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Supporting Information

Diversifying Chemical Space of DNA-Encoded Libraries: Synthesis of 2-Oxa-1-azaspiro(bicyclo[3.2.0])heptanes On-DNA via Visible Light-Mediated Energy Transfer Catalysis

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1 General Considerations

1.1 General

All reagents were purchased and used as received from their respective suppliers unless otherwise noted. The synthesis of new compounds used in the study are described in the appropriate sections in this Supporting Information. The reactions were monitored by ^{1}H NMR or TLC using silica gel F254 plates (60 Å porosity, 250 µm thickness). TLC analysis was performed using EtOAc/hexanes and visualized using permanganate stain, ceric ammonium molybdate (Hanessian's) stain, and/or UV light. Flash chromatography was accomplished using an automated system (monitoring at 254 nm and 280 nm in conjunction with an evaporative light scattering detector) with silica cartridges (60 Å porosity, 20-40 µm). Accurate mass measurement analyses were conducted using electrospray ionization (ESI). The signals were mass-measured against an internal lock mass reference of leucine enkephalin for ESI-LCMS. The utilized software calibrates the instruments and reports measurements by use of neutral atomic masses. The mass of the electron is not included. IR spectra were recorded using either neat oil or solid products. Data are presented as follows: wavenumber (cm^{-1}) peak shape/intensity (w = weak, m = medium, s = strong, vs = very strong, br = broad). Melting points (°C) are uncorrected. ^{1}H NMR (400 MHz) chemical shifts are referenced to residual, non-deuterated $CHCl_3$ (δ 7.26). ^{13}C (^{1}H decoupled) NMR (101 MHz) chemical shifts are reported relative to $CDCl_3$ (δ 77.2). Data are presented as follows: chemical shift (ppm), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad, dd = doublet of doublet, td = triplet of doublets, dt = doublet of triplets), coupling constant *J* (Hz) and integration.

1.2 Materials for On-DNA Synthesis

The DNA headpiece (DNA-HP) with PEG4 linker (5'-/Phos/GAGTCA/iSp9-PEG/iAm C7_CO-PEG4-NH2/iSp9-PEG/TGACTCCC-3') used for the preparation of the functionalized headpieces (HP-1 to HP-17) was obtained from WuXi AppTec, Shanghai, China, and its structure is represented below:

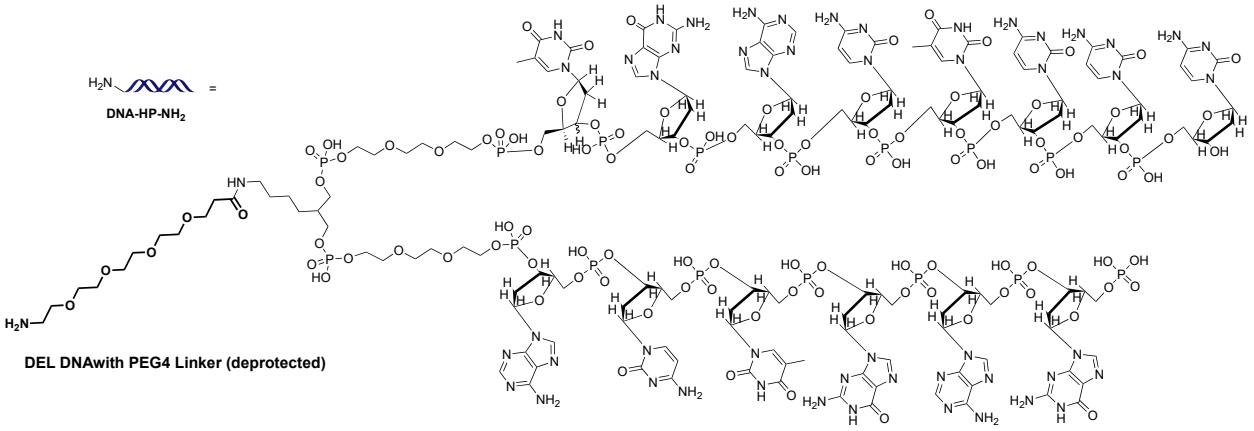


Figure S1. Molecular structure of the **DNA-HP-NH$_2$** employed in this study. The DNA duplex is composed of six self-complementary nucleotide pairs and two additional overhang nucleotides with application for DNA ligation.

1.3 On-DNA Photoreactions

For the visible light-mediated photoreactions, one Kessil H150-Blue lamp (19 V DC 40 W Max) was placed 1.5 inches away from PCR tubes (see details in the experimental section, General Procedure IV), and the light intensity was set to 100%. The photoreactions on-DNA were performed in Eppendorf PCR 8-strip microcentrifuge tubes (Fisher Ref. 781320) with PCR strips of 8 caps (Fisher Ref. 781340). HyPureTM Molecular Biology Grade water was purchased and used as received without further manipulation. Reaction mixtures were vortexed with a Fisherbrand™ Mini Vortex Mixer.

1.4 Analysis of On-DNA Products

For the scope of the developed transformation, an ethanol precipitation method was employed upon reaction completion. Subsequently, the samples were centrifuged with a Corning™ Mini Microcentrifuge (max. speed: 6,000 rpm). The samples for analysis were prepared in HyPureTM Molecular Biology Grade water at approximately 0.05–0.13 mM. For the reaction scope, the conversion was determined using Intact MassTM by Protein Metrics Inc. (version 3.7- 32x64). Data was scanned and deconvoluted between 4,500 to 6,500/7,500 Da, with a mass tolerance window of 2 – 6 Da, with 10% of base peak threshold set for reporting. Na, K, NH_4, Cu, Ni and HFIP adducts were included in the product percentage. Detailed parameters can be found later in the Supporting Information.

The analysis was conducted via LC/MS using a reverse-phase chromatography column (Clarity 2.6 µm Oligo-MS 100 Å 2.1x50 mm) and eluted (10-90% B over 4 min at 0.5 mL/min flow rate; Solvent A: 0.75% v/v/ HFIP / 0.038% Et_3N in H_2O; Solvent B: 0.75% HFIP, 0.038% Et_3N in 90/10 MeOH/deionized H_2O) with no UV monitoring. Effluent was analyzed on a Waters SQ Detector 2 ACQUITY UPLC System in Thermo Exactive Plus LC-esiMS with a Vanquish UHPLC. For the functionalized headpiece samples, % conversion was determined based on reported peak intensities following deconvolution (between 4,500-6,500 Da) of the DNA charge states using Intact MassTM by Protein Metrics Inc. (version 3.7-32x64).

2 Synthetic Protocols

2.1 Preparation of the DNA-tagged Olefin Headpieces (HP-1 to HP-17)

The DNA-tagged olefin headpieces employed in the scope of the reported transformation (Figure S2) were prepared according to **General Procedure I** from the coupling between the DNA-HP-NH_2 tag and the corresponding carboxylic acids, and following the protocols described in Reference 1.

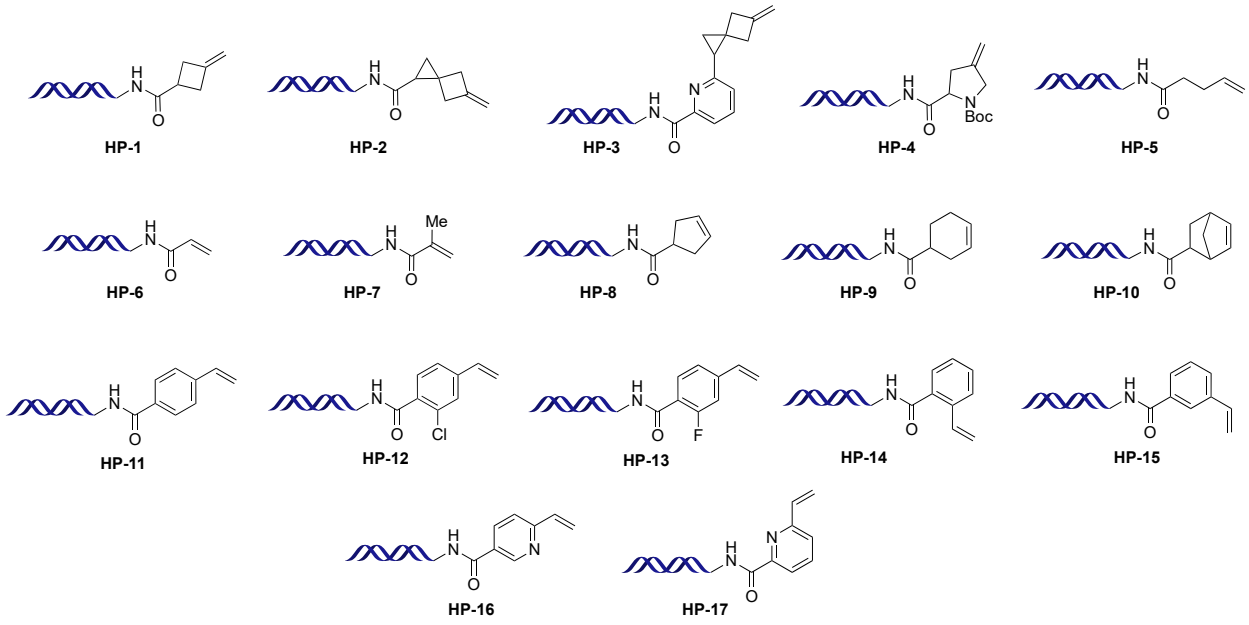
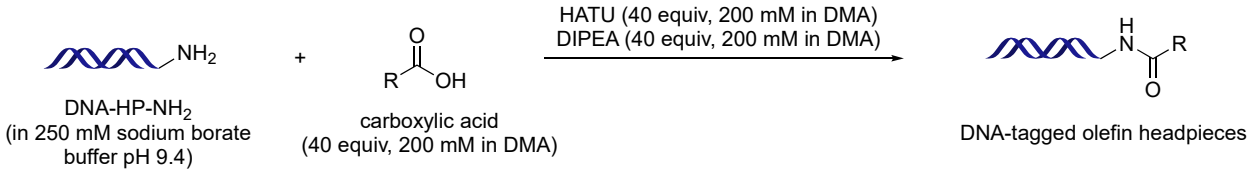


Figure S2. Molecular structures of the DNA-tagged olefin headpieces employed in the scope.

2.1.1 General Procedure I: Acylation of DNA-HP-NH_2 and Purification Method



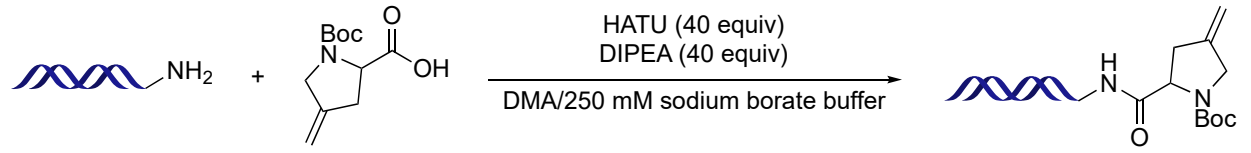
DNA-HP-NH_2 (in 250 mM sodium borate buffer pH 9.4)

carboxylic acid (40 equiv, 200 mM in DMA)

DNA-tagged olefin headpieces

Solutions of HATU (200 mM in DMA, 40.0 equiv), DIPEA (200 mM in DMA, 40.0 equiv), and the corresponding carboxylic acid (200 mM in DMA, 40.0 equiv) were cooled to 4 ºC. Subsequently, the solutions of carboxylic acid, DIPEA, and HATU were combined and vortexed with a Fisherbrand™ Mini Vortex Mixer. The mixture was allowed to react at 4 ºC for 20 min. To this solution, the DNA-HP-NH_2 in sodium borate buffer (1 mM in 250 mM pH 9.4) was added and vigorously vortexed. The reaction mixture was left to react at rt for 1 h, and after this period, worked-up by EtOH precipitation method as follows:

A solution of 5 M NaCl in H_2O (10% of the reaction volume) was added, followed by the addition of cold absolute EtOH (200 Proof, 2.5 reaction volumes). The mixture was vortexed and kept overnight at −20 °C in the freezer before being centrifuged a 4,000 rpm for 30 min at 4 ºC. The supernatant was discarded, and the obtained solid was dried under reduced pressure in the lyophilizer for 1 h. The DNA pellet was then re-dissolved in HyPureTM Molecular Biology Grade H_2O to a final concentration of 2 mM. The title compounds were analyzed via LC/MS and used for the visible light-mediated reactions without further purification. UPLC/MS of DNA headpieces were consistent with those reported in the literature.[1]



DNA-HP-NH_2 (5 μmol, 1.0 equiv)

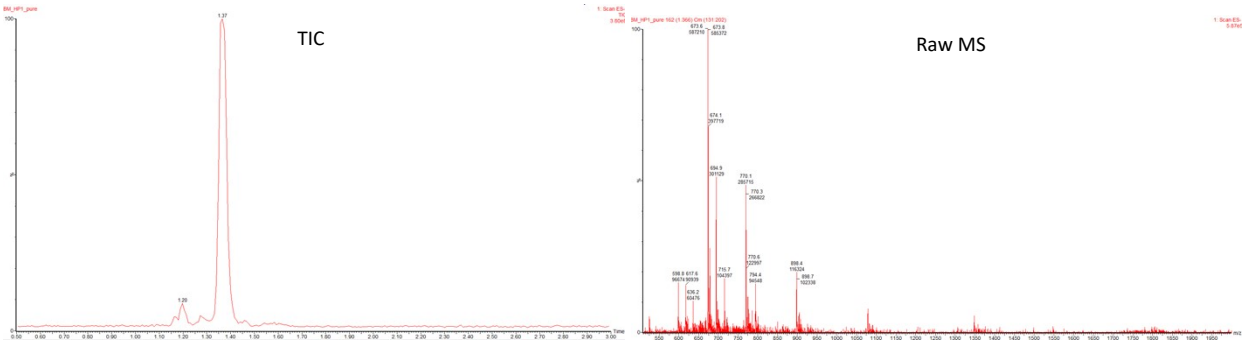
carboxylic acid (40 equiv)

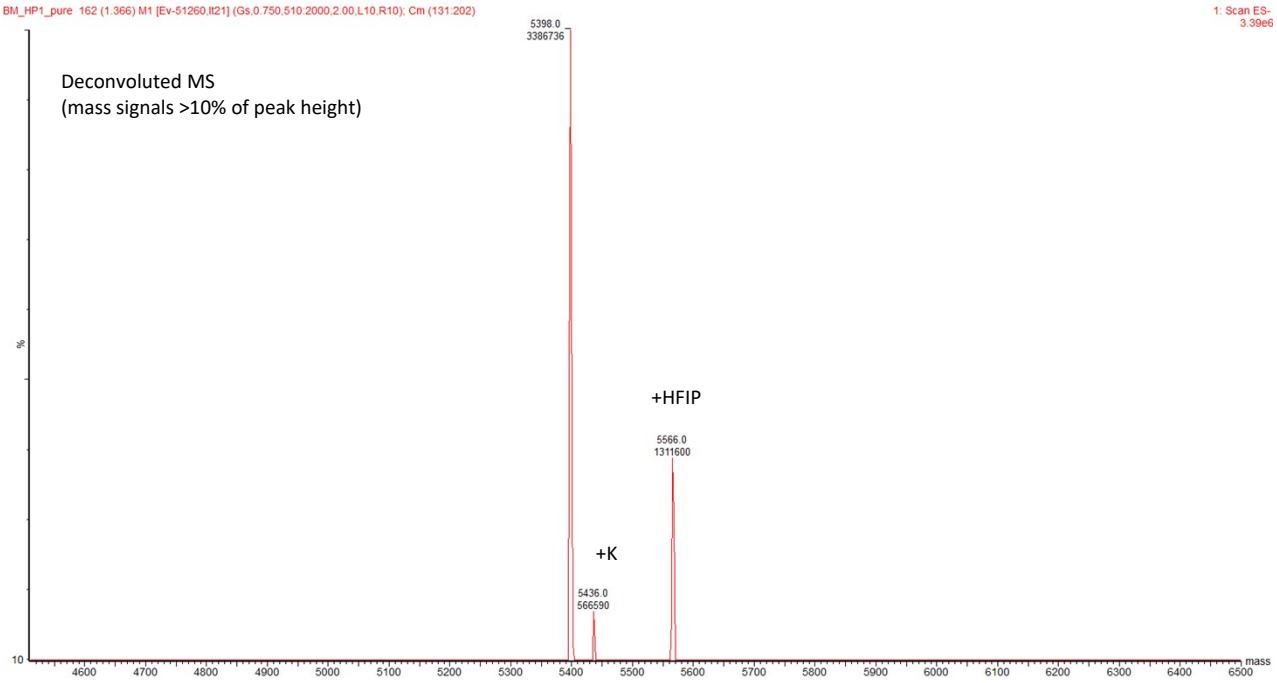
HP-4, > 95% conversion

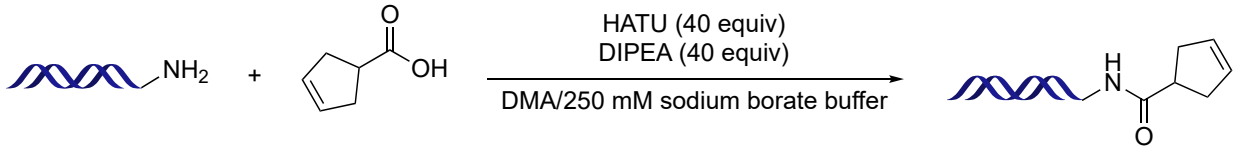
Molecular Weight: 5393.77

Prepared according to **GP-I** using DNA-HP-NH_2 (0.0259 g, 5.0 μmol, 1.0 equiv), HATU (0.076 g, 0.2 mmol, 40.0 equiv, 200 mM in DMA), DIPEA (0.035 mL, 0.2 mmol, 40.0 equiv, 200 mM in

DMA), and 1-(*tert*-butoxycarbonyl)-4-methylenepyrrolidine-2-carboxylic acid (0.045 g, 0.2 mmol, 40.0 equiv, 200 mM in DMA). **HP-4** was obtained with > 95% conversion.





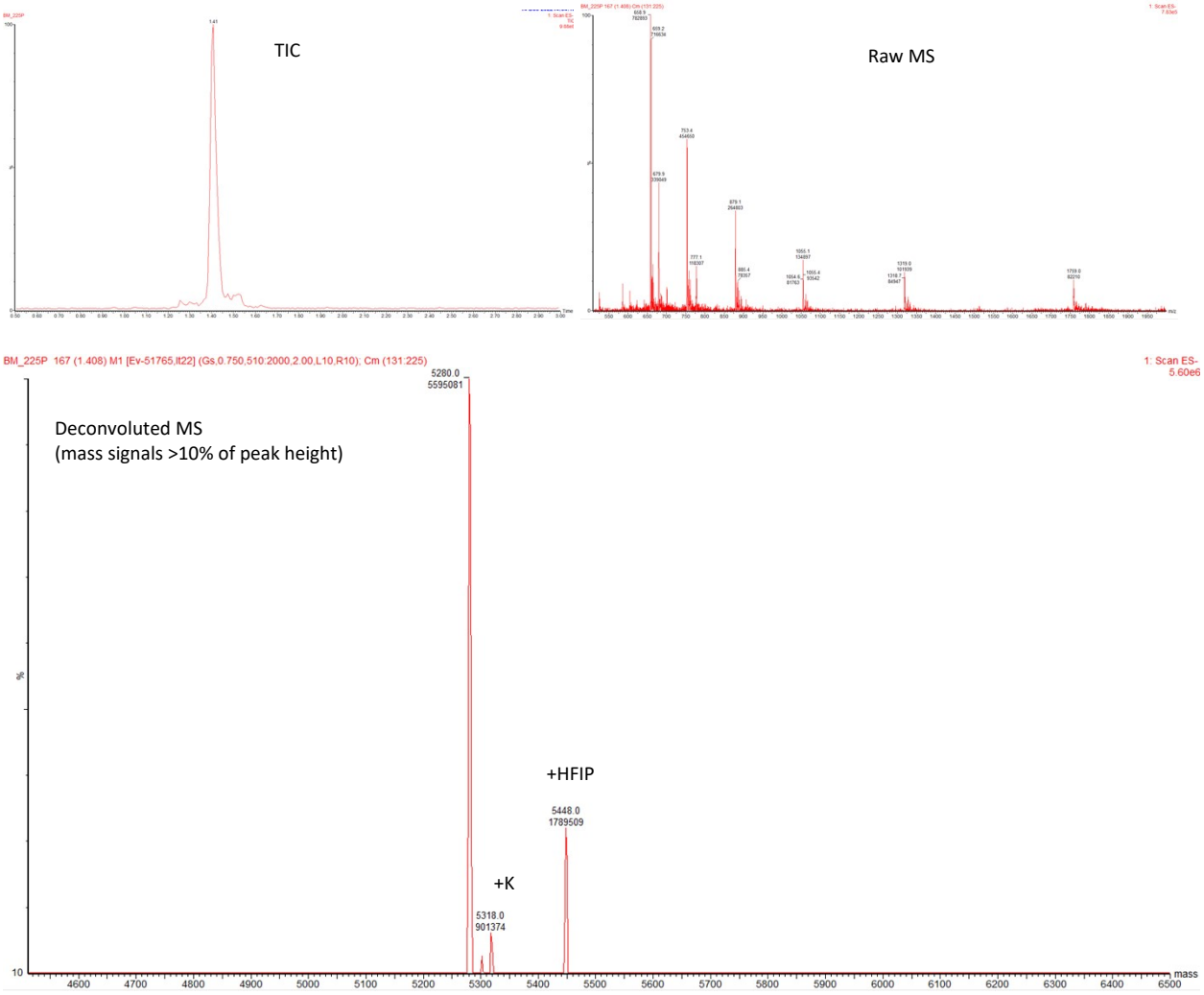


DNA-HP-NH_2
(5 μmol, 1.0 equiv)

carboxylic acid
(40 equiv)

HP-8, > 95% conversion
Molecular Weight: 5278.63

Prepared according to **GP-I** using DNA-HP-NH_2 (0.0104 g, 2.0 μmol, 1.0 equiv), HATU (0.030 g, 0.08 mmol, 40.0 equiv, 200 mM in DMA), DIPEA (0.014 mL, 0.2 mmol, 40.0 equiv, 200 mM in DMA), and cyclopent-3-ene-1-carboxylic acid (0.008 mL, 0.2 mmol, 40.0 equiv, 200 mM in DMA). **HP-8** was obtained with > 95% conversion.



