Click reaction-aided enzymatic kinetic resolution of secondary alcohols

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Supplementary material

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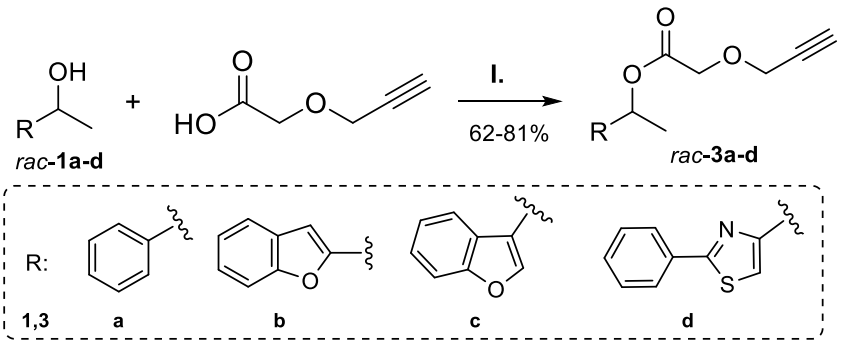
1. Chemical synthesis and spectral characterization of compounds

1.1. Chemical synthesis of racemic (hetero)aryl-propynyloxy-acetates *rac*-3a-d [1]

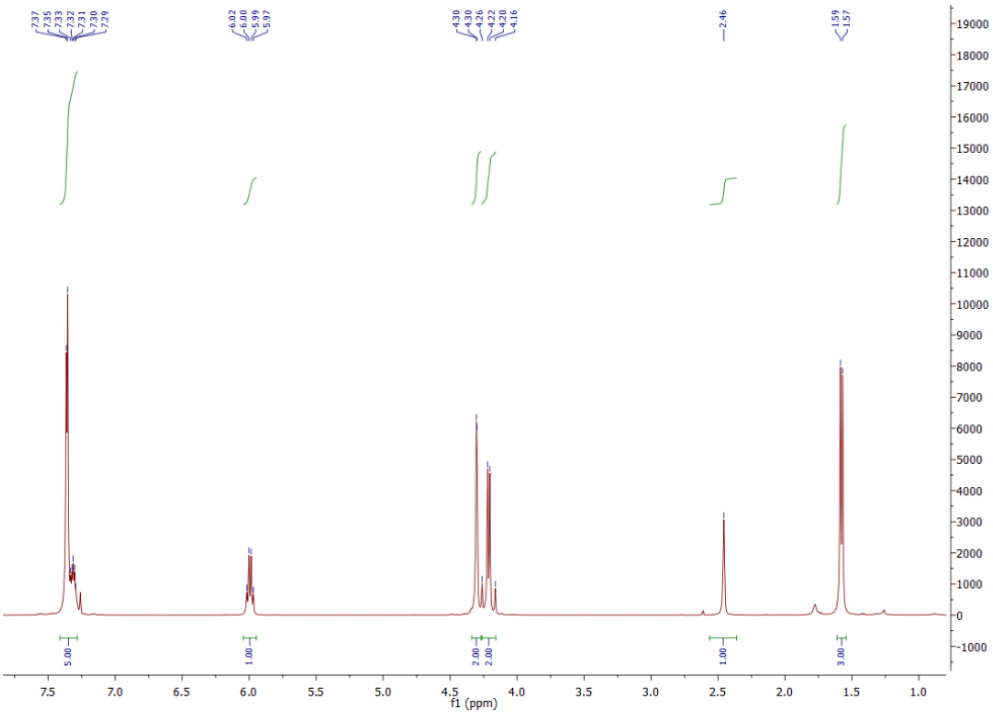


Scheme S1. Chemical esterification of *rac*-**1a-d** with 2-(prop-2-yn-1-yloxy)acetic acid*.*
Reagents and reaction conditions: **I.** DCC, DMAP, dry CH_2Cl_2, rt, 3 h

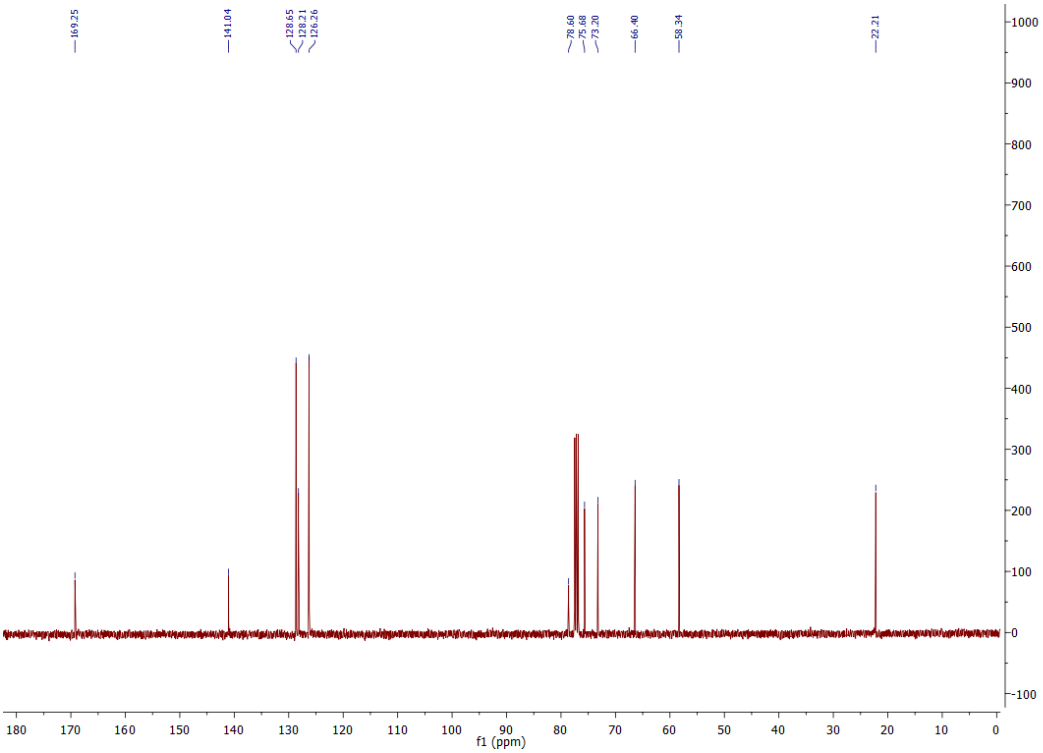
In a dry round-bottom flask one of the racemic alcohols (*rac*-**1a-d**, 1.5 mmol) and 2-(prop-2-yn-1-yloxy)acetic acid (1.1 equiv., 1.65 mmol, 188 mg) were dissolved in dry CH_2Cl_2 (20 mL) and the mixture was cooled to 0 °C. Next, DMAP (0.1 equiv., 18 mg) and DCC (1.1 equiv., 1.8 mmol, 370 mg) were added and the mixture was stirred for 10 minutes at 0 °C and then for 3 h at room temperature. After the completion of the reaction (monitored by TLC using CH_2Cl_2 as eluent), the formed DCU was filtered off and extractions were performed, first with 10% HCl (3 × 15 mL), then with 2M Na_2CO_3 (20 mL) and finally with distilled water (20 mL). The collected organic phases were dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The products (light yellow viscous liquids) were purified on silica gel column chromatography using CH_2Cl_2 as eluent. Yields: 62-81%.

(±)-1-Phenylethyl 2-(prop-2-yn-1-yloxy)acetate (*rac*-3a): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37 - 7.29 (m, 5H), 5.99 (q, *J* = 6.6 Hz, 1H), 4.30 (overlapped dd, 2H), 4.21 (d, *J* = 6.9 Hz, 2H) 2.46 (overlapped dd, 1H), 1.58 (d, *J* = 6.6 Hz, 3H) ppm; ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.25, 141.04, 128.65, 128.21, 126.26, 78.60, 75.68, 73.20, 66.40, 58.34, 22.21 ppm. HRMS (ESI$^+$) m/z: $C_{13}H_{14}O_3$ [M+Na$^+$] calc. 241.0835, found 241.0843.

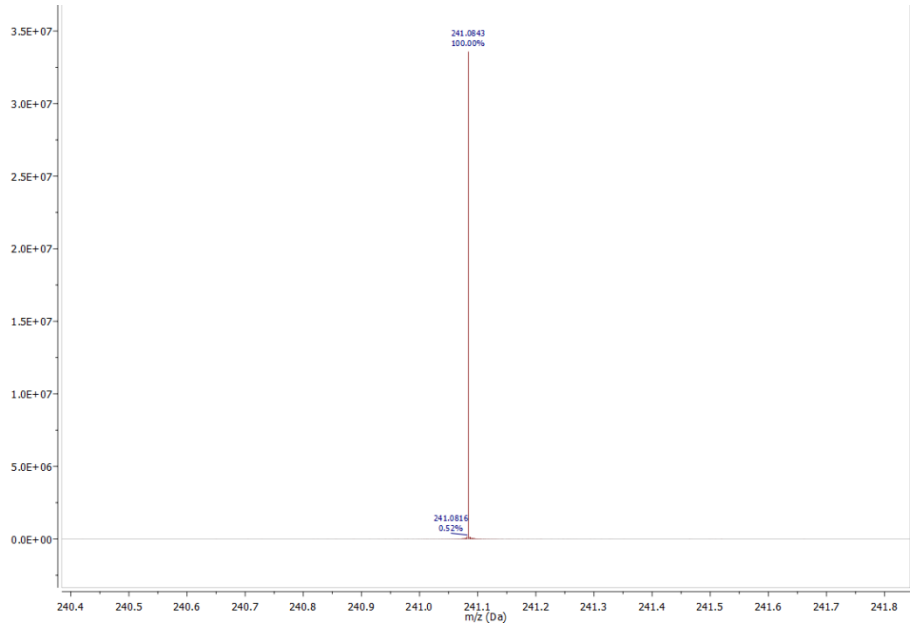
^{1}H NMR: *rac*-**3a** (400 MHz, $CDCl_3$)



^{13}C NMR: *rac*-**3a** (101 MHz, $CDCl_3$)

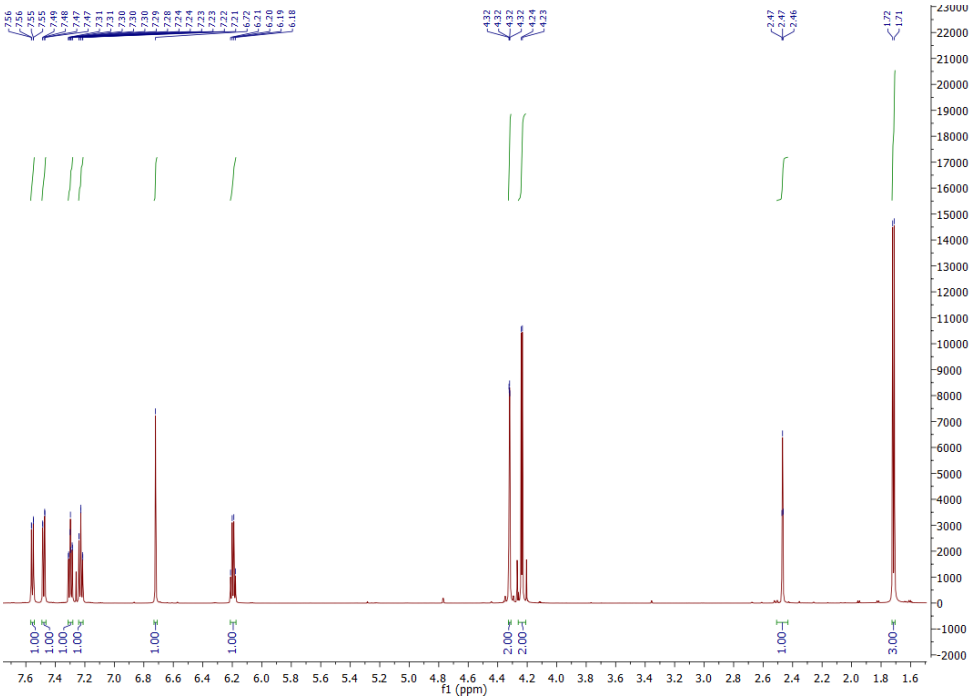


HRMS (ESI$^+$): *rac*-**3a**

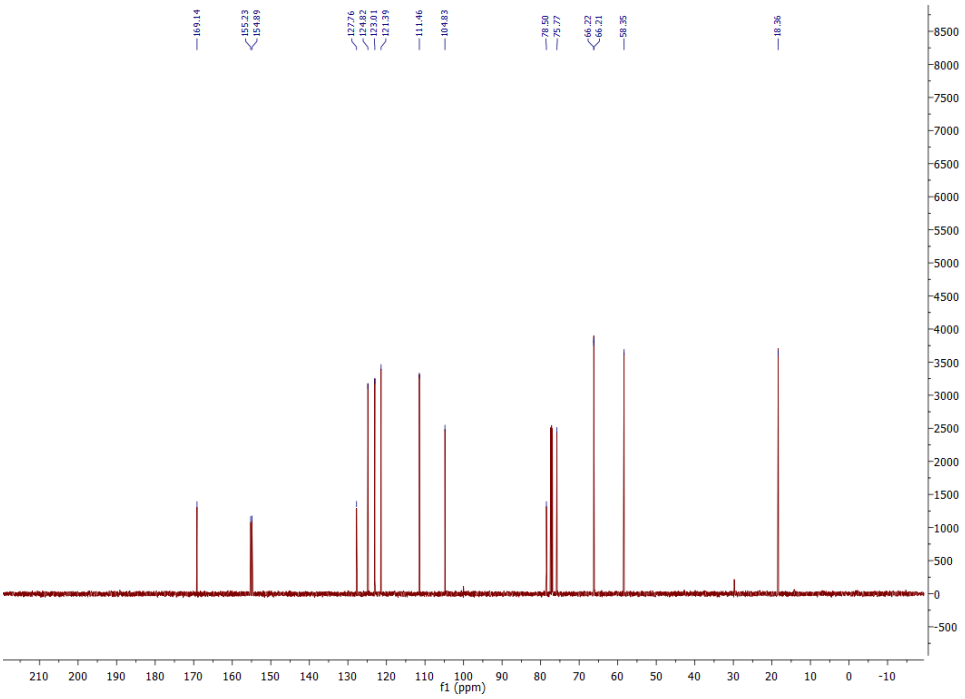


(±)-1-(Benzofuran-2-yl)ethyl 2-(prop-2-yn-1-yloxy)acetate (*rac*-3b): ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.58 – 7.52 (m, 1H), 7.51 – 7.44 (m, 1H), 7.33 – 7.27 (m, 1H), 7.24 -7.21 (m, 1H), 6.72 (s, 1H), 6.20 (q, *J* = 6.7 Hz, 1H), 4.32 (dd, *J* = 2.4, 0.8 Hz, 2H), 4.24 (d, *J* = 4.9, 2H), 2.47 (overlapped dd, 1H), 1.72 (d, *J* = 6.7 Hz, 3H) ppm; ^{13}C NMR (151 MHz, $CDCl_3$) δ 169.14, 155.23, 154.89, 127.76, 124,82, 123.01, 121.39, 111.46, 104.83, 78.50, 75.77, 66.22, 66.21, 58.35, 18.36 ppm. HRMS (ESI$^+$) m/z: $C_{15}H_{14}O_4$ [M+Na$^+$] calc. 281.0784, found 281.0794.

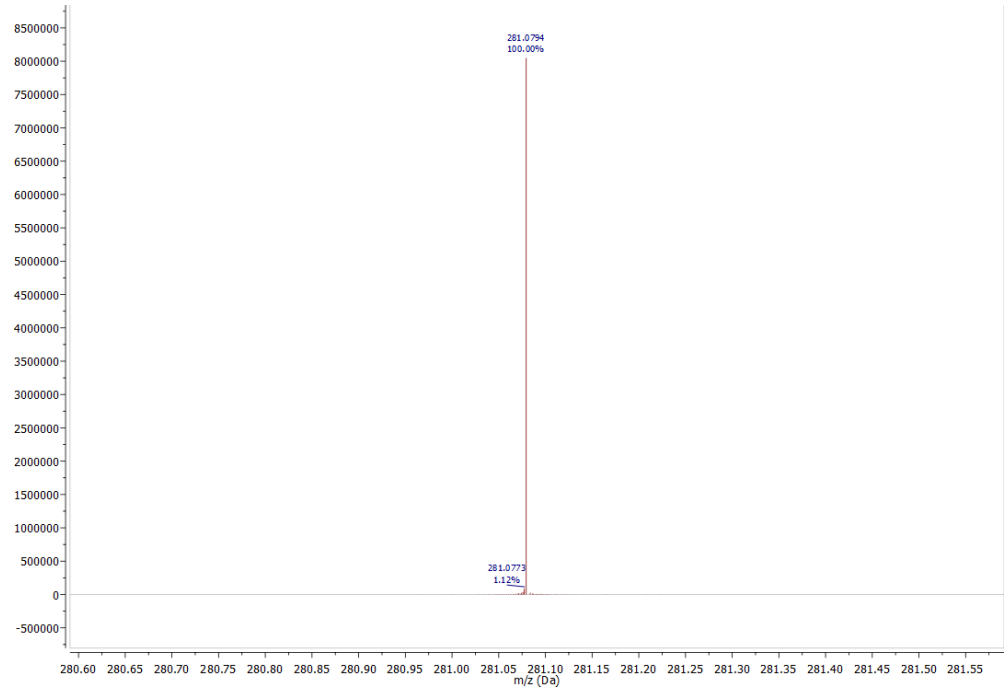
^{1}H NMR: *rac*-**3b** (600 MHz, $CDCl_3$)



^{13}C NMR: *rac*-**3b** (151 MHz, $CDCl_3$)

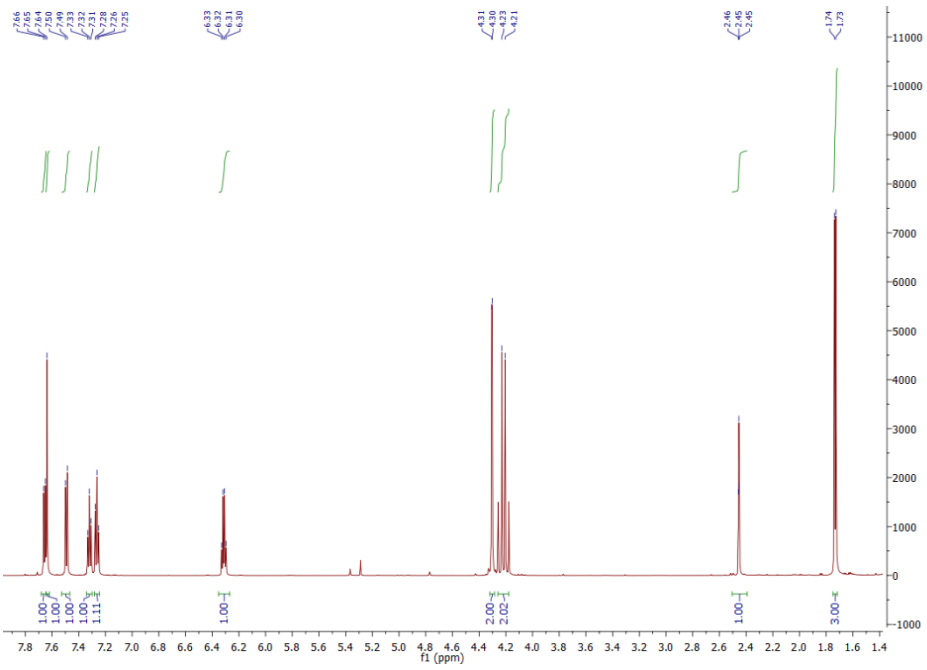


HRMS (ESI$^+$): *rac*-**3b**

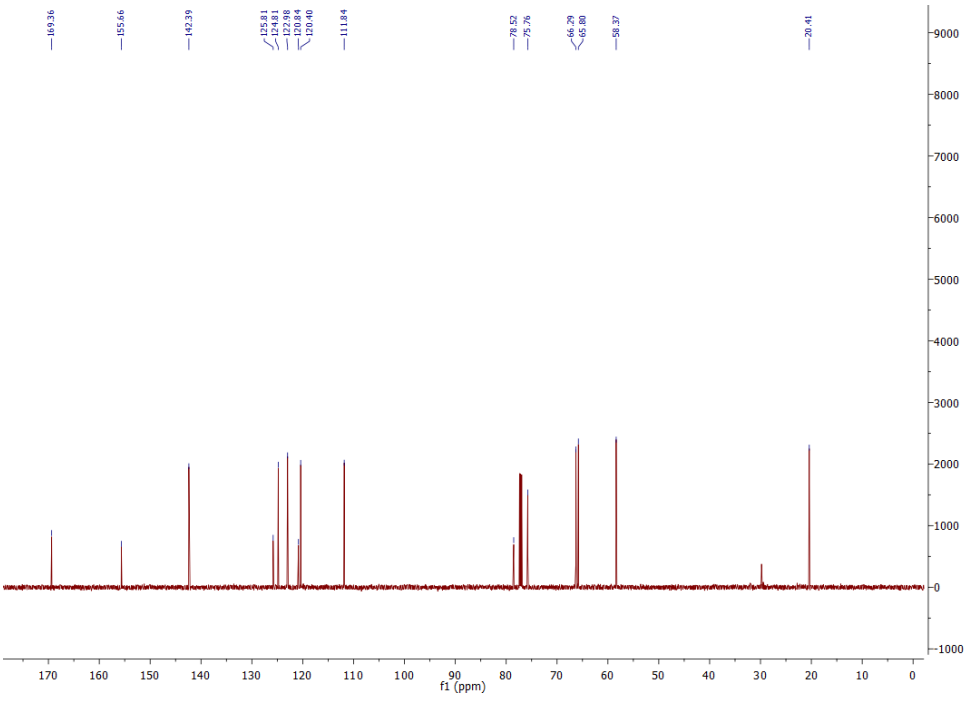


(±)-1-(Benzofuran-3-yl)ethyl 2-(prop-2-yn-1-yloxy)acetate (*rac*-3c): ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.66 (d, *J* = 7.7 Hz, 1H), 7.64 (s, 1H), 7.49 (d, *J* = 8.2 Hz, 1H), 7.33 -7.31 (m, 1H), 7.28 - 7.25 (m, 1H), 6.31 (q, *J* = 6.7 Hz, 1H), 4.30 (overlapped dd, 2H), 4.22 (d, *J* = 14.8 Hz, 2H), 2.45 (overlapped dd, 1H), 1.73 (d, *J* = 6.7 Hz, 3H) ppm; ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.36, 155.66, 142.39, 125.81, 124.81, 122.98, 120.84, 120.4, 111.84, 78.52, 75.76, 66.29, 65.80, 58.37, 20.41 ppm. HRMS (ESI^+) m/z: $C_{15}H_{14}O_4$ [$M+Na^+$] calc. 281.0784, found 281.0794.

