7, 29.04, 29.0, 28.8, 28.6, 22.5, 19.2, 13.9; IR (NaCl): 2955, 2928, 2856, 2235, 1598, 1490 cm^{-1}; MS (EI 70eV) *m/z*: 214 (M), 171, 157, 143, 129, 117, 102, 91.