

Synthesis of new alkynyl containing 9-azabicyclo[4.2.1]nonatrienes from diynes and azepines

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Experimental section

Chromatographic analysis was performed on a Shimadzu GC-9A instrument using a 2000×2 mm column, the SE-30 (5%) stationary phase on Chromaton N-AW-HMDS (0.125-0.160 mm), helium carrier gas (30 ml min⁻¹), temperature programming from 50 to 300 °C at rate of 8 °C min⁻¹ rate. The ¹H, ¹³C spectra were measured in CDCl₃ on a Bruker Avance-500 spectrometer (500 MHz for ¹H, 125 MHz for ¹³C). Mass spectra were obtained with a Shimadzu GC-MS-QP2010 Plus spectrometer at 70 eV and a working temperature 200 °C. High-resolution mass spectra (HRMS) were measured on a instrument using a time-of-flight mass analyzer (TOF) with electrospray ionization (ESI). In experiments on selective collisional activation, the activation energy was set at maximum abundance of fragment peaks. A syringe injection was used for solutions in MeCN/H₂O, 50/50 v/v (flow rate 3 ml min⁻¹). Nitrogen was applied as a dry gas; the interface temperature was set at 180 °C. Elemental analyses were measured with a 1106 Carlo Erba apparatus. IR spectra were recorded on Bruker VE Vertex 70V spectrometer as liquid films and are reported in wavenumbers (cm⁻¹). All solvents were dried and freshly distilled before use. All reactions were carried out under a dry argon atmosphere. ZnI₂ was purchased from commercial sources and used without further purification. Complex Co(acac)₂(dppe), 1,3-diynes were synthesized according to procedures described in literature [S1]. Azepine-1-carboxylates were synthesized according to reported procedures [S2].

Cycloaddition of 1,3-diyne to azepine-1-carboxylates (general reaction procedure). Zinc powder (30 mol%) was added to a solution of Co(acac)₂(dppe) (10 mol%) in ClCH₂CH₂Cl (1.5 ml) for **2a,b,d** (in CF₃CH₂OH for **2c**) in a glass ampoule under a dry argon atmosphere, and the mixture was stirred at room temperature for 2 min. Next, ethyl azepine-1-carboxylate (or phenyl azepine-1-carboxylate) (1.0 mmol), the 1,3-diyne (1.2 mmol) in ClCH₂CH₂Cl (1.5 ml) for **2a,b,d** (in CF₃CH₂OH for **2c**) and dry ZnI₂ (20 mol%) were added successively. The ampoule was sealed and heated at 60 °C for 20 h. The ampoule was cooled, opened, and the mixture was quenched by the addition of petroleum ether and stirring in air for 10 min to deactivate the catalyst. After filtration through a short pad of silica, the volatiles were removed under vacuum. Chromatographic purification over SiO₂ (petroleum ether → petroleum ether / ethyl acetate 15:1 → 10:1 → 5:1 as eluent for **3a,b,d,e,g**, petroleum ether → petroleum ether / ethyl acetate 10:1 → 5:1 → 2:1 for **3c,f**) afforded the target products **3a-g**.

Ethyl 7-butyl-8-(hex-1-yn-1-yl)-9-azabicyclo[4.2.1]nona-2,4,7-triene-9-carboxylate (3a): Yield 92% (0.301 g), yellowish oil, exists as two N-(CO)OEt rotamers. *R*_f = 0.41 (petroleum ether / ethyl acetate 5:1). ¹H NMR (500 MHz, CDCl₃): δ 6.18-6.42 (m, 4H), 5.84-5.94 (m, 4H), 4.94 (d, *J* = 4.7 Hz, 1H), 4.86 (d, *J* = 2.9 Hz, 2H), 4.81 (d, *J* = 4.4 Hz, 1H), 4.00-4.14 (m, 4H), 2.33 (t, *J* = 6.9 Hz, 4H), 2.23-2.30 (m, 4H), 1.24-1.56 (m, 16H), 1.19 (t, *J* = 6.7 Hz, 6H), 0.90 (q, *J* = 7.1 Hz, 12H) ppm. ¹³C NMR (125 MHz, CDCl₃): δ 153.4, 153.38, 142.3, 142.2, 139.1, 138.9, 138.1, 138.0, 124.9, 124.8, 124.1 (2C), 113.9, 113.6, 95.1, 94.9, 72.8 (2C), 62.8, 62.6, 61.9 (2C), 61.0 (2C), 30.9, 30.8, 30.7 (2C), 25.9, 25.7, 22.4, 22.35, 21.9 (2C), 19.2 (2C), 14.6 (2C), 13.7 (2C), 13.5 (2C) ppm. IR (liquid film): 3027, 2931, 2861, 1701, 1420, 1319, 1227, 1114, 1040. MS (EI, 70 eV) (%) = 327 [M]⁺ (100), 298 (19), 270 (63), 254 (26), 227 (58), 209 (19), 198 (28), 167 (29), 129 (19), 81 (16), 57 (11), 41 (17). HRMS (ESI-TOF): calcd for C₂₁H₂₉NO₂ [M + H]⁺ 328.2276, found 328.2274. Anal. Calcd for C₂₁H₂₉NO₂: C, 77.02; H, 8.93; N, 4.28. Found: C, 76.92; H, 8.88; N, 4.21.

Ethyl 7-phenyl-8-(2-phenylethynyl)-9-azabicyclo[4.2.1]nona-2,4,7-triene-9-carboxylate (3b): Yield 85% (0.312 g), yellowish oil, exists as two N-(CO)OEt rotamers. *R*_f = 0.45 (petroleum ether / ethyl acetate 5:1). ¹H NMR (500 MHz, CDCl₃): δ 7.97 (dd, *J* = 8.0 Hz, *J* = 2.6 Hz, 4H), 7.51 (dd, *J* = 6.4 Hz, *J* = 2.8 Hz, 4H), 7.32-7.53 (m, 12H), 6.36-6.59 (m, 4H), 5.95-6.06 (m, 4H), 5.69 (d, *J* = 5.3 Hz, 1H), 5.60 (d, *J* = 5.3 Hz, 1H), 5.26 (d, *J* = 5.4 Hz, 1H), 5.21 (d, *J* = 5.4 Hz, 1H), 4.15-4.25 (m, 4H), 1.30 (td, *J* = 7.1 Hz, *J* = 4.5 Hz, 6H) ppm. ¹³C NMR (125 MHz, CDCl₃): δ 153.5, 153.4, 139.0, 138.9, 138.4, 138.2, 131.63, 131.6 (3C), 128.7 (2C), 128.67, 128.6, 128.56 (2C), 128.5 (2C), 128.47 (4C), 128.03 (2C), 128.0 (2C), 125.3 (2C), 124.9 (2C), 122.9 (2C), 112.5, 112.4, 96.1, 95.9, 83.6, 83.5, 64.2, 64.0, 62.2, 62.1, 61.4 (2C), 14.8, 14.7 ppm. IR (liquid film): 3029, 2979, 2929, 1712, 1427, 1321, 1246, 1112, 1037. MS (EI, 70 eV) (%) = 367 [M]⁺ (100), 293 (18), 270 (44), 254 (32), 227 (63), 207 (15), 193 (32), 167 (51), 131 (23), 91 (19), 55 (16), 41 (13). HRMS (ESI-TOF): calcd for C₂₅H₂₁NO₂ [M + H]⁺ 368.1650, found 368.1649. Anal. Calcd for C₂₅H₂₁NO₂: C, 81.72; H, 5.76; N, 3.81. Found: C, 81.60; H, 5.72; N, 3.75.