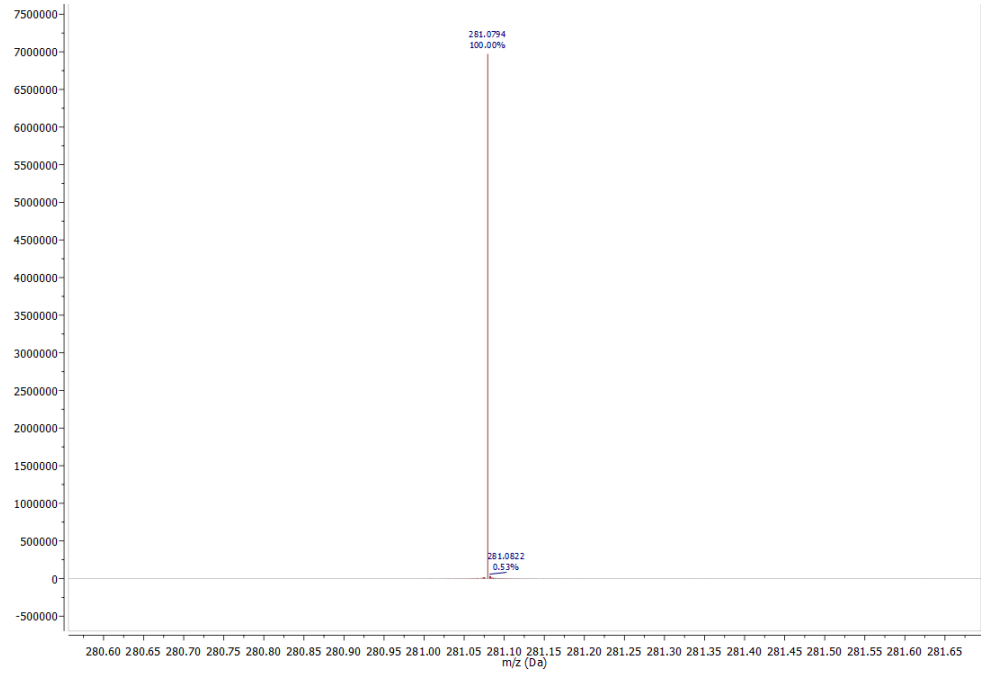
^{1}H NMR: *rac*-**3c** (600 MHz, $CDCl_3$)



^{13}C NMR: *rac*-**3c** (101 MHz, $CDCl_3$)

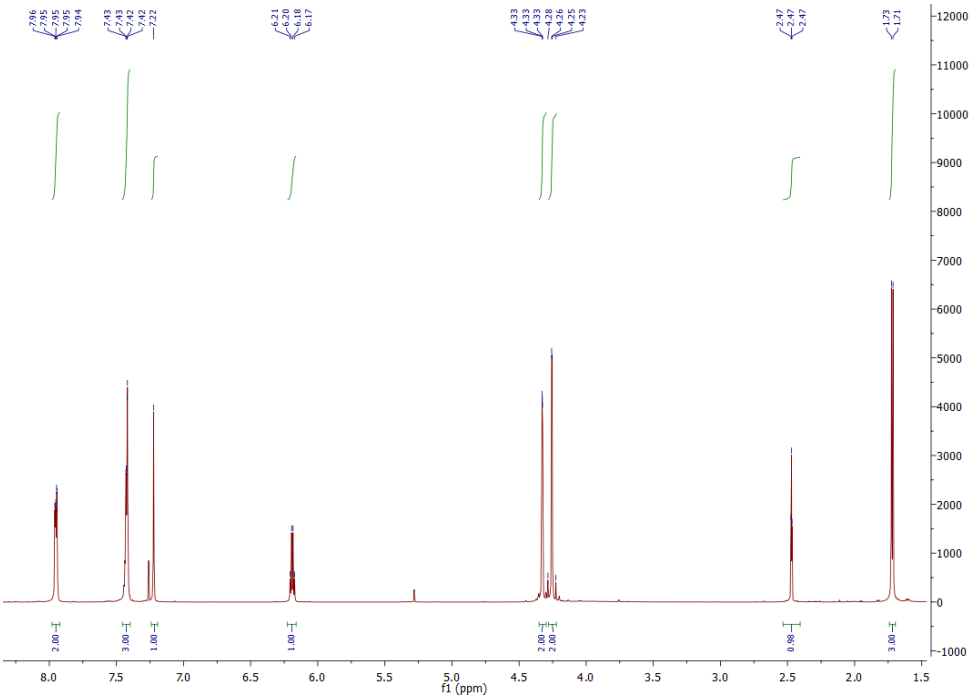


HRMS (ESI$^+$): *rac*-**3c**

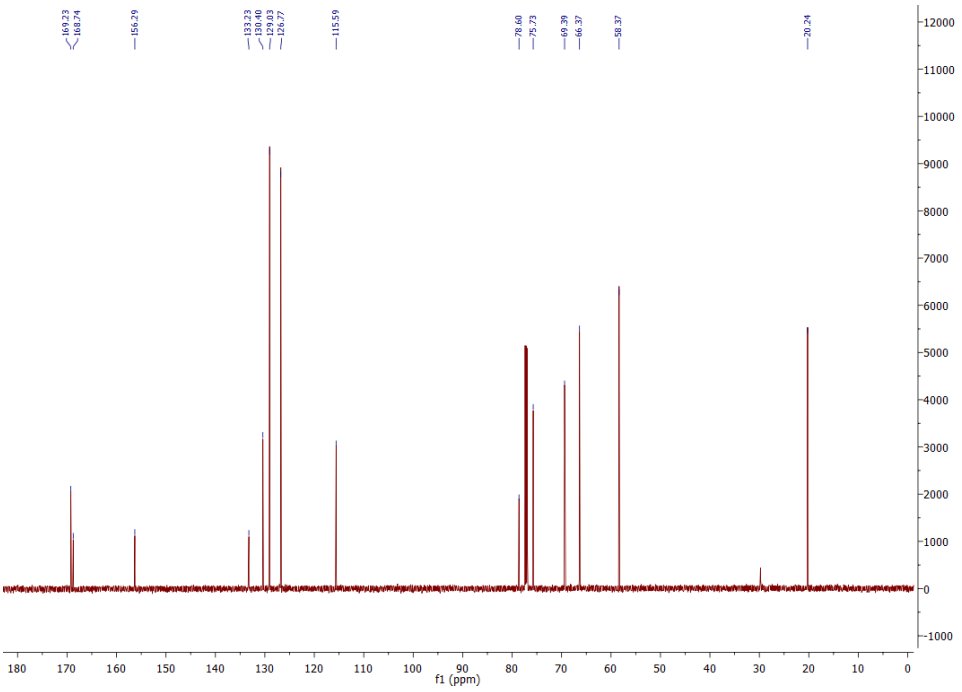


(±)-1-(2-Phenylthiazol-4-yl)ethyl 2-(prop-2-yn-1-yloxy)acetate (*rac*-3d): ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.98 – 7.92 (m, 2H), 7.43 - 7.42 (m, 3H), 7.22 (s, 1H), 6.19 (q, *J* = 6.6 Hz, 1H), 4.33 (overlapped dd, 2H), 4.26 (d, *J* = 2.4 Hz, 2H), 2.47 (overlapped dd, 1H), 1.72 (d, *J* = 6.6 Hz, 3H) ppm; ^{13}C NMR (151 MHz, $CDCl_3$) δ 169.23, 168.74, 156.29, 133.23, 130.40, 129.03, 126.77, 115.59, 78.60, 75.73, 69.39, 66.37, 58.37, 20.24 ppm. HRMS (APCI$^+$) m/z: $C_{16}H_{15}NO_3S$ [M+H$^+$] calc. 302.0850, found 302.0845.

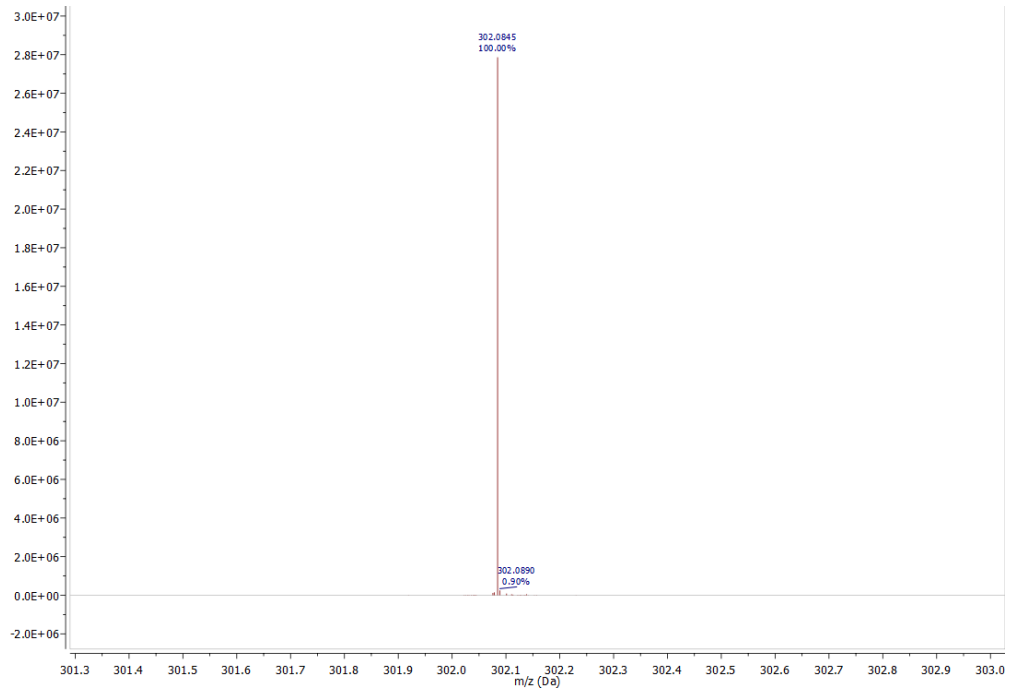
^{1}H NMR: *rac*-**3d** (600 MHz, $CDCl_3$)



^{13}C NMR: *rac*-**3d** (151 MHz, $CDCl_3$)

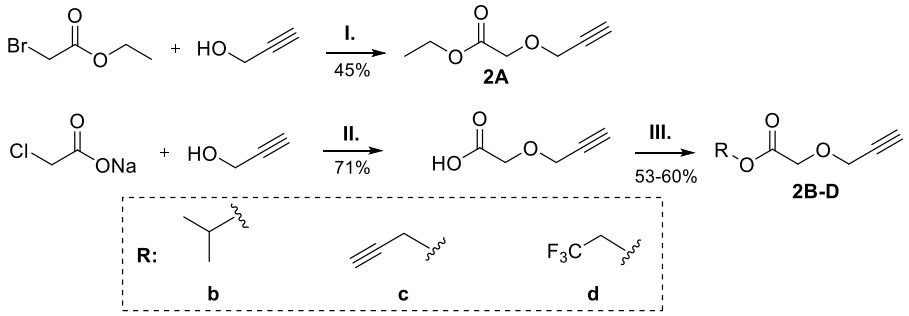


HRMS (APCI$^+$): *rac*-**3d**



1.2. Chemical synthesis of acylating agents 2A-D

2-(Prop-2-yn-1-yloxy)acetic acid and the corresponding ethyl, isopropyl, propargyl and 2,2,2-trifluoroethyl esters **2A-D** were synthesized according to **Scheme S2** [2] and further tested in the enzymatic transesterification reactions.

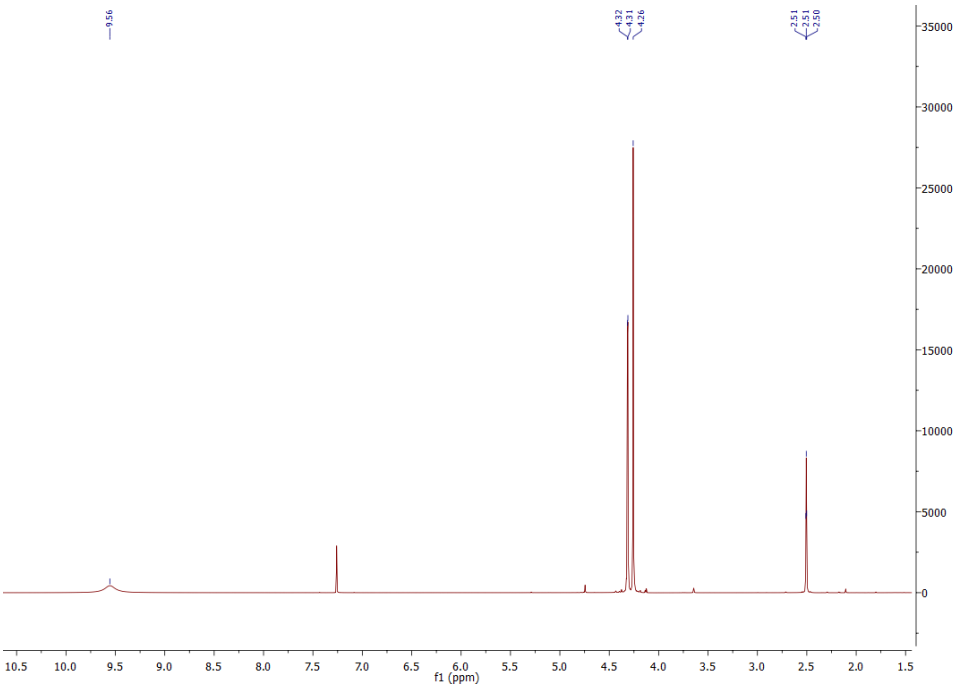


Scheme S2. Synthetic route of activated esters **2A-D.** *Reagents and reaction conditions:* **I.** NaH, dry THF, Ar, rt, 12 h; **II.** MTBE, NaOH, TBAB, rt; **III.** R-OH, DCC, DMAP, dry CH_2Cl_2, rt, 3 h.

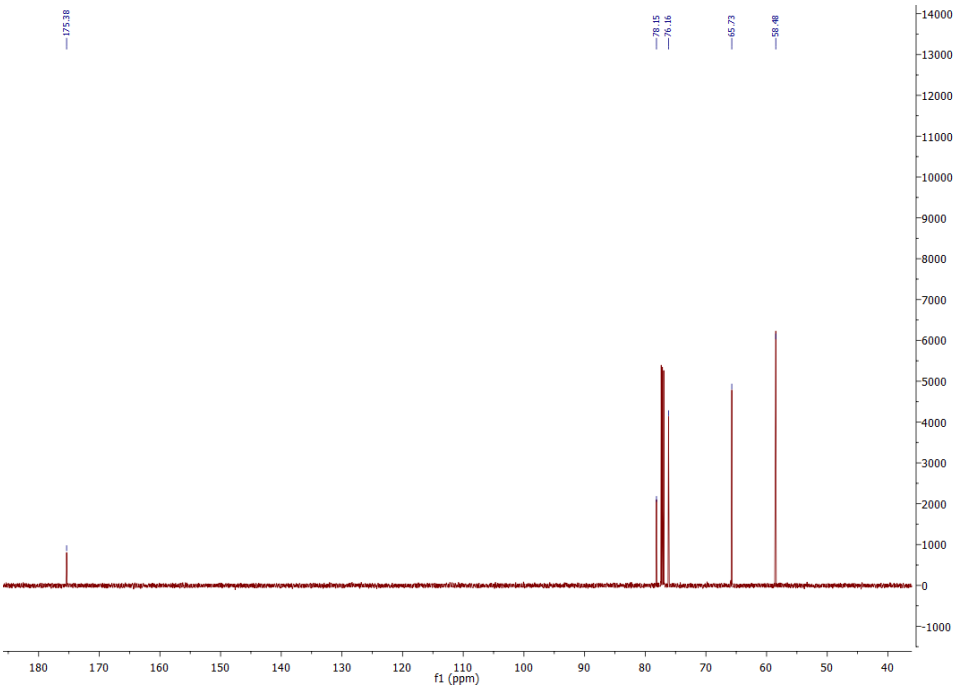
1.2.1. Synthesis of 2-(prop-2-yn-1-yloxy)acetic acid

Into the solution of propargyl alcohol (1.2 equiv., 34 mmol, 2 mL) in MTBE (10 mL), NaOH (1.2 equiv., 34 mmol, 1.39 g) dissolved in distilled water (10 mL) was added. Chloracetic acid sodium salt (1 equiv., 28.9 mmol, 3.37 g) and tetrabutylammonium bromide (10 mol%, 0.93 g) as phase transfer catalyst were added and the mixture was stirred at room temperature for 48 hours. After the completion of the reaction, the two phases were separated, the aqueous phase was washed twice with MTBE (2 × 10 mL) and then the pH was adjusted to 2 using concentrated H_2SO_4. Next, extractions with ethyl acetate (3 × 10 mL) were performed, the combined organic phases were dried over anhydrous Na_2SO_4, filtered and concentrated in *vacuo*. The untransformed propargyl alcohol was removed through vacuum distillation affording the product as a brown oil. Yield: 71%.
2-(Prop-2-yn-1-yloxy)acetic acid: ^{1}H NMR (600 MHz, $CDCl_3$) δ 9.56 (bs, 1H), 4.31 (d, *J* = 2.4 Hz, 2H), 4.26 (s, 2H), 2.51 (t, *J* = 2.4 Hz, 1H) ppm; ^{13}C NMR (151 MHz, $CDCl_3$) δ 175.39, 78.15, 76.16, 65.73, 58.48 ppm.

^{1}H NMR: 2-(Prop-2-yn-1-yloxy)acetic acid (600 MHz, $CDCl_3$)



^{13}C NMR: 2-(Prop-2-yn-1-yloxy)acetic acid (151 MHz, $CDCl_3$)

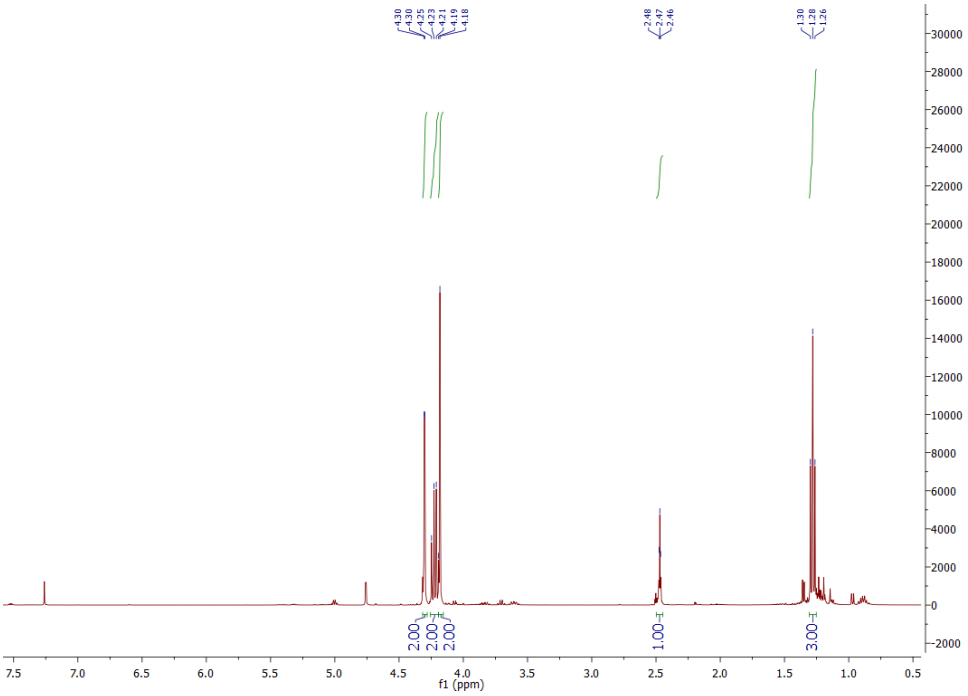


1.2.2. Synthesis of ethyl 2-(prop-2-yn-1-yloxy)acetate (2A)

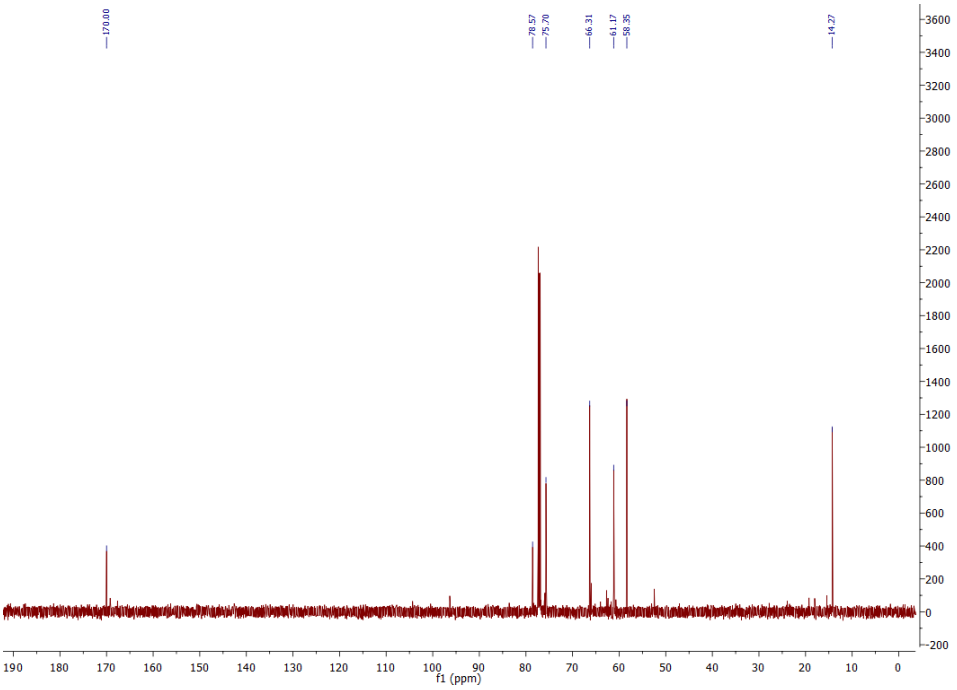
NaH (60% in mineral oil, 1.1 equiv., 22 mmol, 0.88 g) was added to the solution of propargyl alcohol (1 equiv., 20 mmol, 1.2 mL) in anhydrous THF (15 mL) under Ar atmosphere and the mixture was left stirring at room temperature for 1 hour. Ethyl bromoacetate (1.2 equiv., 24 mmol, 2.75 mL) was added afterwards and the reaction was stirred for another 12 hours. The solvent was removed using a rotary evaporator, the remaining yellow oil was dissolved in diethyl ether (10 mL) and extracted with distilled water (3 × 10 mL). The organic phase was dried over anhydrous Na_2SO_4, filtered and concentrated in *vacuo*. Finally, using silica gel column chromatography and first petroleum ether, then petroleum ether:diethyl ether 96:4 (v/v) as eluent, the product was obtained as a colourless viscous liquid. Yield: 45%.

Ethyl 2-(prop-2-yn-1-yloxy)acetate (2A): ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.30 (d, *J* = 2.4 Hz, 2H), 4.22 (q, *J* = 7.1 Hz, 2H), 4.18 (s, 2H), 2.47 (t, *J* = 2.4 Hz, 1H), 1.28 (t, *J* = 7.1 Hz, 3H) ppm; ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.00, 78.57, 75.70, 66.31, 61.17, 58.35, 14.27 ppm. HRMS ($APCI^+$) m/z: $C_7H_{10}O_3$ [$M+H^+$] calc. 143.0703, found 143.0706.