2.2 Synthesis of Small Molecules

2-Isoxazoline-3-carboxylates employed in the scope of the reported transformation (Figure S3) were prepared according to reported literatures, which are described in **General Procedures II** (**S1-S14**) and **III** (**S15-S17).** Compound **S12** (isoxadifen-ethyl 98.0+%) was purchased from TCI America™ and used as received. Compounds **S18-S20** were obtained from AbbVie, Inc.

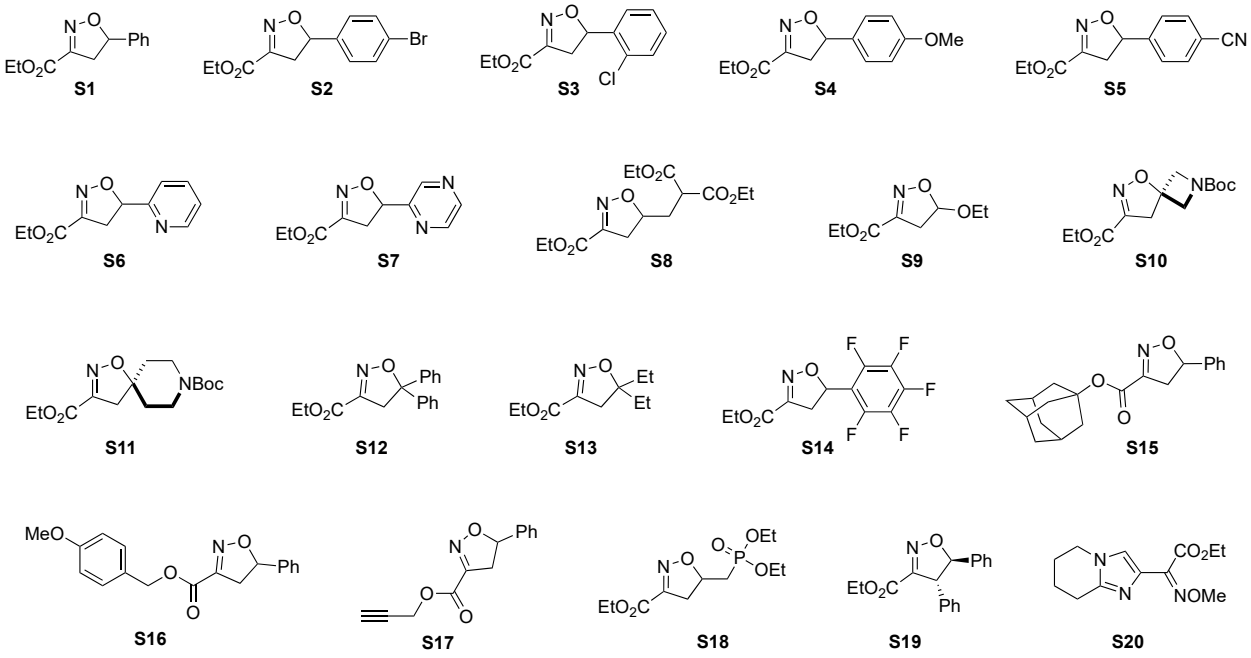
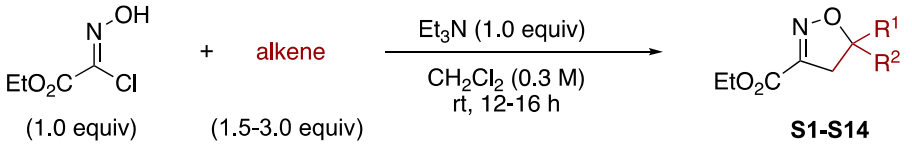
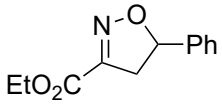


Figure S3. Molecular structures of the 2-isoxazoline-3-carboxylates explored in the transformation scope.

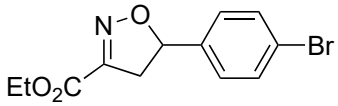
2.2.1 General Procedure II: Synthesis of 2-Isoxazoline-3-Carboxylates (S1-S14)



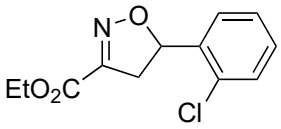
In a round-bottom flask charged with ethyl 2-chloro-2-(hydroxyamino)acetate (1.0 equiv) in CH_2Cl_2 (0.3 M) under argon atmosphere, the corresponding alkene (1.5-3.0 equiv) was added dropwise. Et_3N (1.0 equiv) was slowly added dropwise, and the reaction was stirred overnight (12-16 h). The reaction was washed with 2 M HCl (aq), and the aqueous layer was extracted with CH_2Cl_2 (3x). The combined organic layer was washed with brine, dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The crude mixture was purified by flash column chromatography (EtOAc/hexanes) to afford the pure title compound.



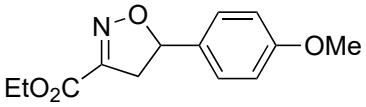
Ethyl 5-Phenyl-4,5-dihydroisoxazole-3-carboxylate (S1): Prepared according to GP-II using 2-chloro-2-(hydroxyamino) acetate (0.302 g, 2.0 mmol, 1.0 equiv), Et_3N (0.200 g, 0.279 mL, 2.0 mmol, 1.0 equiv) and styrene (0.417 g, 0.458 mL, 4.0 mmol, 2.0 equiv). Purification by flash column chromatography (5-50% EtOAc/hexanes) afforded the pure title compound as a colorless oil (0.475 g, 1.6 mmol, 80%). Spectroscopic data were consistent with those reported in the literature.[2]



Ethyl 5-(4-Bromophenyl)-4,5-dihydroisoxazole-3-carboxylate (S2): Prepared according to GP-II using 2-chloro-2-(hydroxyamino) acetate (0.500 g, 3.30 mmol, 1.0 equiv), Et_3N (0.334 g, 0.460 mL, 3.30 mmol, 1.0 equiv) and 1-bromo-4-vinylbenzene (1.81 g, 9.90 mmol, 3.0 equiv). Purification by flash column chromatography (0-20% EtOAc/hexanes) afforded the pure title compound as a white solid (0.550 g, 1.85 mmol, 56%). Spectroscopic data were consistent with those reported in the literature.[3]

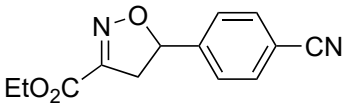


Ethyl 5-(2-Chlorophenyl)-4,5-dihydroisoxazole-3-carboxylate (S3): Prepared according to GP-II using 2-chloro-2-(hydroxyamino) acetate (0.455 g, 3.0 mmol, 1.0 equiv), Et_3N (0.303 g, 0.420 mL, 3.0 mmol, 1.0 equiv) and 1-chloro-2-vinylbenzene (0.831 g, 0.770 mL, 6.0 mmol, 2.0 equiv). Purification by flash column chromatography (5-30% EtOAc/hexanes) afforded the pure title compound as a colorless liquid (0.686 g, 2.7 mmol, 90%). Spectroscopic data were consistent with those reported in the literature.[3]

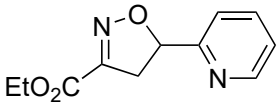


Ethyl 5-(4-Methoxyphenyl)-4,5-dihydroisoxazole-3-carboxylate (S4): Prepared according to GP-II using 2-chloro-2-(hydroxyamino) acetate (0.500 g, 3.30 mmol, 1.0 equiv), Et_3N (0.334 g,

0.460 mL, 3.30 mmol, 1.0 equiv) and 1-methoxy-4-vinylbenzene (1.33 g, 9.90 mmol, 3.0 equiv). Purification by flash column chromatography (0-25% EtOAc/hexanes) afforded the pure title compound as a colorless oil (0.420 g, 1.7 mmol, 51%). Spectroscopic data were consistent with those reported in the literature.[3]

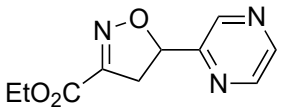


Ethyl 5-(4-Cyanophenyl)-4,5-dihydroisoxazole-3-carboxylate (S5): Prepared according to GP-II using 2-chloro-2-(hydroxyamino) acetate (0.500 g, 3.30 mmol, 1.0 equiv), Et_3N (0.334 g, 0.460 mL, 3.30 mmol, 1.0 equiv) and 4-vinylbenzonitrile (1.07 g, 8.25 mmol, 2.5 equiv). Purification by flash column chromatography (0-25% EtOAc/hexanes) afforded the pure title compound as a colorless oil (0.350 g, 1.4 mmol, 43%). Spectroscopic data were consistent with those reported in the literature.[3]



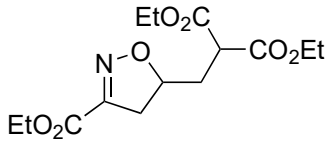
Ethyl 5-(Pyridin-2-yl)-4,5-dihydroisoxazole-3-carboxylate (S6): Prepared according to GP-II using 2-chloro-2-(hydroxyamino) acetate (0.230 g, 1.50 mmol, 1.0 equiv), Et_3N (0.152 g, 0.210 mL, 1.50 mmol, 1.0 equiv) and 2-vinylpyridine (0.315 g, 0.322 mL, 3.00 mmol, 2.0 equiv). Purification by flash column chromatography (5-50% EtOAc/hexanes) afforded the pure title compound as a pale-yellow solid (0.123 g, 0.56 mmol, 37%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.58 (ddd, J = 4.9, 1.8, 1.0 Hz, 1H), 7.71 (td, J = 7.7, 1.8 Hz, 1H), 7.47 – 7.44 (m, 1H), 7.24 (ddd, J = 7.6, 4.8, 1.2 Hz, 1H), 5.86 (dd, J = 11.0, 8.2 Hz, 1H), 4.33 (q, J = 7.1 Hz, 2H), 3.68 – 3.55 (m, 2H), 1.35 (t, J = 7.1 Hz, 3H).
^{13}C NMR (101 MHz, $CDCl_3$) δ 160.5, 158.3, 151.7, 149.8, 137.1, 123.4, 121.0, 84.8, 62.2, 39.9, 14.2.
FT-IR (cm^{-1}, neat, ATR) $\tilde{\nu}$ = 2980, 1717, 1589, 1572, 1473, 1436, 1405, 1379, 1347, 1333, 1295, 1249, 1145, 1121, 1016, 998, 918, 860, 829, 769, 749. **HRMS** (ESI) calcd for $C_{11}H_{13}N_2O_3$ [M + H]$^+$: 221.0921, found: 221.0926. **MP** (°C) 79.3 – 80.0.

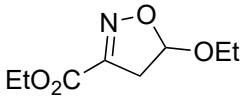


Ethyl 5-(Pyrazin-2-yl)-4,5-dihydroisoxazole-3-carboxylate (S7): Prepared according to GP-II using 2-chloro-2-(hydroxyamino) acetate (0.230 g, 1.50 mmol, 1.0 equiv), Et_3N (0.152 g, 0.210 mL, 1.50 mmol, 1.0 equiv) and 2-vinylpyrazine (0.318 g, 0.306 mL, 3.00 mmol, 2.0 equiv). Purification by flash column chromatography (5-70% EtOAc/hexanes) afforded the pure title compound as a colorless oil (0.213 g, 0.75 mmol, 50%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.73 (d, J = 1.3 Hz, 1H), 8.55 – 8.54 (m, 2H), 5.89 (t, J = 9.5 Hz, 1H), 4.33 (q, J = 7.2 Hz, 2H), 3.64 (d, J = 9.5 Hz, 2H), 1.34 (t, J = 7.2 Hz, 3H).
^{13}C NMR (101 MHz, $CDCl_3$) δ 160.2, 153.3, 151.7, 144.9, 144.3, 143.3, 82.8, 62.4, 39.3, 14.2.
FT-IR (cm^{-1}, neat, ATR) $\tilde{\nu}$ = 2984, 1717, 1594, 1472, 1405, 1379, 1347, 1334, 1296, 1247, 1120, 1057, 1017, 913, 848, 826, 801, 765, 745, 647. **HRMS** (ESI) calcd for $C_{10}H_{12}N_3O_3$ $[M + H]^+$: 222.0873, found: 222.0879.

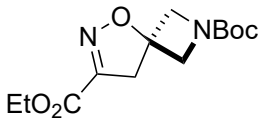


Diethyl 2-((3-(Ethoxycarbonyl)-4,5-dihydroisoxazol-5-yl)methyl)malonate (S8): Prepared according to GP-II using 2-chloro-2-(hydroxyamino) acetate (0.500 g, 3.30 mmol, 1.0 equiv), Et_3N (0.334 g, 0.460 mL, 3.30 mmol, 1.0 equiv) and diethyl 2-allylmalonate (1.65 g, 8.25 mmol, 2.5 equiv). Purification by flash column chromatography (0-30% EtOAc/hexanes) afforded the pure title compound as a colorless oil (0.624 g, 1.96 mmol, 60%). Spectroscopic data were consistent with those reported in the literature.[2]



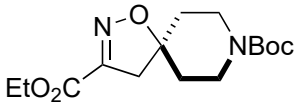
Ethyl 5-Ethoxy-4,5-dihydroisoxazole-3-carboxylate (S9): Prepared according to GP-II using 2-chloro-2-(hydroxyamino) acetate (0.500 g, 3.30 mmol, 1.0 equiv), Et_3N (0.334 g, 0.460 mL, 3.30

mmol, 1.0 equiv) and ethoxyethene (0.595 g, 8.25 mmol, 2.5 equiv). Purification by flash column chromatography (0-25% EtOAc/hexanes) afforded the pure title compound as colorless oil (0.408 g, 1.96 mmol, 66%). Spectroscopic data were consistent with those reported in the literature.[2]

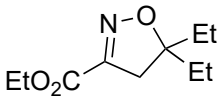


2-(*tert*-Butyl) 7-Ethyl 5-Oxa-2,6-diazaspiro[3.4]oct-6-ene-2,7-dicarboxylate (S10): Prepared according to GP-II using 2-chloro-2-(hydroxyamino) acetate (0.230 g, 1.50 mmol, 1.0 equiv), Et_3N (0.152 g, 0.210 mL, 1.50 mmol, 1.0 equiv) and *tert*-butyl 3-methyleneazetidine-1-carboxylate (0.508 g, 3.00 mmol, 2.0 equiv). Purification by flash column chromatography (5-50% EtOAc/hexanes) afforded the pure title compound as a white solid (0.213 g, 0.75 mmol, 50%).

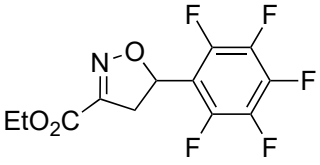
^{1}H NMR (400 MHz, $CDCl_3$) δ 4.34 (q, *J* = 7.1 Hz, 2H), 4.29 (dd, *J* = 9.9, 1.2 Hz, 2H), 4.03 (dd, *J* = 9.9, 1.3 Hz, 2H), 3.43 (s, 2H), 1.43 (s, 9H), 1.35 (t, *J* = 7.1 Hz, 3H).
^{13}C NMR (101 MHz, CDCl3) δ 160.2, 156.0, 151.7, 82.9, 80.4, 62.9, 62.5, 43.2, 28.4, 14.2.
FT-IR (cm^{-1}, neat, ATR) $\tilde{\nu}$ = 2979, 2879, 1702, 1593, 1478, 1453, 1394, 1368, 1274, 1236, 1158, 1077, 1017, 929, 855, 816, 770, 744, 608. **HRMS** (ESI) calcd for $C_{13}H_{21}N_2O_5$ $[M + H]^+$: 285.1445, found: 285.1450. **MP** (°C) 71.9 – 72.8.



8-(*tert*-Butyl) 3-Ethyl 1-Oxa-2,8-diazaspiro[4.5]dec-2-ene-3,8-dicarboxylate (S11): Prepared according to GP-II using 2-chloro-2-(hydroxyamino) acetate (0.380 g, 2.5 mmol, 1.0 equiv), Et_3N (0.253 g, 0.350 mL, 2.5 mmol, 1.0 equiv) and *tert*-butyl 4-methylenepiperidine-1-carboxylate (0.740 g, 3.8 mmol, 1.5 equiv). Purification by flash column chromatography (0-30% EtOAc/hexanes) afforded the pure title compound as a white solid (0.200 g, 0.64 mmol, 25%). Spectroscopic data were consistent with those reported in the literature.[2]

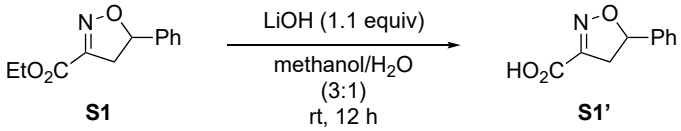


Ethyl 5,5-Diethyl-4,5-dihydroisoxazole-3-carboxylate (S13): Prepared according to GP-II using 2-chloro-2-(hydroxyamino) acetate (0.500 g, 3.30 mmol, 1.0 equiv), Et_3N (0.334 g, 0.460 mL, 3.30 mmol, 1.0 equiv) and 2-ethyl-1-butene (0.833 g, 9.90 mmol, 3.0 equiv). Purification by flash column chromatography (0-5% EtOAc/hexanes) afforded the pure title compound as a colorless oil (0.100 g, 0.5 mmol, 15%). Spectroscopic data were consistent with those reported in the literature.[4]



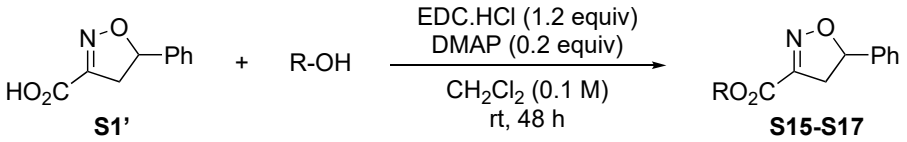
Ethyl 5-(Perfluorophenyl)-4,5-dihydroisoxazole-3-carboxylate (S14): Prepared according to GP-II using 2-chloro-2-(hydroxyamino) acetate (0.455 g, 3.0 mmol, 1.0 equiv), Et_3N (0.304 g, 0.420 mL, 3.0 mmol, 1.0 equiv) and 1,2,3,4,5-pentafluoro-6-vinylbenzene (1.16 g, 0.830 mL, 6.0 mmol, 2.0 equiv). Purification by flash column chromatography (0-20% EtOAc/hexanes) afforded the pure title compound as a white solid (0.698 g, 2.2 mmol, 73%). Spectroscopic data were consistent with those reported in the literature.[3]

2.2.2 General Procedure III: Synthesis of 2-Isoxazoline-3-carboxylates (S15-S17)

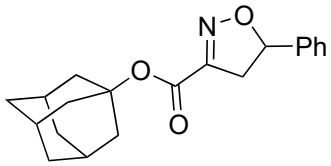


5-Phenyl-4,5-dihydroisoxazole-3-carboxylic Acid (S1'): To a round-bottom flask equipped with a magnetic stir bar was added ethyl 5-phenyl-4,5-dihydroisoxazole-3-carboxylate **S1** (1.0 g, 4.50 mmol, 1.0 equiv) and a 3:1 mixture of MeOH/H_2O (20 mL). LiOH (0.119 g, 4.95 mmol, 1.1 equiv) was added, and the reaction mixture was stirred for 12 h at rt. The soln was acidified with 2 M HCl

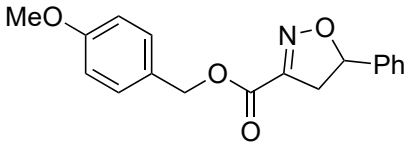
(aq) and subsequently extracted with CH_2Cl_2 (5 x 20 mL). The combined organic layers were dried ($MgSO_4$), filtered, and concentrated in vacuo, then dried using high vac to afford the pure title compound as a light-yellow oil (0.740 g, 4.87 mmol, 86%). Spectroscopic data were consistent with those reported in the literature.[2]



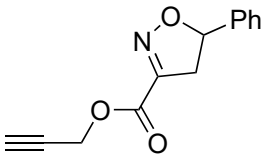
To a round-bottom flask equipped with a magnetic stir bar containing **S1'** (1.0 equiv) and CH_2Cl_2 (0.1 M), was added the corresponding alcohol (2.0 equiv), EDC•HCl (1.2 equiv), and DMAP (0.2 equiv). The resulting reaction mixture was stirred at rt for 48 h. Then, 2 M HCl (aq) was added, the organic layer separated, and the aqueous layer was extracted with CH_2Cl_2 (3x). The combined organic layers were washed with brine, dried ($MgSO_4$), filtered, and concentrated in vacuo. The crude product was purified by flash column chromatography to afford the pure 2-isoxazoline-3-carboxylate.