Ethyl 7-(4-hydroxybutyl)-8-(6-hydroxyhex-1-yn-1-yl)-9-azabicyclo[4.2.1]nona-2,4,7-triene-9-carboxylate (3c): Yield 76% (0.273 g), yellowish oil, exists as two N-(CO)OEt rotamers. $R_f = 0.55$ (petroleum ether / ethyl acetate 2:1). $^1\text{H NMR}$ (500 MHz, CDCl_3): δ 6.28-6.38 (m, 2H), 6.16-6.27 (m, 2H), 5.82-5.92 (m, 4H), 4.90 (d, $J = 5.0$ Hz, 1H), 4.84 (d, $J = 5.0$ Hz, 1H), 4.81 (d, $J = 5.1$ Hz, 1H), 4.79 (d, $J = 5.1$ Hz, 1H), 3.97-4.12 (m, 4H), 3.59 (dd, $J = 12.3$ Hz, $J = 5.8$ Hz, 8H), 2.21-2.39 (m, 8H), 1.43-1.72 (m, 16H), 1.17 (t, $J = 7.1$ Hz, 6H) ppm. $^{13}\text{C NMR}$ (125 MHz, CDCl_3): δ 153.5, 153.46, 142.0, 141.9, 138.9, 138.7, 137.9 (2C), 125.0, 124.95, 124.2 (2C), 114.0, 113.7, 94.9, 94.7, 73.1 (2C), 62.7, 62.5, 62.0 (2C), 61.9 (2C), 61.89 (2C), 61.2 (2C), 32.0, 31.9, 31.7 (2C), 25.8, 25.6, 24.9, 24.86 (2C), 24.8, 19.3 (2C), 14.6 (2C) ppm. IR (liquid film): 3417, 3027, 2938, 2865, 1685, 1429, 1322, 1254, 1115, 1041. MS (EI, 70 eV) (%) = 359 $[\text{M}]^+$ (15), 342 (54), 295 (22), 282 (39), 254 (29), 207 (75), 193 (38), 167 (48), 131 (30), 115 (29), 91 (36), 75 (100), 55 (22), 41 (22). HRMS (ESI-TOF): calcd for $\text{C}_{21}\text{H}_{29}\text{NO}_4$ $[\text{M} + \text{H}]^+$ 360.2174, found 360.2173. Anal. Calcd for $\text{C}_{21}\text{H}_{29}\text{NO}_4$: C, 70.17; H, 8.13; N, 3.90. Found: C, 70.04; H, 8.08; N, 3.85.

Ethyl 7-(4-*tert*-butylthiobutyl)-8-(6-*tert*-butylthiohex-1-yn-1-yl)-9-azabicyclo[4.2.1]nona-2,4,7-triene-9-carboxylate (3d): Yield 79% (0.397 g), yellowish oil, exists as two N-(CO)OEt rotamers. $R_f = 0.43$ (petroleum ether / ethyl acetate 5:1). $^1\text{H NMR}$ (500 MHz, CDCl_3): δ 6.38 (dd, $J = 10.5$ Hz, $J = 4.8$ Hz, 1H), 6.33 (dd, $J = 10.4$ Hz, $J = 5.1$ Hz, 1H), 6.26 (dd, $J = 10.4$ Hz, $J = 5.3$ Hz, 1H), 6.21 (dd, $J = 10.6$ Hz, $J = 5.1$ Hz, 1H), 5.84-5.95 (m, 4H), 4.94 (d, $J = 5.2$ Hz, 1H), 4.86 (dd, $J = 11.3$ Hz, $J = 5.1$ Hz, 2H), 4.81 (d, $J = 5.1$ Hz, 1H), 4.00-4.14 (m, 4H), 2.49-2.58 (m, 8H), 2.35 (t, $J = 6.5$ Hz, 4H), 2.23-2.33 (m, 4H), 1.51-1.74 (m, 16H), 1.31 (s, 36H), 1.19 (t, $J = 7.1$ Hz, 6H) ppm. $^{13}\text{C NMR}$ (125 MHz, CDCl_3): δ 153.4 (2C), 141.8, 141.6, 139.0, 138.9, 138.0 (2C), 125.0, 124.99, 124.2 (2C), 114.1, 113.8, 94.7, 94.5, 73.1, 73.0, 62.7, 62.6, 61.8 (2C), 61.1 (2C), 41.9 (4C), 31.0 (12C), 29.4, 29.3, 29.0 (2C), 28.1 (4C), 27.9, 27.89, 27.7 (2C), 25.8, 25.6, 19.2 (2C), 14.6 (2C) ppm. IR (liquid film): 3026, 2940, 2861, 1709, 1422, 1364, 1318, 1166, 1110, 1041. HRMS (ESI-TOF): calcd for $\text{C}_{29}\text{H}_{45}\text{NO}_2\text{S}_2$ $[\text{M} + \text{H}]^+$ 504.2969, found 504.2965. Anal. Calcd for $\text{C}_{29}\text{H}_{45}\text{NO}_2\text{S}_2$: C, 69.14; H, 9.00; N, 2.78. Found: C, 68.98; H, 8.93; N, 2.75.

Phenyl 7-butyl-8-(hex-1-yn-1-yl)-9-azabicyclo[4.2.1]nona-2,4,7-triene-9-carboxylate (3e): Yield 89% (0.334 g), yellowish oil, exists as two N-(CO)OPh rotamers. $R_f = 0.46$ (petroleum ether / ethyl acetate 5:1). $^1\text{H NMR}$ (500 MHz, CDCl_3): δ 7.35 (t, $J = 7.7$ Hz, 4H), 7.19 (t, $J = 7.3$ Hz, 2H), 7.10 (d, $J = 7.5$ Hz, 4H), 6.44-6.52 (m, 2H), 6.31-6.40 (m, 2H), 5.99-6.07 (m, 4H), 5.13 (d, $J = 5.1$ Hz, 1H), 5.09 (d, $J = 5.1$ Hz, 1H), 5.06 (d, $J = 5.1$ Hz, 1H), 4.99 (d, $J = 5.0$ Hz, 1H), 2.32-2.44 (m, 8H), 1.32-1.62 (m, 16H), 0.98 (t, $J = 7.0$ Hz, 12H) ppm. $^{13}\text{C NMR}$ (125 MHz, CDCl_3): δ 151.5, 151.4, 151.2 (2C), 142.2 (2C), 138.8, 138.78, 137.9, 137.8, 129.2 (4C), 125.2 (2C), 125.15, 125.1, 124.4, 124.2, 121.7 (4C), 113.9, 113.7, 95.4, 95.2, 72.7 (2C), 63.2, 63.1, 62.6, 62.4, 31.0, 30.9, 30.8 (2C), 26.0, 25.8, 22.5, 22.4, 21.9 (2C), 19.3 (2C), 13.8 (2C), 13.6 (2C) ppm. IR (liquid film): 3030, 2928, 2863, 1701, 1421, 1320, 1228, 1112, 1040. MS (EI, 70 eV) (%) = 375 $[\text{M}]^+$ (100), 318 (68), 282 (23), 227 (45), 196 (34), 182 (29), 167 (29), 141 (37),

115 (32), 77 (68), 57 (43), 41 (72). HRMS (ESI-TOF): calcd for C₂₅H₂₉NO₂ [M + H]⁺ 376.2276, found 376.2273. Anal. Calcd for C₂₅H₂₉NO₂: C, 79.96; H, 7.78; N, 3.73. Found: C, 79.78; H, 7.70; N, 3.69.