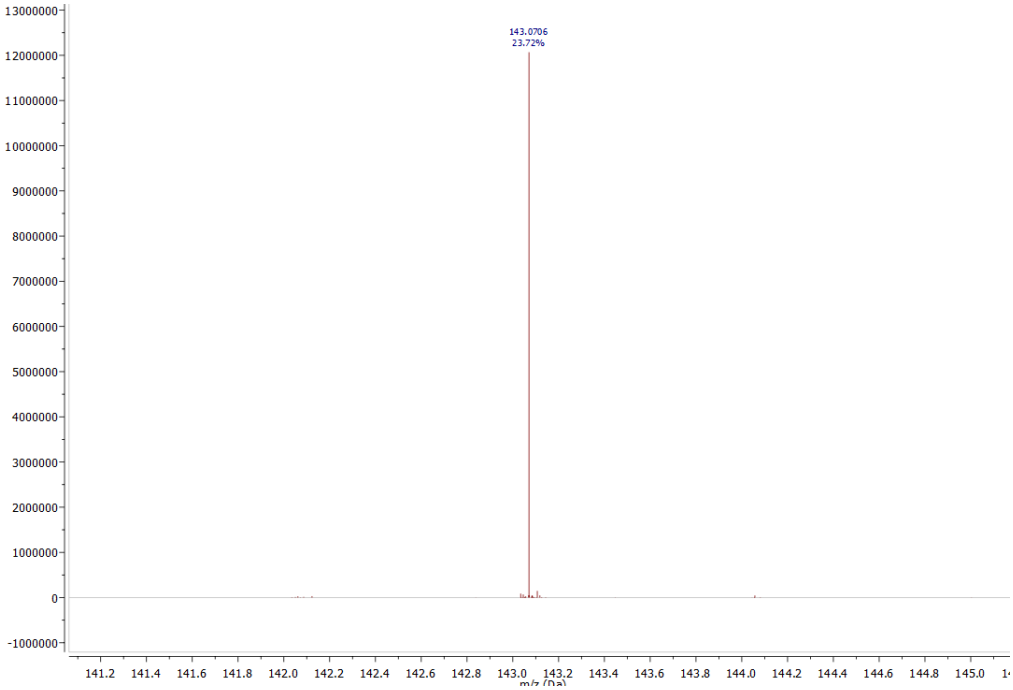
^{1}H NMR: 2A (400 MHz, $CDCl_3$)



^{13}C NMR: 2A (101 MHz, $CDCl_3$)



HRMS (APCI$^+$): 2A

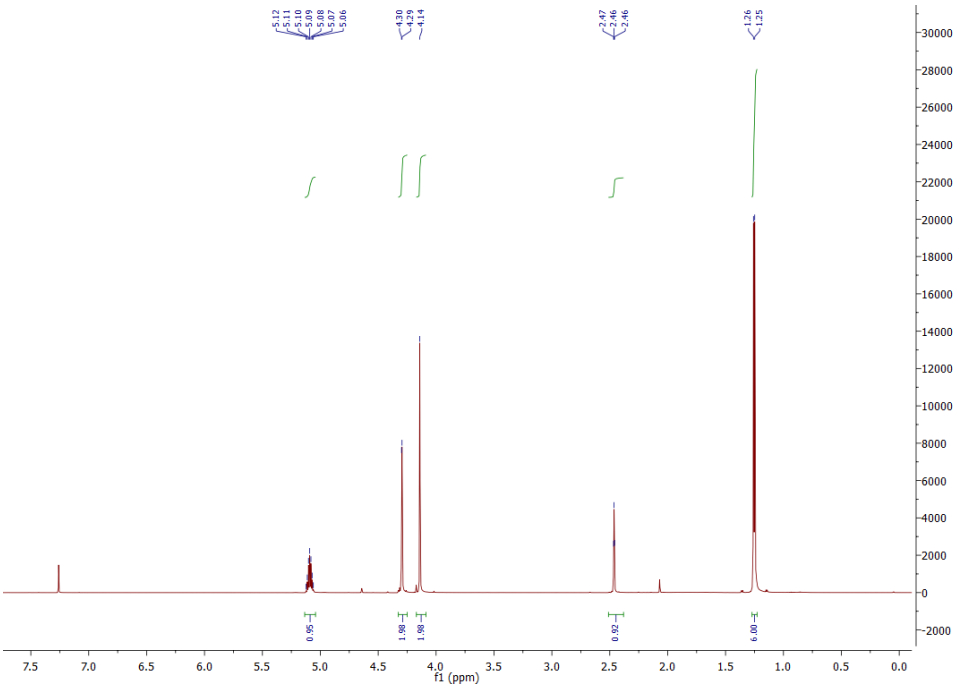


1.2.3. Synthesis of isopropyl, propargyl and 2,2,2-trifluoroethyl 2-(prop-2-yn-1-yloxy)acetates (2B-D)

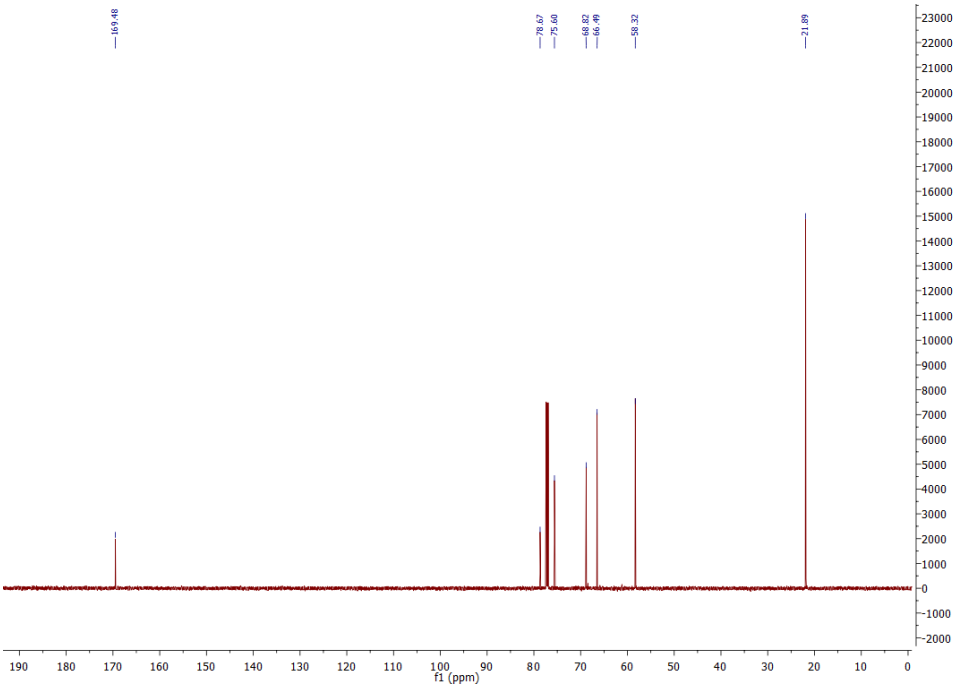
In a dry round-bottom flask one of the three alcohols (2 equiv., 8 mmol) – 2-propanol, propargyl alcohol or 2,2,2-trifluoroethanol and 2-(prop-2-yn-1-yloxy)acetic acid (1 equiv., 4 mmol, 456 mg) were added in dry CH_2Cl_2 (10 mL). After cooling the mixture to 0 °C, DMAP (0.1 equiv., 0.4 mmol, 49 mg) and DCC (1.1 equiv, 4.4 mmol, 908 mg) were added. The reaction mixture was stirred for 10 minutes at 0 °C and then for 3 hours at room temperature. When the reaction was completed (monitored by TLC using *n*-hexane:ethyl acetate 7:3 (v/v) as eluent and potassium permanganate solution as developing agent), the precipitated DCU was filtered off and the organic solution was extracted with 10% HCl (2 × 10 mL), then with 2M Na_2CO_3 (2 × 10 mL) and finally with distilled water (2 × 10 mL). The collected organic phases were dried over anhydrous Na_2SO_4, filtered and concentrated in *vacuo*. The products (light yellow viscous liquids) were purified using silica gel column chromatography and a mixture of *n*-hexane: ethyl acetate 7:3 (v/v) as eluent. Yields: 53-60%.

Isopropyl 2-(prop-2-yn-1-yloxy)acetate (2B): ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.09 (m, 1H), 4.30 (d, *J* = 2.4 Hz, 2H), 4.14 (s, 2H), 2.46 (t, *J* = 2.4 Hz, 1H), 1.25 (d, *J* = 6.3 Hz, 6H) ppm; ^{13}C NMR (151 MHz, $CDCl_3$) δ 169.48, 78.67, 75.60, 68.82, 66.49, 58.32, 21.89 ppm. HRMS (APCI$^+$) m/z: $C_8H_{12}O_3$ [M+H$^+$] calc. 157.0859, found 157.0862.

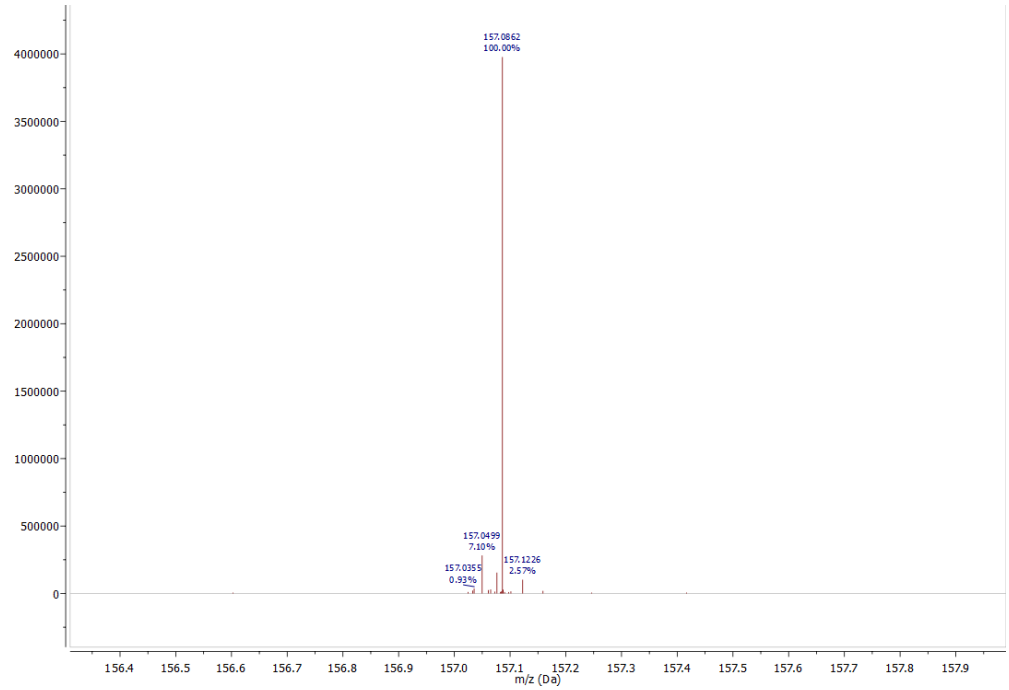
^{1}H NMR: 2B (600 MHz, $CDCl_3$)



^{13}C NMR: 2B (151 MHz, $CDCl_3$)

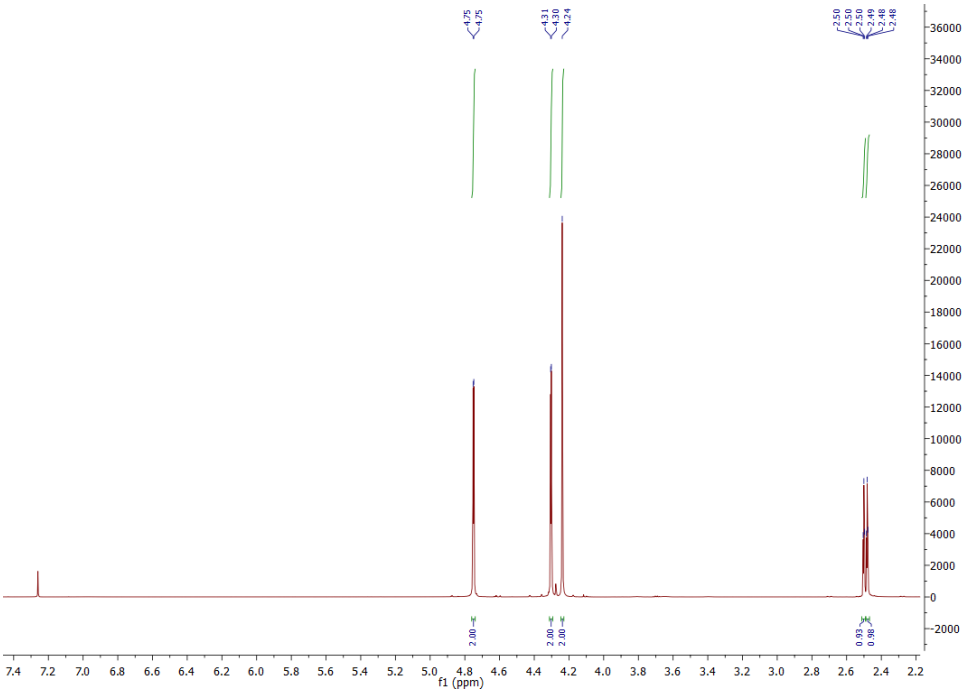


HRMS (APCI$^+$): 2B

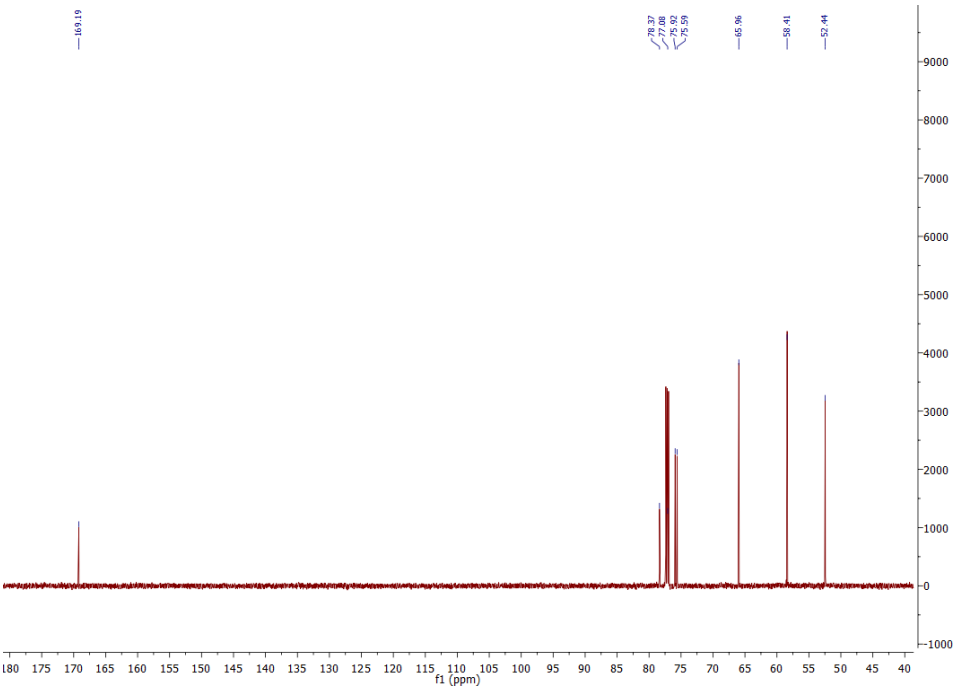


Prop-2-yn-1-yl 2-(prop-2-yn-1-yloxy)acetate (2C): ^{1}H NMR (600 MHz, $CDCl_3$) δ 4.75 (d, *J* = 2.5 Hz, 1H), 4.30 (d, *J* = 2.4 Hz, 1H), 4.24 (s, 1H), 2.50 (t, *J* = 2.5 Hz, 1H), 2.48 (t, *J* = 2.4 Hz, 1H) ppm; ^{13}C NMR (151 MHz, $CDCl_3$) δ 169.19, 78.37, 77.08, 75.92, 75.59, 65.96, 58.41, 52.44 ppm. HRMS (APCI$^+$) m/z: $C_8H_8O_3$ [M+H$^+$] calc. 153.0546, found 153.0550.

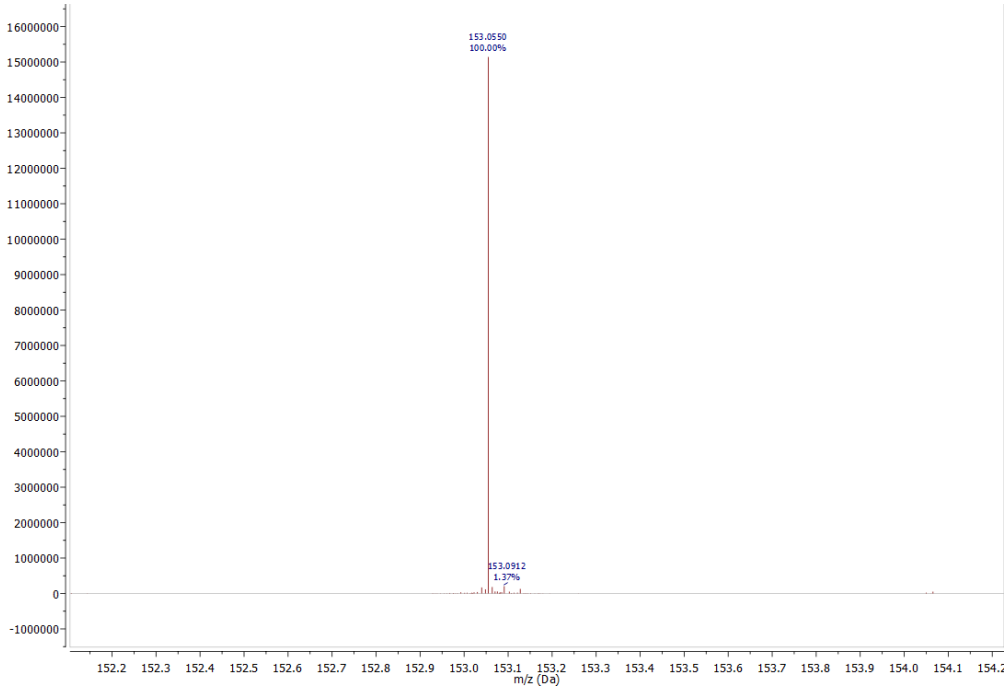
^{1}H NMR: 2C (600 MHz, $CDCl_3$)



^{13}C NMR: 2C (151 MHz, $CDCl_3$)

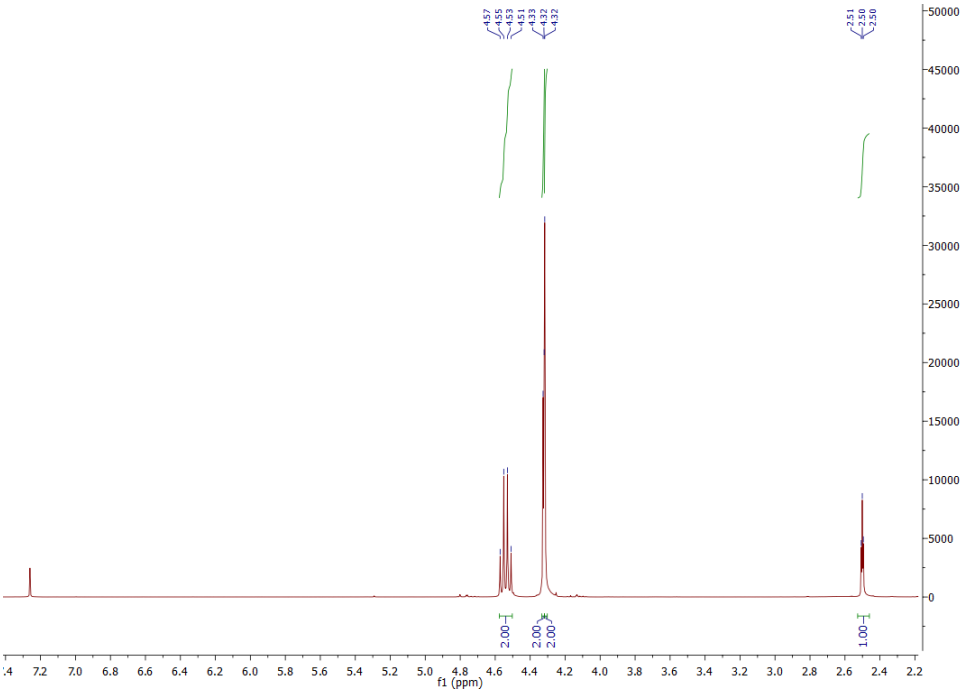


HRMS (APCI$^+$): 2C

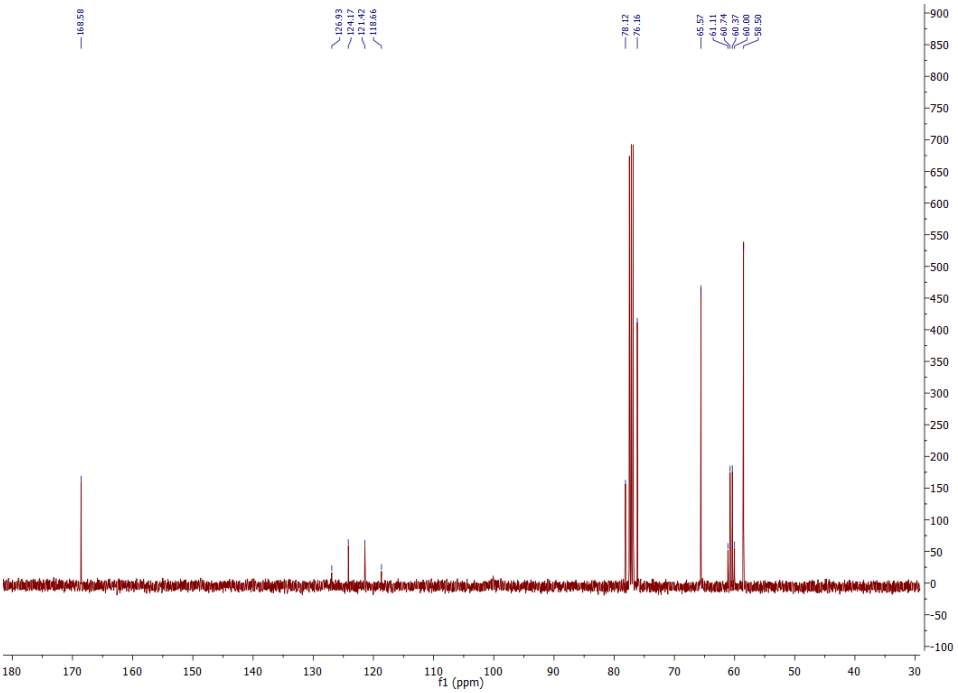


2,2,2-Trifluoroethyl 2-(prop-2-yn-1-yloxy)acetate (2D): ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.54 (q, *J* = 8.3 Hz, 2H), 4.32 (d, *J* = 2.4 Hz, 2H), 4.32 (s, 2H), 2.50 (t, *J* = 2.4 Hz, 1H) ppm; ^{13}C NMR (101 MHz, $CDCl_3$) δ 168.58, 126.93, 124.17, 121.42, 118.66, 78.12, 76.16, 65.57, 61.11, 60.74, 60.37, 60.00, 58.50 ppm. HRMS (APCI$^+$) m/z: $C_7H_7F_3O_3$ [M+H$^+$] calc. 197.0420, found 197.0427.

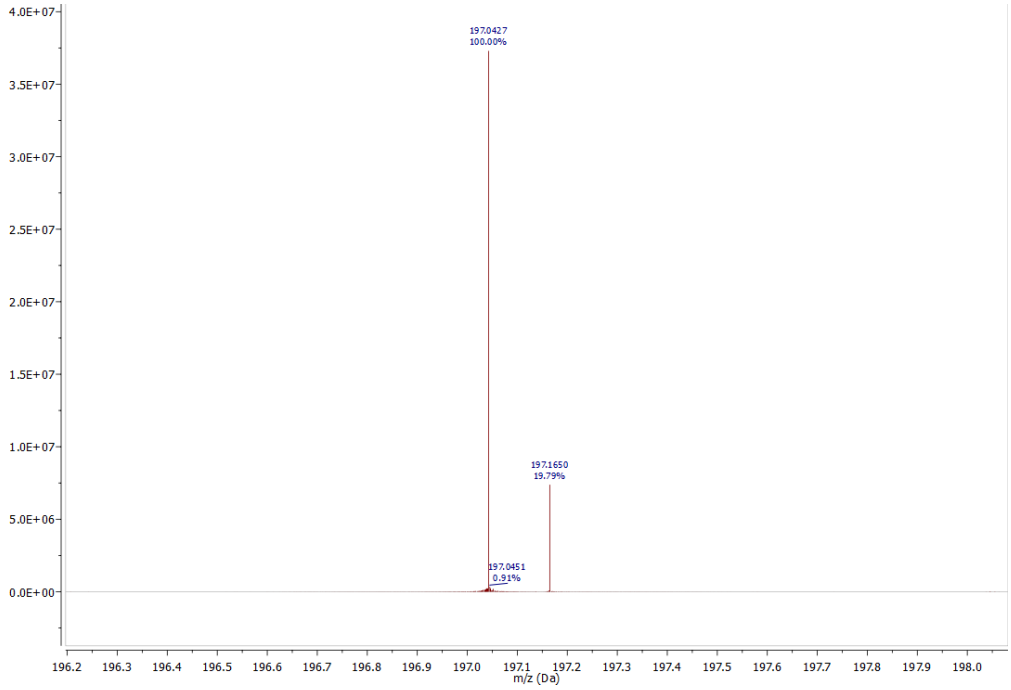
^{1}H NMR: 2D (400 MHz, $CDCl_3$)



^{13}C NMR: 2D (101 MHz, $CDCl_3$)

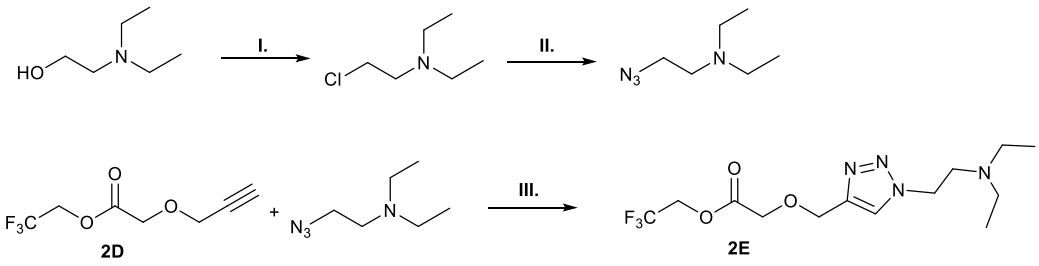


HRMS (APCI$^+$): 2D



1.3. Chemical synthesis of 2-azido-*N,N*-diethylethan-1-amine and of 2,2,2-trifluoroethyl 2-((1-(2-(diethyl-amino)ethyl)-1*H*-1,2,3-triazol-4-yl)methoxy)acetate (2E)

A tertiary amine functionalized with an azide group, 2-azido-*N*,*N*-diethylethan-1-amine, was selected for the click reaction-based separation protocol of EKR products due to its property of being extractable in an aqueous acidic solution. The click-reaction of 2-azido-*N*,*N*-diethylethan-1-amine with **2D** generated an acylating agent bearing an amino-triazole group **2E**. The synthesis were performed according to **Scheme S3**. [3]



Scheme S3. Synthesis of 2-azido-*N*,*N*-diethylethan-1-amine *Reagents and reaction conditions:* **I.** $SOCl_2$, $CHCl_3$, 0 °C - reflux, 4 h; **II.** NaN_3, H_2O, 80 °C; **III.** CuI, CH_2Cl_2.