(3s,5s,7s)-Adamantan-1-yl 5-Phenyl-4,5-dihydroisoxazole-3-carboxylate (S15): Prepared according to GP-III using **S1'** (0.500 g, 2.6 mmol, 1.0 equiv), 1-adamantanol (0.792 g, 5.2 mmol, 2.0 equiv), EDC•HCl (0.598 g, 3.12 mmol, 1.2 equiv), and DMAP (0.063 g, 0.52 mmol, 0.2 equiv). The crude product was purified by flash column chromatography (0-30% EtOAc/hexanes) to afford the pure title compound as a colorless oil (0.770 g, 2.4 mmol, 91%). Spectroscopic data were consistent with those reported in the literature.[2]



4-Methoxybenzyl 5-Phenyl-4,5-dihydroisoxazole-3-carboxylate (S16): Prepared according to GP-III using **S1'** (0.500 g, 2.6 mmol, 1.0 equiv), (4-methoxyphenyl)methanol (0.718 g, 5.2 mmol, 2.0 equiv), EDC•HCl (0.598 g, 3.12 mmol, 1.2 equiv), and DMAP (0.063 g, 0.52 mmol, 0.2 equiv). The crude product was purified by flash column chromatography (0-30% EtOAc/hexanes) to afford the pure title compound as a colorless oil (0.583 g, 1.9 mmol, 72%). Spectroscopic data were consistent with those reported in the literature.[2]



Prop-2-yn-1-yl 5-Phenyl-4,5-dihydroisoxazole-3-carboxylate (S17): Prepared according to GP-III using **S1'** (0.500 g, 2.6 mmol, 1.0 equiv), prop-2-yn-1-ol (0.291 g, 5.2 mmol, 2.0 equiv), EDC•HCl (0.598 g, 3.12 mmol, 1.2 equiv), and DMAP (0.063 g, 0.52 mmol, 0.2 equiv). The crude product was purified by flash column chromatography (0-35% EtOAc/hexanes) to afford the pure title compound as a colorless oil (0.358 g, 1.9 mmol, 60%). Spectroscopic data were consistent with those reported in the literature.[2]

3 Optimization: Visible-light Mediated [2+2] Photocycloaddition On-DNA

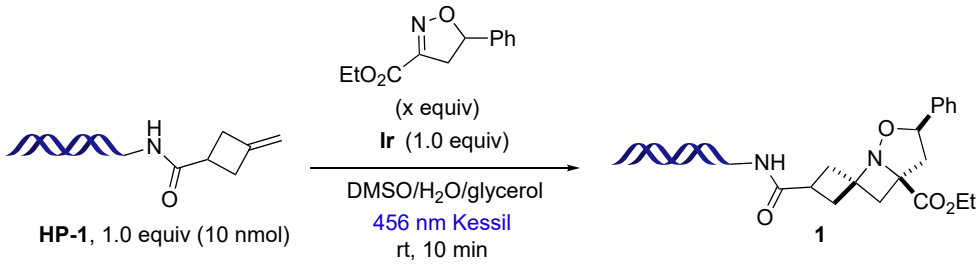
For the optimization of the on-DNA [2+2] photocycloaddition reaction, we elected to use a photo-inert exomethylene cyclobutane headpiece (**HP-1**), which would afford valuable spirocyclic compounds after the cycloaddition with 2-isoxazoline-3-carboxylates. With the reaction partner ethyl 5-phenyl-4,5-dihydroisoxazole-3-carboxylate, **HP-1** provided **1** with 35% conversion when one equivalent of photosensitizer $[Ir\{dF(CF_3)ppy\}_2(dtbbpy)]PF_6$ was used in the presence of glycerol as a radical scavenging additive and DMSO as solvent (**Table S1, entry 1**). Lower yields were observed with a larger excess of the isoxazoline reactant, increased loading of the photosensitizer, reaction time, or irradiation source were employed (**entries 2-8**).

However, when **HP-1** was combined with ethyl 5-phenyl-4,5-dihydroisoxazole-3-carboxylate in the presence of photosensitizer $Ir(dFppy)_3$, **1** was formed with 35% conversion (**entry 9**). In this case, a clean reaction profile was observed with only the formation of the desired product and recovery of the alkene starting material. This commercially available photosensitizer has a triplet

energy of approximately ET= 60 kcal.mol^{-1} and was previously demonstrated to participate in an efficient triplet-energy-transfer process with a range of 2-isoxazoline-3-carboxylates. [2] Doubling the amount of the isoxazoline directly boosted conversion to 68% in a still very clean transformation (**entry 10**). The maximum conversion (95%) was observed when 100 equivalents of isoxazoline was employed (**entry 11**). Lowering the amount of catalyst to 0.5 equivalents or decreasing the reaction time still led to excellent results, albeit in slightly lower yield than the previous conditions (entries **12** and **13**).

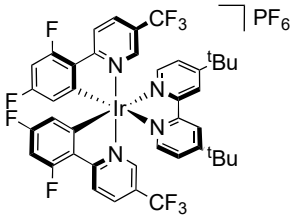
Demonstrating the operational simplicity of the protocol, the reaction was performed under open-to-air conditions, which did not lead to any erosion in the conversion (**entry 14**). The use of glycerol was crucial to the reaction outcome (**entry 15**), as was the iridium photocatalyst (**entry 16**) and the irradiation source (**entry 17**). Regarding the selectivity for this transformation, off-DNA experiments revealed a unique regioselectivity, in contrast to transformations using singlet-state aryl isoxazolines, which is independent of the electronic properties of the alkene.[2] The triplet-state isoxazolines were found to form the exo-diastereomer as the major isomer when reacted with alkenes, likely driven by the minimization of interactions between the larger alkene substituent and the isoxazoline ring.

***Table S1**. Optimization of the [2+2] cycloaddition reaction.*

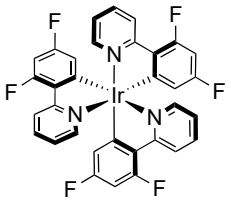


HP-1, 1.0 equiv (10 nmol) — **1**

Entry	Reaction Condition	Conversion (%)
1	**Ir-I**, 25 equiv of isoxazoline	35%
2	**Ir-I**, 50 equiv of isoxazoline	28%
3	**Ir-I**, 100 equiv of isoxazoline	44%
4[a]	**Ir-I**, 100 equiv of isoxazoline	degradation
5	**Ir-I**, 100 equiv of isoxazoline, 20 min	degradation
6	**Ir-I**, 200 equiv of isoxazoline	26% (7% rsm)
7[b]	**Ir-I**, 50 equiv of isoxazoline	degradation
8[c]	**Ir-I**, 50 equiv of isoxazoline	degradation
9	**Ir-II**, 25 equiv of isoxazoline	35% (54% rsm)
10	**Ir-II**, 50 equiv of isoxazoline	68% (32% rsm)
11	**Ir-II**, 100 equiv of isoxazoline	>95%
12	0.5 equiv of **Ir-II**	75% (15% rsm)
13	**Ir-II**, t = 5 min	73% (19% rsm)
14	**Ir-II**, **no degassing**	**>95%**
15	**Ir-II**, no glycerol	15% (71% rsm)
16	no catalyst	only rsm
17	**Ir-II**, no light (heating at 60 °C)	only rsm



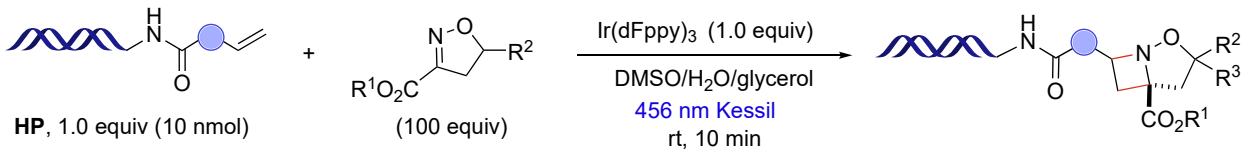
$[Ir\{dF(CF_3)ppy\}_2(dtbbpy)]PF_6$ (**Ir-I**)



$Ir(dFppy)_3$ (**Ir-II**)

Conditions: **HP-1** (2 mM in H_2O, 10 nmol, 1.0 equiv), isoxazoline, Ir catalyst (2 mM in DMSO, 1.0 equiv), glycerol (10 µL of a 20% solution in DMSO), rt, 10 min, 456 nm blue Kessil. Conversions determined by LC-MS analysis after ethanol precipitation work-up. Rsm = recovered starting material. All reactions were performed under degassed conditions, unless otherwise noticed. [a] Using 2.0 equiv of **Ir-I** catalyst. [b] Under UV irradiation, 10 minutes. [c] Under UV irradiation, 5 min.

4 General Procedure IV: Visible-light Mediated [2+2] Photocycloaddition On-DNA

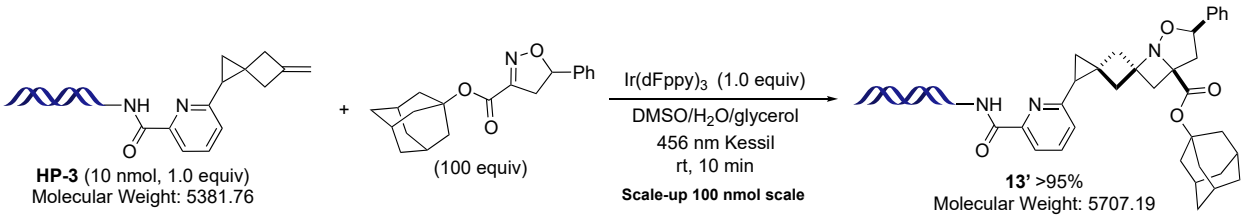


HP, 1.0 equiv (10 nmol) — (100 equiv)

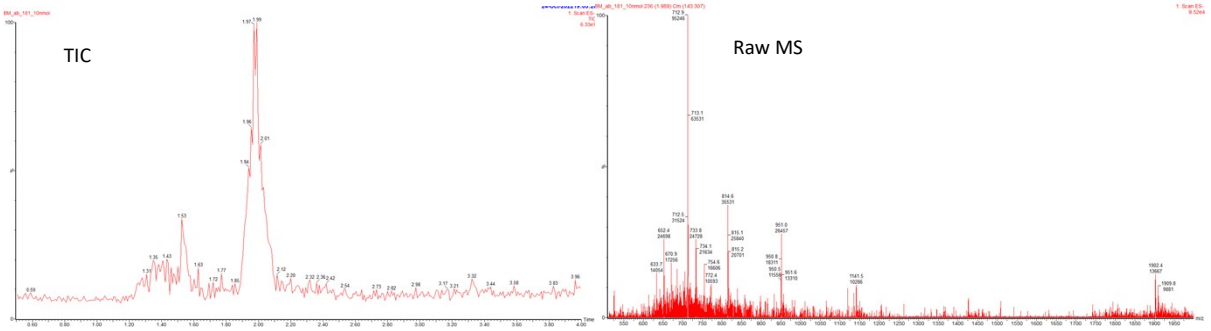
To an Eppendorf PCR microcentrifuge tube was added $Ir(dFppy)_3$ (5 µL of a 2 nmol/µL soln in DMSO, 10 nmol, 1.0 equiv), 2-isoxazoline-3-carboxylate (10 µL of a 100 nmol/µL soln in DMSO, 1000 nmol, 100 equiv), glycerol solution (10 µL of a soln 20% glycerol in DMSO), and DNA-tethered alkene (5 µL of a 2 nmol/µL soln in H_2O, 10 nmol, 1.0 equiv). The PCR tube was capped, and the reaction mixture was vortexed and placed at 1.5 inches from the irradiation source (Kessil H150-456 nm blue lamp) for 10 min. For the work-up, a solution of 5 M NaCl in HyPureTM Molecular Biology Grade H_2O (3 µL) was added, followed by the addition of cold EtOH (75 µL). The mixture was left at −20 °C for 1 h and then centrifuged at 6,000 rpm for 15 min. The supernatant was discarded, and the DNA pellet was re-dissolved in H_2O (100 µL), and analyzed via LC/MS.

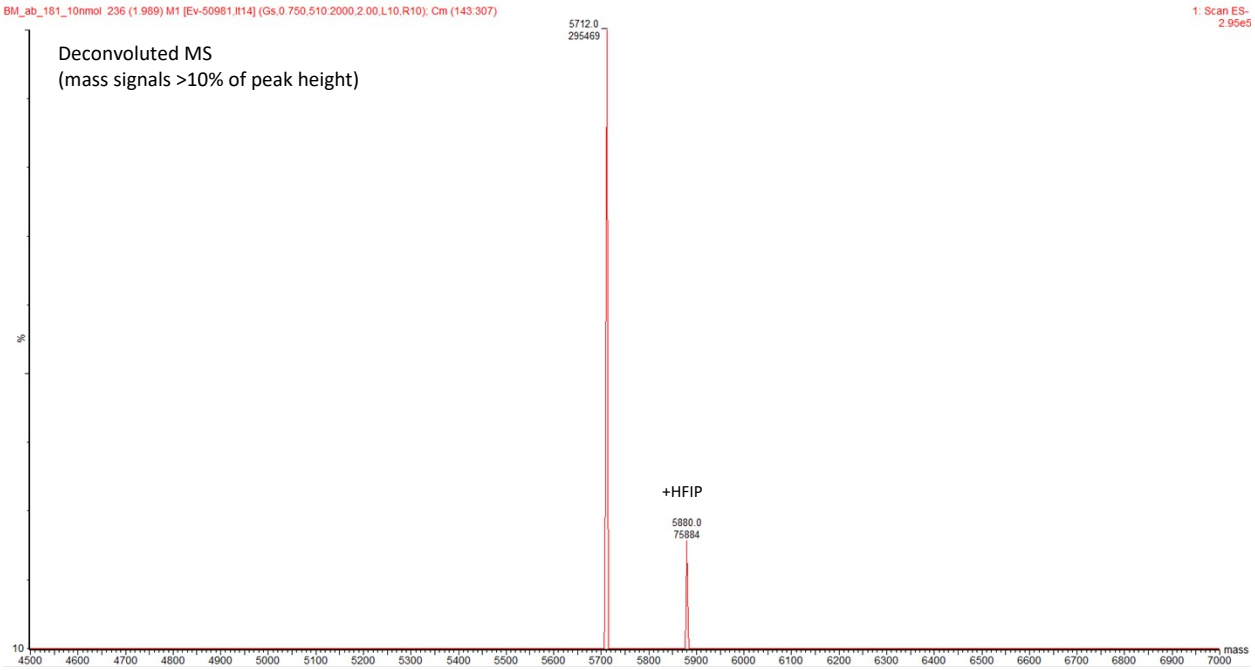
4.1 General Procedure V: 100 nmol Scale-Up Reaction

To a 0.6 mL Eppendorf tube was added $Ir(dFppy)_3$ (50 µL of a 2 nmol/µL soln in DMSO, 10 nmol, 1.0 equiv), 2-isoxazoline-3-carboxylate (100 µL of a 100 nmol/µL soln in DMSO, 1000 nmol, 100 equiv), glycerol solution (100 µL of a soln 20% glycerol in DMSO), and DNA-tethered alkene (50 µL of a 2 nmol/µL soln in H_2O, 10 nmol, 1.0 equiv). The Eppendorf tube was capped, and the reaction mixture was vortexed and placed at 1.5 inches from the irradiation source (Kessil H150-456 nm blue lamp) for 10 min. For the work-up, a solution of NaCl in HyPureTM Molecular Biology Grade H_2O (5 M, 30 µL) was added, followed by the addition of cold EtOH (750 µL). The mixture was left at −20 °C for 1 h and then centrifuged at 6,000 rpm for 15 min. The supernatant was discarded, and the DNA pellet was re-dissolved in H_2O (1.0 mL). An aliquot (10 µL) was taken, diluted in HyPureTM Molecular Biology Grade H_2O (100 µL), and analyzed via LC/MS.

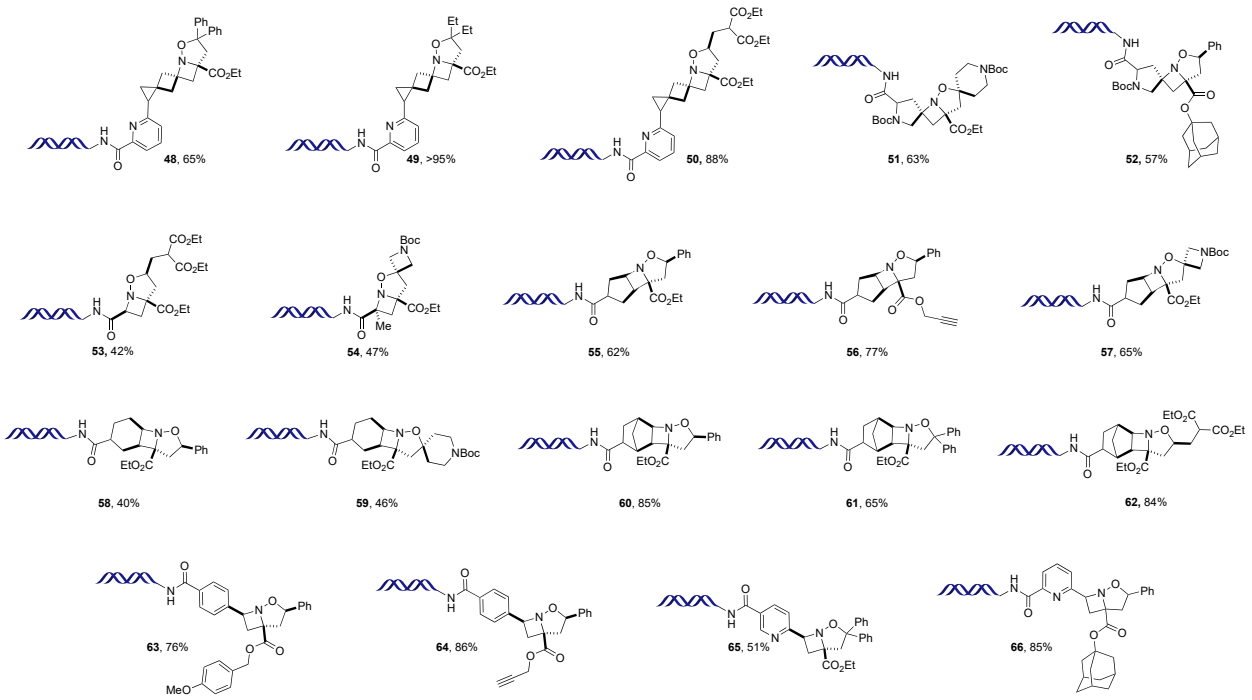


The title compound **13'** was synthesized according to **GP-IV** from (3s,5s,7s)-adamantan-1-yl 5-phenyl-4,5-dihydroisoxazole-3-carboxylate and the DNA-tagged alkene **HP-3**. The product was formed with > 95% conversion.