Phenyl 7-(4-hydroxybutyl)-8-(6-hydroxyhex-1-yn-1-yl)-9-azabicyclo[4.2.1]nona-2,4,7-triene-9-carboxylate (3f): Yield 74% (0.301 g), yellowish oil, exists as two N-(CO)OPh rotamers. *R*_f = 0.58 (petroleum ether / ethyl acetate 2:1). ¹H NMR (500 MHz, CDCl₃): δ 7.33 (t, *J* = 7.7 Hz, 4H), 7.16-7.20 (m, 2H), 7.06 (d, *J* = 7.6 Hz, 4H), 6.40-6.49 (m, 2H), 6.28-6.37 (m, 2H), 5.97-6.06 (m, 4H), 5.11 (d, *J* = 5.2 Hz, 1H), 5.06 (d, *J* = 5.2 Hz, 1H), 5.02 (d, *J* = 5.2 Hz, 1H), 4.95 (d, *J* = 5.2 Hz, 1H), 3.61-3.69 (m, 8H), 2.41 (t, *J* = 6.6 Hz, 4H), 2.32-2.39 (m, 4H), 1.52-1.77 (m, 16H) ppm. ¹³C NMR (125 MHz, CDCl₃): δ 151.5, 151.47, 151.1 (2C), 141.9 (2C), 138.7, 138.6, 137.7, 137.6, 129.2 (4C), 125.3 (3C), 125.2, 124.5, 124.3, 121.6 (4C), 114.0, 113.9, 95.2, 95.0, 73.1 (2C), 63.1, 63.0, 62.6, 62.3, 62.2 (2C), 62.1 (2C), 32.0, 31.97, 31.8 (2C), 25.9, 25.7, 25.0, 24.9 (3C), 19.4 (2C) ppm. IR (liquid film): 3415, 3028, 2940, 2865, 1684, 1427, 1321, 1254, 1116, 1040. HRMS (ESI-TOF): calcd for C₂₅H₂₉NO₄ [M + H]⁺ 408.2174, found 408.2172. Anal. Calcd for C₂₅H₂₉NO₄: C, 73.69; H, 7.17; N, 3.44. Found: C, 73.58; H, 7.11; N, 3.40.

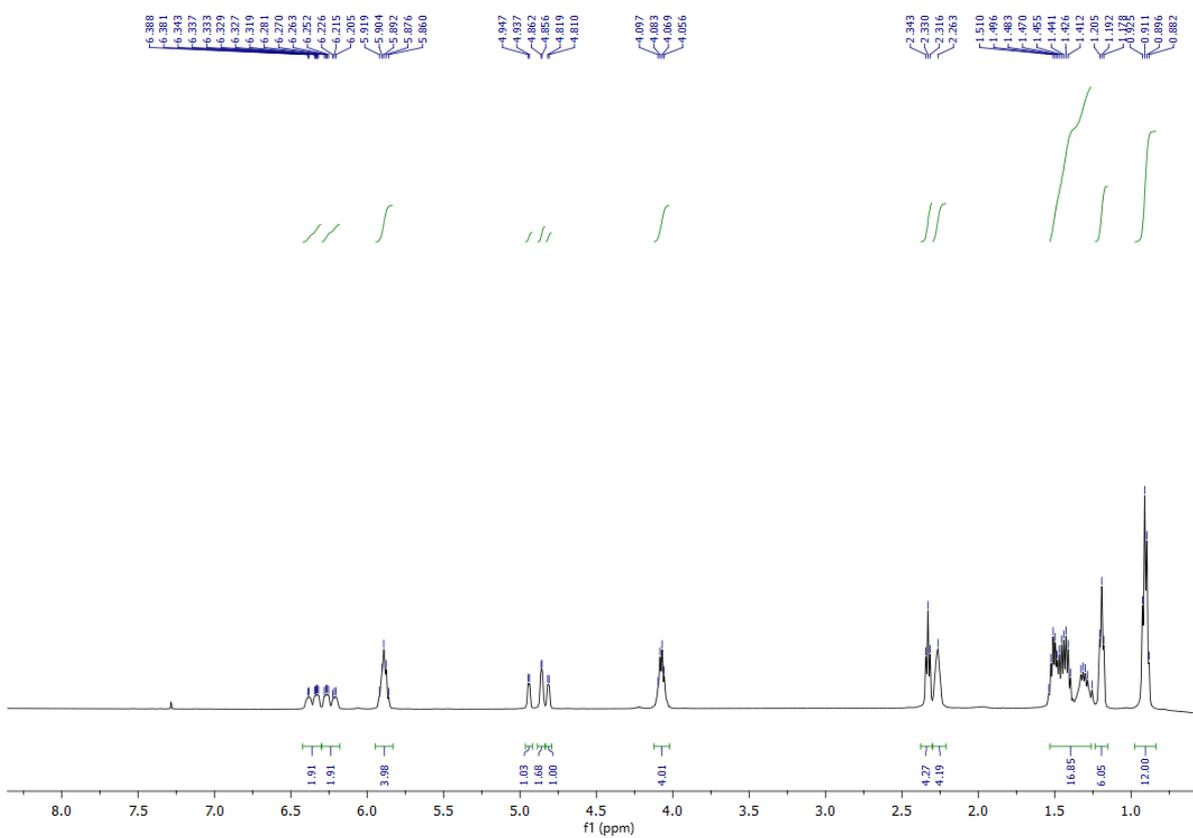
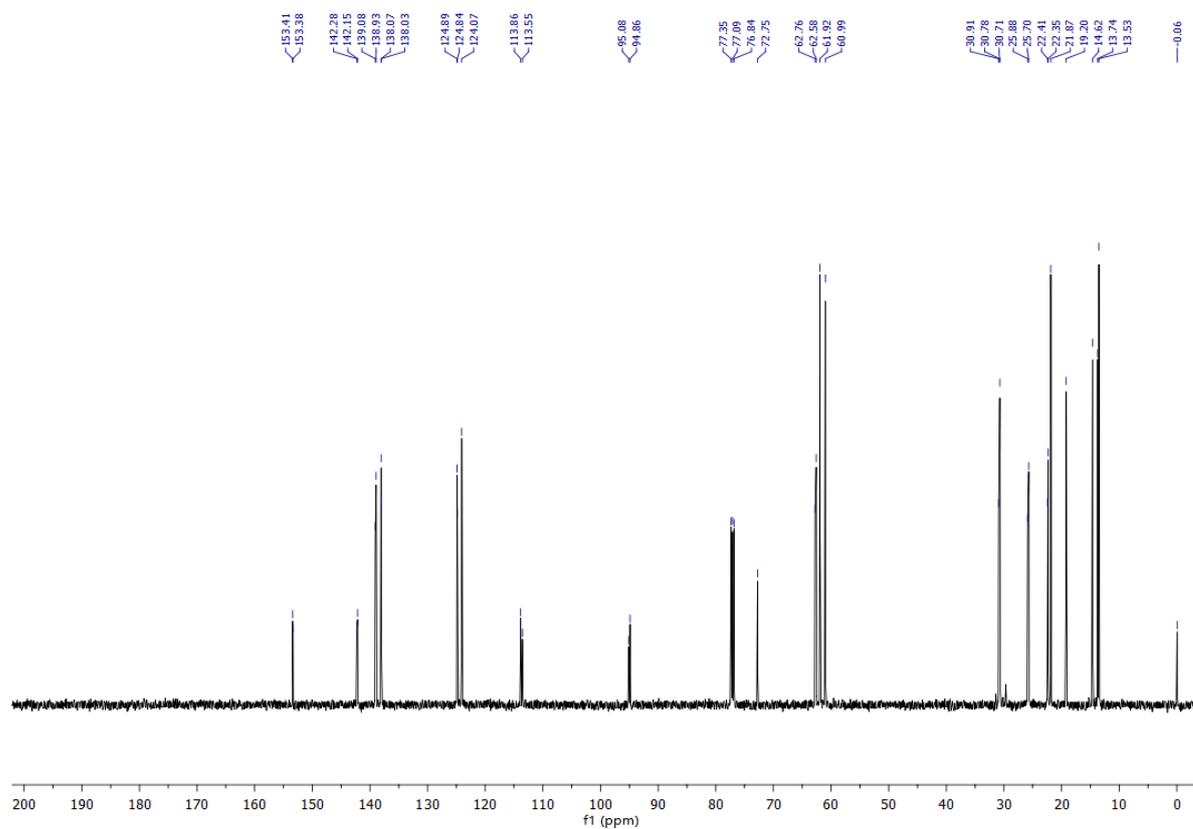
Phenyl 7-(4-*tert*-butylthiobutyl)-8-(6-*tert*-butylthiohex-1-yn-1-yl)-9-azabicyclo[4.2.1]nona-2,4,7-triene-9-carboxylate (3g): Yield 80% (0.441 g), yellowish oil, exists as two N-(CO)OPh rotamers. *R*_f = 0.49 (petroleum ether / ethyl acetate 5:1). ¹H NMR (500 MHz, CDCl₃): δ 7.33 (t, *J* = 7.9 Hz, 4H), 7.18 (t, *J* = 7.4 Hz, 2H), 7.07 (d, *J* = 8.4 Hz, 4H), 6.40-6.49 (m, 2H), 6.28-6.38 (m, 2H), 5.96-6.08 (m, 4H), 5.12 (d, *J* = 5.2 Hz, 1H), 5.04 (dd, *J* = 11.4 Hz, *J* = 5.3 Hz, 2H), 4.95 (d, *J* = 5.2 Hz, 1H), 2.57 (t, *J* = 6.9 Hz, 8H), 2.29-2.42 (m, 8H), 1.56-1.77 (m, 16H), 1.34 (s, 36H) ppm. ¹³C NMR (125 MHz, CDCl₃): δ 151.4 (2C), 151.2 (2C), 141.7, 141.6, 138.8, 138.7, 137.8, 137.7, 129.2 (4C), 125.3, 125.2 (2C), 125.18, 124.5, 124.3, 121.6 (4C), 114.0, 113.9, 95.0, 94.8, 72.9 (2C), 63.2, 63.1, 62.5, 62.2, 41.9 (4C) 31.0 (12C), 29.4, 29.35, 29.0 (2C), 28.1 (2C), 28.07, 28.06, 28.0, 27.9, 27.8 (2C), 25.8, 25.7, 19.3 (2C) ppm. IR (liquid film): 3028, 2924, 2857, 1734, 1458, 1384, 1318, 1181, 1074, 1026. HRMS (ESI-TOF): calcd for C₃₃H₄₅NO₂S₂ [M + H]⁺ 552.2969, found 552.2965. Anal. Calcd for C₃₃H₄₅NO₂S₂: C, 71.82; H, 8.22; N, 2.54. Found: C, 71.67; H, 8.14; N, 2.51.