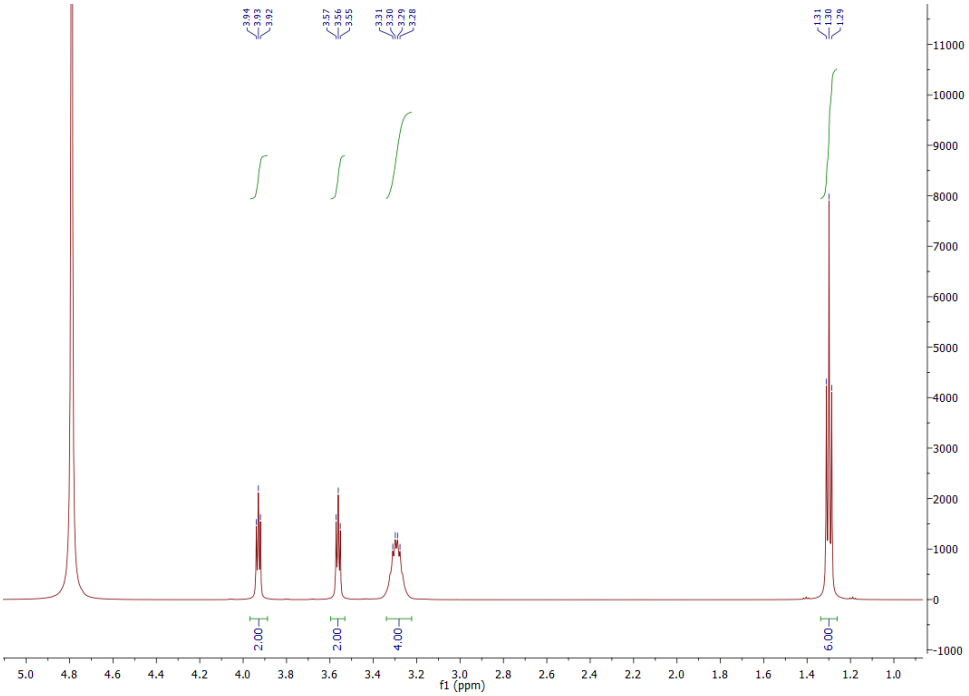
1.3.1. Synthesis of 2-chloro-N,N-diethylethan-1-amine hydrochloride

Into the ice-cold solution of 2-(diethylamino)ethan-1-ol (10 mmol, 1.3 mL) in $CHCl_3$ (20 mL) $SOCl_2$ (1.5 equiv., 15 mmol, 1.088 mL) in dry $CHCl_3$ (5 mL) was added dropwise under magnetic stirring. The reaction mixture was next refluxed for 4 hours. The solvent was evaporated under reduced pressure, the remaining residue was redissolved in ethanol (10 mL) and the product was obtained through precipitation by pouring diethyl ether (50

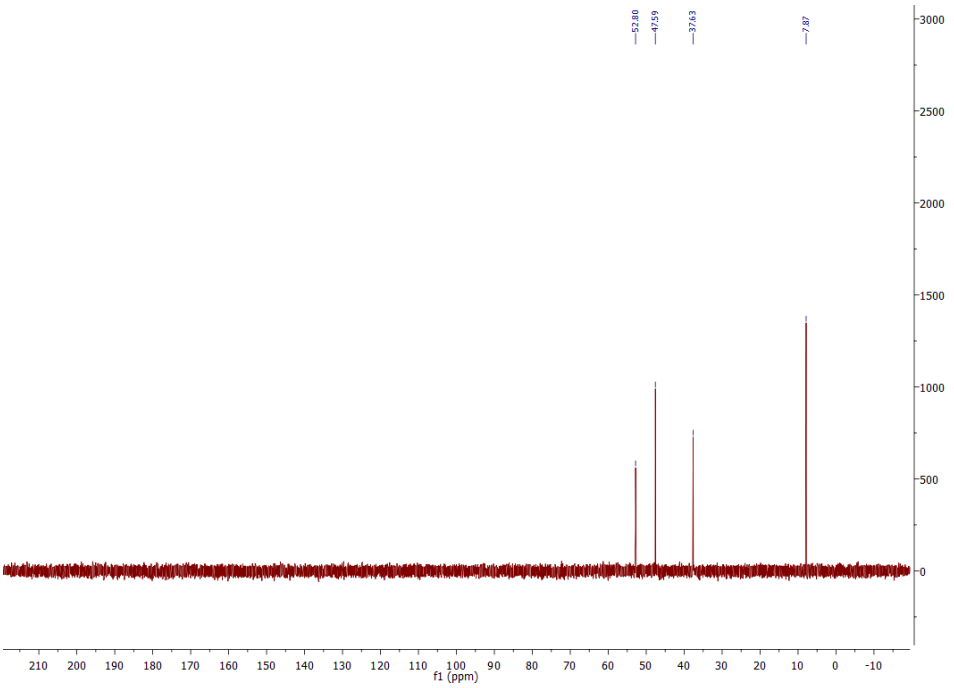
mL) into the ethanolic solution. The precipitate was then filtered and washed with diethyl ether, resulting a white solid. Yield: 88%.

2-Chloro-*N*,*N*-diethylethan-1-amine hydrochloride: ^{1}H NMR (600 MHz, D_2O) δ 3.92 (t, *J* = 5.7 Hz, 2H), 3.56 (t, *J* = 5.7 Hz, 2H), 3.37 – 3.22 (m, 4H), 1.30 (t, *J* = 7.3 Hz, 6H) ppm; ^{13}C NMR (151 MHz, D_2O) δ 52.80, 47.59, 37.63, 7.87 ppm.

^{1}H NMR: 2-Chloro-*N*,*N*-diethylethan-1-amine hydrochloride (600 MHz, D_2O)



^{13}C NMR: 2-Chloro-*N*,*N*-diethylethan-1-amine hydrochloride (151 MHz, D_2O)

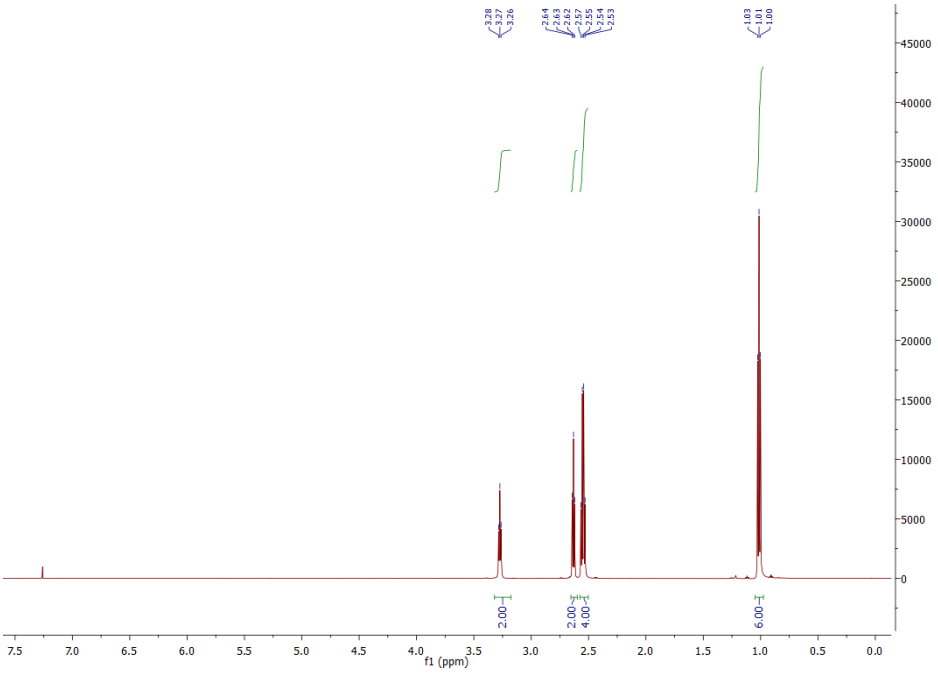


1.3.2. Synthesis of 2-azido-N,N-diethylethan-1-amine

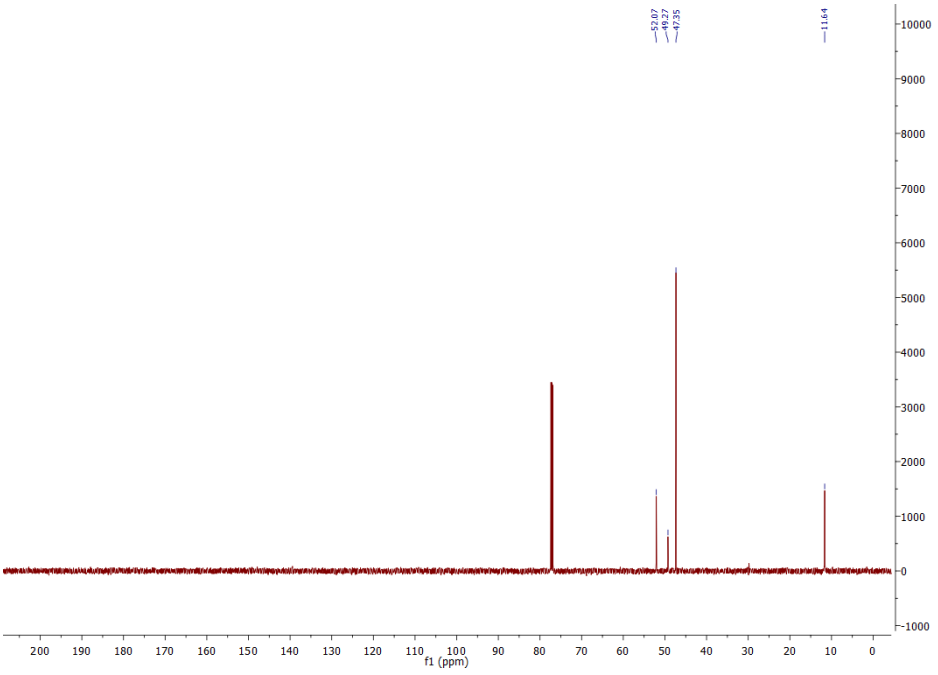
2-Chloro-*N*,*N*-diethylethan-1-amine hydrochloride (8 mmol, 1.36 g) was dissolved in deionized water (20 mL) and NaN_3 (3 equiv., 24 mmol, 1.56 g) was added. The mixture was stirred at 80 °C for 24 hours. After cooling the reaction mixture, the pH of the aqueous solution was adjusted to 10 using 10% KOH and extractions with diethyl ether were performed (3 × 20 mL). The organic phase was dried over anhydrous Na_2SO_4, filtered and concentrated in *vacuo,* affording the desired azide as a light yellow viscous liquid. Yield: 83%.

2-azido-*N*,*N*-diethylethan-1-amine: ^{1}H NMR (600 MHz, $CDCl_3$) δ 3.27 (t, *J* = 6.3 Hz, 2H), 2.63 (t, *J* = 6.3 Hz, 2H), 2.55 (q, *J* = 7.2 Hz, 4H), 1.01 (t, *J* = 7.2 Hz, 6H) ppm; ^{13}C NMR (151 MHz, $CDCl_3$) δ 52.07, 49.27, 47.35, 11.64 ppm.

^{1}H NMR: 2-azido-*N*,*N*-diethylethan-1-amine (600 MHz, $CDCl_3$)



^{13}C NMR: 2-azido-*N*,*N*-diethylethan-1-amine (151 MHz, $CDCl_3$)

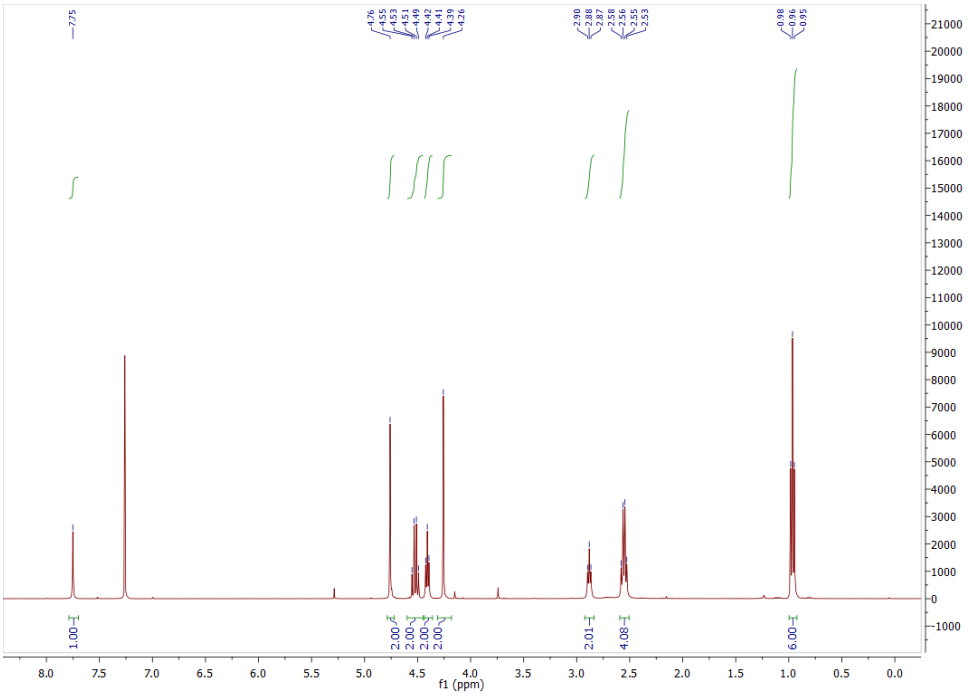


1.3.3. Synthesis of 2,2,2-trifluoroethyl 2-((1-(2-(diethyl-amino)ethyl)-1H-1,2,3-triazol-4-yl)methoxy)acetate (2E)

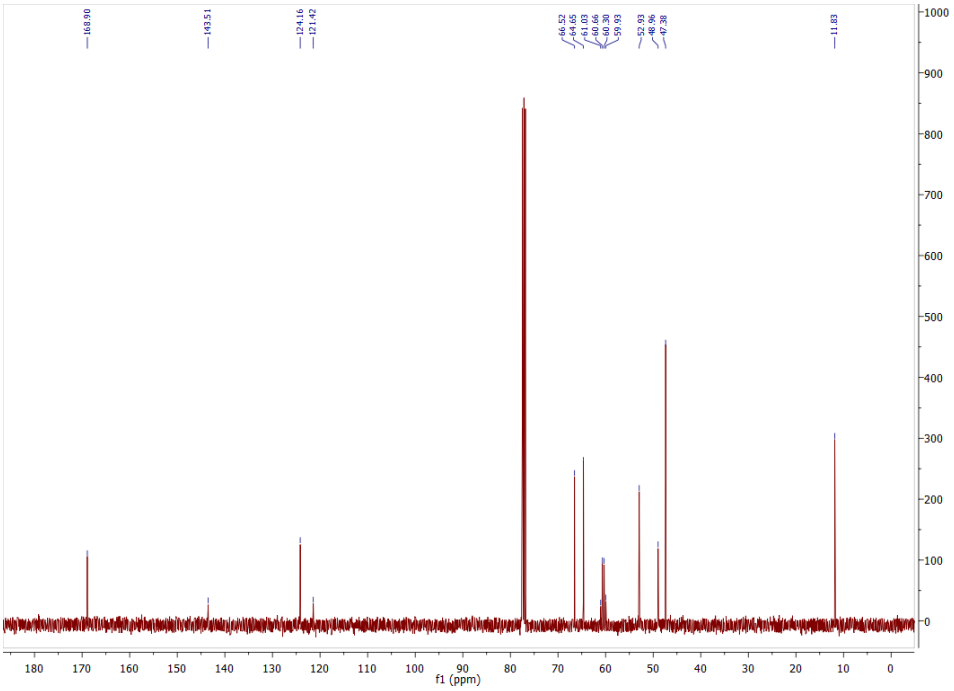
Into the solution of 2-azido-*N*,*N*-diethylethan-1-amine (0.51 mmol, 72.5 mg) and 2,2,2-trifluoroethyl 2-(prop-2-yn-1-yloxy)acetate (**2D**) (1 equiv., 0.51 mmol, 100 mg) in *n*-hexane (5 mL), CuI (0.1 equiv., 0.05 mmol, 9.7 mg) was added and the reaction mixture was stirred at room temperature for 12 h. When the reaction was completed (monitored by TLC using CH_2Cl_2: MeOH 9:1 (v/v) as mobile phase), the catalyst was removed by filtration. Next, extraction was performed with aqueous acetic acid solution (0.1 M, pH 2, 3 × 5 mL). The product, ester **2E,** was obtained from the aqueous phase (after adjusting the pH to 10 with 2 M Na_2CO_3) by extraction with CH_2Cl_2 (2 × 10 mL) followed by drying (over Na_2SO_4), filtration and removal of the solvent under reduced pressure.

2,2,2-trifluoroethyl 2-((1-(2-(diethyl-amino)ethyl)-1H-1,2,3-triazol-4-yl)methoxy)acetate (2E): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.75 (s, 1H), 4.76 (s, 2H), 4.52 (q, J = 8.4 Hz, 2H), 4.41 (t, J = 6.3 Hz, 2H), 4.26 (s, 2H), 2.88 (t, J = 6.2 Hz, 2H), 2.55 (q, J = 7.1 Hz, 4H), 0.96 (t, J = 7.1 Hz, 6H) ppm; ^{13}C NMR (101 MHz, $CDCl_3$) δ 168.90, 145.51, 124.16, 66.52, 64.65, 60.66, 60.30, 59.93, 52.93, 48.96, 47.38, 11.83.

^{1}H NMR: 2E: (400 MHz, $CDCl_3$)



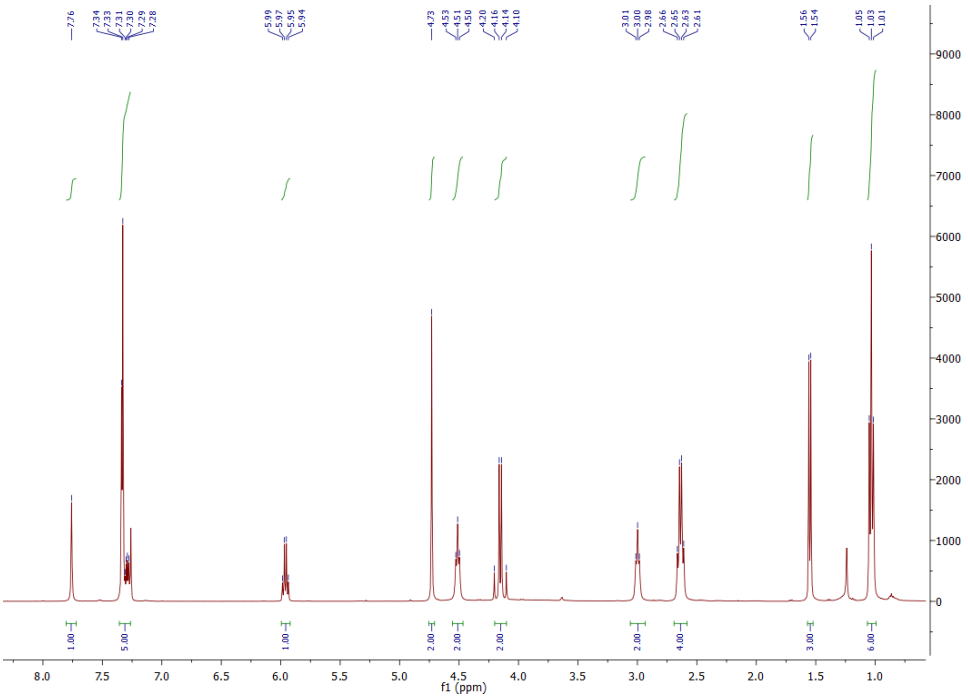
^{13}C NMR: 2,2,2-trifluoroethyl 2-((1-(2-(diethyl-amino)ethyl)-1*H*-1,2,3-triazol-4-yl)methoxy)acetate (101 MHz, $CDCl_3$)



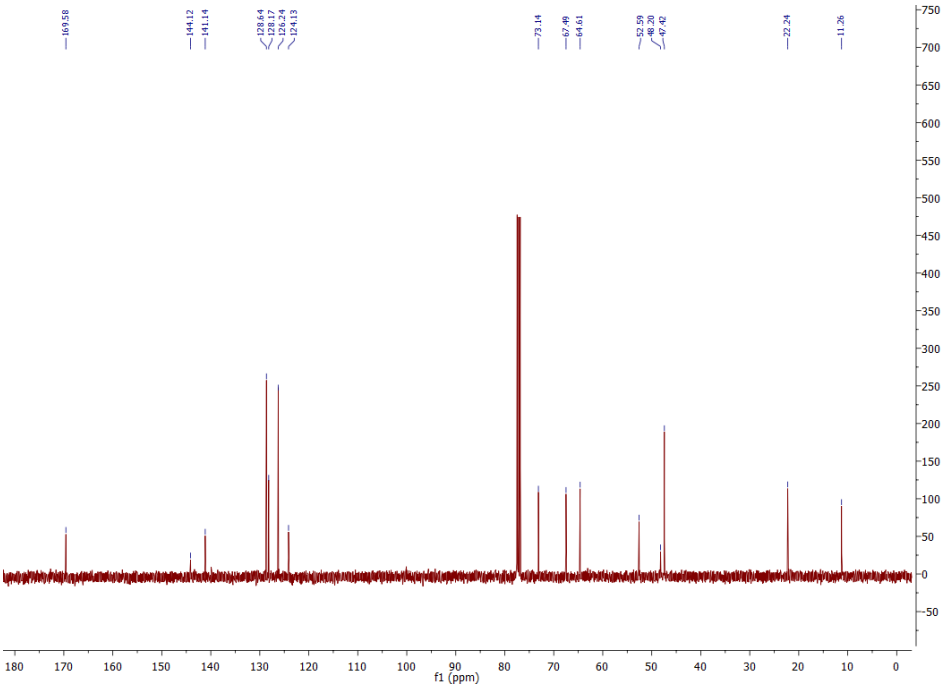
1.4. Spectral characterization of racemic heteroaromatic triazoles *rac*-4a-d