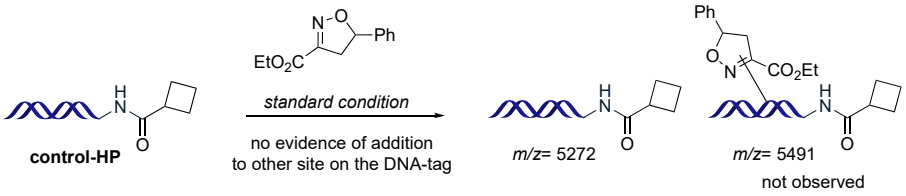


5 Additional Scope of 2-Oxa-1-azaspiro(bicyclo[3.2.0])heptanes On-DNA



6 Control Experiments

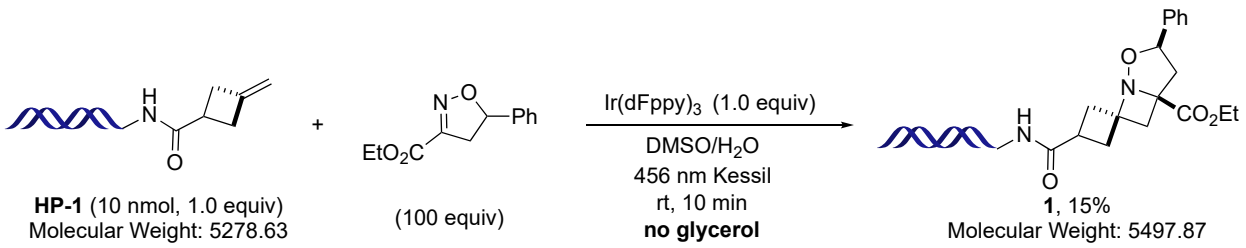
6.1 DNA-Tag Reactivity Under the Developed Reaction Condition



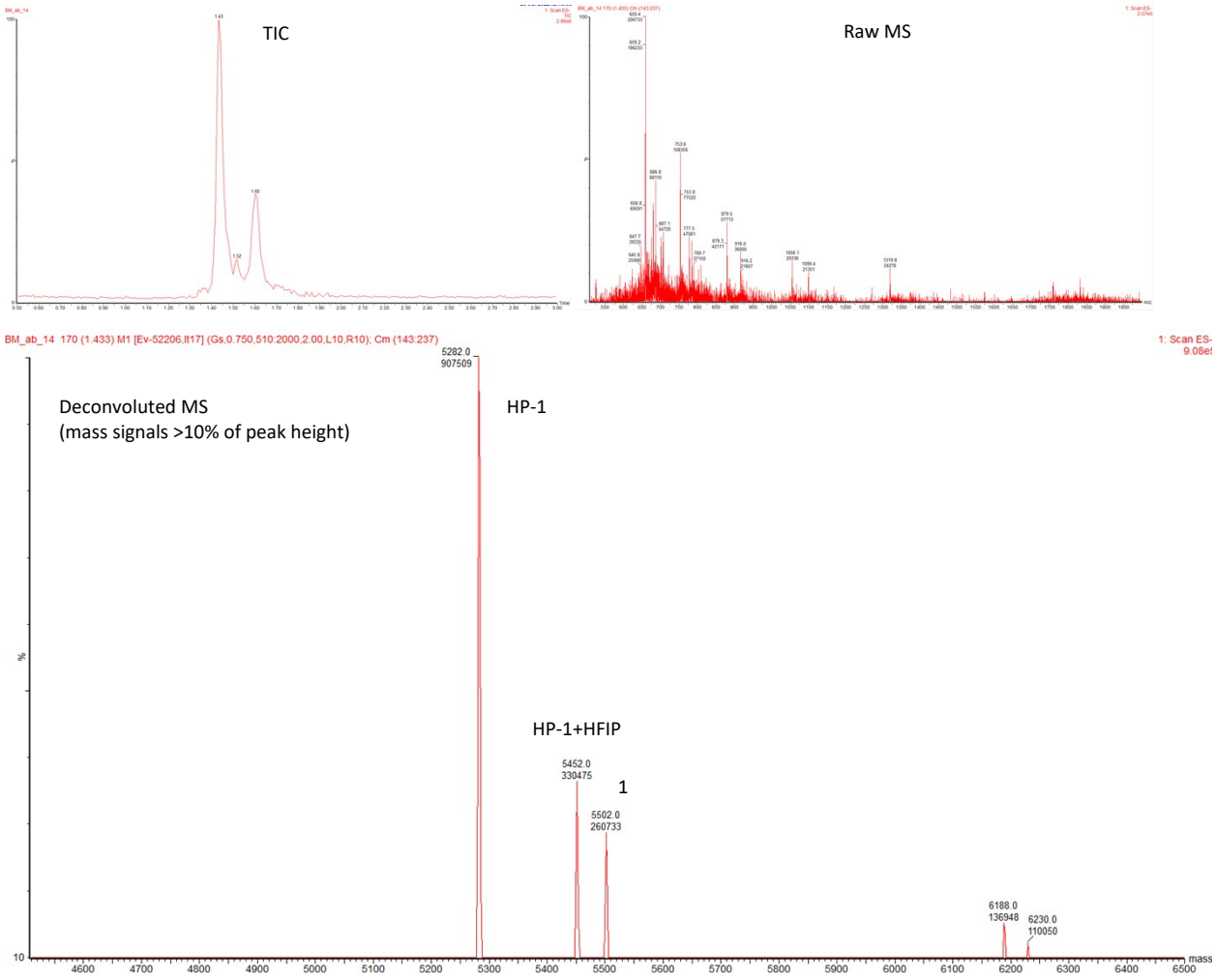
The control experiment was performed following **GP-IV** with the control-HP lacking the alkene moiety. As result, no product from a side [2+2] cycloaddition reaction with the ethyl 5-phenyl-4,5-dihydroisoxazole-3-carboxylate (**S1**) was observed, and only the control-HP was recovered. This result indicates that there is no evidence of reaction on the structure of the DNA-tag, and the only reactive site toward the photocycloaddition is the attached olefin.

6.2 Control Reactions: Photocatalyst, Light, Glycerol

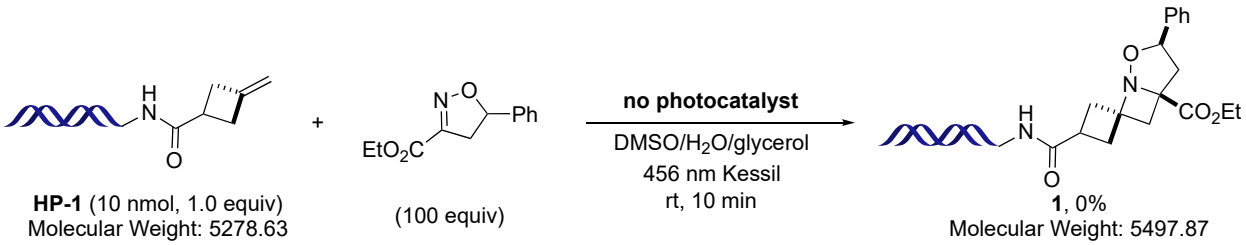
Absence of glycerol:



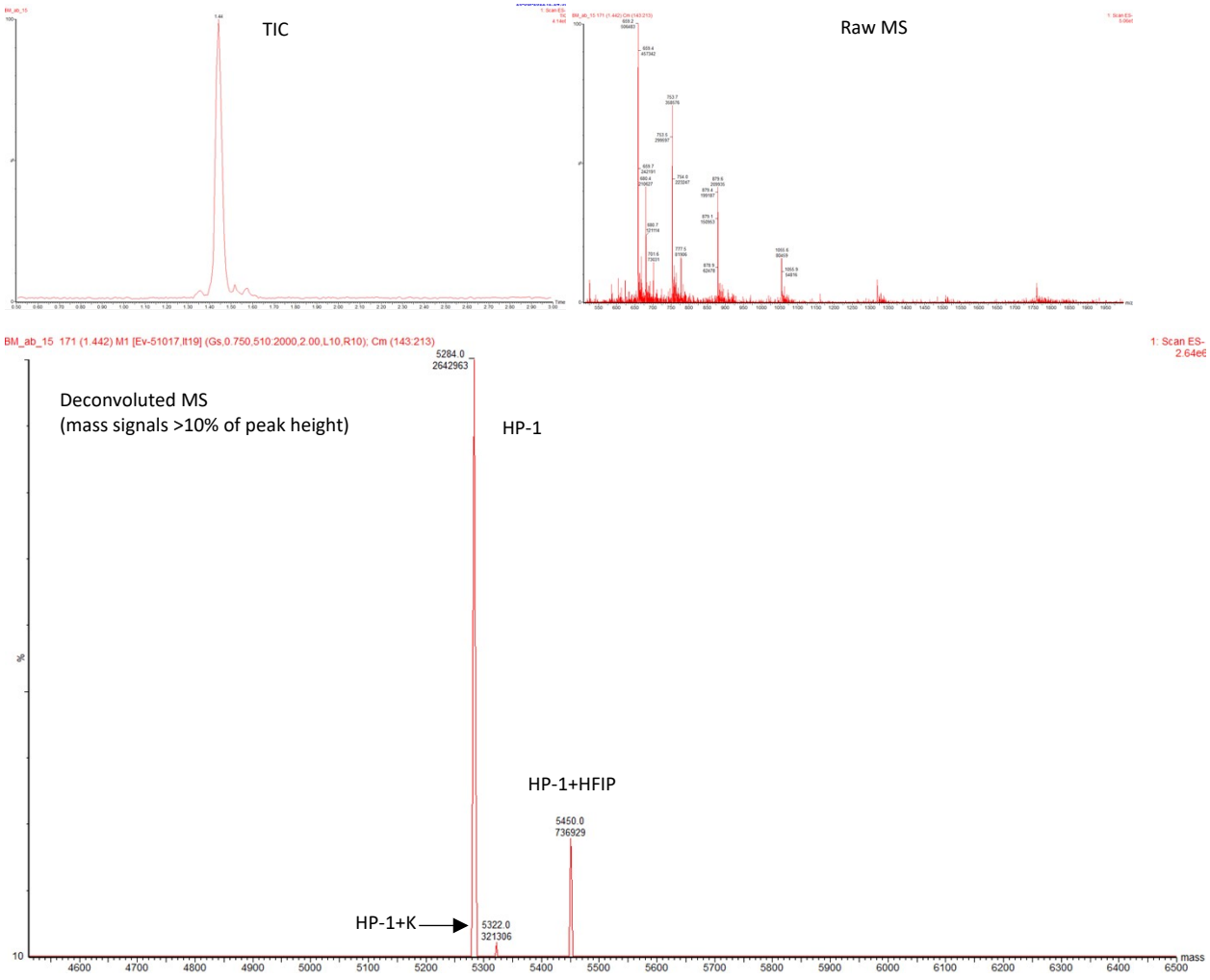
The reaction was performed in absence of glycerol, according to the **GP-IV** from ethyl 5-phenyl-4,5-dihydroisoxazole-3-carboxylate and the DNA-tagged alkene **HP-1**. The product was formed with 15% conversion (recovered **HP-1** = 71%).



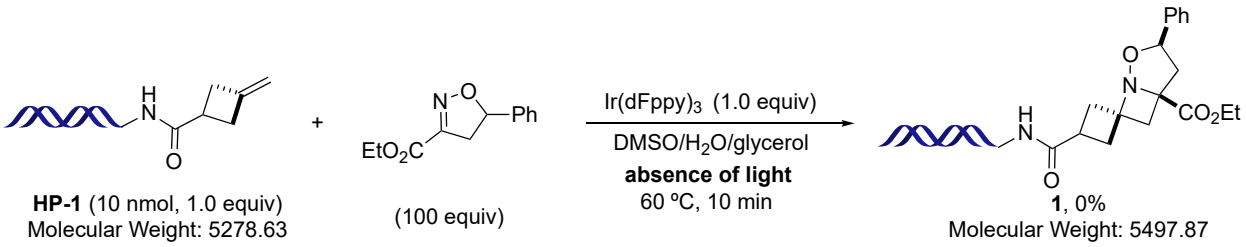
Absence of photocatalyst:



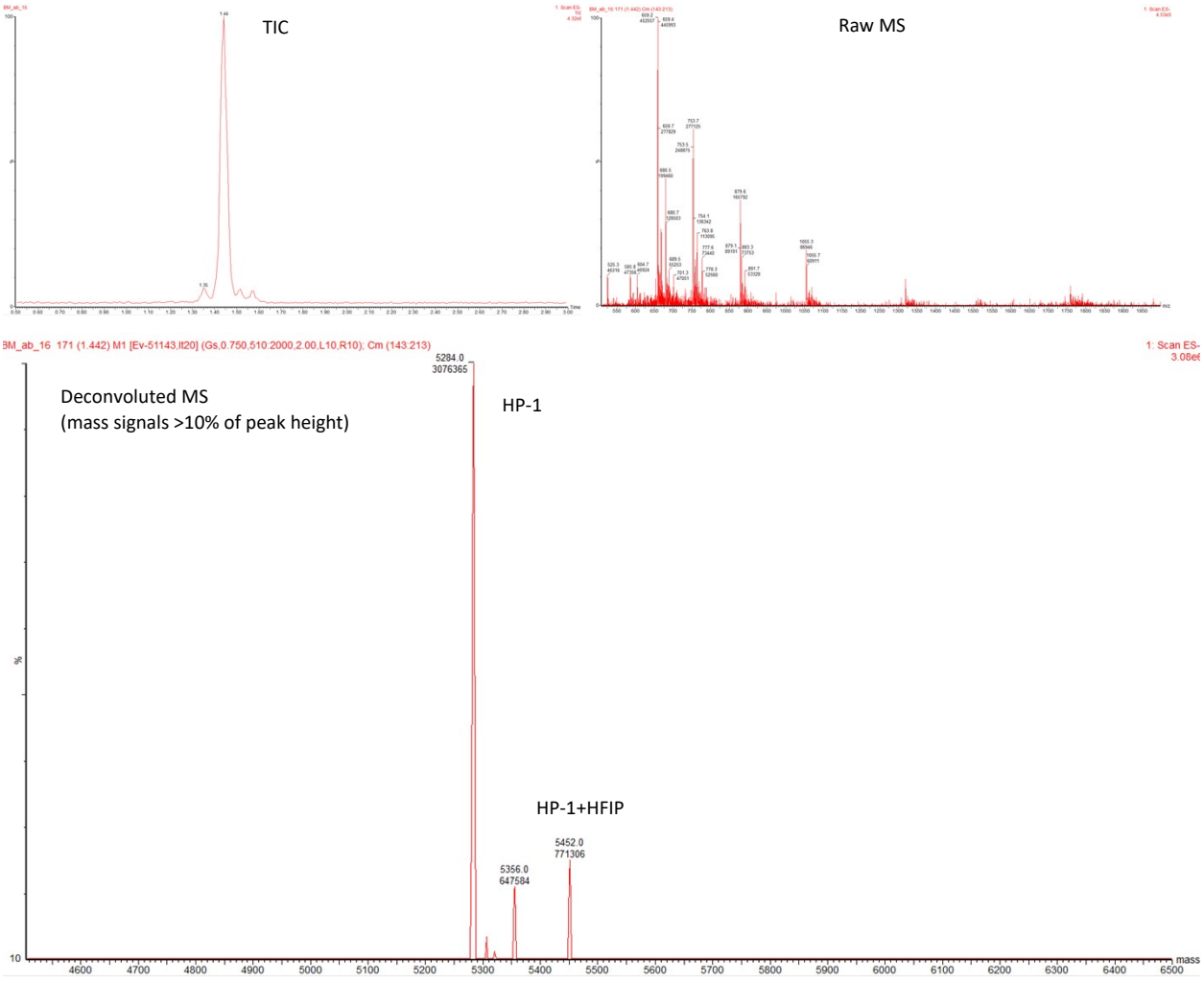
The reaction was performed in absence of photocatalyst, according to the **GP-IV** from ethyl 5-phenyl-4,5-dihydroisoxazole-3-carboxylate, and the DNA-tagged alkene **HP-1**. No product formation was observed, and **HP-1** was recovered.



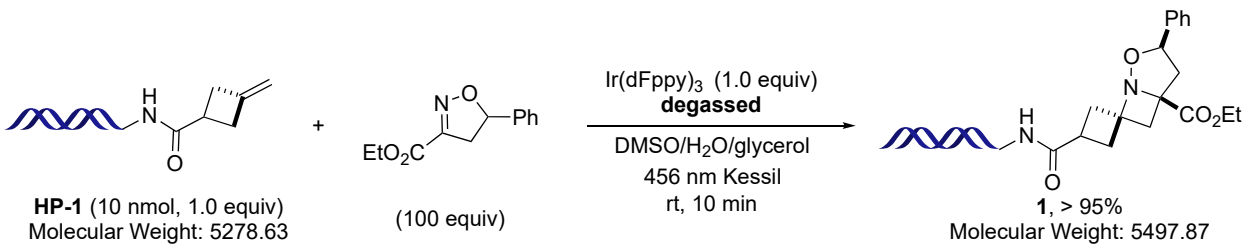
In the dark:



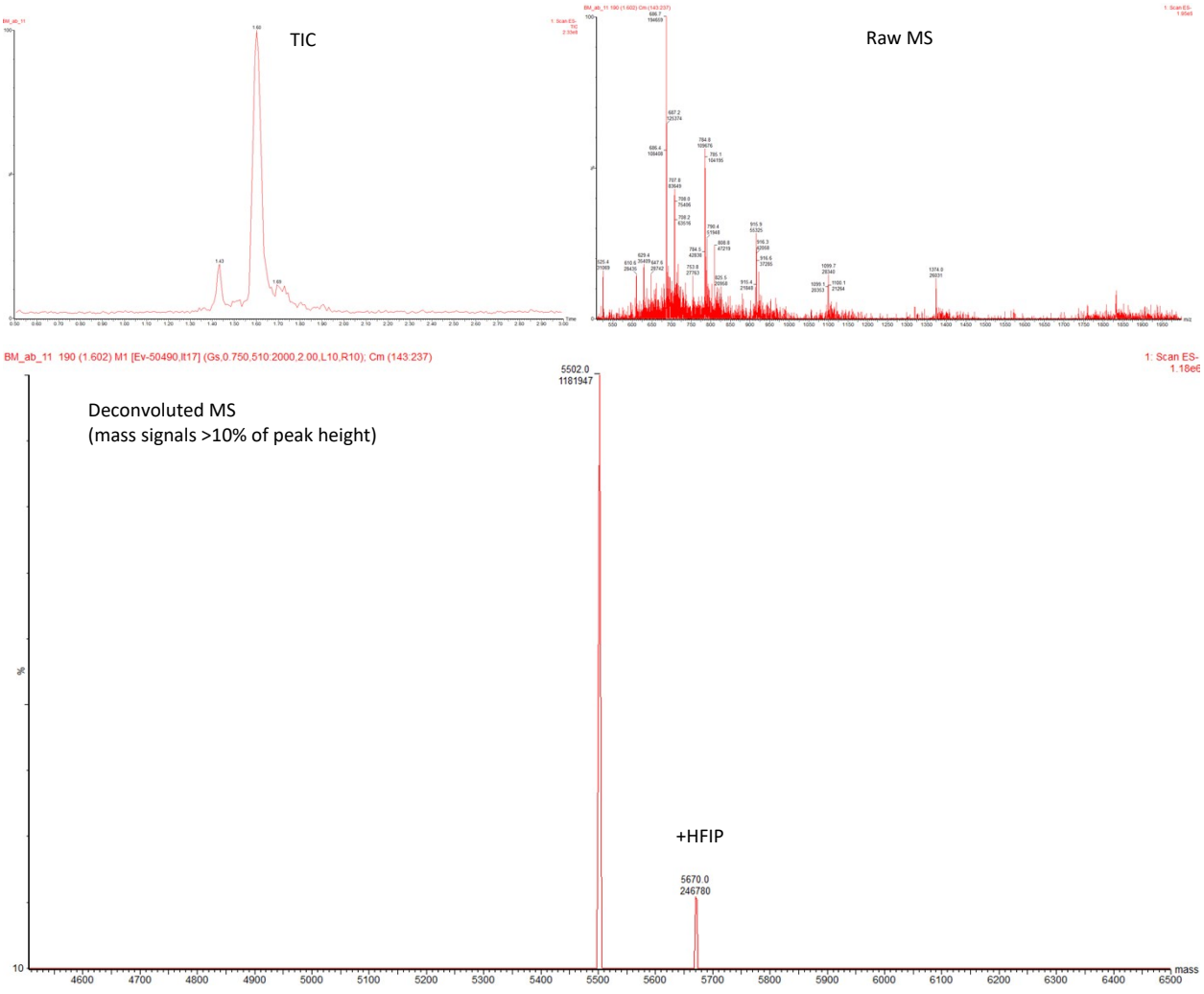
The reaction was performed in absence of light at ~ 60 °C using an oil bath, according to **GP-IV** from ethyl 5-phenyl-4,5-dihydroisoxazole-3-carboxylate, and the DNA-tagged alkene **HP-1**. No product formation was observed, and **HP-1** was recovered.