References

- [S1] (a) F. A. Cotton, O. D. Faut, M. L. Goodgame and R. H. Holm, *J. Am. Chem. Soc.*, 1961, **83**, 1780; (b) L. Brandsma, *Synthesis of Acetylenes, Allenes and Cumulenes: Methods and Techniques*, Elsevier Academic Press, Bilthoven, the Netherlands, 2004.
- [S2] R. J. Cotter and W. F. Beach, *J. Org. Chem.*, 1964, **29**, 751.

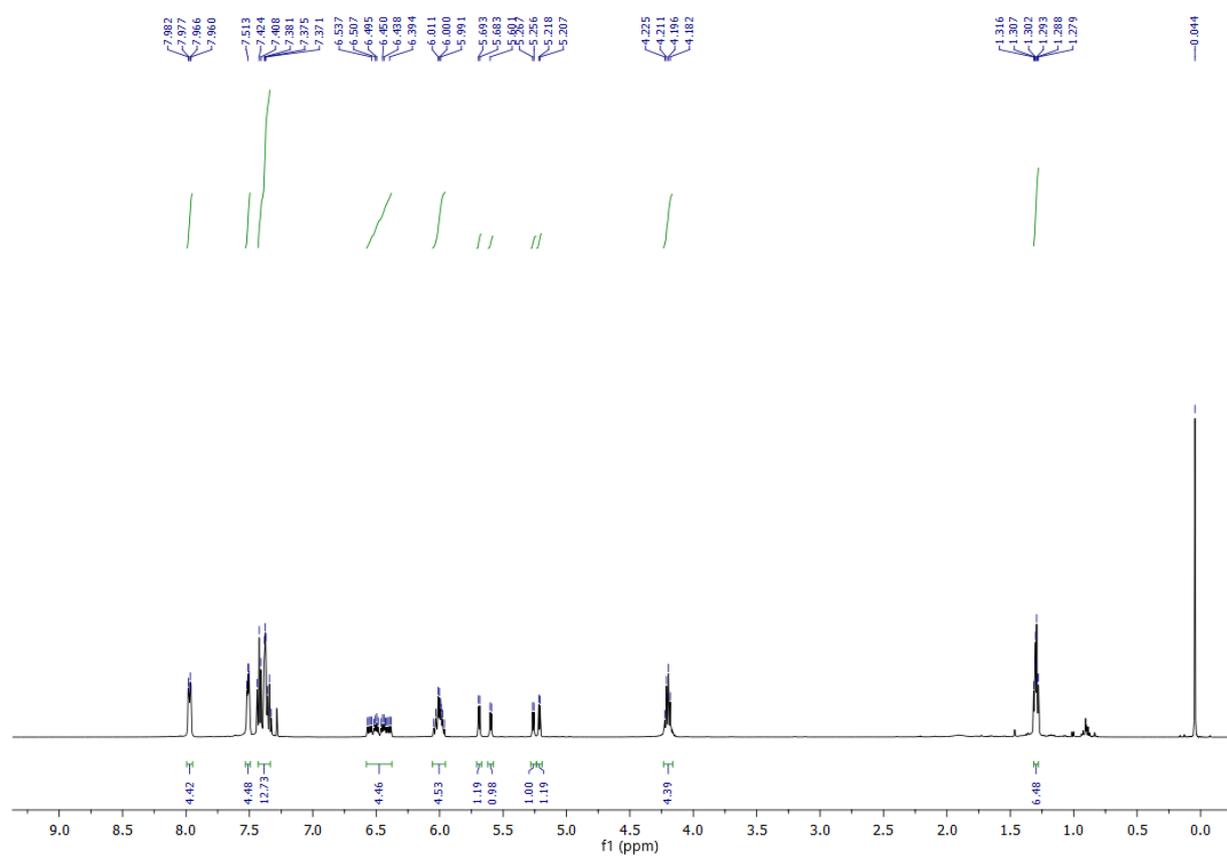
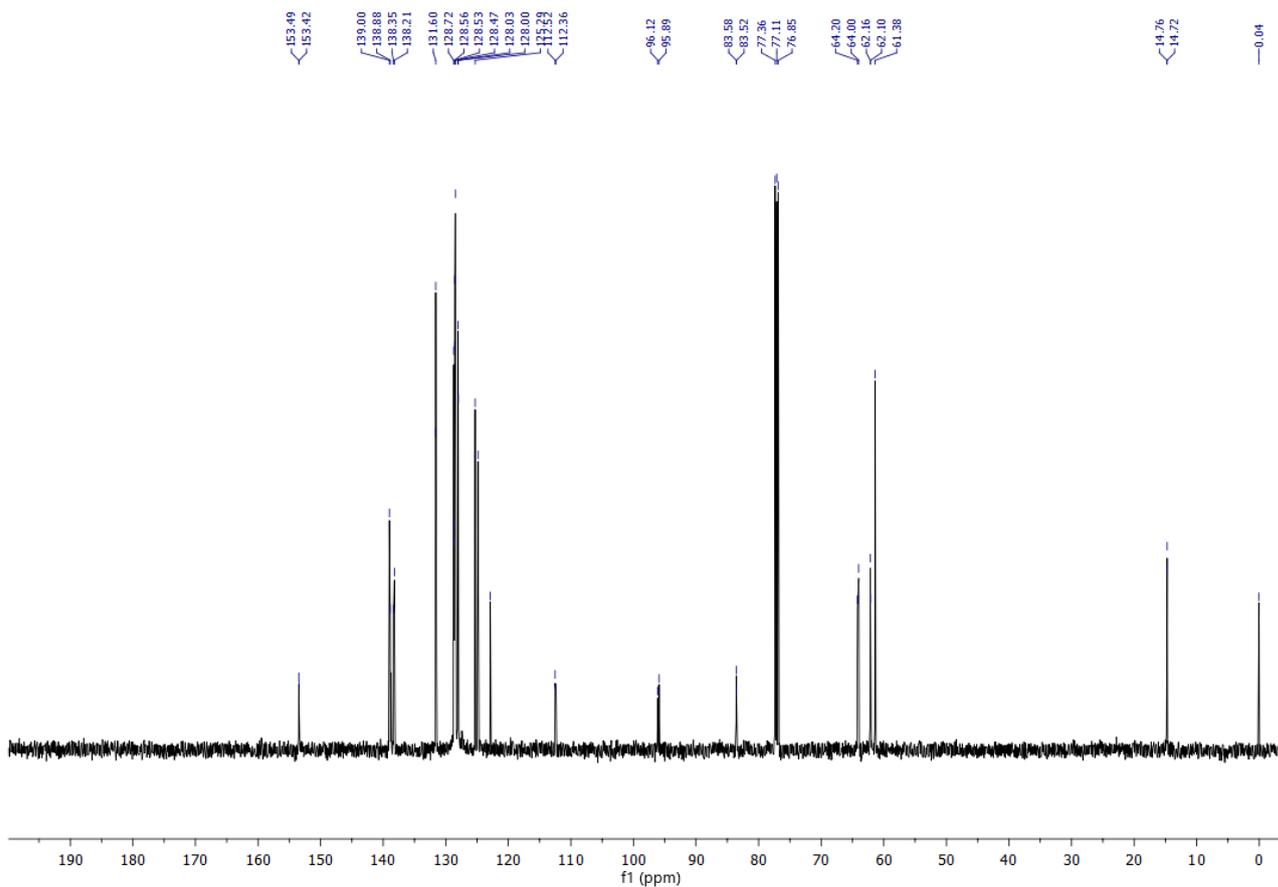
Copies of NMR spectra for the compounds

Ethyl 7-butyl-8-(hex-1-yn-1-yl)-9-azabicyclo[4.2.1]nona-2,4,7-triene-9-carboxylate (3a):