1.4.1. (±)-1-Phenylethyl 2-((1-(2-(diethylamino)ethyl)-1H-1,2,3-triazol-4-yl)methoxy)acetate (rac-4a)

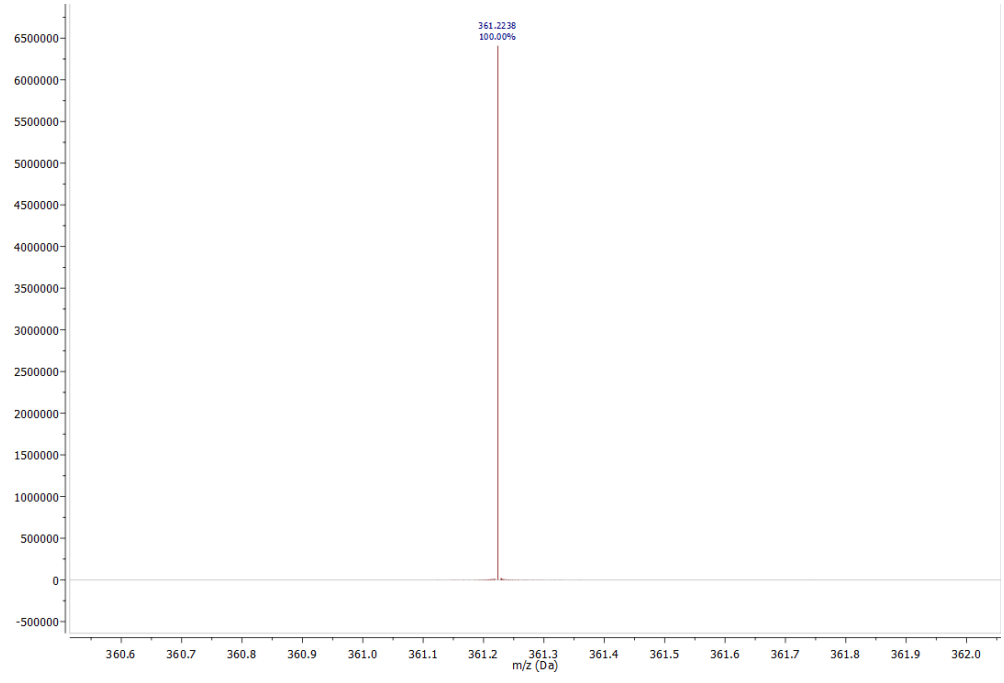
^{1}H NMR: ***rac*-4a** (400 MHz, $CDCl_3$): δ 7.76 (s, 1H), 7.36 – 7.26 (m, 5H), 5.96 (q, J = 6.6 Hz, 1H), 4.73 (s, 2H), 4.51 (t, J = 6.0 Hz, 2H), 4.15 (d, J = 7.7 Hz, 2H), 3.00 (t, J = 6.0 Hz, 2H), 2.64 (q, J = 7.1 Hz, 4H), 1.55 (d, J = 6.6 Hz, 3H), 1.03 (t, J = 7.1 Hz, 6H)



^{13}C NMR: ***rac*-4a** (101 MHz, $CDCl_3$): δ 169.58, 144.12, 141.14, 128.64, 128.17, 126.24, 124.13, 73.14, 67.49, 64.61, 52.59, 48.20, 47.42, 22.24, 11.26

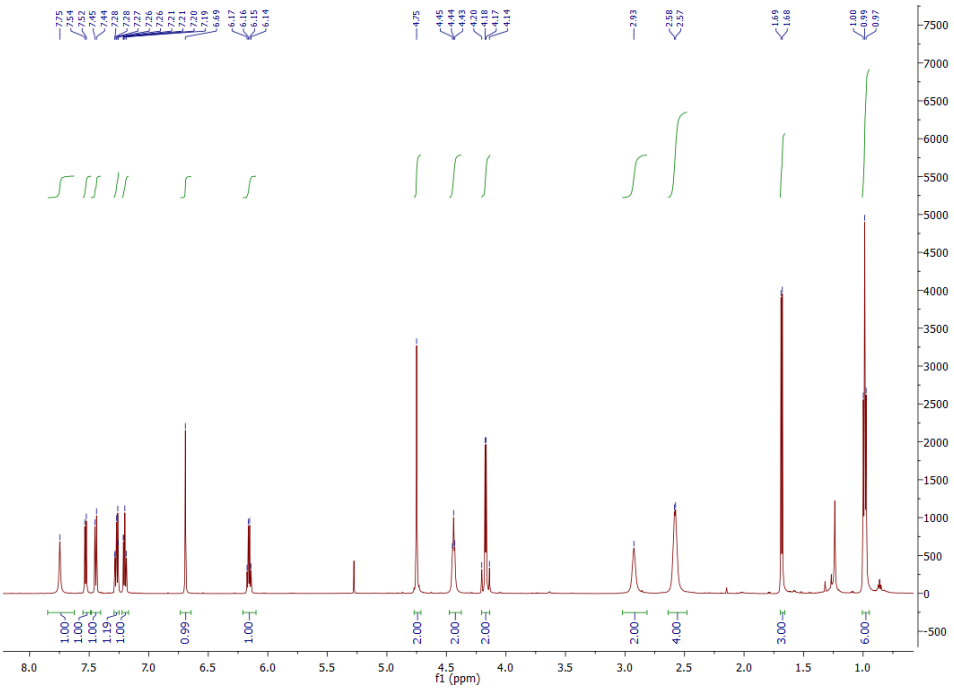


HRMS (ESI$^+$): *rac*-4a: m/z: $C_{19}H_{28}N_4O_3$ [M+H$^+$] calc. 361.2234, found 361.2238.

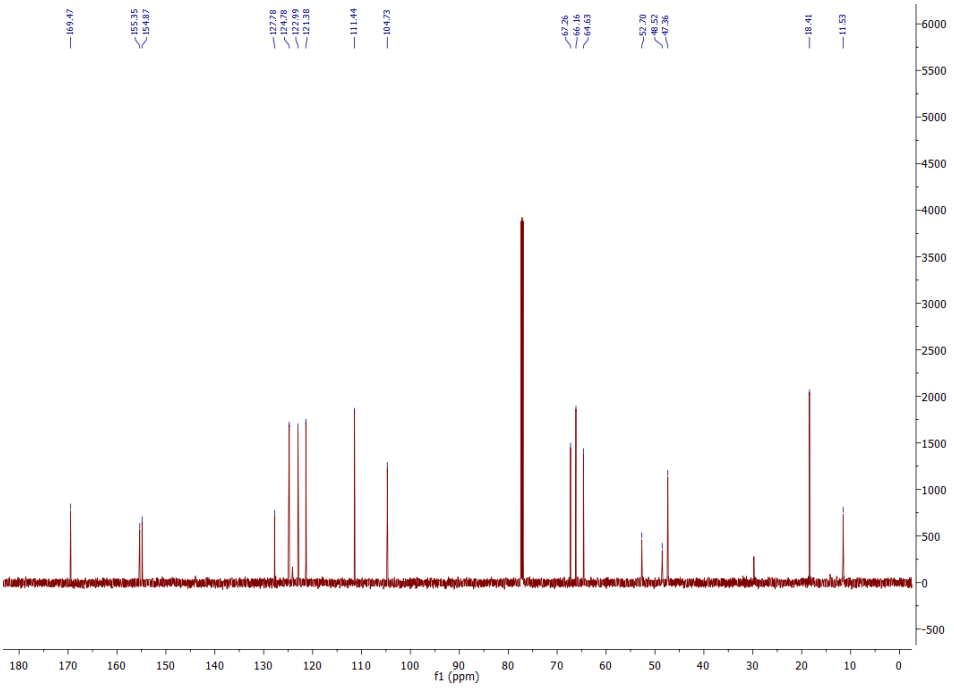


1.4.2. (±)-1-(Benzofuran-2-yl)ethyl 2-((1-(2-(diethylamino)ethyl)-1H-1,2,3-triazol-4-yl)methoxy)acetate (rac-4b)

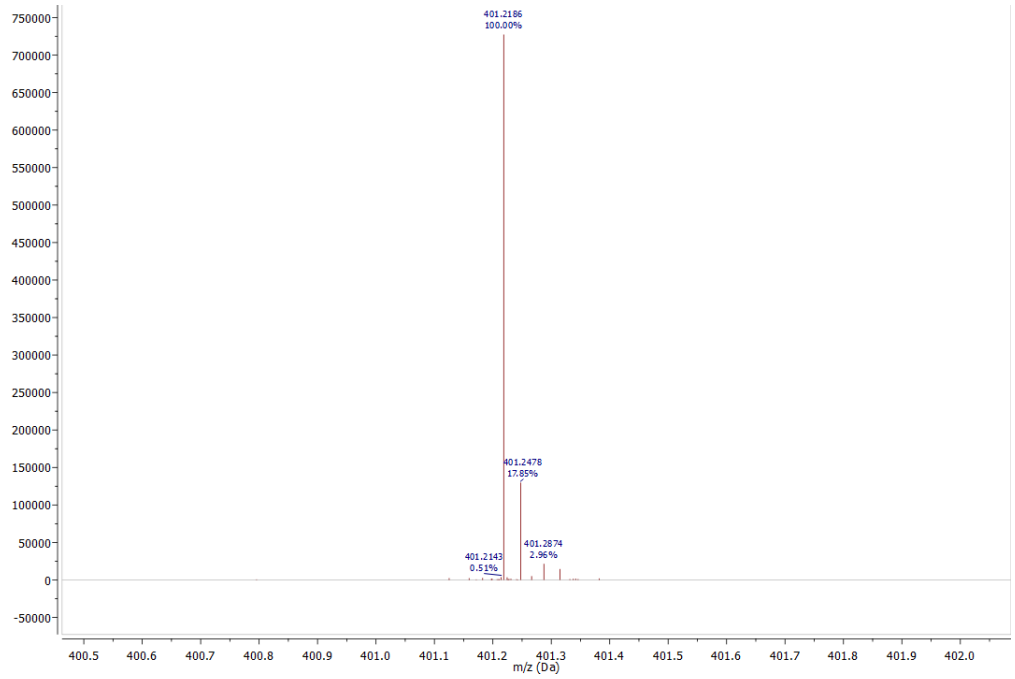
^{1}H NMR: ***rac*-4b** (600 MHz, $CDCl_3$): δ 7.75 (s, 1H), 7.53 (d, J = 7.7 Hz, 1H), 7.45 (d, J = 8.2 Hz, 1H), 7.29 – 7.25 (m, 1H), 7.22 – 7.18 (m, 1H), 6.69 (s, 1H), 6.16 (q, J = 6.7 Hz, 1H), 4.75 (s, 2H), 4.44 (t, J = 5.9 Hz, 2H), 4.17 (d, J = 5.4 Hz, 2H), 2.93 (overlapped t, 2H), 2.58 (overlapped q, 4H), 1.68 (d, J = 6.7 Hz, 3H), 0.99 (t, J = 7.1 Hz, 6H).



^{13}C NMR: ***rac*-4b** (151 MHz, $CDCl_3$): δ 169.47, 155.35, 154.87, 127.78, 124.78, 122.99, 121.38, 111.44, 104.73, 67.26, 66.16, 64.63, 52.70, 48.52, 47.36, 18.41, 11.53.

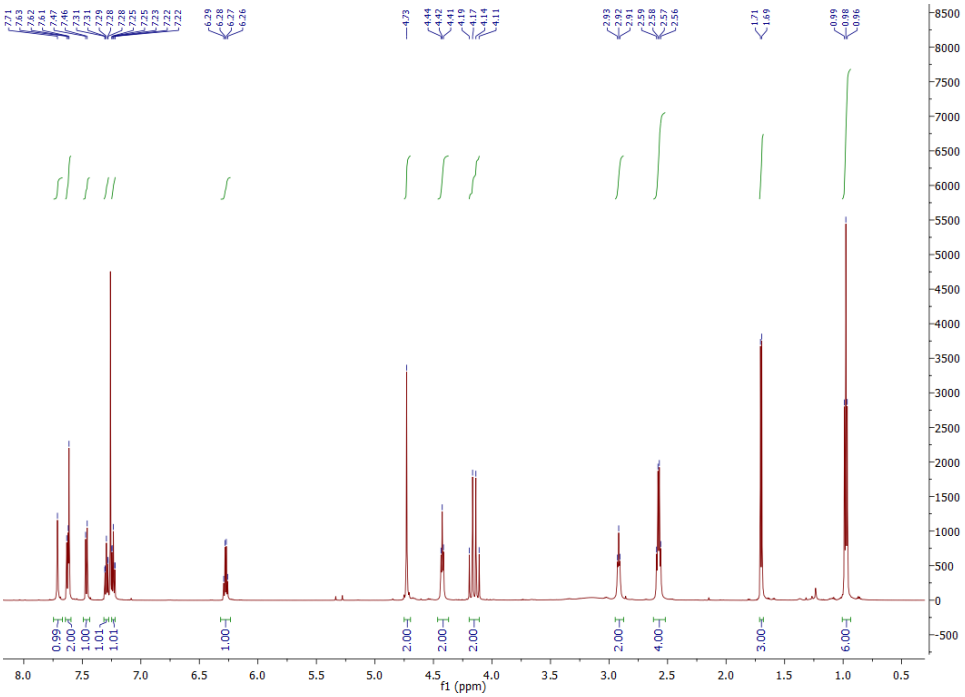


HRMS (ESI$^+$): *rac*-4b: m/z: $C_{21}H_{28}N_4O_4$ [M+H$^+$] calc. 401.2183, found 401.2186.

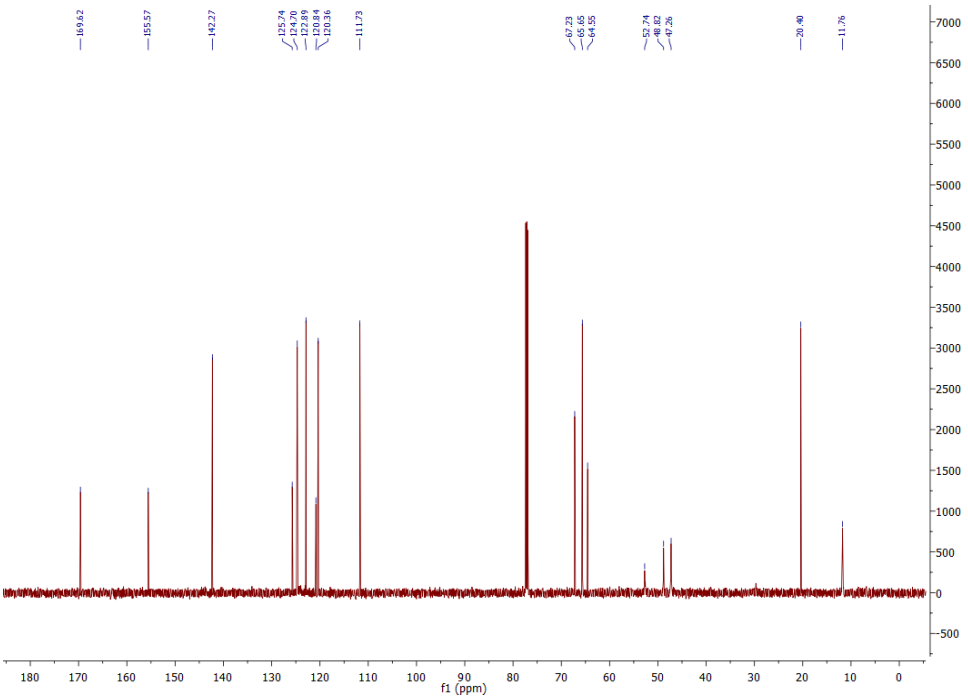


1.4.3. (±)-1-(Benzofuran-3-yl)ethyl 2-((1-(2-(diethylamino)ethyl)-1H-1,2,3-triazol-4-yl)methoxy)acetate (rac-4c)

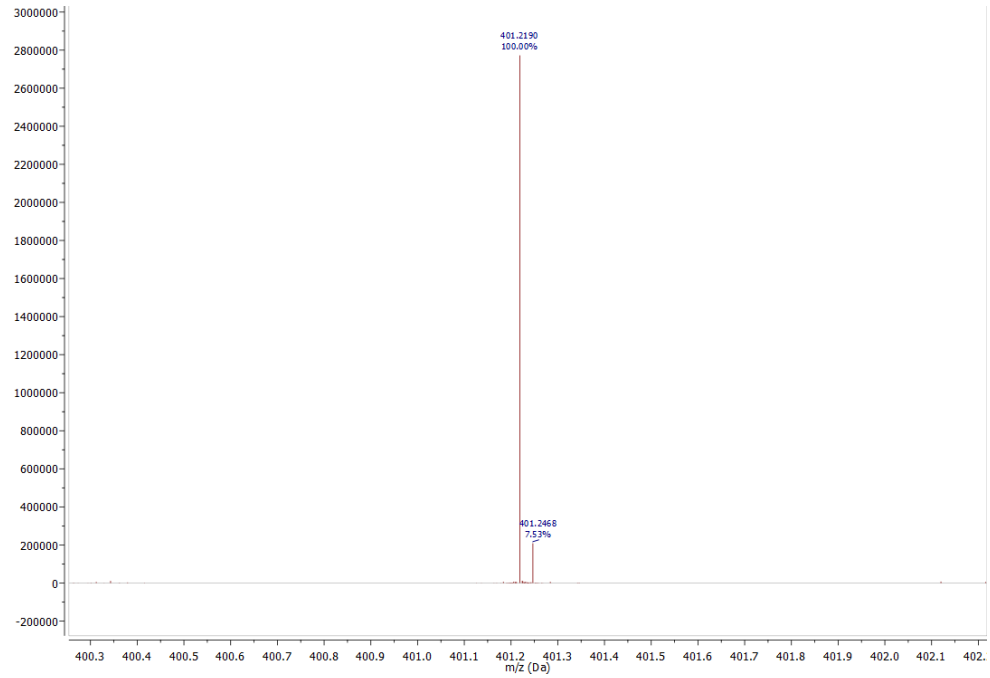
^{1}H NMR: *rac*-4c (600 MHz, $CDCl_3$): δ 7.71 (s, 1H), 7.62 (d, J = 7.8 Hz, 1H), 7.61 (s, 1H), 7.47 (d, J = 8.2 Hz, 1H), 7.32 – 7.27 (m, 1H), 7.25 – 7.22 (m, 1H), 6.28 (q, J = 6.6 Hz, 1H), 4.73 (s, 2H), 4.42 (t, J = 6.2 Hz, 2H), 4.15 (d, J = 16.5 Hz, 2H), 2.92 (t, J = 6.2 Hz, 2H), 2.58 (q, J = 7.1 Hz, 4H) 1.70 (d, J = 6.6 Hz, 3H), 0.98 (t, J = 7.1 Hz, 6H).



^{13}C NMR: ***rac*-4c** (151 MHz, $CDCl_3$): δ 169.62, 155.57, 142.27, 125.74, 124.70, 122.89, 120.84, 120.36, 111.73, 67.23, 65.65, 64.55, 52.74, 48.82, 47.26, 20.40, 11.76.

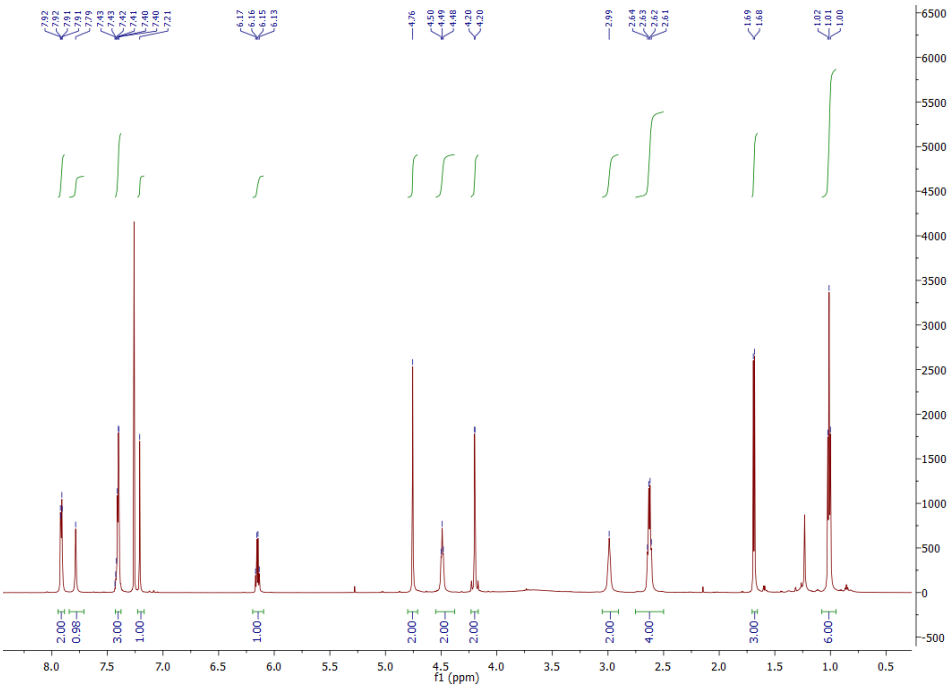


HRMS (ESI$^+$): ***rac*-4c**: m/z: $C_{21}H_{28}N_4O_4$ [M+H$^+$] calc. 401.2183, found 401.2190.

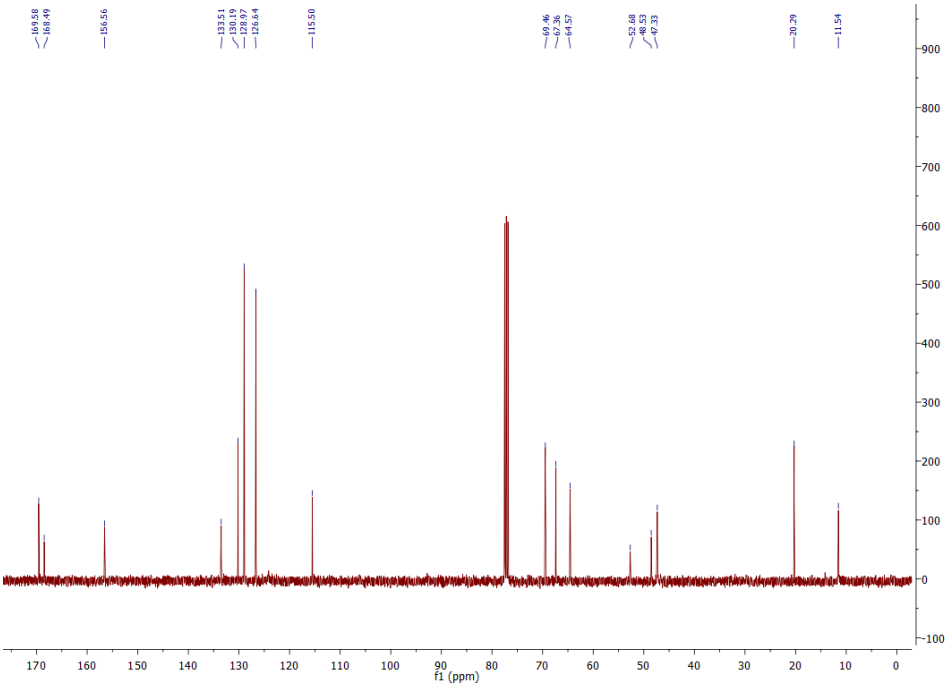


1.4.4. (±)-1-(2-phenylthiazol-4-yl)ethyl 2-((1-(2-(diethylamino)ethyl)-1H-1,2,3-triazol-4-yl)methoxy)acetate (rac-4d)

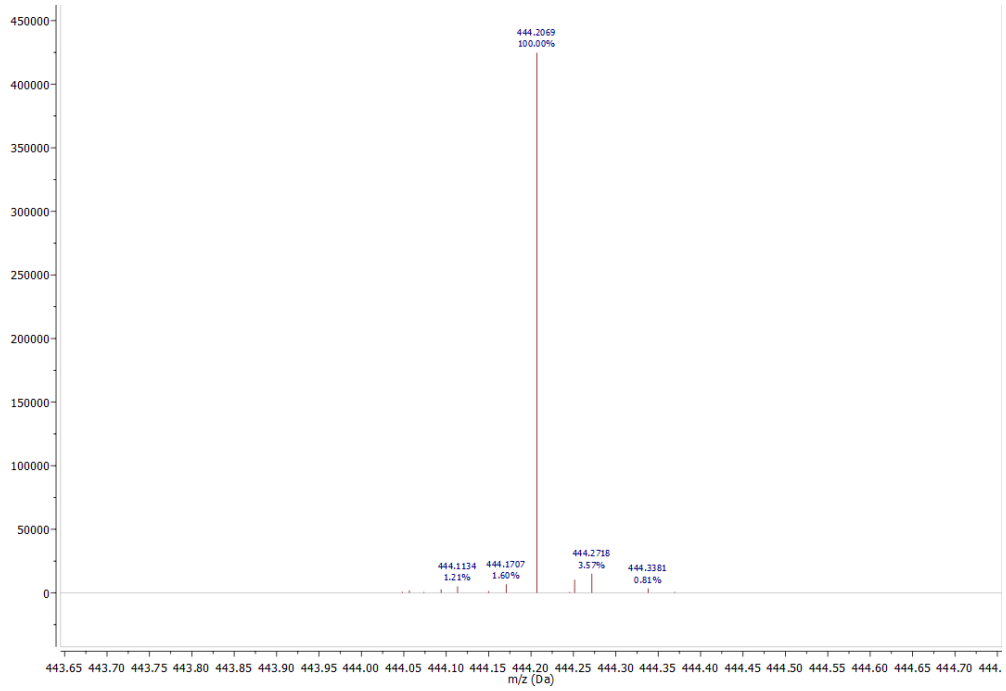
^{1}H NMR: ***rac*-4d** (600 MHz, $CDCl_3$):) δ 7.92 - 7.90 (m, 2H), 7.79 (s, 1H), 7.43 – 7.38 (m, 3H), 7.21 (s, 1H), 6.15 (q, J = 6.6 Hz, 1H), 4.76 (s, 2H), 4.49 (t, J = 5.9 Hz, 2H), 4.20 (s, 2H), 2.99 (overlapped t, 2H), 2.63 (q, J = 6.9 Hz, 4H), 1.69 (d, J = 6.6 Hz, 3H), 1.01 (t, J = 6.9 Hz, 6H).