Degassed condition:



The title compound **1** was synthesized according to **GP-IV** from ethyl 5-phenyl-4,5-dihydroisoxazole-3-carboxylate under degassed condition, and the DNA-tagged alkene **HP-1**. The product was formed in > 95% conversion.



7 Unsuccessful Examples

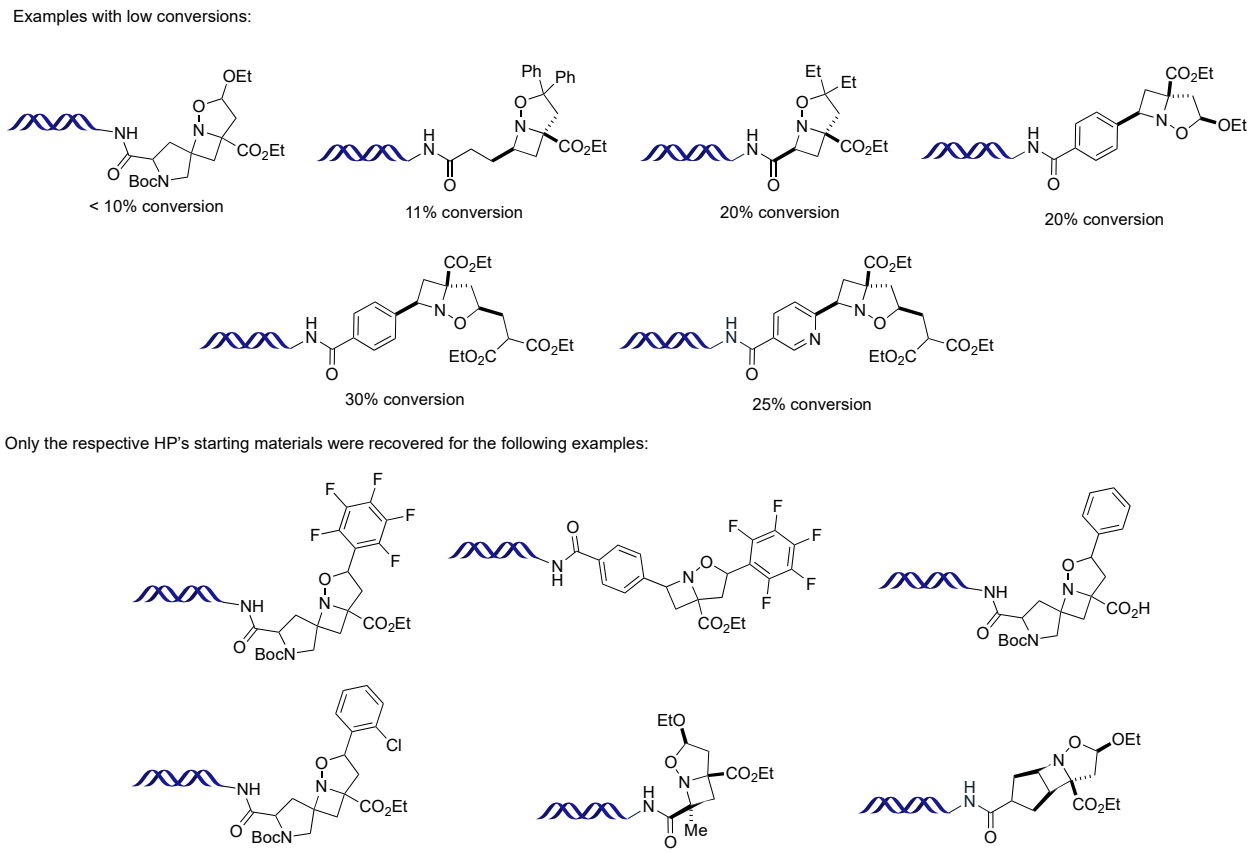


Figure S4. Unsuccessful examples during the scope evaluation of the [2+2] photocycloaddition reaction.

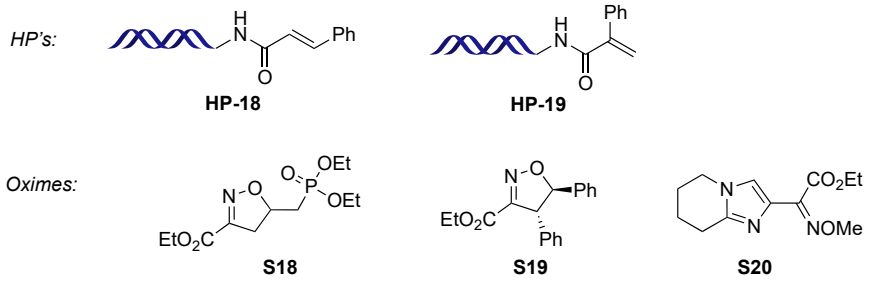
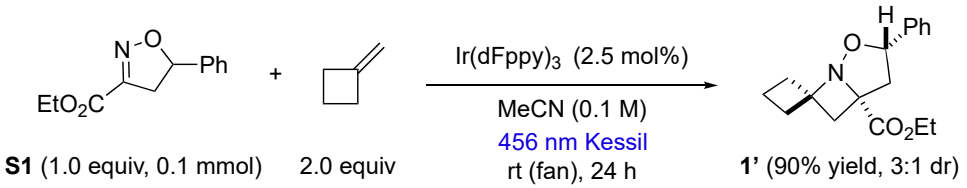


Figure S5. Unreactive headpieces (HP's) and 2-isoxazoline-3-carboxylates.

8 Off-DNA Reaction

The dr values for a range of alkenes off-DNA were reported to be from 1.2:1 to >20:1, depending on the structure of the substrate.[2] as additional proof of concept, the off-dna reaction of methylenecyclobutane and ethyl 5-phenyl-4,5-dihydroisoxazole-3-carboxylate was performed, and the corresponding cycloadduct **1'** was obtained in 90% yield and 3:1 dr.

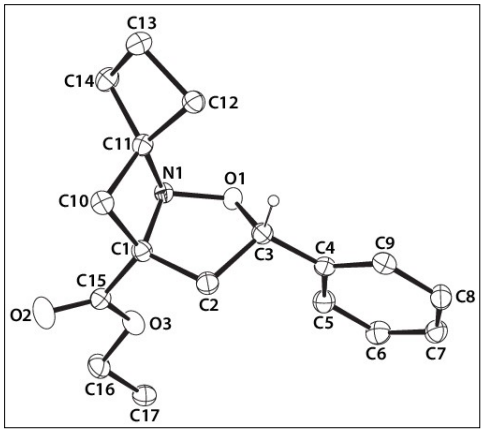


S1 (1.0 equiv, 0.1 mmol) 2.0 equiv **1'** (90% yield, 3:1 dr)

A 1-dram vial was charged with ethyl 5-phenyl-4,5-dihydroisoxazole-3-carboxylate **S1** (0.219 g, 0.10 mmol, 1.0 equiv) and $Ir(dFppy)_3$ (0.002 g, 0.0025 mmol, 2.5 mol %). The vial was subsequently capped, and reaction mixture was degassed with argon gas (3x). Next, anhyd CH_3CN (0.1 M, 1.0 mL), and methylenecyclobutane (0.136 mg, 0.20 mmol, 2.0 equiv) were added. The sealed vial was irradiated by one 456 nm Kessil H150-Blue lamp (19 V DC 40 W Max) placed at a distance of 1.5 inches with a cooling fan to ensure reactions remained at or near rt for 24 h. The solvent was removed in vacuo, and the residue was purified by SiO_2 column chromatography (EtOAc/hexanes) to give the corresponding product **1'** as mixture of diastereomers (3:1 dr; determined by crude ^1H NMR analysis) as a light-yellow solid (0.258 mg, 0.09 mmol, 90% yield).

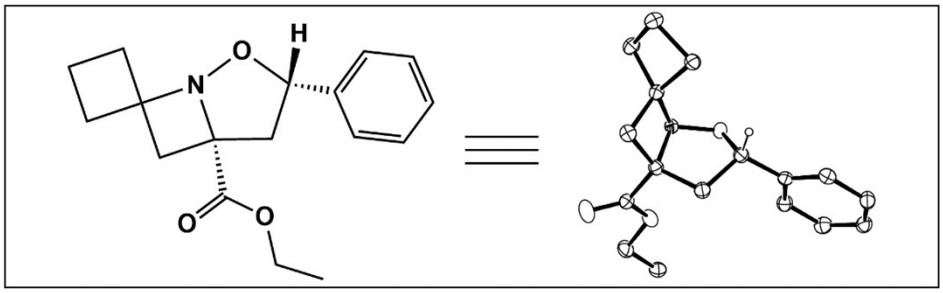
^1H NMR (400 MHz, $CDCl_3$) δ 7.44 – 7.41 (m, 2.3H), 7.38 – 7.28 (m, 3.5H), 5.25 (dd, J = 11.5, 4.8 Hz, 0.15H), 5.15 (dd, J = 9.9, 6.2 Hz, 1H), 4.32 – 4.17 (m, 2.3H), 2.90 – 2.78 (m, 0.34H), 2.88 (d, J = 12.4 Hz, 1H), 2.78 (dt, J = 11.5, 9.3 Hz, 1H), 2.49 – 2.48 (m, 1.2H), 2.51 – 2.49 (m, 1H), 2.44 (d, J = 12.5 Hz, 1H), 2.43 – 2.37 (m, 1H), 2.13 (ddt, J = 12.4, 8.4, 3.8 Hz, 1H), 1.99 (ddt, J = 12.2, 8.4, 4.1 Hz, 1H), 1.79 – 1.70 (m, 2.4H), 1.35 (t, J = 7.1 Hz, 1.25H), 1.31 (t, J = 7.1 Hz, 3H).
^{13}C NMR (101 MHz, $CDCl_3$) δ 173.1, 138.3, 128.6, 128.4, 127.0, 84.2, 72.4, 68.7, 61.7, 47.6, 39.4, 36.3, 30.9, 14.2, 13.7.
FT-IR (cm^{-1}, neat, ATR) $\tilde{\nu}$ = 2981, 2932, 1730, 1495, 1448, 1368, 1325, 1306, 1252, 1201, 1178, 1136, 1101, 1072, 1024, 899, 865, 760, 699, 581. **HRMS** (ESI) calcd for $C_{17}H_{22}NO_3$ $[M + H]^+$: 288.1594, found: 288.1600. **MP** (°C) 94.5 – 95.8.

The structure of **1'** was further confirmed by X-ray crystallography, see details in **Section 8**.



Note: Solvents used for recrystallization: ethyl acetate/DCM.

9 X-ray Structure Determination of Compound 1'



Compound 9262, $C_{17}H_{21}NO_3$, crystallizes in the orthorhombic space group $Pna2_1$ (systematic absences h0l: h=odd, 0kl: k+l=odd) with a=8.78560(10)Å, b=32.0930(3)Å, c=5.26760(10)Å, α=90°, β=90°, γ=90°, V=1485.23(4)Å^3, Z=4, and d_{calc}=1.285 g/cm^3. X-ray intensity data were collected on a Rigaku XtaLAB Synergy-S diffractometer [1] equipped with an HPC area detector (HyPix-6000HE) and employing confocal multilayer optic-monochromated Cu-Kα radiation (λ=1.54184 Å) at a temperature of 100K. Preliminary indexing was performed from a series of sixty 0.5° rotation frames with exposures of 0.25 seconds for θ = ±47.156° and 1 second for θ = 113.25°. A total of 3836 frames (41 runs) were collected employing ω scans with a crystal to detector distance of 34.0 mm, rotation widths of 0.5° and exposures of 1 second.

Rotation frames were integrated using CrysAlisPro [2], producing a listing of unaveraged F^2 and $\sigma(F^2)$ values. A total of 19650 reflections were measured over the ranges $5.508 \leq 2\theta \leq 148.876°$, $-10 \leq h \leq 10$, $-39 \leq k \leq 40$, $-6 \leq l \leq 6$ yielding 3023 unique reflections ($R_{int} = 0.0349$). The intensity data were corrected for Lorentz and polarization effects and for absorption using SCALE3 ABSPACK [3] (minimum and maximum transmission 0.47344, 1.00000). The structure was solved by dual methods - SHELXT [4]. Refinement was by full-matrix least squares based on F^2 using SHELXL [5]. All reflections were used during refinement. The weighting scheme used was $w=1/[\sigma^2(F_o^2) + (0.0425P)^2 + 0.3159P]$ where $P = (F_o^2 + 2F_c^2)/3$. Non-hydrogen atoms were refined anisotropically and hydrogen atoms were refined using a riding model. Refinement converged to R1=0.0302 and wR2=0.0755 for 2942 observed reflections for which $F > 4\sigma(F)$ and R1=0.0316 and wR2=0.0771 and GOF =1.050 for all 3023 unique, non-zero reflections and 191 variables. The maximum Δ/σ in the final cycle of least squares was 0.001 and the two most prominent peaks in the final difference Fourier were +0.18 and -0.18 e/Å^3.

Table S2. lists cell information, data collection parameters, and refinement data. Final positional and equivalent isotropic thermal parameters are given in Tables 2. and 3. Anisotropic thermal parameters are in Table 4. Tables 5. and 6. list bond distances and bond angles. Figure 1. is an ORTEP representation of the molecule with 50% probability thermal ellipsoids displayed.

Figure S6. ORTEP drawing of the title compound with 50% thermal ellipsoids.

Table S2. Summary of Structure Determination of Compound 9262

Empirical formula	$C_{17}H_{21}NO_3$
Formula weight	287.35
Diffractometer	Rigaku XtaLAB Synergy-S (HyPix-6000HE)
Temperature/K	100
Crystal system	orthorhombic
Space group	$Pna2_1$
a	8.78560(10)Å
b	32.0930(3)Å
c	5.26760(10)Å
α	90°
β	90°
γ	90°
Volume	1485.23(4)$Å^3$
Z	4
d_{calc}	1.285 g/cm^3
μ	0.708 mm^{-1}
F(000)	616.0
Crystal size, mm	0.47 × 0.04 × 0.04
2θ range for data collection	5.508 - 148.876°
Index ranges	-10 ≤ h ≤ 10, -39 ≤ k ≤ 40, -6 ≤ l ≤ 6
Reflections collected	19650
Independent reflections	3023[R(int) = 0.0349]
Data/restraints/parameters	3023/1/191
Goodness-of-fit on F^2	1.050
Final R indexes [I>=2σ (I)]	R_1 = 0.0302, wR_2 = 0.0755
Final R indexes [all data]	R_1 = 0.0316, wR_2 = 0.0771
Largest diff. peak/hole	0.18/-0.18 $eÅ^{-3}$
Flack parameter	-0.08(10)

Table S3. Refined Positional Parameters for Compound 9262

Atom	*x*	*y*	*z*	U(eq)
O1	0.63131(14)	0.60099(4)	0.4746(2)	0.0192(3)
O2	0.55622(17)	0.73682(4)	0.6668(3)	0.0349(4)
O3	0.43846(15)	0.69200(4)	0.4085(3)	0.0256(3)
N1	0.70560(16)	0.63996(4)	0.5240(3)	0.0178(3)
C1	0.6086(2)	0.66358(5)	0.7113(3)	0.0187(4)
C2	0.4925(2)	0.63175(5)	0.8121(4)	0.0216(4)
C3	0.5443(2)	0.58982(5)	0.7005(3)	0.0191(4)
C4	0.4150(2)	0.56156(5)	0.6240(3)	0.0192(4)
C5	0.3208(2)	0.57109(6)	0.4191(3)	0.0232(4)
C6	0.1970(2)	0.54590(6)	0.3623(4)	0.0258(4)
C7	0.1663(2)	0.51094(6)	0.5087(4)	0.0247(4)
C8	0.2598(2)	0.50102(5)	0.7114(4)	0.0245(4)
C9	0.3847(2)	0.52619(6)	0.7676(4)	0.0220(4)
C10	0.7505(2)	0.67190(6)	0.8747(4)	0.0203(4)
C11	0.83191(19)	0.63820(5)	0.7178(3)	0.0186(4)
C12	0.8820(2)	0.59781(5)	0.8542(4)	0.0208(4)
C13	1.0458(2)	0.61539(6)	0.8528(4)	0.0236(4)
C14	0.9980(2)	0.64465(6)	0.6332(4)	0.0226(4)
C15	0.5335(2)	0.70183(5)	0.5957(3)	0.0202(4)
C16	0.3608(2)	0.72704(6)	0.2881(4)	0.0288(4)
C17	0.2366(2)	0.71001(6)	0.1248(4)	0.0255(4)

Table S4. Positional Parameters for Hydrogens in Compound 9262.

Atom	*x*	*y*	*z*	U(eq)
H2A	0.493792	0.630918	0.999989	0.026
H2B	0.388381	0.638775	0.754421	0.026
H3	0.611826	0.575151	0.824454	0.023
H5	0.341408	0.594929	0.317838	0.028
H6	0.133005	0.552614	0.222723	0.031
H7	0.081166	0.49383	0.469792	0.03
H8	0.238905	0.47713	0.811984	0.029
H9	0.449581	0.519102	0.905165	0.026
H10A	0.793588	0.700195	0.854117	0.024
H10B	0.73767	0.664715	1.056218	0.024
H12A	0.838135	0.594361	1.02598	0.025
H12B	0.868987	0.572241	0.751327	0.025
H13A	1.124318	0.59471	0.804944	0.028
H13B	1.074119	0.630192	1.010759	0.028
H14A	1.034825	0.673639	0.651758	0.027
H14B	1.020838	0.633546	0.461991	0.027
H16A	0.433513	0.743215	0.183716	0.035
H16B	0.317641	0.745747	0.419053	0.035

H17A	0.279682	0.690281	0.002295	0.038
H17B	0.18694	0.732891	0.033577	0.038
H17C	0.161593	0.695669	0.231271	0.038

Table S5. Refined Thermal Parameters (U's) for Compound 9262

Atom	U_{11}	U_{22}	U_{33}	U_{23}	U_{13}	U_{12}
O1	0.0206(6)	0.0182(6)	0.0187(6)	-0.0023(5)	0.0029(5)	-0.0032(5)
O2	0.0423(9)	0.0188(6)	0.0438(9)	-0.0027(6)	-0.0199(7)	0.0007(6)
O3	0.0280(7)	0.0197(6)	0.0291(8)	-0.0023(6)	-0.0101(6)	0.0039(5)
N1	0.0181(7)	0.0160(6)	0.0192(7)	-0.0015(6)	0.0002(6)	-0.0008(5)
C1	0.0199(8)	0.0180(8)	0.0180(8)	-0.0008(7)	0.0006(7)	0.0004(6)
C2	0.0224(9)	0.0205(8)	0.0220(9)	-0.0013(7)	0.0058(7)	-0.0009(7)
C3	0.0195(8)	0.0191(8)	0.0185(8)	0.0012(7)	0.0025(7)	-0.0001(6)
C4	0.0176(8)	0.0187(8)	0.0215(9)	-0.0033(7)	0.0036(7)	0.0011(7)
C5	0.0241(9)	0.0228(8)	0.0226(10)	0.0028(7)	0.0012(7)	-0.0007(7)
C6	0.0239(9)	0.0297(9)	0.0238(9)	-0.0026(8)	-0.0028(8)	-0.0003(7)
C7	0.0225(9)	0.0237(9)	0.0277(10)	-0.0064(8)	0.0021(8)	-0.0031(7)
C8	0.0266(9)	0.0190(8)	0.0280(9)	0.0003(8)	0.0025(8)	-0.0026(7)
C9	0.0221(8)	0.0215(8)	0.0225(8)	0.0004(7)	-0.0002(8)	0.0034(7)
C10	0.0227(9)	0.0207(8)	0.0175(8)	0.0000(7)	-0.0013(7)	0.0003(6)
C11	0.0185(8)	0.0185(8)	0.0187(9)	0.0010(7)	-0.0005(7)	-0.0015(6)
C12	0.0202(9)	0.0198(8)	0.0224(8)	0.0020(7)	0.0002(7)	0.0007(7)
C13	0.0188(9)	0.0278(9)	0.0243(9)	0.0014(8)	0.0003(7)	-0.0005(7)
C14	0.0210(9)	0.0258(9)	0.0209(9)	0.0015(7)	0.0013(7)	-0.0034(7)
C15	0.0182(8)	0.0203(9)	0.0219(9)	-0.0011(7)	-0.0005(7)	-0.0011(6)
C16	0.0276(10)	0.0205(8)	0.0383(11)	0.0027(8)	-0.0085(9)	0.0029(7)
C17	0.0224(9)	0.0271(9)	0.0270(9)	0.0012(8)	-0.0019(8)	0.0031(7)

Table S6. Bond Distances in Compound 9262, Å

O1-N1	1.4345(18)	O1-C3	1.459(2)	O2-C15	1.200(2)
O3-C15	1.330(2)	O3-C16	1.460(2)	N1-C1	1.508(2)
N1-C11	1.509(2)	C1-C2	1.538(2)	C1-C10	1.538(2)
C1-C15	1.521(2)	C2-C3	1.537(2)	C3-C4	1.508(2)
C4-C5	1.395(3)	C4-C9	1.390(2)	C5-C6	1.388(3)
C6-C7	1.388(3)	C7-C8	1.384(3)	C8-C9	1.394(3)
C10-C11	1.538(2)	C11-C12	1.546(2)	C11-C14	1.540(2)
C12-C13	1.545(2)	C13-C14	1.548(3)	C16-C17	1.493(3)

Table S7. Bond Angles in Compound 9262, °

N1-O1-C3	107.74(12)	C15-O3-C16	115.62(14)	O1-N1-C1	107.44(12)
O1-N1-C11	115.13(12)	C1-N1-C11	89.54(12)	N1-C1-C2	105.47(13)
N1-C1-C10	89.74(13)	N1-C1-C15	112.90(14)	C10-C1-C2	117.35(15)
C15-C1-C2	112.73(15)	C15-C1-C10	115.82(15)	C3-C2-C1	104.64(14)
O1-C3-C2	104.61(13)	O1-C3-C4	108.95(15)	C4-C3-C2	113.91(15)
C5-C4-C3	121.47(16)	C9-C4-C3	119.38(16)	C9-C4-C5	119.09(17)
C6-C5-C4	120.30(17)	C5-C6-C7	120.23(18)	C8-C7-C6	119.94(17)
C7-C8-C9	119.84(17)	C4-C9-C8	120.59(18)	C11-C10-C1	87.38(13)
N1-C11-C10	89.71(13)	N1-C11-C12	123.73(14)	N1-C11-C14	119.74(15)
C10-C11-C12	118.19(15)	C10-C11-C14	120.14(15)	C14-C11-C12	88.70(13)
C13-C12-C11	87.53(13)	C12-C13-C14	88.42(14)	C11-C14-C13	87.66(13)
O2-C15-O3	123.91(17)	O2-C15-C1	123.89(17)	O3-C15-C1	112.19(15)
O3-C16-C17	108.04(15)				

This report has been created with Olex2 [6], compiled on 2022.04.07 svn.rca3783a0 for OlexSys.