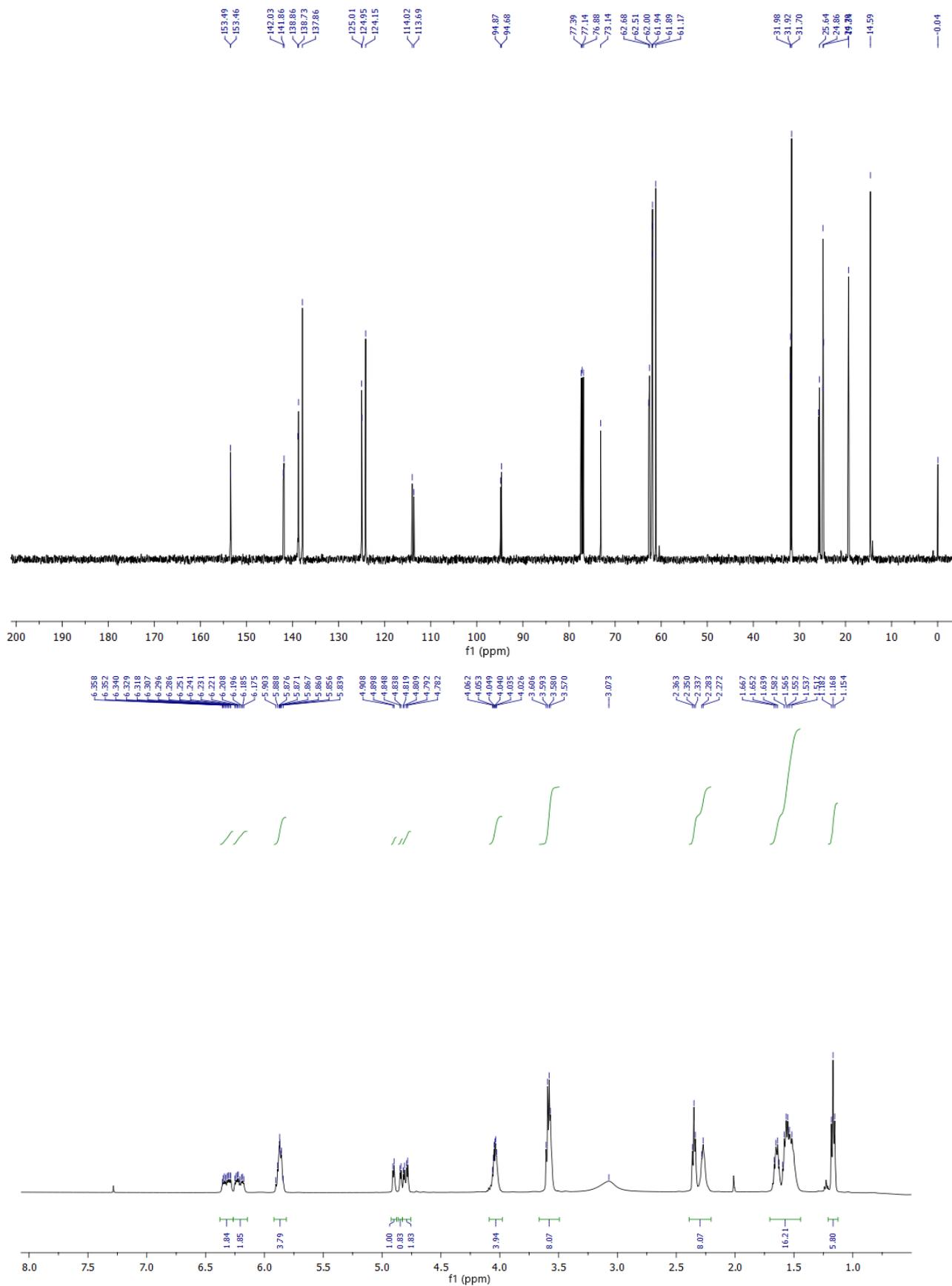


Ethyl
(3b):

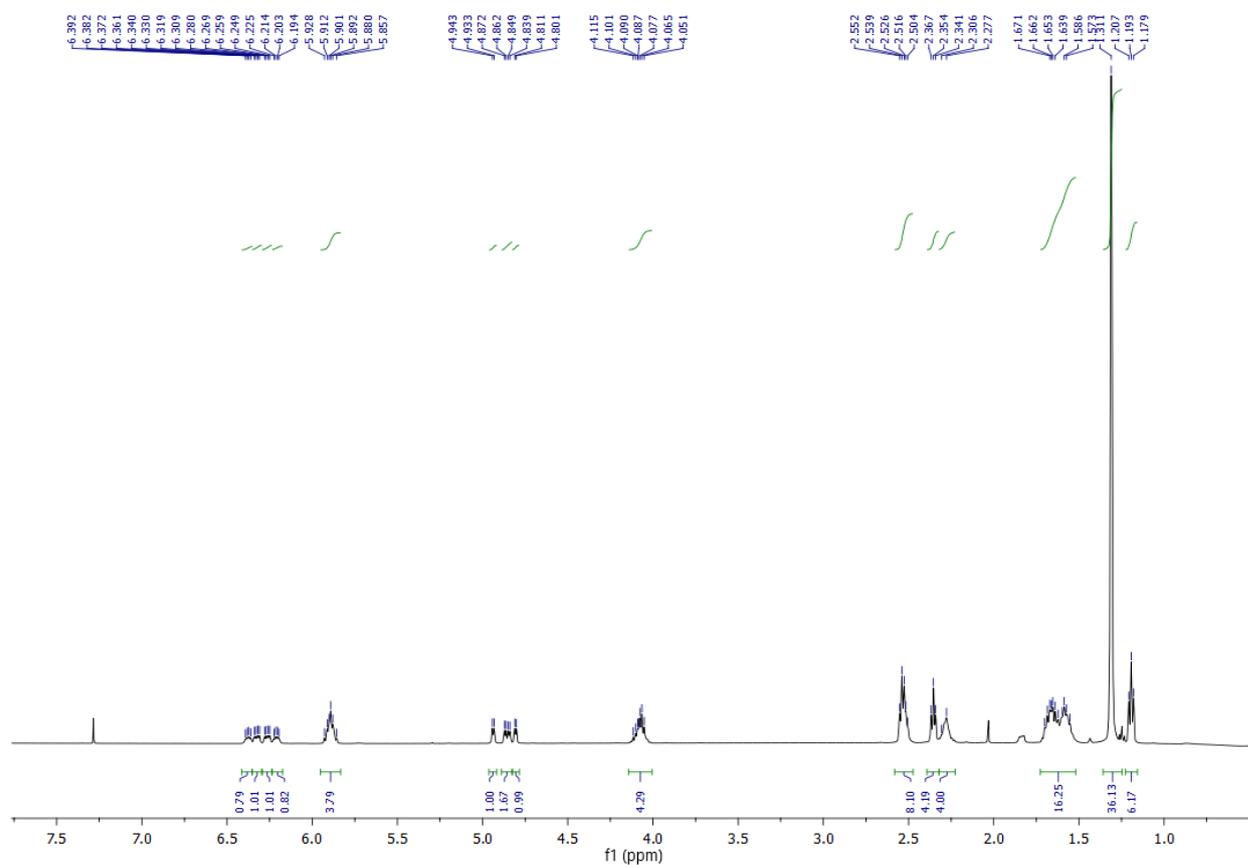