^{13}C NMR: ***rac*-4d** (101 MHz, $CDCl_3$): δ 169.58, 169.49, 156.56, 133.51, 130.19, 128.97, 126.64, 115.50, 69.46, 67.36, 64.57, 52.68, 48.53, 47.33, 20.29, 11.54.



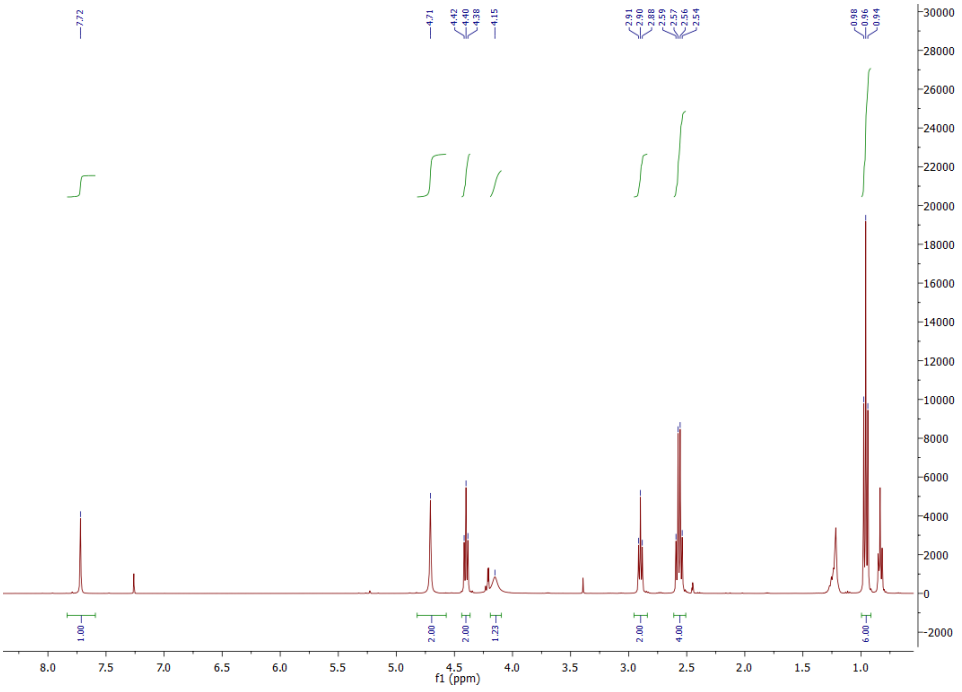
HRMS (ESI$^+$): *rac*-4d: m/z: $C_{22}H_{29}N_5O_3S$ [M+H$^+$] calc. 444.2064, found 444.2069.



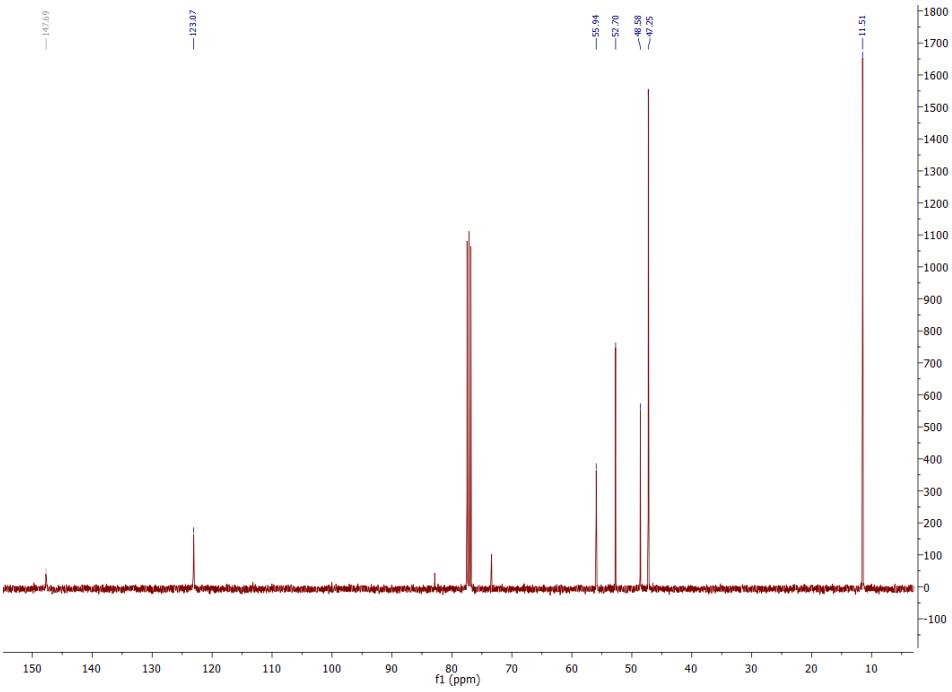
1.5. Copper(I) source screening for the click reaction

(1-(2-(diethylamino)ethyl)-1*H*-1,2,3-triazol-4-yl)methanol:

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.72 (s, 1H), 4.71 (s, 2H), 4.40 (t, *J* = 6.5 Hz, 2H), 4.15 (bs, 1H), 2.90 (t, *J* = 6.5 Hz, 2H), 2.57 (q, *J* = 7.1 Hz, 4H), 0.96 (t, *J* = 7.1 Hz, 6H) ppm



^{13}C NMR (101 MHz, $CDCl_3$): δ 147.69, 123.07, 55.94, 52.70, 48.58, 47.25, 11.51 ppm



2. Solvent screening for the acylation reaction of racemic ethanols *rac*-1a-d with ethyl 2-(prop-2-yn-1-yloxy)acetate 2A

Table S1. Solvent screening for the enzymatic acylation reaction of *rac*-**1a-d** (10 mM) with ethyl 2-(prop-2-yn-1-yloxy)acetate **2A** (1 equiv.), at 30 °C (substrate:biocatalyst *ratio* 1:1, w/w)

Solvent	$c_{rac\text{-}1a}$ [a,b] **(%)**	ee_{3a} [a,b] **(%)**	$c_{rac\text{-}1b}$ [c,d] **(%)**	ee_{3b} [c,d] **(%)**	$c_{rac\text{-}1c}$ [e,b] **(%)**	ee_{3c} [e,b] **(%)**	$c_{rac\text{-}1d}$ [f,b] **(%)**	ee_{3d} [f,b] **(%)**
n-Hexane	17	>99	17	>99	10	>99	n.d.	<5
CH_2Cl_2	n.d.	n.d.	n.d.	n.d.	11	>99	15	>99
ACN	n.d.	n.d.	n.d.	n.d.	18	>99	n.d.	n.d.
THF	n.d.	n.d.	n.d.	n.d.	15	>99	n.d.	n.d.
MTBE	n.d.	n.d.	n.d.	n.d.	18	>99	n.d.	<5
DIPE	n.d.	n.d.	n.d.	n.d.	21	>99	4	30
Cyclohexane	16	>99	n.d.	n.d.	30	>99	25	39
Toluene	n.d.	n.d.	n.d.	n.d.	26	>99	18	86

[a]after 4 h; [b]Lipase B from *Candida antarctica* (Novozym 435, $CaLB_{N435}$); [c]after 120 h; [d]Lipase from *Pseudomonas fluorescens* (L-AK); [e]after 15 h; [f]after 130 h; n.d. not detected

3. Equilibrium effect for the acylation reaction of racemic ethanols *rac*-1a-d with ethyl 2-(prop-2-yn-1-yloxy)acetate 2A in the optimal solvents

Table S2. Equilibrium effect for the lipase-mediated acylation reaction of *rac*-**1a-d** (10 mM) with **2A** (1 equiv.) in the optimal solvents, at 30 °C (substrate:biocatalyst *ratio* 1:1 w/w)

Entry	Substrate	Solvent	Reaction time (h)	*c* (%)
1	*rac*-**1a**[a]	*n*-Hexane	4	17
2			20	20
3		Cyclohexane	4	16
4			20	16
5	*rac*-**1b**[b]	*n*-Hexane	120	17
6			168	18
7	*rac*-**1c**[a]	Cyclohexane	15	30
8			50	31
9	*rac*-**1d**[a]	CH_2Cl_2	130	15
10			190	16

[a] Lipase B from *Candida antarctica* (Novozym 435, $CaLB_{N435}$); [b] Lipase from *Pseudomonas fluorescens* (L-AK)

4. Optimization of lipase-mediated *O*-acylation of *rac*-1c

Table S3. Temperature and substrate:biocatalyst *ratio* (w/w) screening for the acylation reaction of *rac*-**1c** with ethyl 2-(prop-2-yn-1-yloxy)acetate **2A** (1 equiv.) in cyclohexane, catalysed by Novozym 435 after 6 h reaction time

Conversion (%)			
Substrate:biocatalyst *ratio* (w/w)	Temperature (°C)		
	30	40	50
1:1	11	8	12
1:2	12	11	10
1:3	9	8	9

Table S4. Acyl donor quantity screening for the Novozym 435-mediated reaction of *rac*-**1c** with ethyl 2-(prop-2-yn-1-yloxy)acetate **2A** in cyclohexane at 30 °C (substrate:biocatalyst *ratio* 1:1, w/w)

Conversion (%)				
Equiv. of acyl donor	Reaction time (h)			
	12	30	50	140
1	26	28	31	31
2	30	32	33	29
3	31	35	35	36

5. HPLC separation conditions for racemic compounds *rac*-1,3,4a-d

Table S5. Retention times and chromatographic separation methods of racemic compounds *rac*-**1,3,4a–d**

Entry	Compound	t_R (min)	Separation method	
			Column[a]	Mobile phase[b]
1	*rac*-**1a**	6.5/7.1	Phenomenex LUX-3	*n*-hexane:2-propanol 9:1
	rac-**3a**	15.4/18.0		
	rac-**4a**	11.1/16.3		*n*-hexane:2-propanol 8:2
2	*rac*-**1b**	10.6/11.0	Phenomenex LUX-3	*n*-hexane:2-propanol 9:1
	rac-**3b**	21.8/24.5		
	rac-**4b**	35.3/38.4		*n*-hexane:2-propanol 9:1
3	*rac*-**1c**	10.2/11.3	Phenomenex LUX-3	*n*-hexane:2-propanol 9:1
	rac-**3c**	9.8/11.2	Chiralpak IA	*n*-hexane:2-propanol 98:2
	rac-**4c**	17.1/18.5	Phenomenex LUX-3	*n*-hexane:2-propanol 8:2
4	*rac*-**1d**	11.9/12.6	Phenomenex LUX-3	*n*-hexane:2-propanol 9:1
	rac-**3d**	21.4/25.6		
	rac-**4d**	19.7/21.7		*n*-hexane:2-propanol 8:2

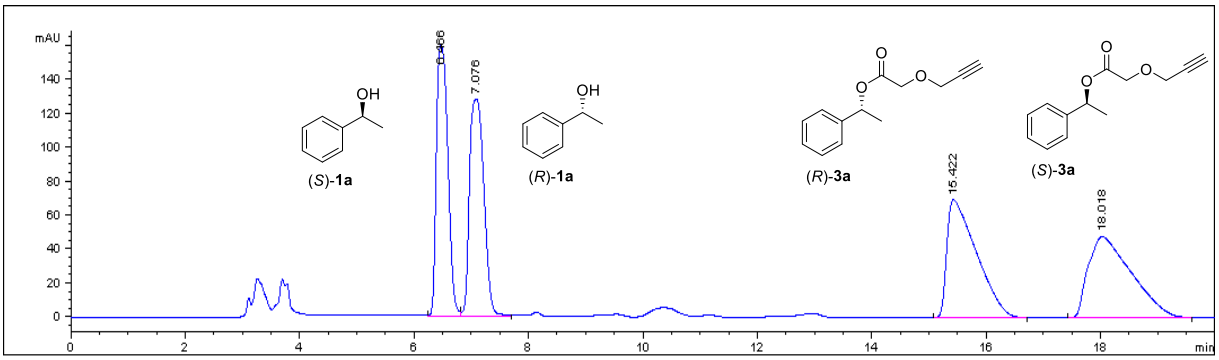
[a] 4.6×250 mm column; [b] 1 mL min^{-1} flow rate, 25 °C.

6. HPLC chromatograms

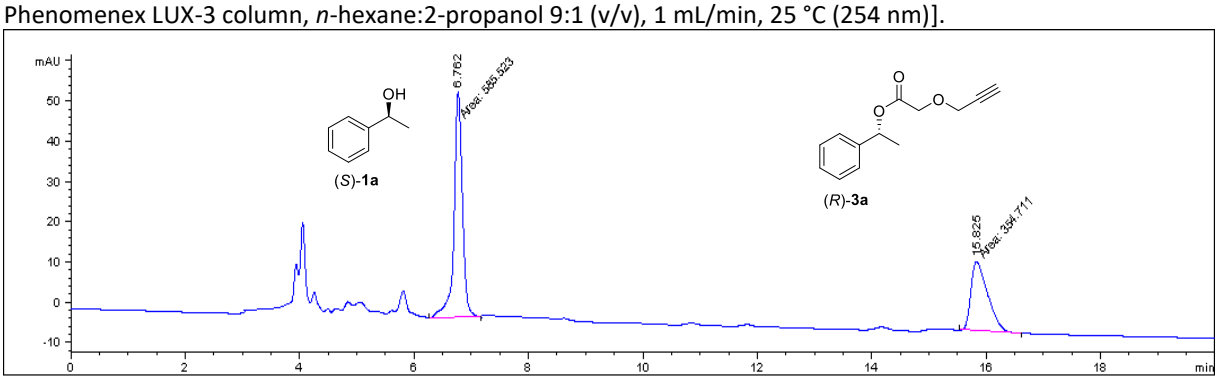
The chromatograms are presented following the synthetic steps, together with those of the racemic compound used as reference.

6.1. Resolution of the racemic 1-phenyl-1-ethanol *rac*-1a

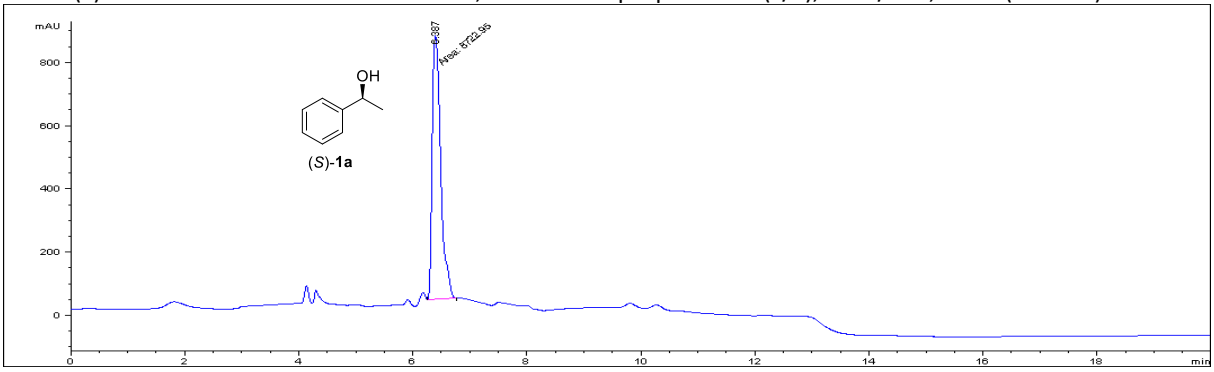
HPLC: *rac*-**1,3a** on Phenomenex LUX-3 column, *n*-hexane:2-propanol 9:1 (v/v), 1 mL/min, 25 °C (254 nm).



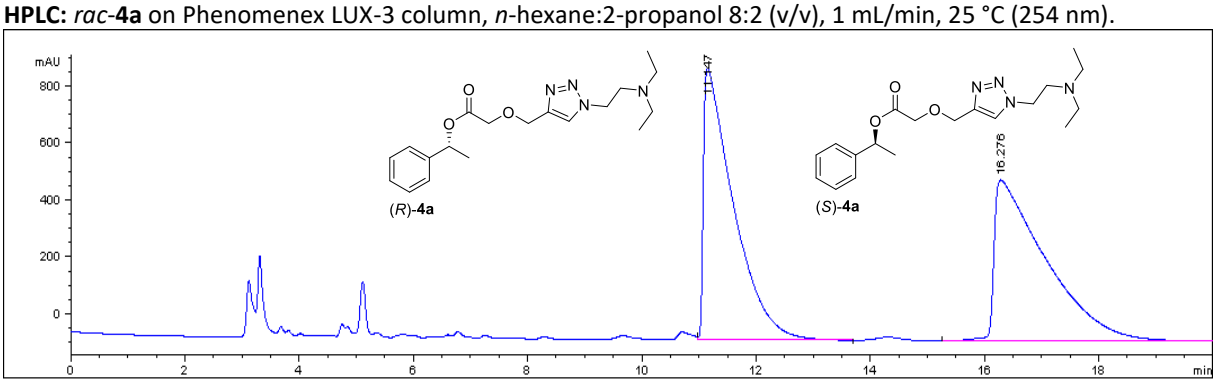
HPLC: $CaLB_{N435}$-mediated kinetic resolution of *rac*-**1a** (10 mM) with 2,2,2-trifluoroethyl 2-(prop-2-yn-1-yloxy)acetate **2D** (1 equiv.), in *n*-hexane at 30 °C (substrate:biocatalyst *ratio* 1:1, w/w) after 10 hours [on Phenomenex LUX-3 column, *n*-hexane:2-propanol 9:1 (v/v), 1 mL/min, 25 °C (254 nm)].



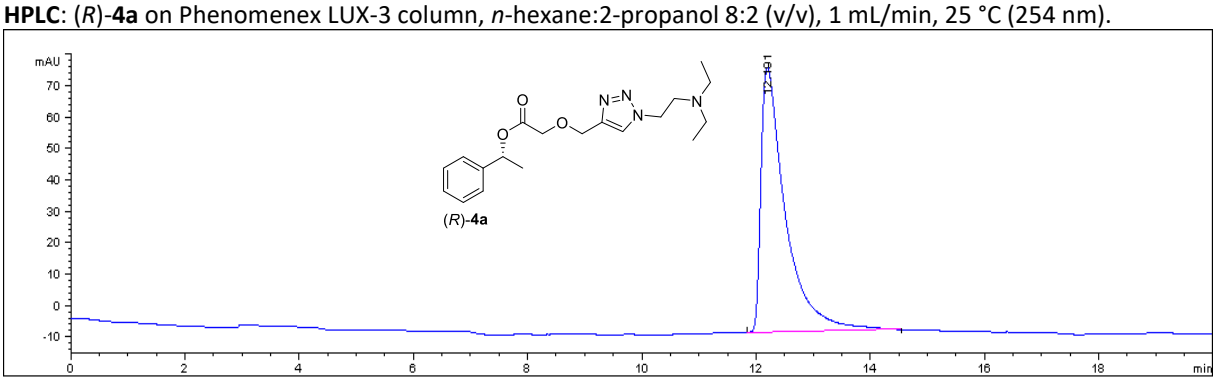
HPLC: (*S*)-**1a** on Phenomenex LUX-3 column, *n*-hexane:2-propanol 9:1 (v/v), 1 mL/min, 25 °C (254 nm).



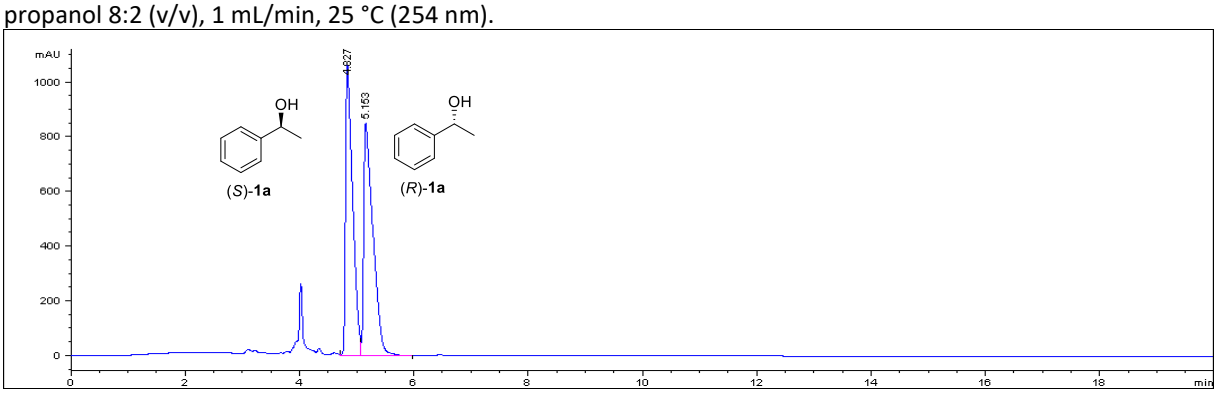
HPLC: *rac*-**4a** on Phenomenex LUX-3 column, *n*-hexane:2-propanol 8:2 (v/v), 1 mL/min, 25 °C (254 nm).



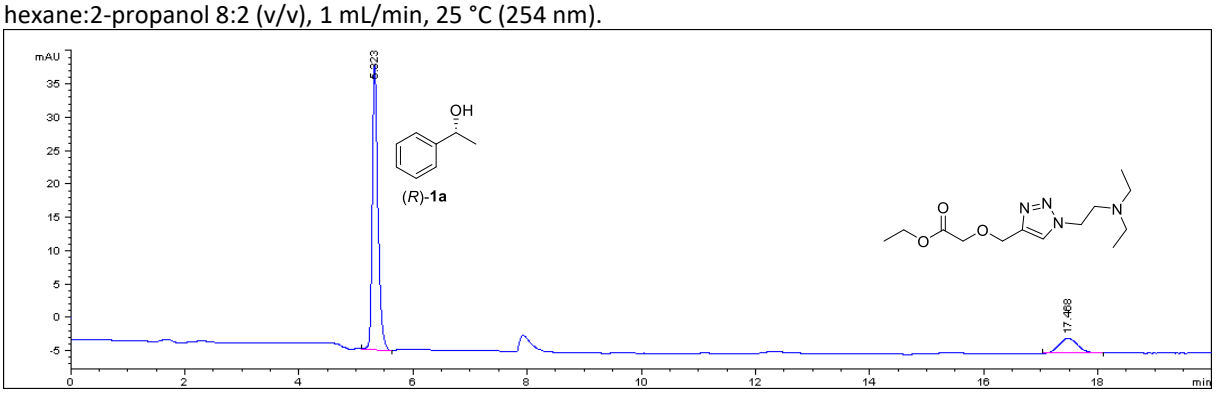
HPLC: (*R*)-**4a** on Phenomenex LUX-3 column, *n*-hexane:2-propanol 8:2 (v/v), 1 mL/min, 25 °C (254 nm).