10 DNA Damage Assessment

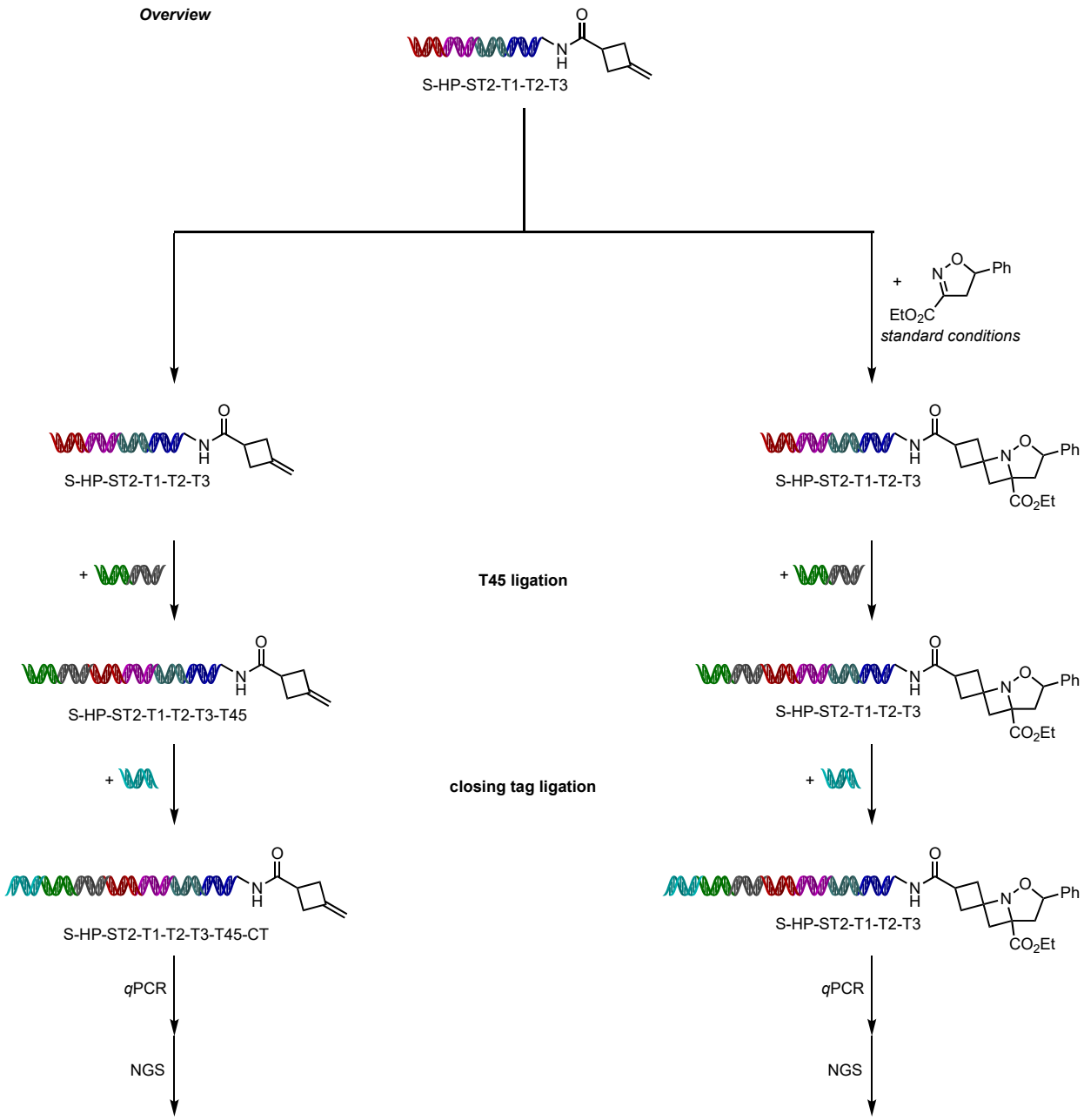


Figure S7. Overview of DNA damage evaluation workflow.

Preparation of 3-exo-methylene cyclobutamide capped cycle 2 DNA tag

Cycle 3 tagged DNA material was defrosted, and 100 µL (500 nmol) was transferred to a 1.5 mL PCR tube. To the 100 µL of DNA was added 400 µL of sodium borate buffer (pH 9.4). To the reaction solution was added 100 µL of DMTMM (40 equiv, 20000 nmol, 200 mM in H_2O), followed by 100 µL of methylene cyclobutene carboxylic acid (40 equiv, 20000 nmol, 200 mM in

DMA). The reaction was equipped with a PTFE coated stir bar and allowed to stir at rt overnight. At the conclusion of the reaction, a 1 μL aliquot was diluted to 40 μL and used for LCMS analysis. The bulk solution was then subjected to an ethanol precipitation. The DNA material was then resuspended in ddH_2O, frozen in a dry ice/acetone bath, and lyophilized to provide a colorless solid.

Synthesis of cycle 3 DNA coupled aza-[2+2] product

To a PCR Eppendorf tube was added $Ir(dFppy)_3$ (5 μL of a 2 nmol/μL soln in DMSO, 10 nmol, 1.0 equiv), 2-isoxazoline-3-carboxylate (10 μL of a 100 nmol/μL soln in DMSO, 1000 nmol, 100 equiv), glycerol solution (10 μL of a soln 20% glycerol in DMSO), and cycle 3 DNA tethered methylene cyclobutenamide (5 μL of a 2 nmol/μL soln in H_2O, 10 nmol, 1.0 equiv). The reaction mixture was vortexed and placed at 1.5 inches from the irradiation source (Kessil PR160-456 nm blue lamp) for 10 min. Reactions were carried out on a 10 nmol scale, 10 reactions were run side-by-side, then combined to provide the 100 nmol sample that was used for DNA damage evaluation. At the conclusion of the reaction, the 10 mmol reaction solutions were combined and a solution of 5 M NaCl in ddH_2O (30 μL) was added, followed by the addition of cold EtOH (750 μL). The mixture was left at -20 °C for 1 h and then centrifuged at 4 ºC and 6,000 rpm for 30 min. The supernatant was discarded, and the DNA pellet was re-dissolved in H_2O (100 μL), and analyzed via LC/MS.

Reactions 567 A – aza-[2+2] reaction sample – Reactions were run as outlined above with no alterations

Reactions 567 B – No photocatalyst control – Reactions were run as outlined above, except no photocatalyst was added

Reactions 567 C – Dark reaction (no light control) – Reaction was wrapped in aluminum foil to protect from light, then run as outlined above

Reactions 567 D – True blank (null) – Sample was taken from substrate preparation and lyophilized

Ligations

Cycle 4 and 5 (T45) ligation

General procedure for T45 ligation: To each tube of 567A-D (12.5 nmol, 1 equiv, 1 mM in $_{dd}H_2O$) was added T45 (8.75 nmol, 0.7 equiv, 1 mM in $_{dd}H_2O$), 10X T4 DNA ligase buffer (5 µL), $_{dd}H_2O$ (39.22 µL) and T4 DNA ligase (1 µL, 30 U/ µL). The tubes were vortexed, centrifuged and stood at 16°C for 4 h. After that the samples were subjected to ethanol precipitation and spin filtration with 30K membrane. The ligation efficiency was detected by gel electrophoresis and LC-MS analysis.

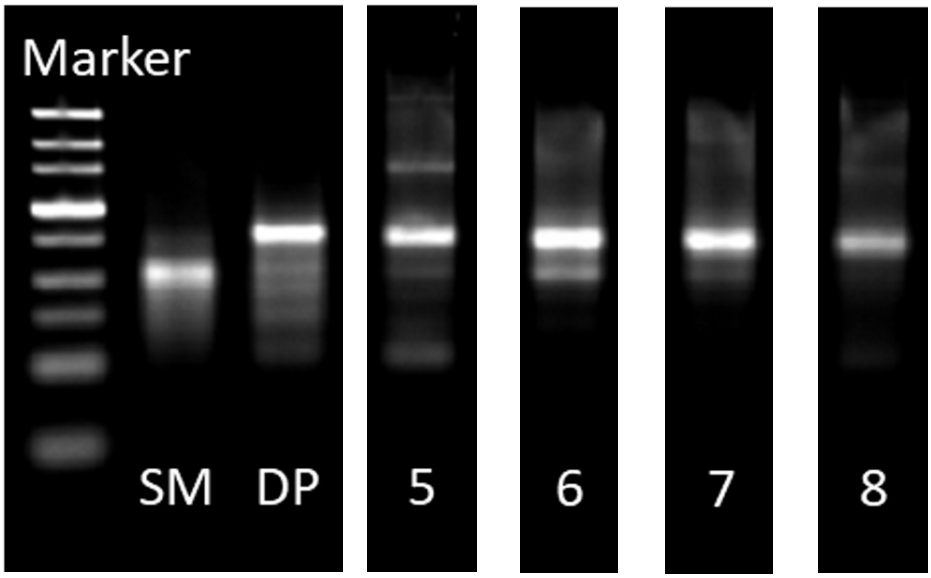


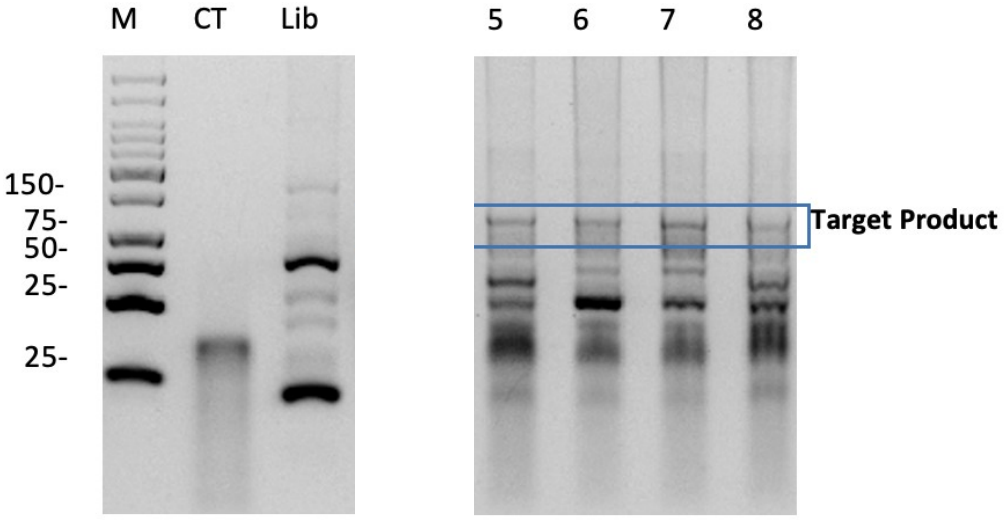
Figure S8. Gel electrophoresis of T45 ligation reaction. SM = starting material. DP = desired product. Lane 5 = sample 567A; lane 6 = sample 567B; lane 7 = sample 567C; lane 8 = sample 567D.

Table S7. LCMS yields of T45 ligations reactions

Entry	Sample	Yield (%)
5	567 A	94%
6	567 B	90%
7	567 C	96%
8	567 D	93%

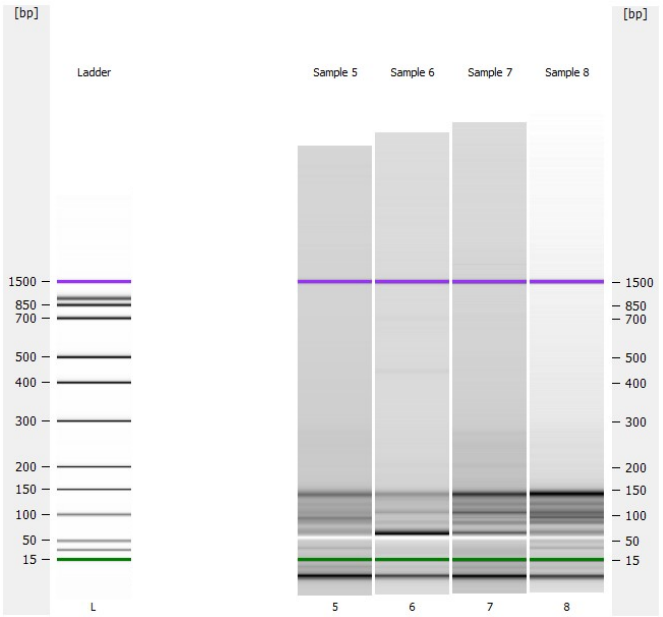
Closing Tag Ligations

General Procedure: To each tube of 567A-D (1 nmol, 1 mM in $_{dd}H_2O$) was added closing tag (1.5 to 4.16 equiv, 1 mM in ddH_2O), 10X T4 DNA ligase buffer (2 µL), $_{dd}H_2O$ (added to adjust total volume to 20 µL) and T4 DNA ligase (0.4 µL, 30 U/ µL). The tubes were vortexed, centrifuged, and allowed to remain at 16 °C for 16 h. After that the samples were subjected to ethanol precipitation and gel electrophoresis. Different closing tag equivalents were caused by different library tag residue. Detailed closing tag equivalents: 567A – 2.83 equiv; 567B – 1.50 equiv; 567C – 1.50 equiv; 567D – 2.30 equiv.



Entry	Sample
5	567 A
6	567 B
7	567 C
8	567 D

Figure S9. Gel electrophoresis of closing tag ligations. Lane 5 – 567A; Lane 6 – 567B; Lane 8 – 567C; Lane 9 -567D.



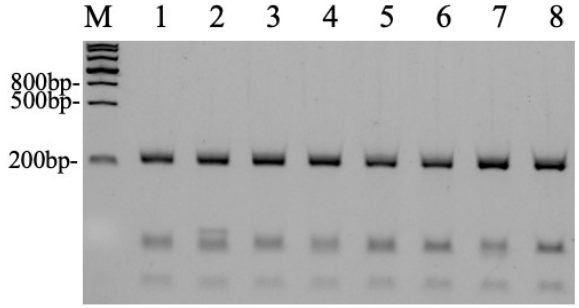
Entry	Sample
5	567 A
6	567 B
7	567 C
8	567 D

qPCR and Next Generation Sequencing (NGS)

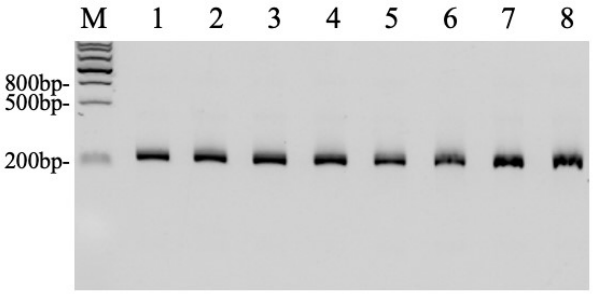
qPCR

qPCR was performed with the SYBR Green Master Mix kit (Thermo) on a Real-Time PCR System (QuantStudio 7 Flex). All samples were subjected to PCR cycles as follows: 50 °C incubation for 2 min, then 95 °C heat activation for 5 min followed by 40 cycles of 95 °C denaturation (10 seconds each), 55 °C annealing (15 seconds each), and 72 °C extension (30 seconds each). To assess the amplification efficiency, the quantity of the full-length DNA templates was first normalized based on the Bioanalyzer result and qPCR with serial dilutions was performed. Linear fitting was then calculated based on the CT values. The quantitative results and the slopes were compared between the eight samples. The quantitative result dictated the complementary situation on forward primer region, while the slope dictated the amplification efficacy for different samples.