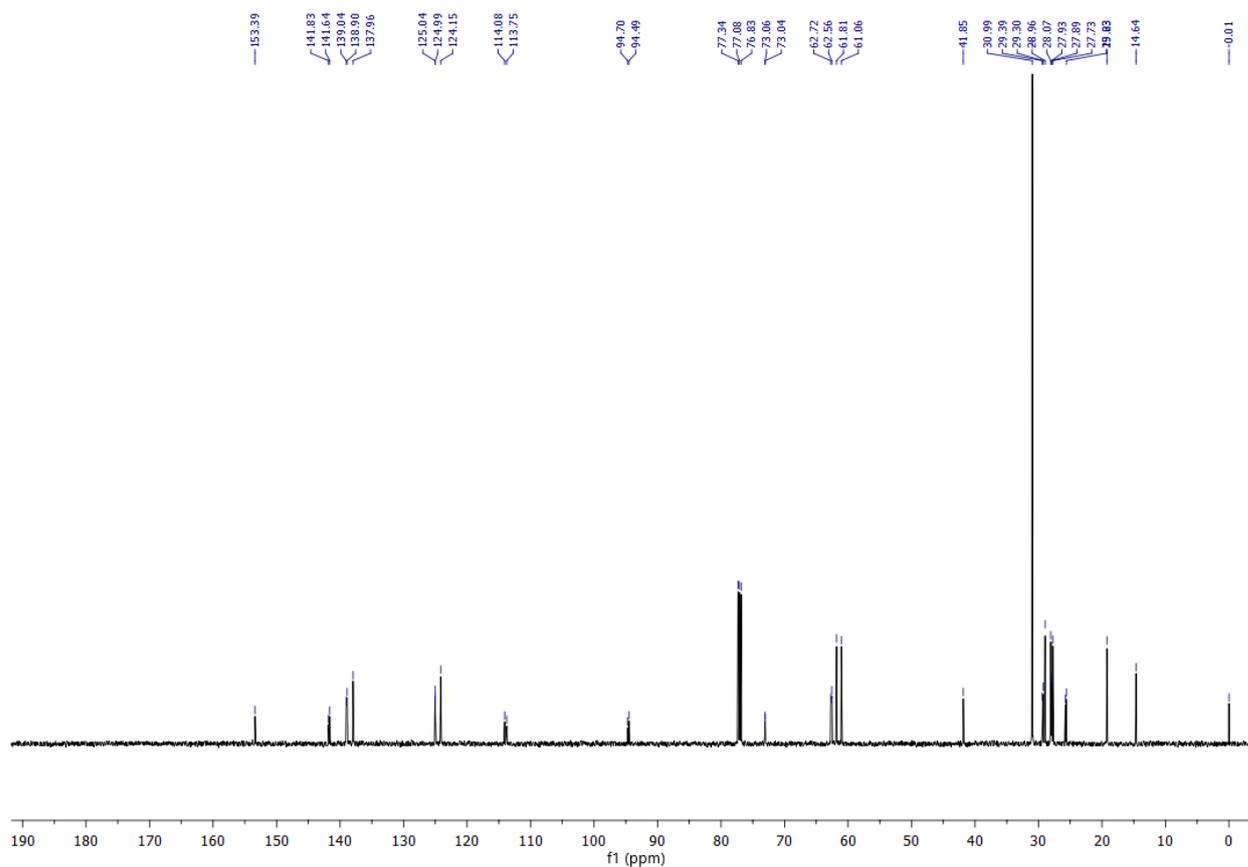
7-phenyl-8-(2-phenylethynyl)-9-azabicyclo[4.2.1]nona-2,4,7-triene-9-carboxylate