HPLC: Chromatographic enantiomeric separation of *rac*-**1a** on Phenomenex LUX-3 column, *n*-hexane:2-propanol 8:2 (v/v), 1 mL/min, 25 °C (254 nm).

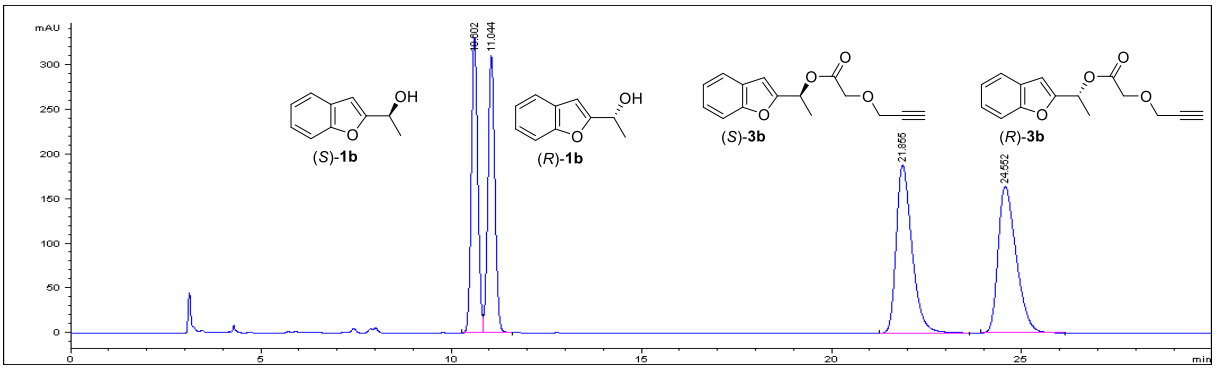


HPLC: $CaLB_{SWCNT}$-mediated alcoholysis of (*R*)-**4a** (10 mM) with ethanol (5 equiv.), in ACN at 60 °C (substrate:biocatalyst *ratio* 1:1, w/w) after 20 hours. HPLC separation on Phenomenex LUX-3 column, *n*-hexane:2-propanol 8:2 (v/v), 1 mL/min, 25 °C (254 nm).

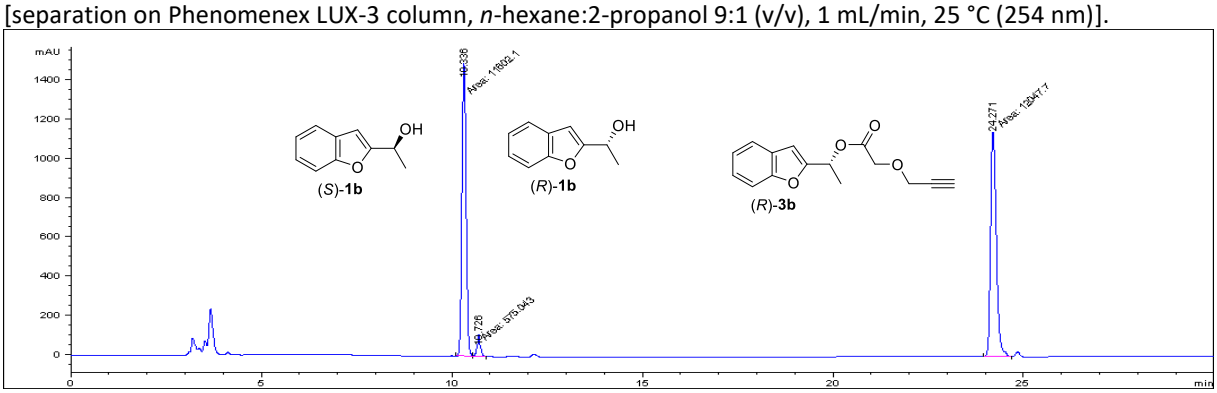


6.2. Resolution of the racemic 1-(Benzofuran-2-yl)ethanol *rac*-1b

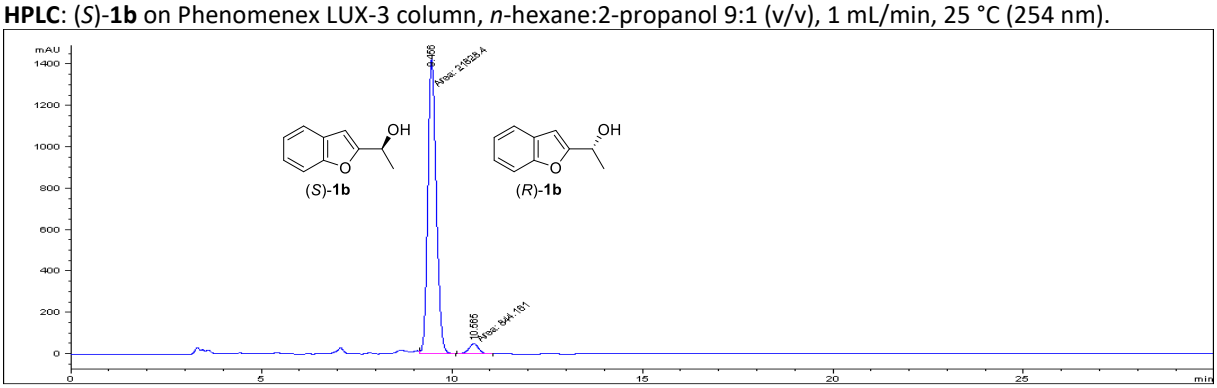
HPLC: *rac*-**1,3b** on Phenomenex LUX-3 column, *n*-hexane:2-propanol 9:1 (v/v), 1 mL/min, 25 °C (254 nm).



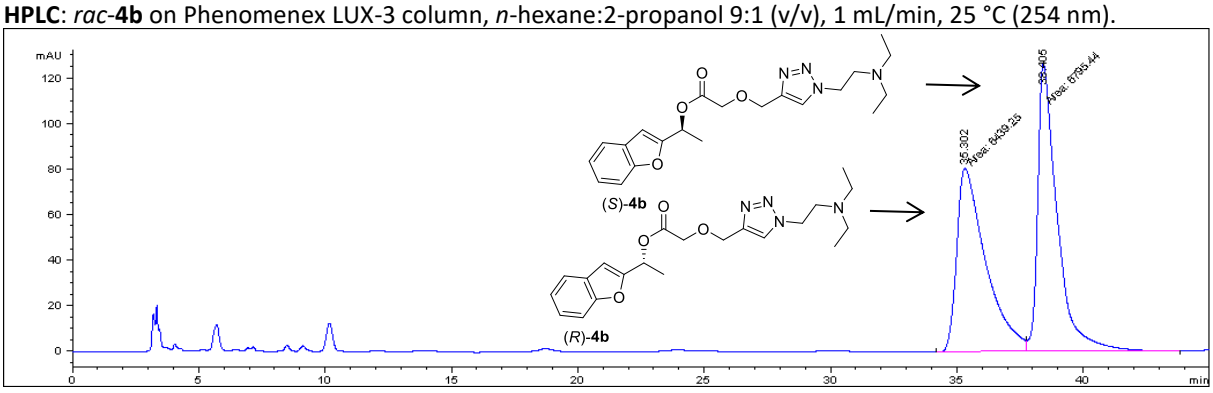
HPLC: L-AK-mediated kinetic resolution of *rac*-**1b** (10 mM) with 2,2,2-trifluoroethyl 2-(prop-2-yn-1-yloxy)acetate **2D** (1 equiv.), in *n*-hexane at 30 °C (substrate:biocatalyst *ratio* 1:1, w/w) after 90 hours [separation on Phenomenex LUX-3 column, *n*-hexane:2-propanol 9:1 (v/v), 1 mL/min, 25 °C (254 nm)].



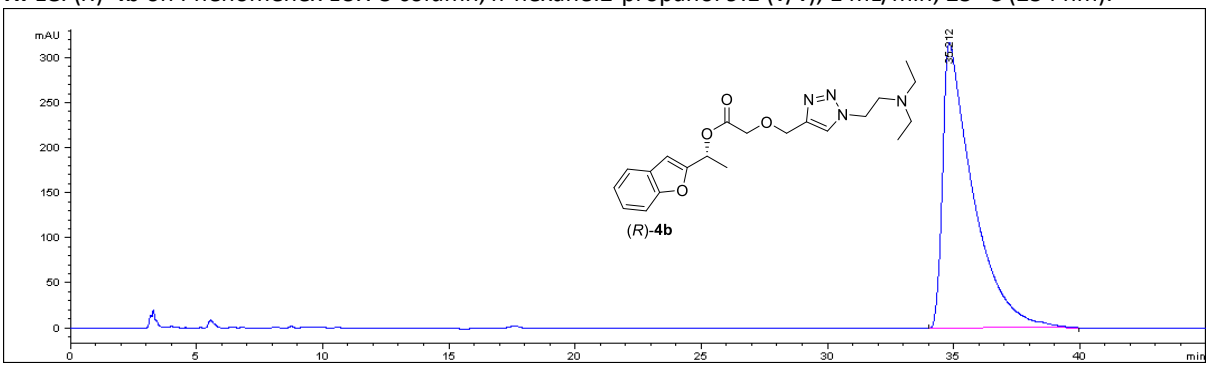
HPLC: (*S*)-**1b** on Phenomenex LUX-3 column, *n*-hexane:2-propanol 9:1 (v/v), 1 mL/min, 25 °C (254 nm).



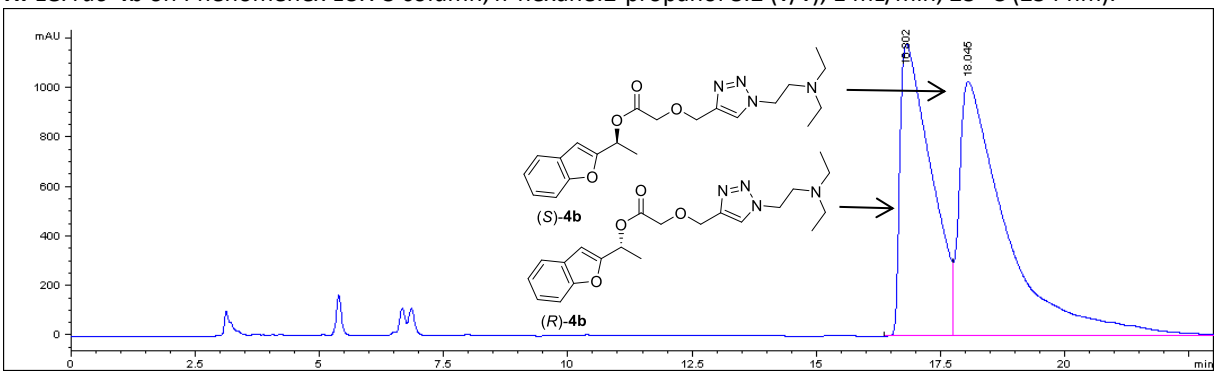
HPLC: *rac*-**4b** on Phenomenex LUX-3 column, *n*-hexane:2-propanol 9:1 (v/v), 1 mL/min, 25 °C (254 nm).



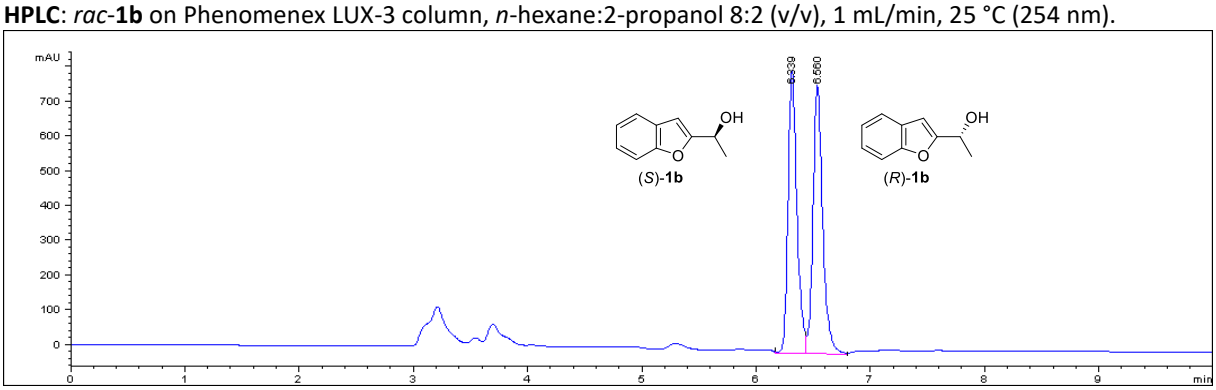
HPLC: (*R*)-**4b** on Phenomenex LUX-3 column, *n*-hexane:2-propanol 9:1 (v/v), 1 mL/min, 25 °C (254 nm).



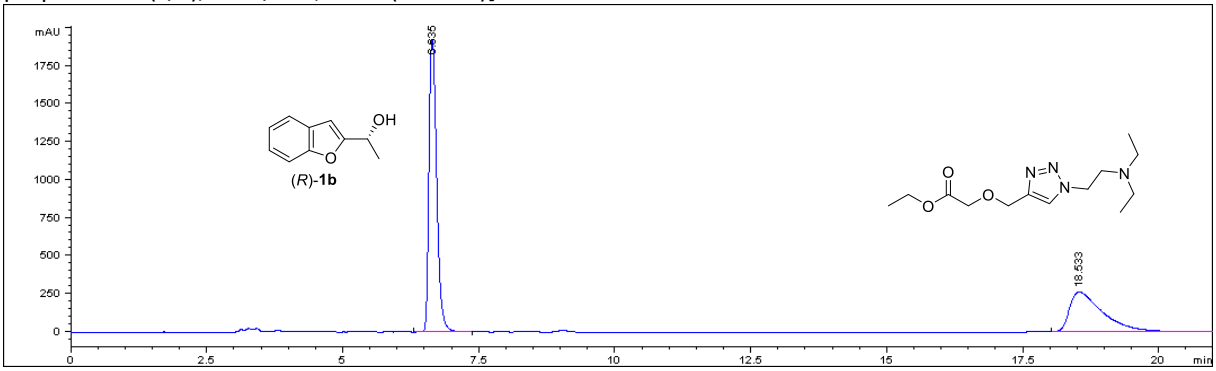
HPLC: *rac*-**4b** on Phenomenex LUX-3 column, *n*-hexane:2-propanol 8:2 (v/v), 1 mL/min, 25 °C (254 nm).



HPLC: *rac*-**1b** on Phenomenex LUX-3 column, *n*-hexane:2-propanol 8:2 (v/v), 1 mL/min, 25 °C (254 nm).



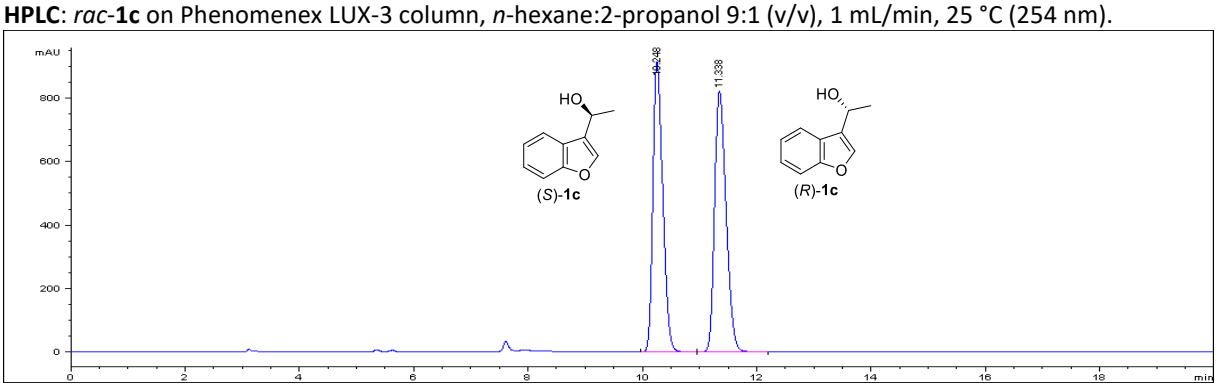
HPLC: $CaLB_{SWCNT}$-mediated alcoholysis of (*R*)-**4b** (10 mM) with ethanol (5 equiv.), in ACN at 60 °C (substrate:biocatalyst *ratio* 1:1, w/w) after 22 hours [separation on Phenomenex LUX-3 column, *n*-hexane:2-propanol 8:2 (v/v), 1 mL/min, 25 °C (254 nm)].



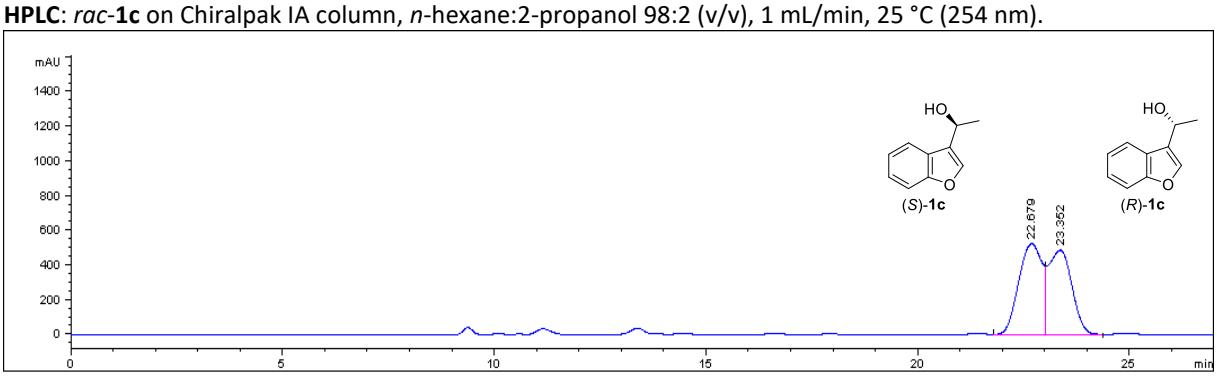
6.3. Resolution of the racemic 1-(Benzofuran-3-yl)ethanol *rac*-1c

Since the base-line separation of the racemic ethanol *rac*-**1c** and racemic ester *rac*-**3c** was obtained on two different chiral columns, the reaction mixture was analyzed on both columns.

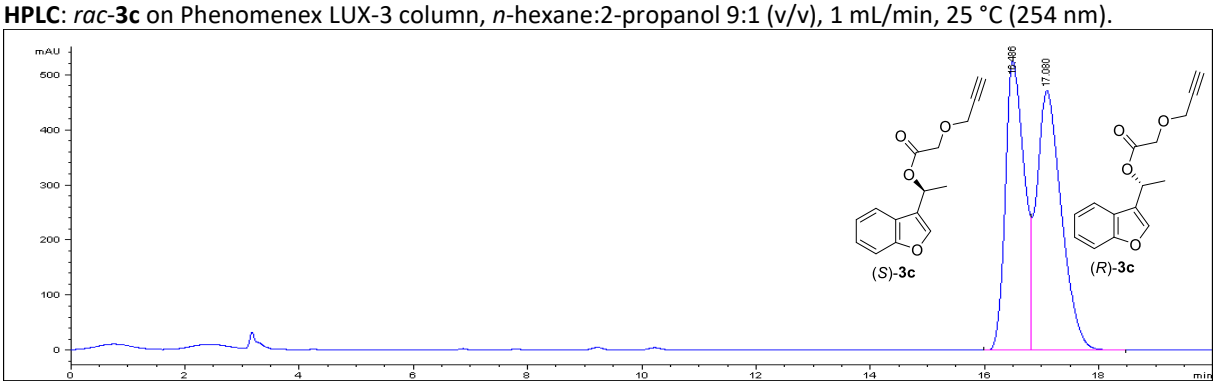
HPLC: *rac*-**1c** on Phenomenex LUX-3 column, *n*-hexane:2-propanol 9:1 (v/v), 1 mL/min, 25 °C (254 nm).



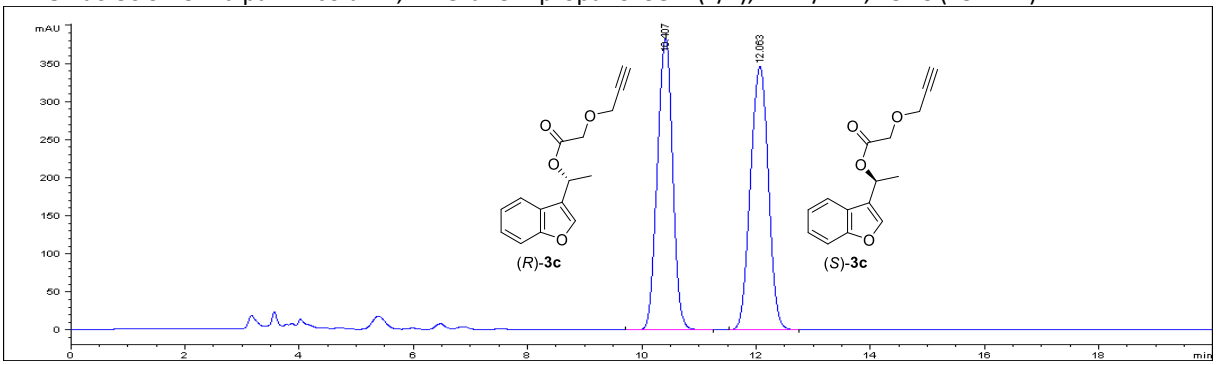
HPLC: *rac*-**1c** on Chiralpak IA column, *n*-hexane:2-propanol 98:2 (v/v), 1 mL/min, 25 °C (254 nm).



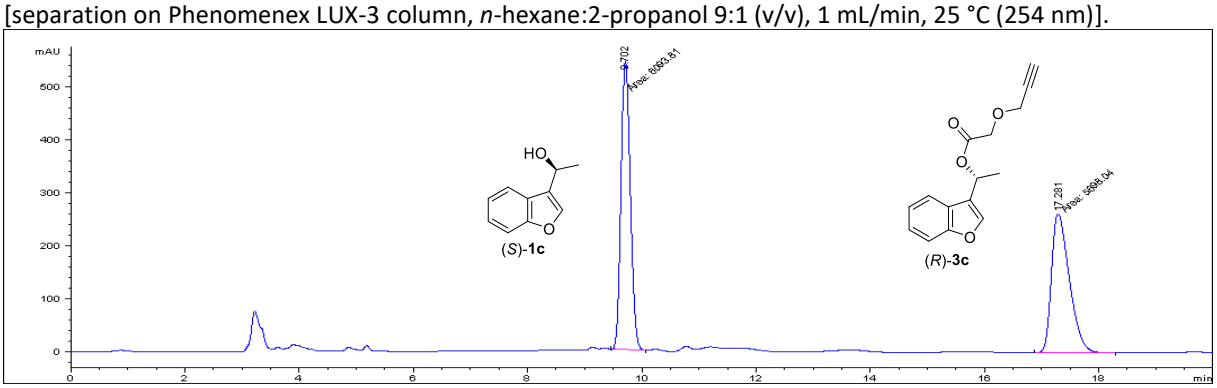
HPLC: *rac*-**3c** on Phenomenex LUX-3 column, *n*-hexane:2-propanol 9:1 (v/v), 1 mL/min, 25 °C (254 nm).