Condition	Quantitive Conc. (1 ng/µL)	Quantitive Conc. (0.1 ng/µL)	Quantitive Conc. (0.01 ng/µL)	Quantitive Conc. (0.001 ng/µL)	Quantitive Conc. (0.0001 ng/µL)	qPCR System Amplification Efficiency
567A	2.96E+08	3.37E+07	1.59E+06	7.40E+04	4.14E+03	74%
567B	2.46E+08	2.58E+07	1.80E+06	6.75E+04	5.48E+03	74%
567C	3.32E+08	3.41E+07	1.00E+06	6.69E+04	4.86E+03	74%
567D	2.47E+08	3.14E+07	1.32E+06	4.44E+04	6.82E+03	74%



PCR Products



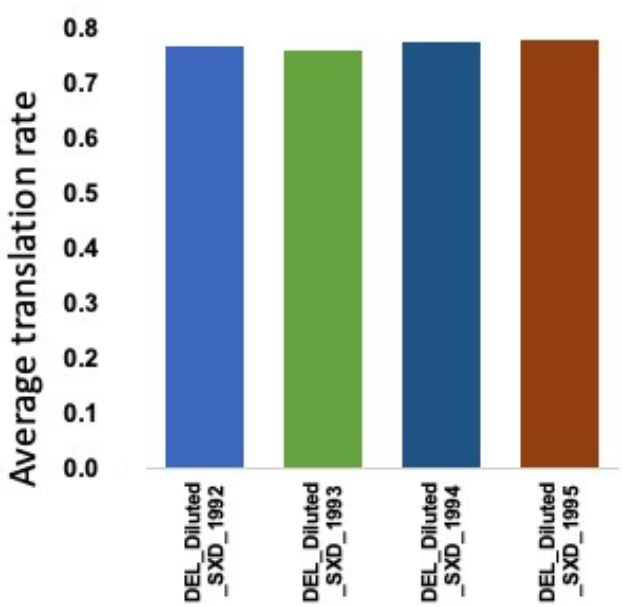
Purified PCR Products

Next Generation Sequencing

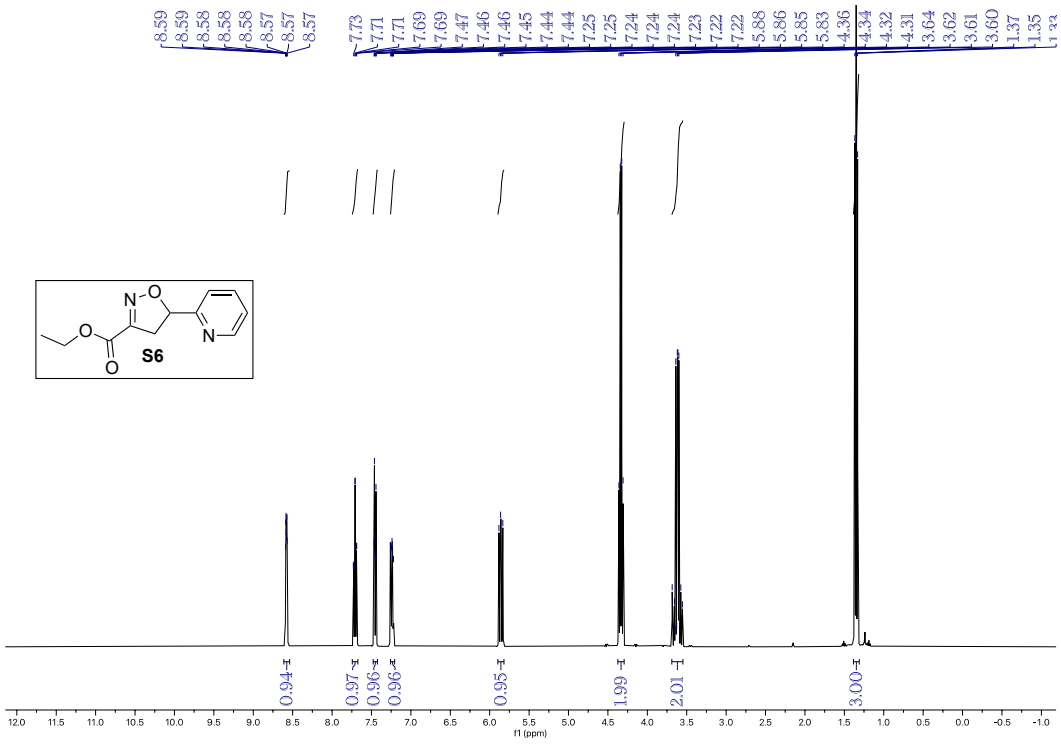
Samples were diluted to 1E+7 copies/35 uL as a template for PCR amplification. To a PCR tube was added diluted sample (35.0 µL), 10x high fidelity PCR buffer (5.0 µL), 50.0 mM MgSO4 (2.0 µL), 10.0 mM dNTP mix (1.0 µL), Platinum Taq DNA Polymerase (0.2 µL), 10.0 µM forward primer (2.0 µL), 10.0 µM reverse primer (2.0 µL), and nuclease-free water (2.8 µL). The PCR products were purified by the Agencourt AMPure XP Beads method. The purified samples were sent for next-generation sequencing (Illumina NovaSeq). Bowtie2 was used to map the sequenced reads to reference by local alignment. The detailed mapping identity were extracted from CIGAR string and XM flag in the SAM format.

The percent of sequences that were a perfect match for each of the six samples was nearly identical and ranged from 75.8% for reaction sample 567 B to 77.7% for the control 567 D. When normalized to the control, reaction sample 567 A was a 98.5% perfect sequence match, indicating just 1% mutated sequences.

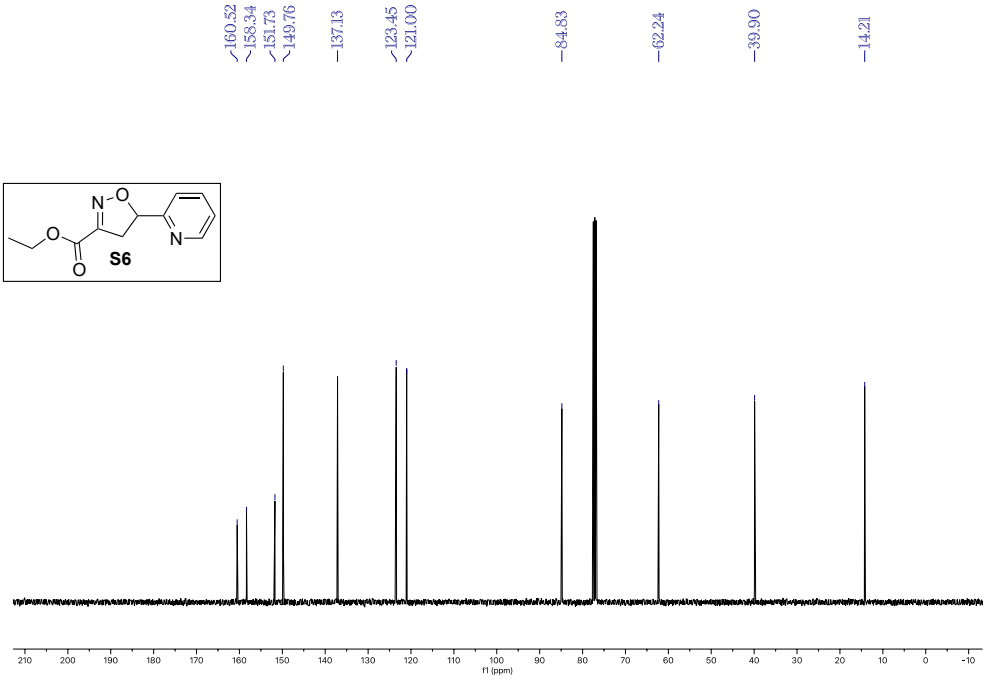
Note	Sample ID	total Reads	perfect match	1bp mismatch	perfect match ratio	identical	1bp mismatch
567a	DEL_Diluted_SXD_1992	65,681,311	50,285,777	3,711,648	76.6%	98.49%	5.65%
567b	DEL_Diluted_SXD_1993	54,526,018	41,309,513	3,213,487	75.8%	97.46%	5.89%
567c	DEL_Diluted_SXD_1994	66,749,047	51,730,923	3,907,841	77.5%	99.70%	5.85%
567d	DEL_Diluted_SXD_1995	61,422,689	47,745,104	3,632,625	77.7%	100.60%	5.91%



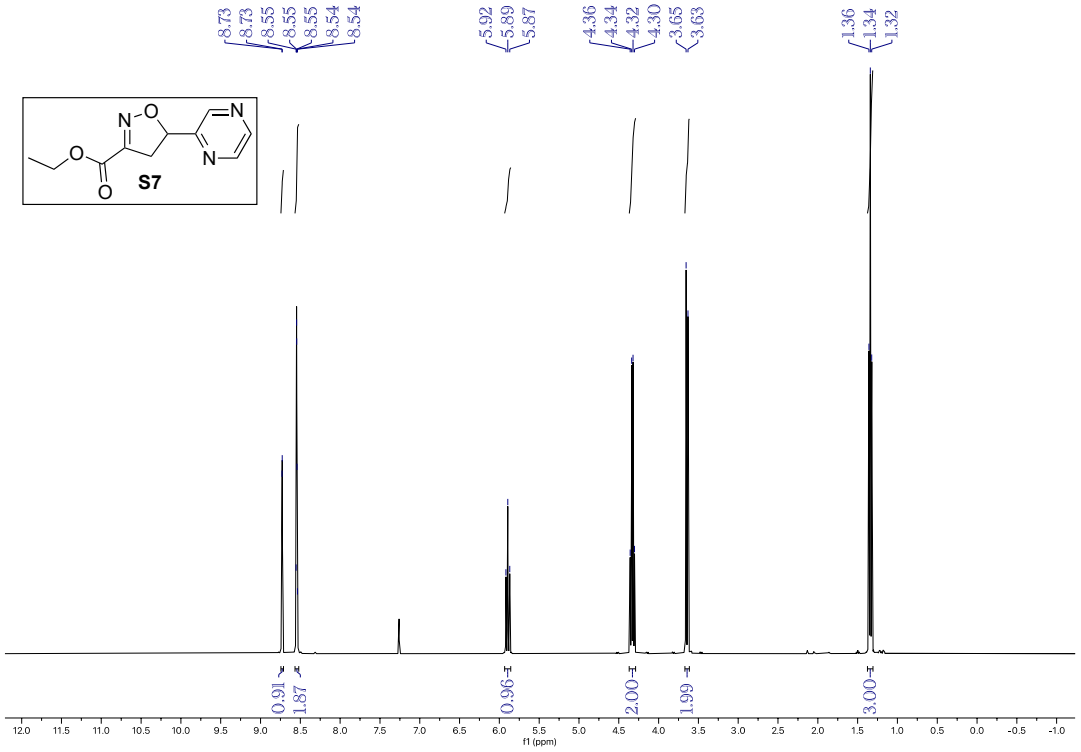
11 NMR Spectra: Small Molecules



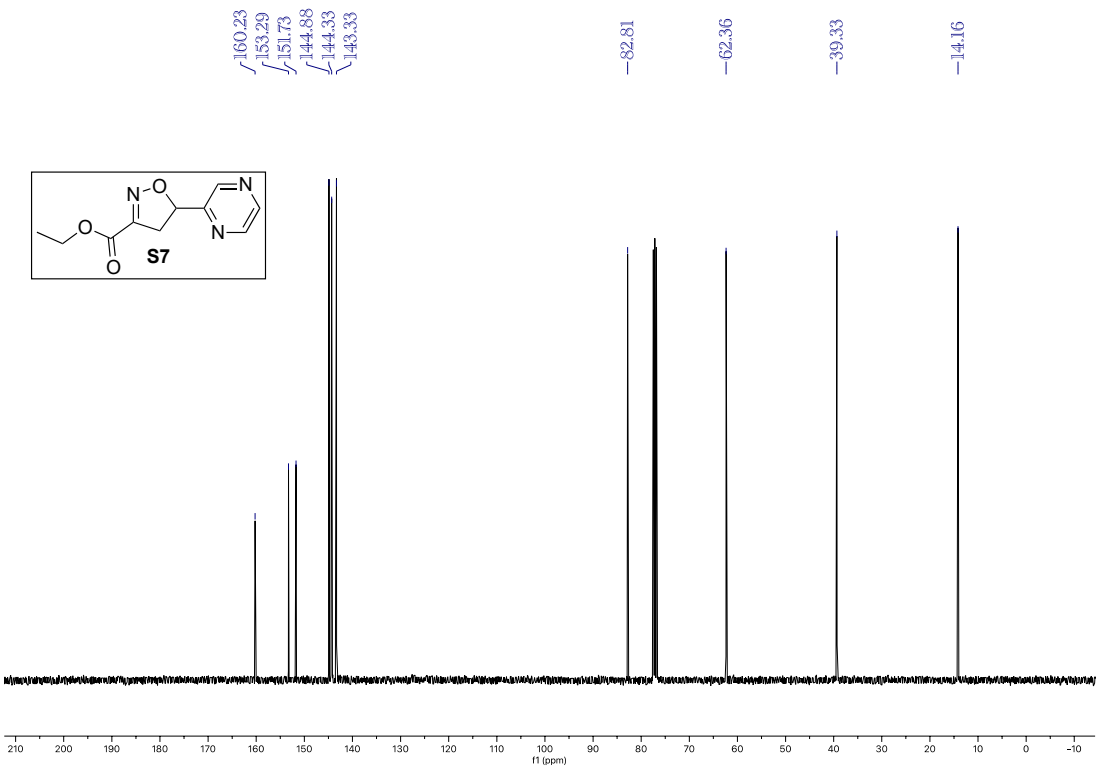
^{1}H NMR (400 MHz, $CDCl_3$) of compound **S6**.



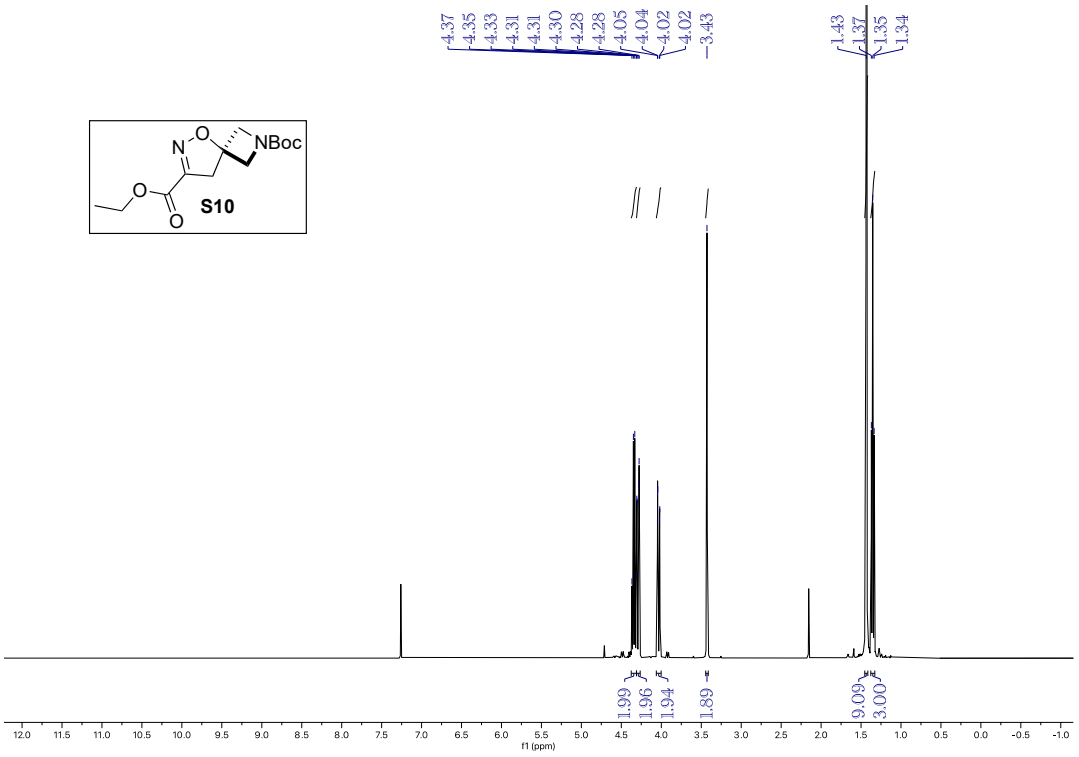
^{13}C NMR (101 MHz, $CDCl_3$) of compound **S6**.



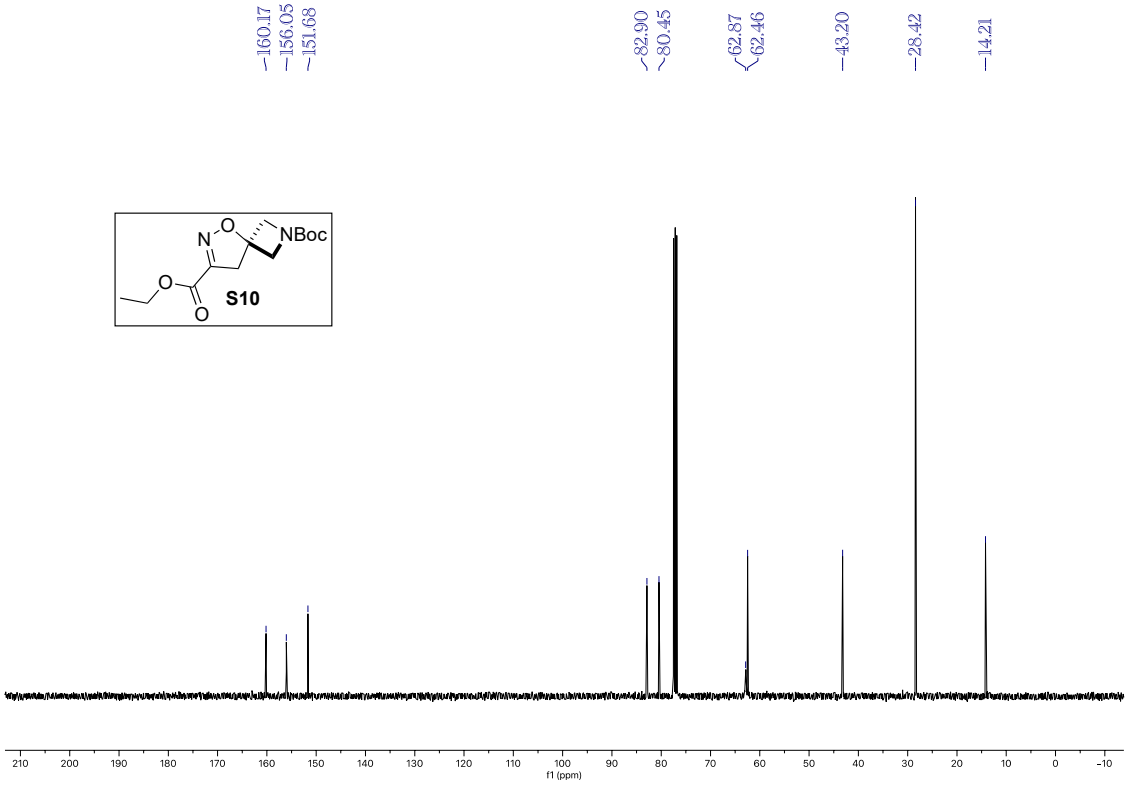
^{1}H NMR (400 MHz, $CDCl_3$) of compound **S7**.



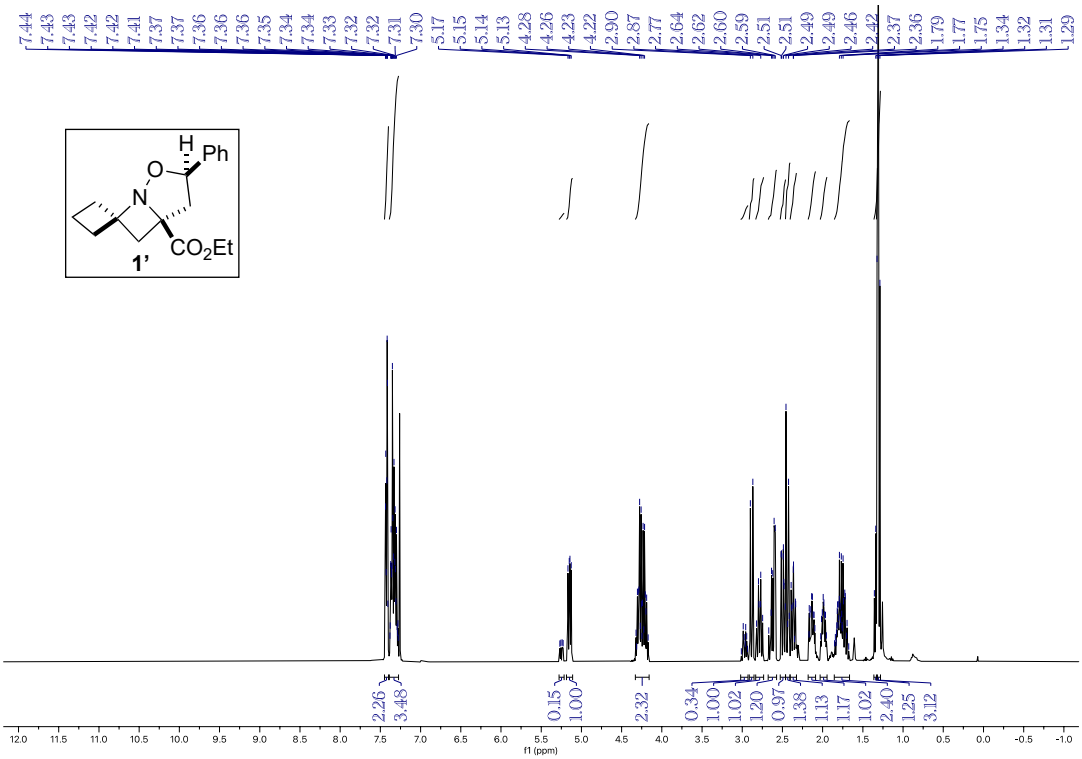
^{13}C NMR (101 MHz, $CDCl_3$) of compound **S7**.