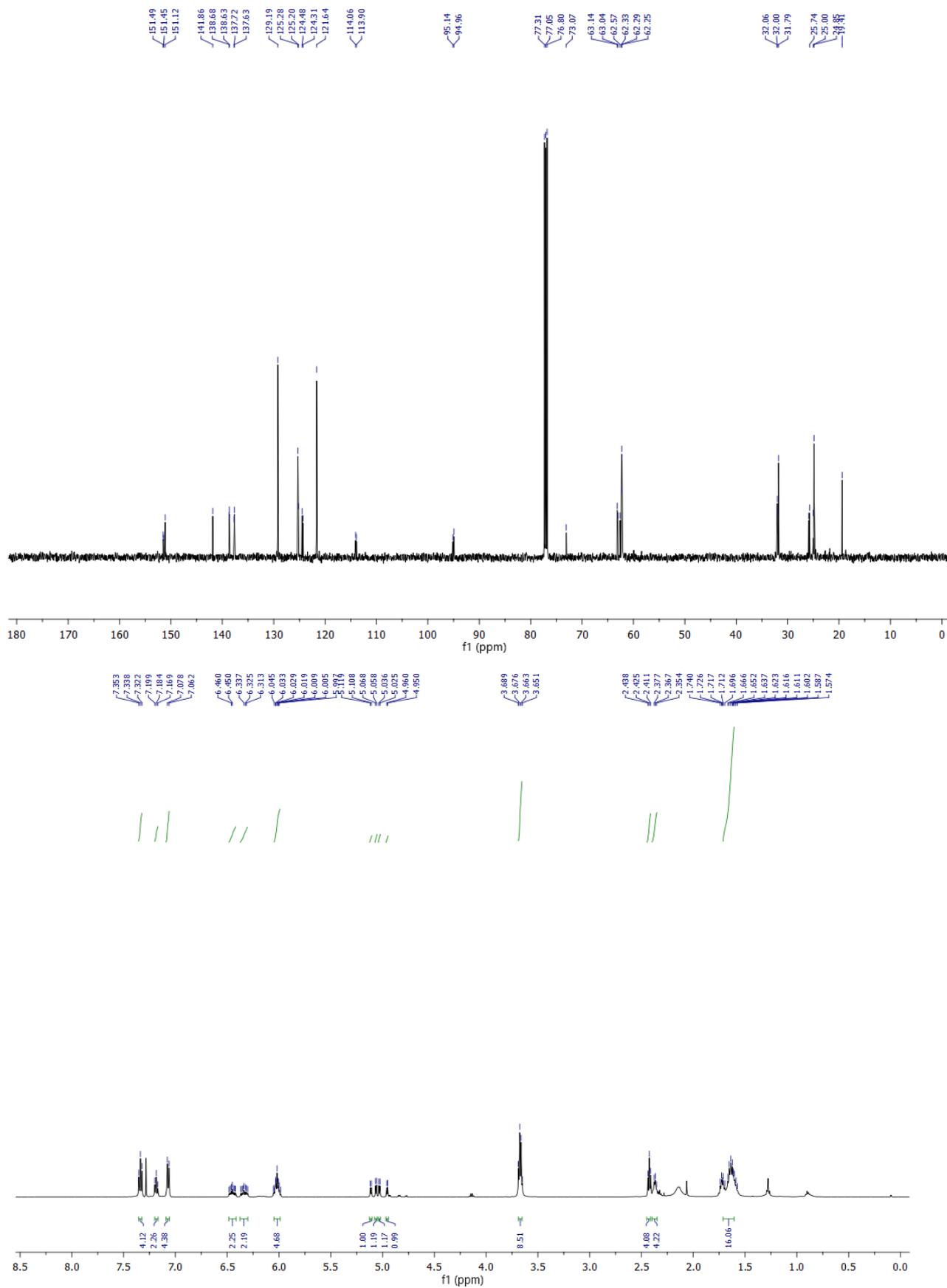
Ethyl 7-(4-hydroxybutyl)-8-(6-hydroxyhex-1-yn-1-yl)-9-azabicyclo[4.2.1]nona-2,4,7-triene-9-carboxylate (3c):