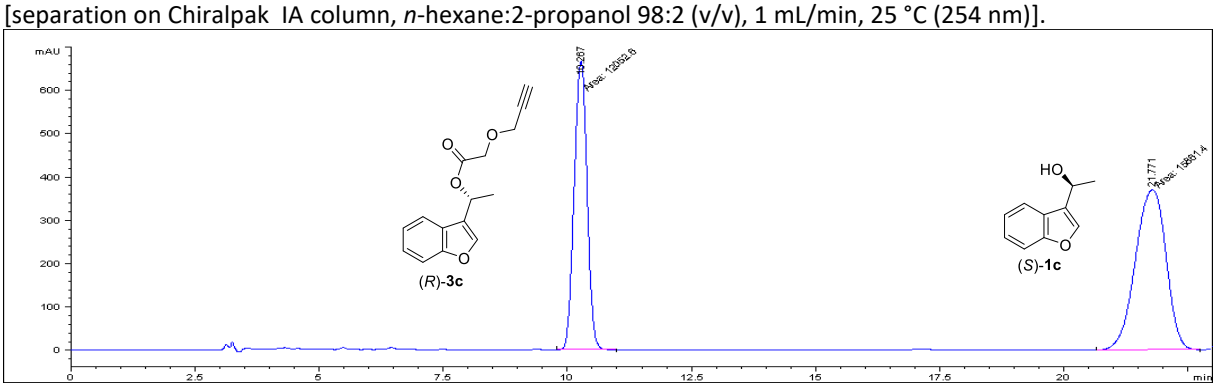
HPLC: *rac*-**3c** on Chiralpak IA column, *n*-hexane:2-propanol 98:2 (v/v), 1 mL/min, 25 °C (254 nm).



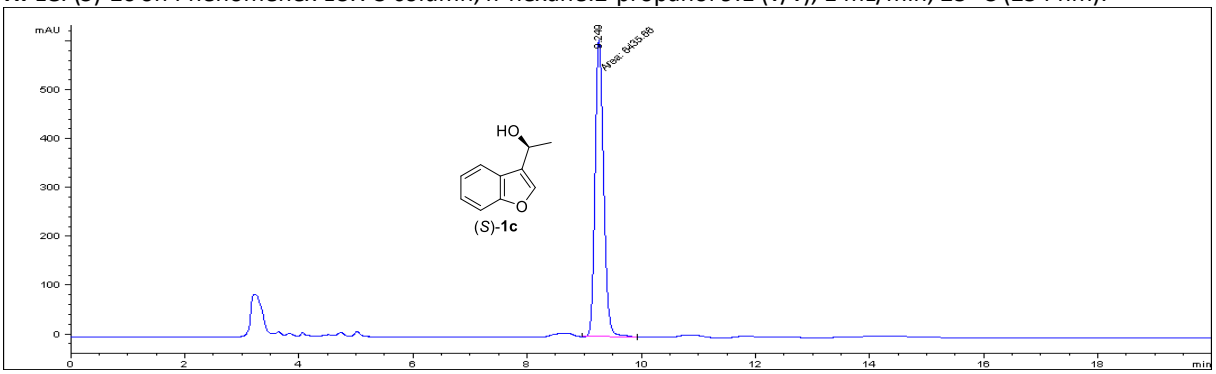
HPLC: $CaLB_{N435}$-mediated kinetic resolution of *rac*-**1c** (10 mM) with 2,2,2-trifluoroethyl 2-(prop-2-yn-1-yloxy)acetate **2D** (1 equiv.), in cyclohexane at 30 °C (substrate:biocatalyst *ratio* 1:1, w/w) after 7 hours [separation on Phenomenex LUX-3 column, *n*-hexane:2-propanol 9:1 (v/v), 1 mL/min, 25 °C (254 nm)].



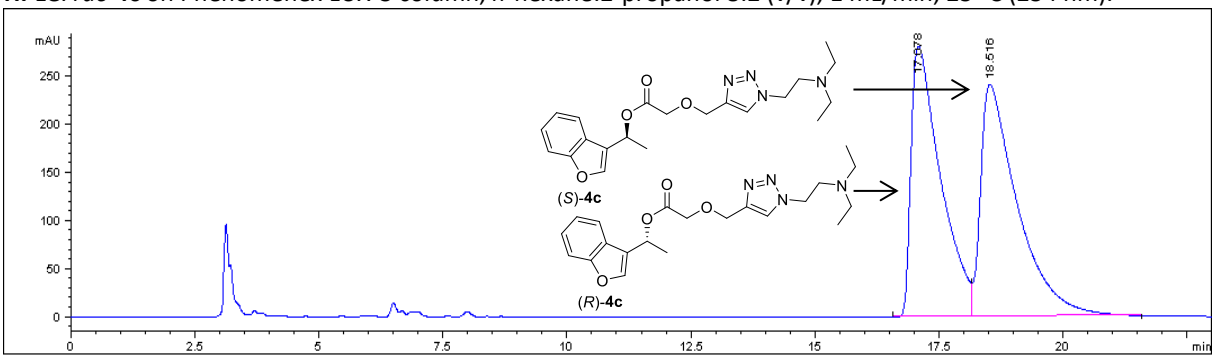
HPLC: $CaLB_{N435}$-mediated kinetic resolution of *rac*-**1c** (10 mM) with 2,2,2-trifluoroethyl 2-(prop-2-yn-1-yloxy)acetate **2D** (1 equiv.), in cyclohexane at 30 °C (substrate:biocatalyst *ratio* 1:1, w/w) after 7 hours [separation on Chiralpak IA column, *n*-hexane:2-propanol 98:2 (v/v), 1 mL/min, 25 °C (254 nm)].



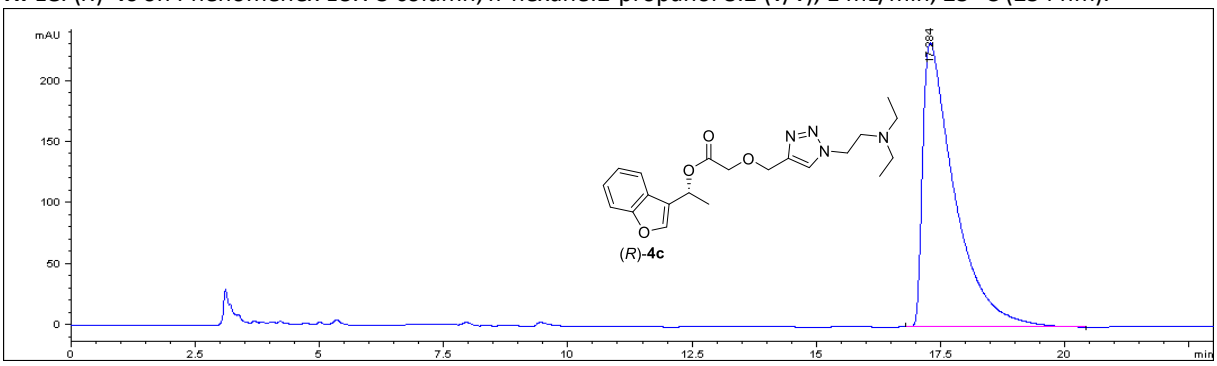
HPLC: (*S*)-**1c** on Phenomenex LUX-3 column, *n*-hexane:2-propanol 9:1 (v/v), 1 mL/min, 25 °C (254 nm).



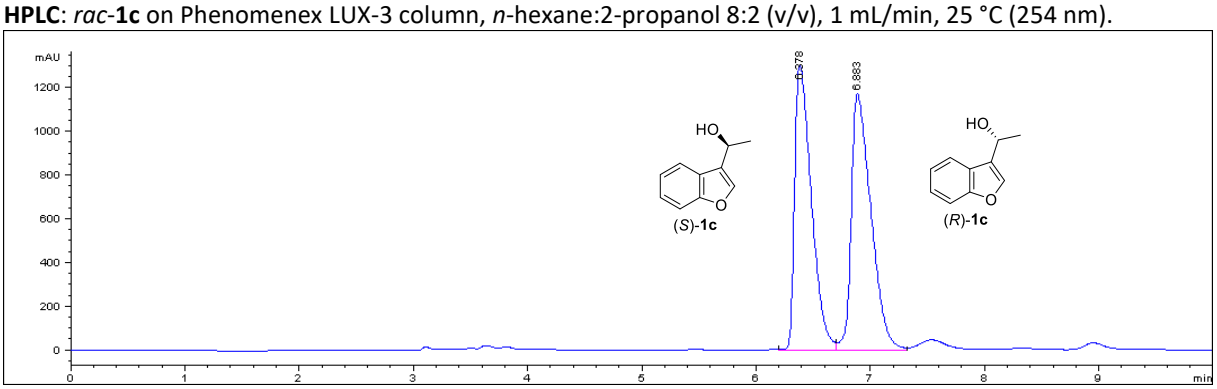
HPLC: *rac*-**4c** on Phenomenex LUX-3 column, *n*-hexane:2-propanol 8:2 (v/v), 1 mL/min, 25 °C (254 nm).



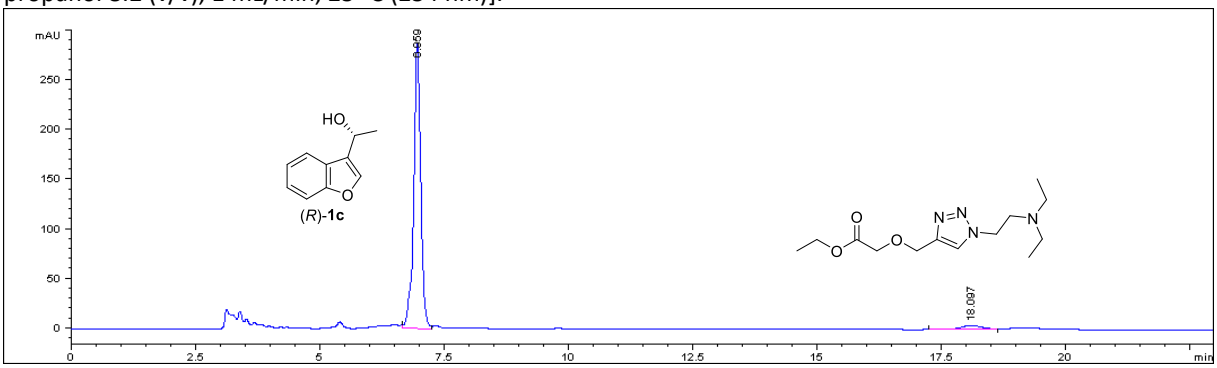
HPLC: (*R*)-**4c** on Phenomenex LUX-3 column, *n*-hexane:2-propanol 8:2 (v/v), 1 mL/min, 25 °C (254 nm).



HPLC: *rac*-**1c** on Phenomenex LUX-3 column, *n*-hexane:2-propanol 8:2 (v/v), 1 mL/min, 25 °C (254 nm).

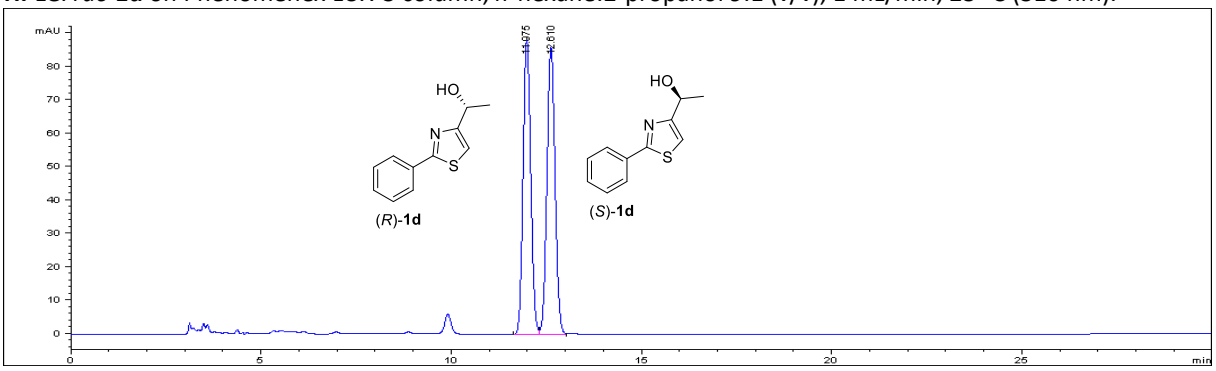


HPLC: $CaLB_{SWCNT}$-mediated alcoholysis of (*R*)-**4c** (10 mM) with ethanol (5 equiv.), in ACN at 60 °C (substrate:biocatalyst *ratio* 1:1, w/w) after 24 hours [separation on Phenomenex LUX-3 column, *n*-hexane:2-propanol 8:2 (v/v), 1 mL/min, 25 °C (254 nm)].



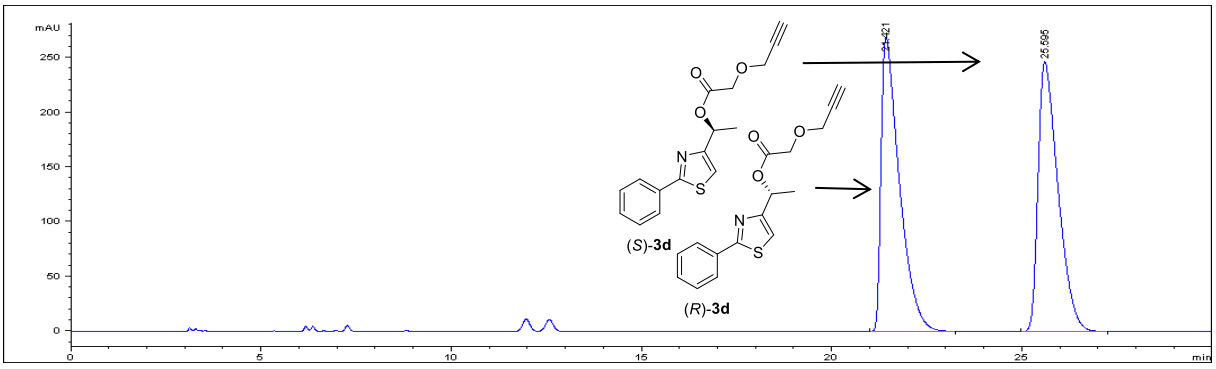
6.4. Resolution of the racemic 1-(2-phenylthiazol-4-yl)ethanol *rac*-1d

HPLC: *rac*-**1d** on Phenomenex LUX-3 column, *n*-hexane:2-propanol 9:1 (v/v), 1 mL/min, 25 °C (310 nm).

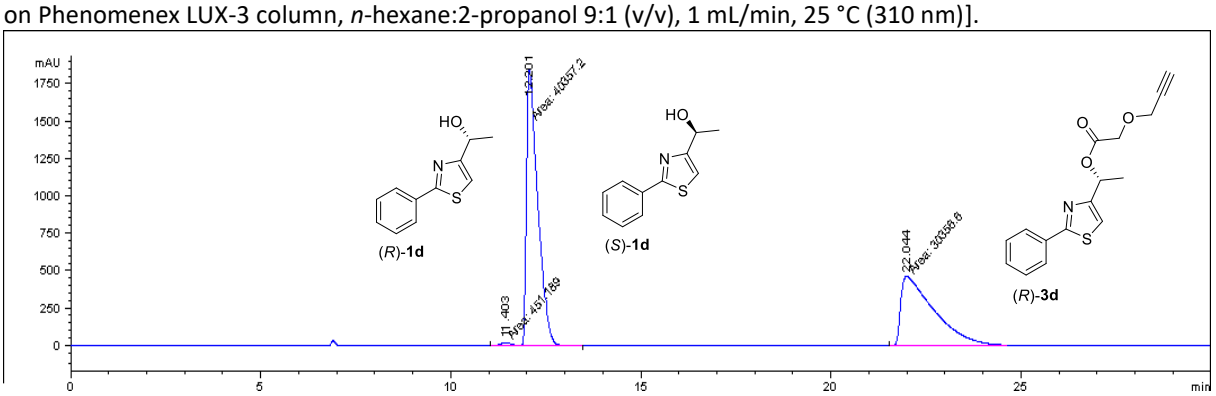


HPLC: *rac*-**3d** on Phenomenex LUX-3 column, *n*-hexane:2-propanol 9:1 (v/v), 1 mL/min, 25 °C (310 nm).

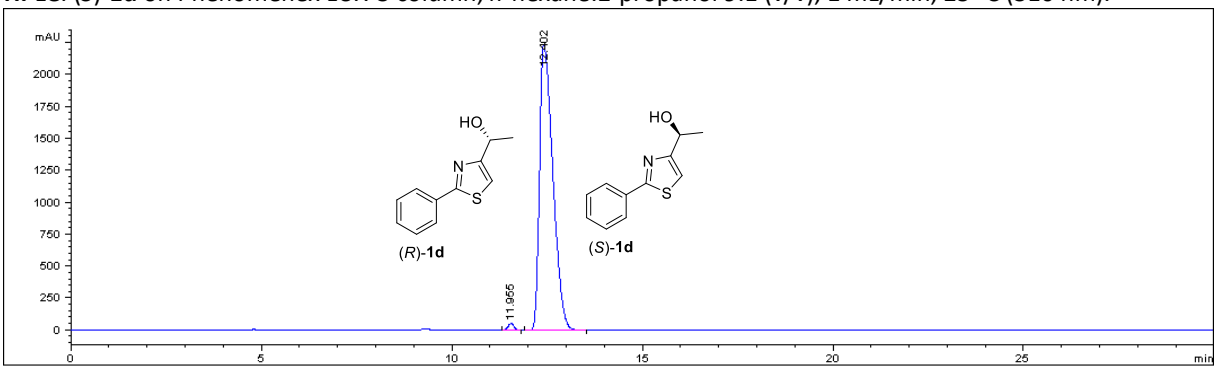
0



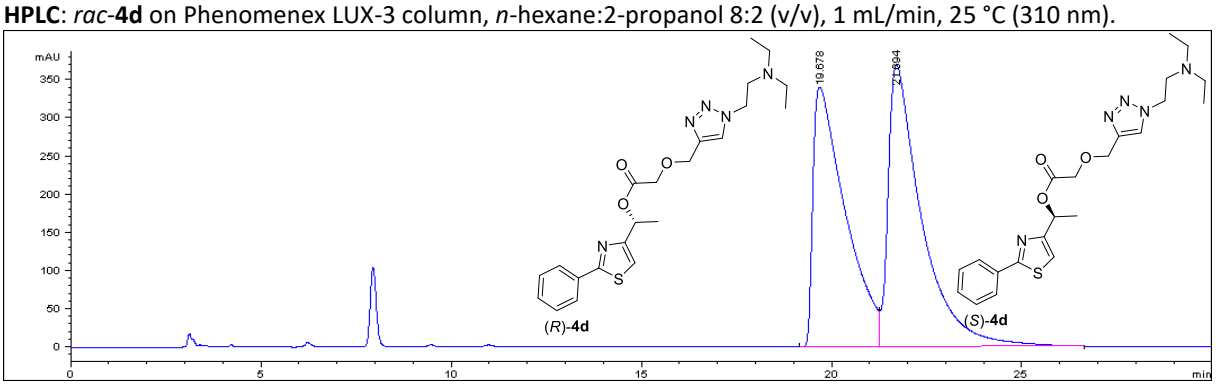
HPLC: $CaLB_{N435}$-mediated kinetic resolution of *rac*-**1d** (10 mM) with 2,2,2-trifluoroethyl 2-(prop-2-yn-1-yloxy)acetate **2D** (1 equiv.), in CH_2Cl_2 at 30 °C (substrate:biocatalyst *ratio* 1:1, w/w) after 30 hours [separation on Phenomenex LUX-3 column, *n*-hexane:2-propanol 9:1 (v/v), 1 mL/min, 25 °C (310 nm)].



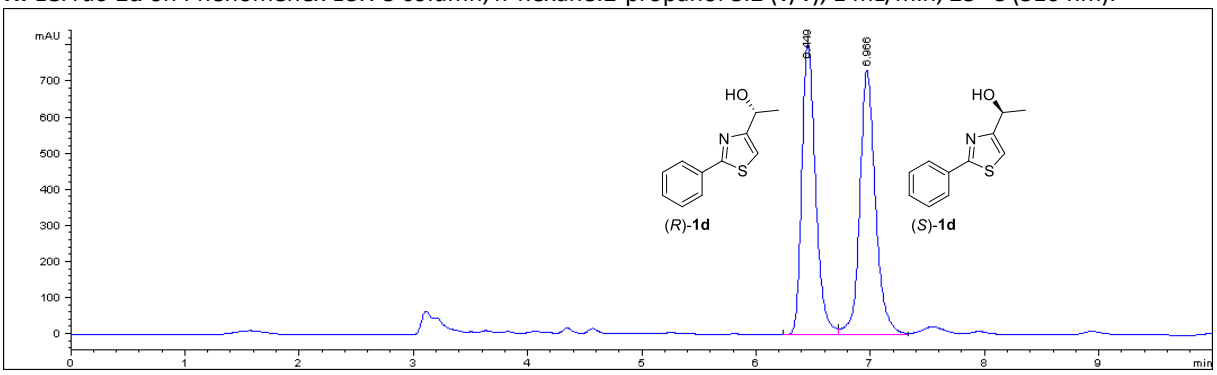
HPLC: (*S*)-**1d** on Phenomenex LUX-3 column, *n*-hexane:2-propanol 9:1 (v/v), 1 mL/min, 25 °C (310 nm).



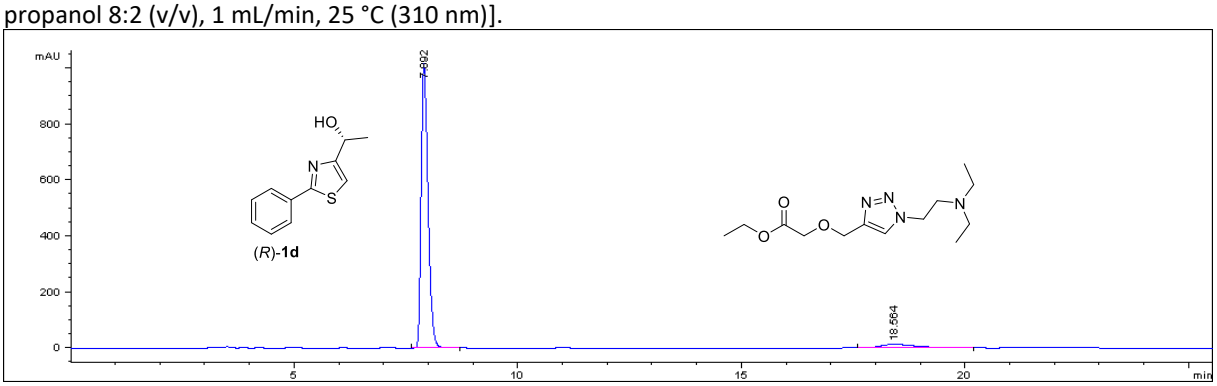
HPLC: *rac*-**4d** on Phenomenex LUX-3 column, *n*-hexane:2-propanol 8:2 (v/v), 1 mL/min, 25 °C (310 nm).



HPLC: *rac*-**1d** on Phenomenex LUX-3 column, *n*-hexane:2-propanol 8:2 (v/v), 1 mL/min, 25 °C (310 nm).



HPLC: $CaLB_{SWCNT}$-mediated alcoholysis of (*R*)-**4d** (10 mM) with ethanol (5 equiv.), in ACN at 60 °C (substrate:biocatalyst *ratio* 1:1, w/w) after 20 hours [separation on Phenomenex LUX-3 column, *n*-hexane:2-propanol 8:2 (v/v), 1 mL/min, 25 °C (310 nm)].



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[1] B. Neises and W. Steglich, *Angew. Chem. Int. Ed.,* 1978, **17**, 522.

[2] M. Starck, P. Kadjane, E. Bois, B. Darboure, A. Incamps, R. Ziessel and L. J. Charbonnière, *Chem. Eur. J.*, 2011, **17**, 9164.

[3] C. Xiao, Y. Cheng, Y. Zhang, J. Ding, C. He. X. Zhuang and Chen X. *J. Polym. Sci. Pol. Chem.,* 2014, **52**, 671.