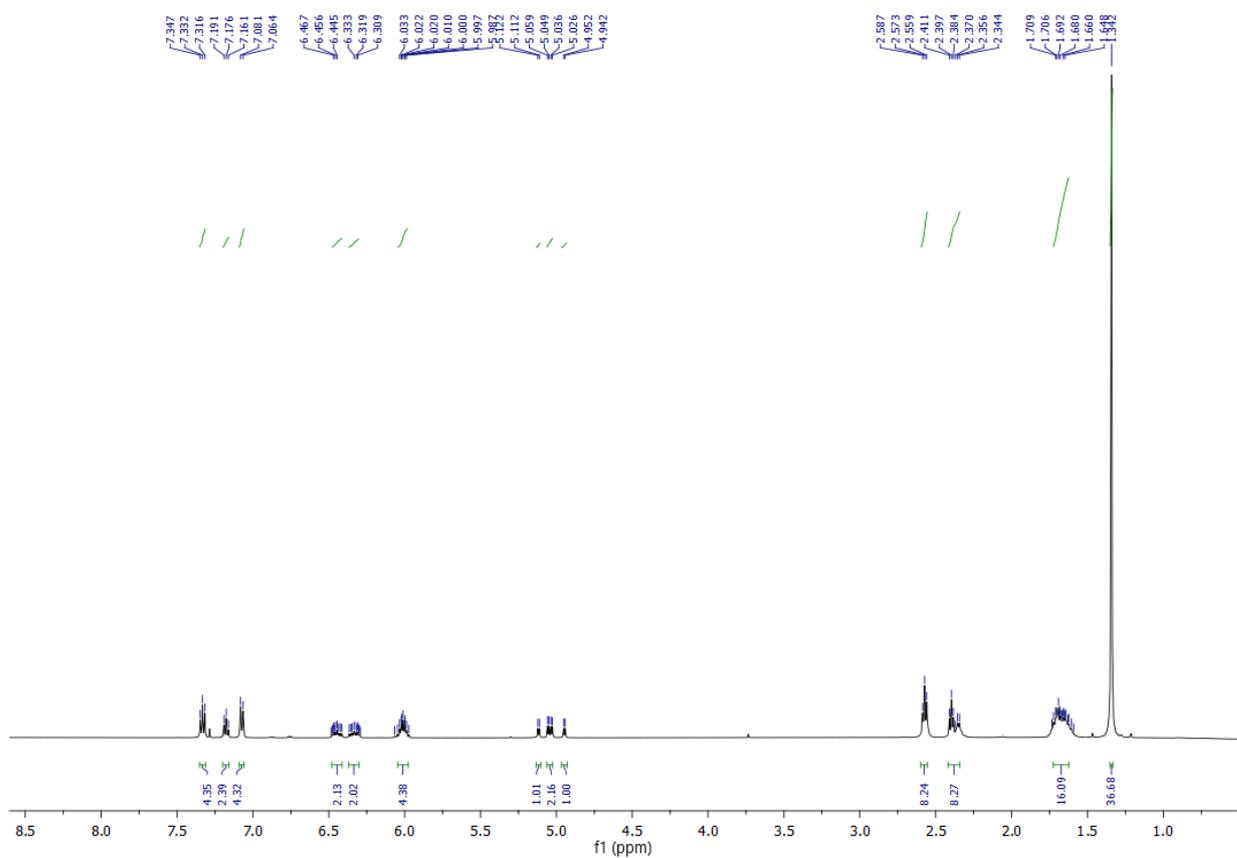
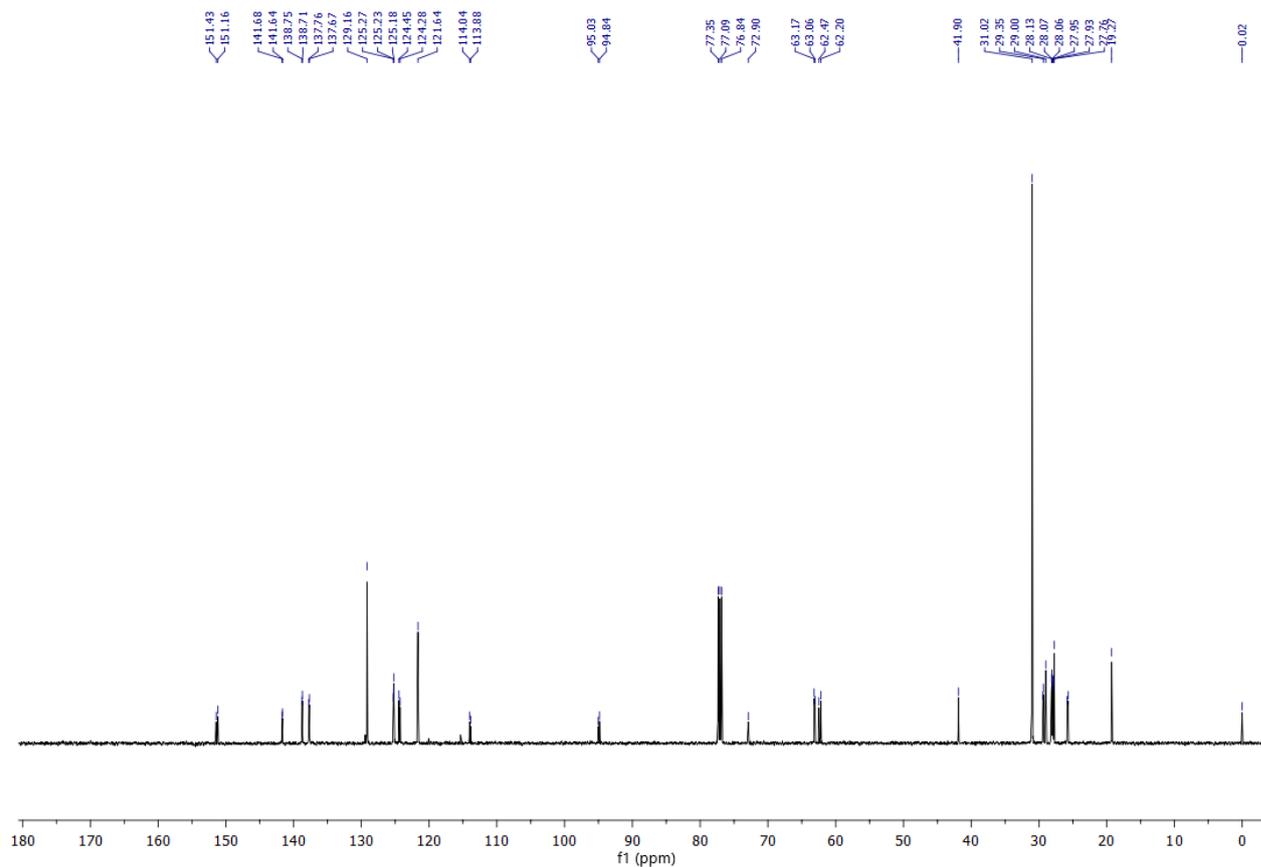
Ethyl 7-(4-*tert*-butylthiobutyl)-8-(6-*tert*-butylthiohex-1-yn-1-yl)-9-azabicyclo[4.2.1]nona-2,4,7-triene-9-carboxylate (3d):



Phenyl 7-(4-hydroxybutyl)-8-(6-hydroxyhex-1-yn-1-yl)-9-azabicyclo[4.2.1]nona-2,4,7-triene-9-carboxylate (3f):



Phenyl 7-(4-(*tert*-butylthio)butyl)-8-(6-(*tert*-butylthio)hex-1-yn-1-yl)-9-azabicyclo[4.2.1]-nona-2,4,7-triene-9-carboxylate (3g):



Biology studies

Cell culturing

Cells (Jurkat, K562, U937, HL-60) were purchased from Russian Cell Culture Collection (Institute of Cytology of the Russian Academy of Sciences) and cultured according to standard mammalian tissue culture protocols and sterile technique. All cell lines used in the study were tested and shown to be free of mycoplasma and viral contamination.

Cells were maintained in RPMI 1640 (Jurkat, K562, U937) (Gibco) supplemented with 4 mM glutamine, 10% FBS (Sigma) and 100 units/ml penicillin-streptomycin (Sigma). All types of cells were grown in an atmosphere of 5 % CO₂ at 37 °C. The cells were subcultured at 2-3 days intervals. Cells were then seeded in 24 well plates at 5x10⁴ cells per well and incubated overnight. Jurkat, K562, U937, HL-60 cells were subcultured at 2 day intervals with a seeding density of 1×10⁵cells per 24 well plates in RPMI with 10% FBS.

Cytotoxicity assay

Viability (Live/dead) assessment was performed by staining cells with 7-AAD (7-Aminoactinomycin D) (Biolegend). After treatment cells were harvested, washed 1-2 times with phosphate-buffered saline (PBS) and centrifuged at 400g for 5 minutes. Cell pellets were resuspended in 200 uL of flow cytometry staining buffer (PBS without Ca²⁺ and Mg²⁺, 2.5% FBS) and stained with 5 uL of 7-AAD staining solution for 15 minutes at room temperature in the dark. Samples were acquired on NovoCyte™ 2000 FlowCytometry System (ACEA) equipped with 488 nm argon laser. Detection of 7-AAD emission was collected through a 675/30 nm filter in FL4 channel.