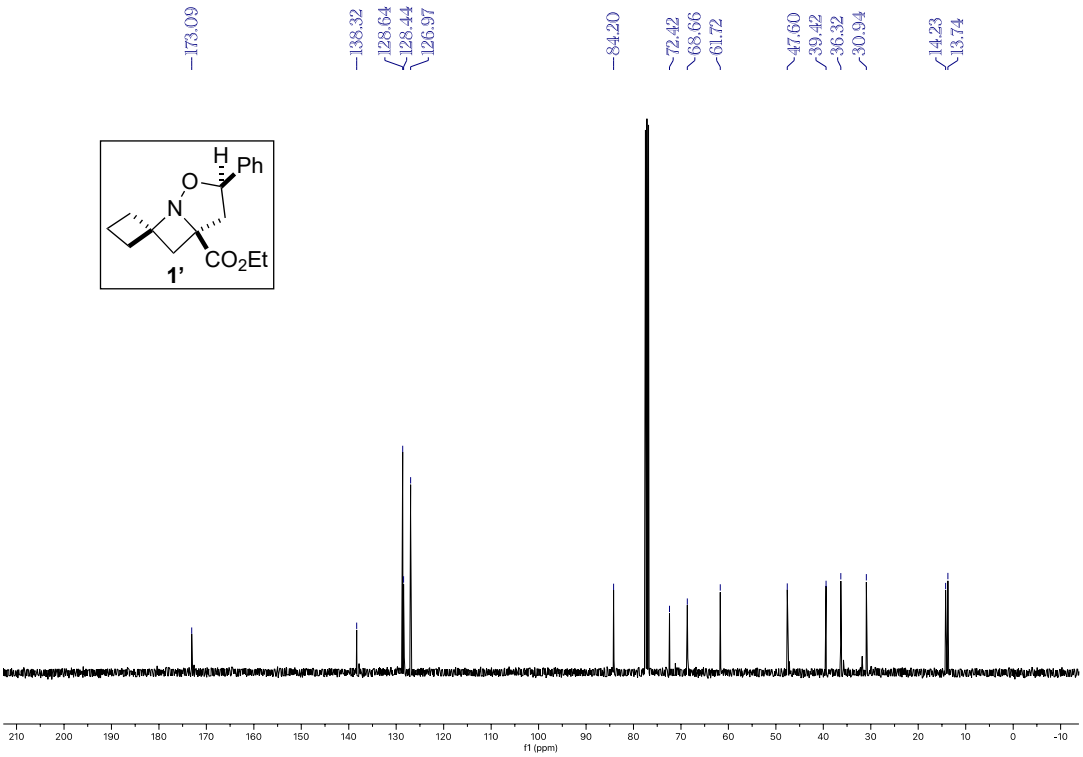
^{1}H NMR (400 MHz, $CDCl_3$) of compound **S10**.



^{13}C NMR (101 MHz, $CDCl_3$) of compound **S10**.



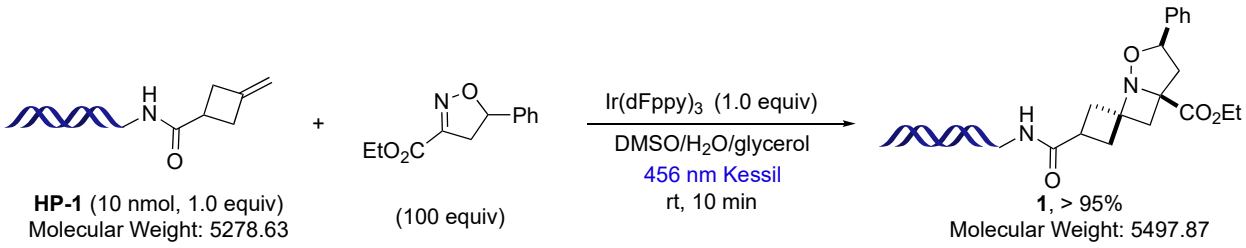
^{1}H NMR (400 MHz, $CDCl_3$) of compound **1'**.



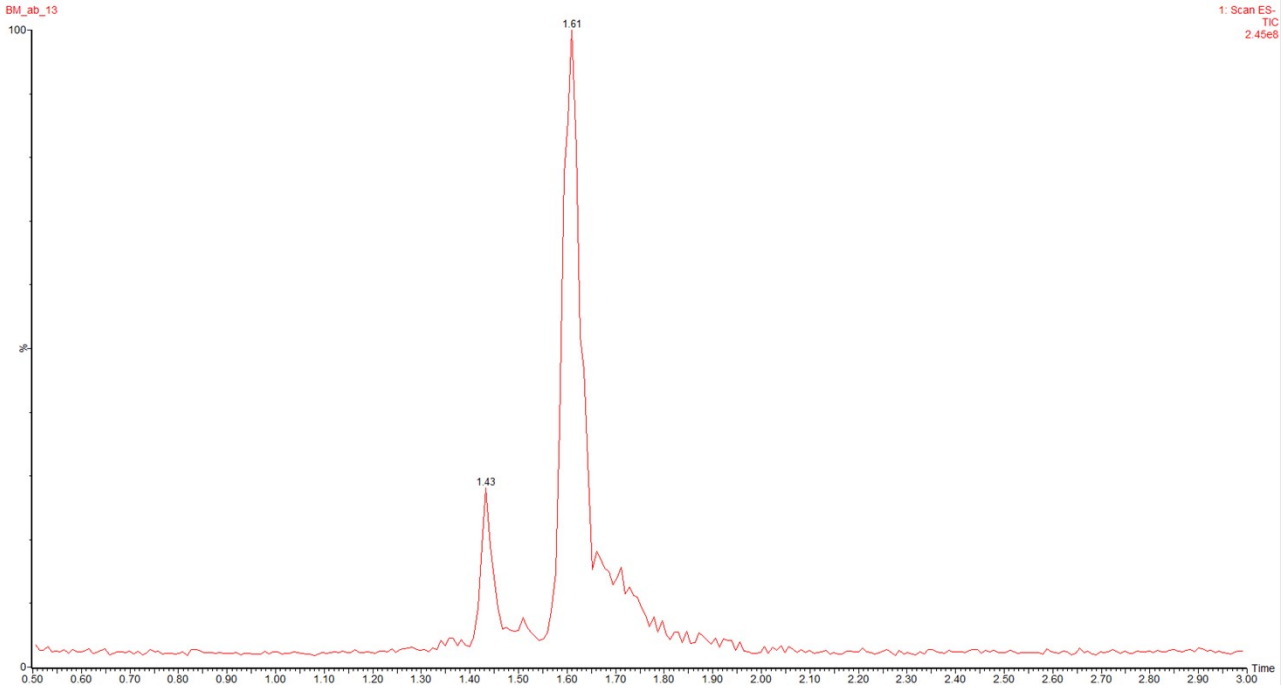
^{13}C NMR (101 MHz, $CDCl_3$) of compound **1'**.

12 UPLC/MS Spectra

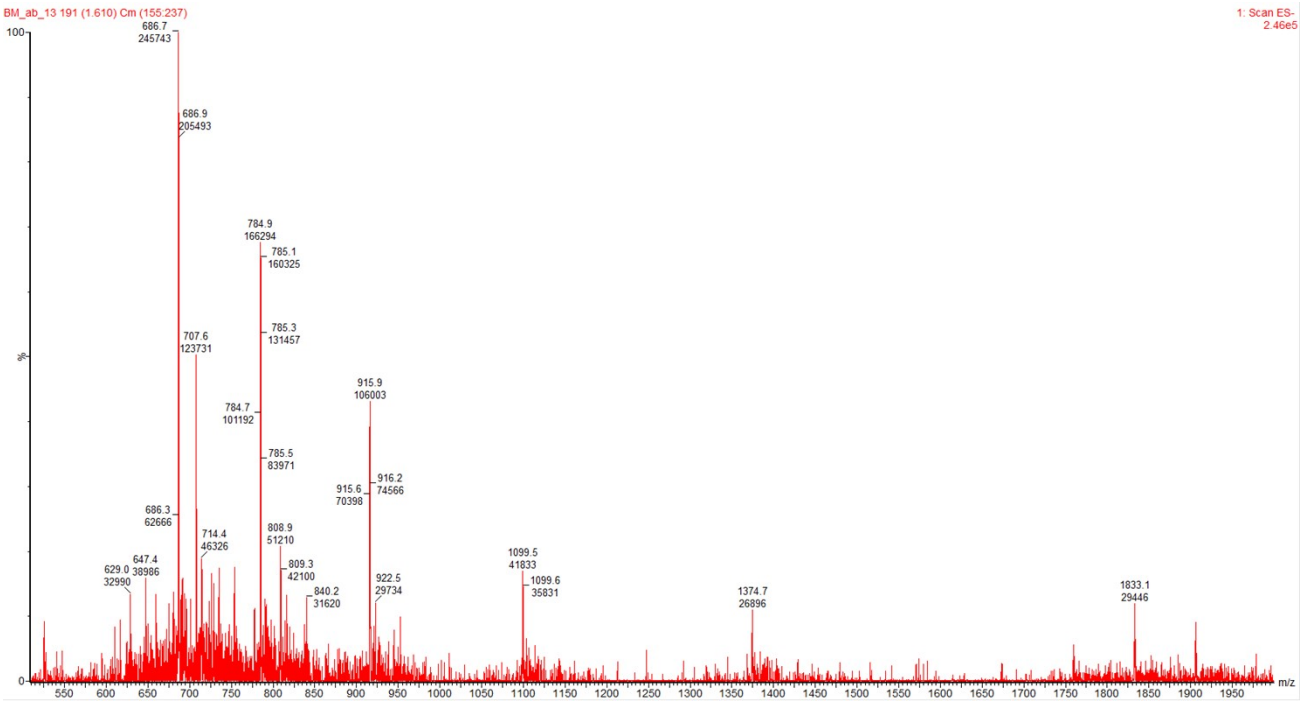
Data was scanned and deconvoluted between 4,500 to 6,500/7,500 Da, with a mass tolerance window of 2-6 Da, with 10% of base peak threshold set for reporting. Conversions are based on all product associated masses, since the analytical methods often results in HFIP (+168), Na (+23), K(+39), NH_4 (+18), Cu (+63), Ni (+58) adducts.



The title compound **1** was synthesized according to the **GP-IV** from ethyl 5-phenyl-4,5-dihydroisoxazole-3-carboxylate, and the DNA-tagged alkene **HP-1**. The product was formed with > 95% conversion.

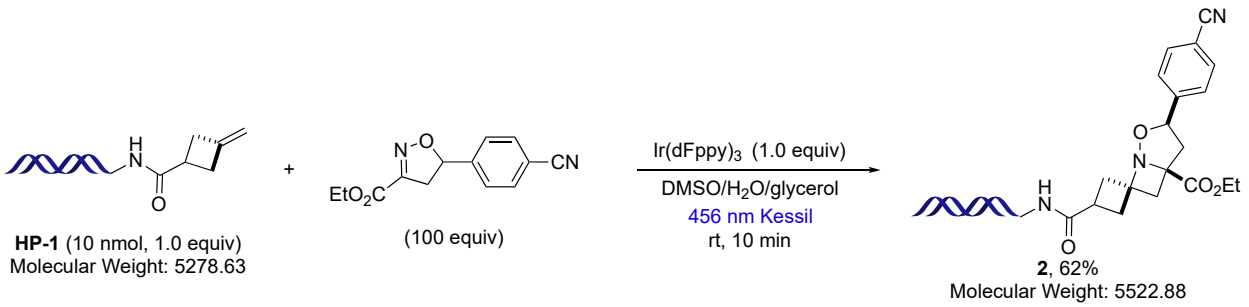


TIC of compound 1.

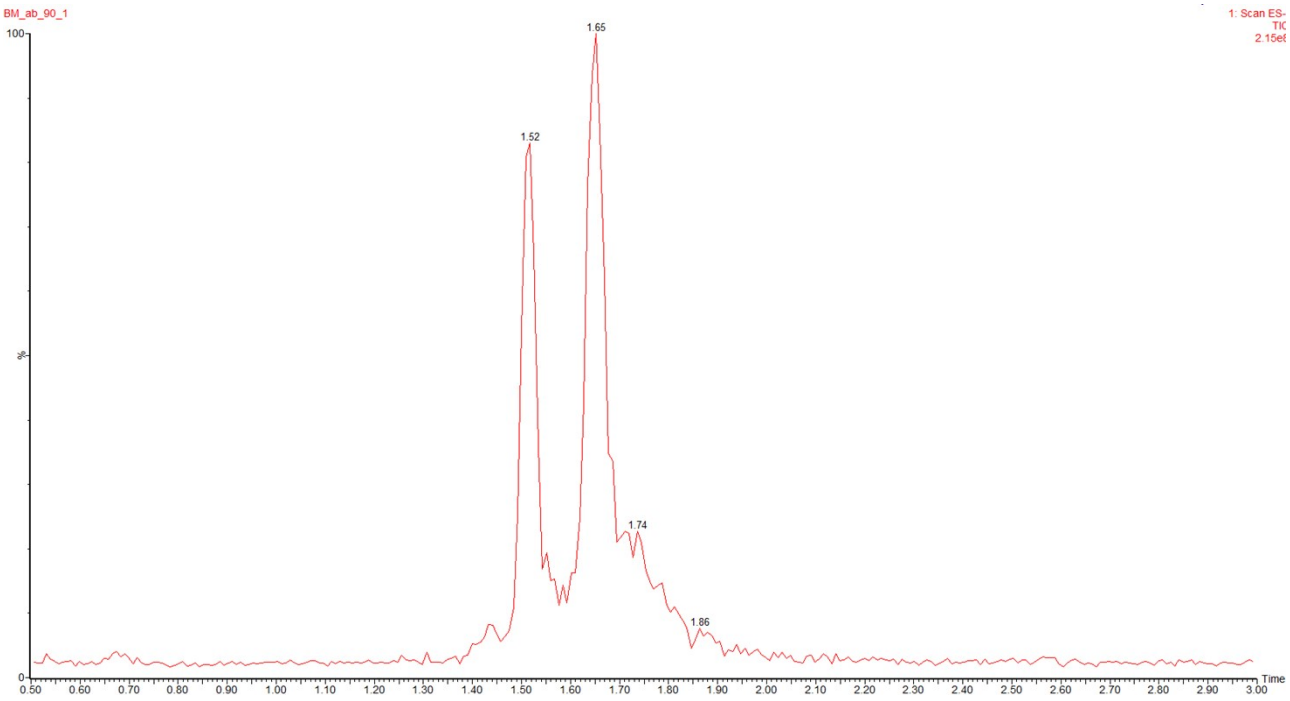


Raw MS of compound 1.

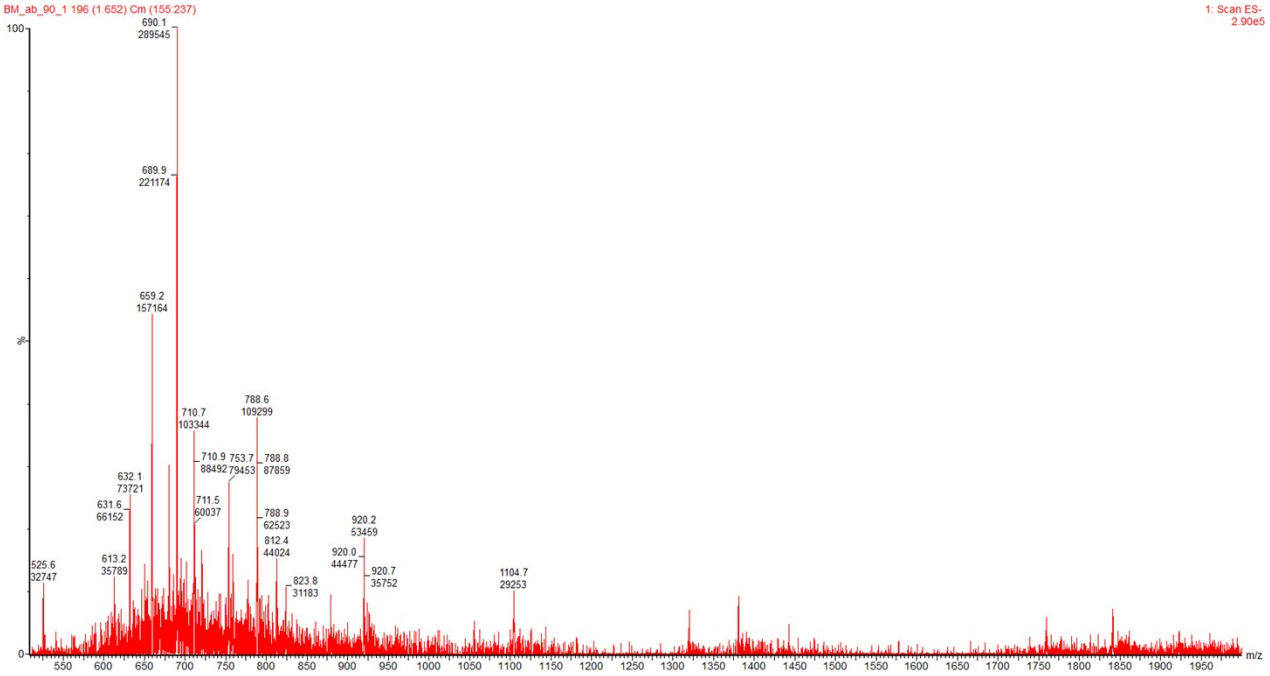
Deconvoluted MS of 1 (mass signals >10% of peak height).



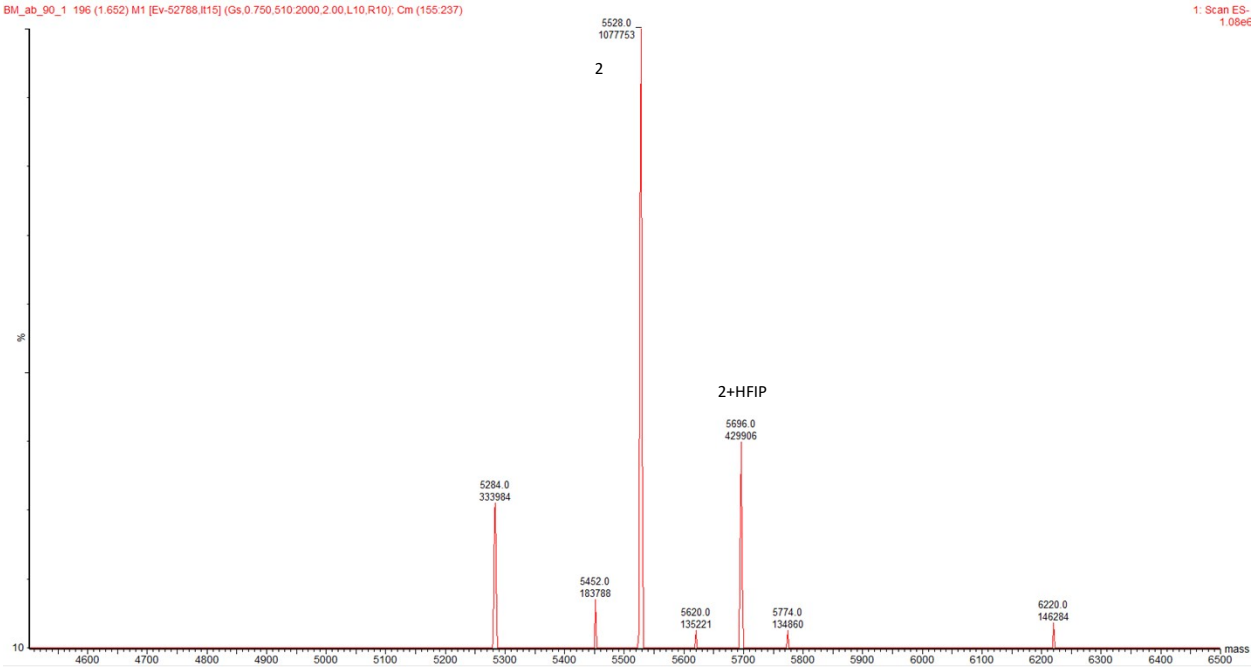
The title compound **2** was synthesized according to **GP-IV** from ethyl 5-(4-cyanophenyl)-4,5-dihydroisoxazole-3-carboxylate and the DNA-tagged alkene **HP-1**. The product was formed with 62% conversion (28% recovered **HP-1**).



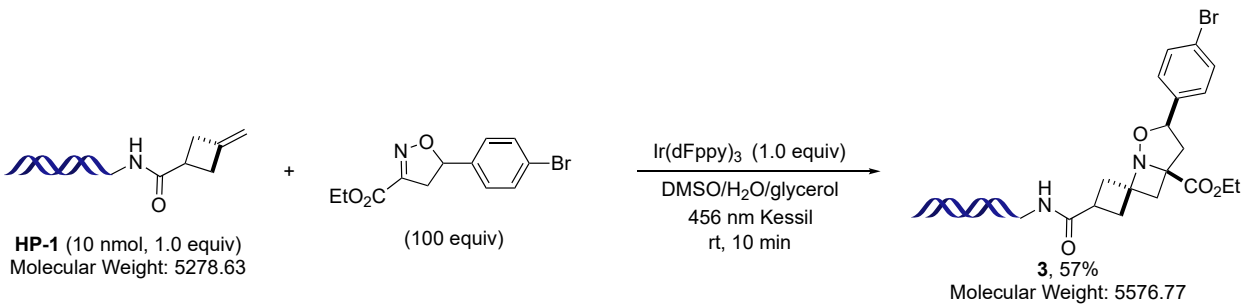
TIC of compound 2.



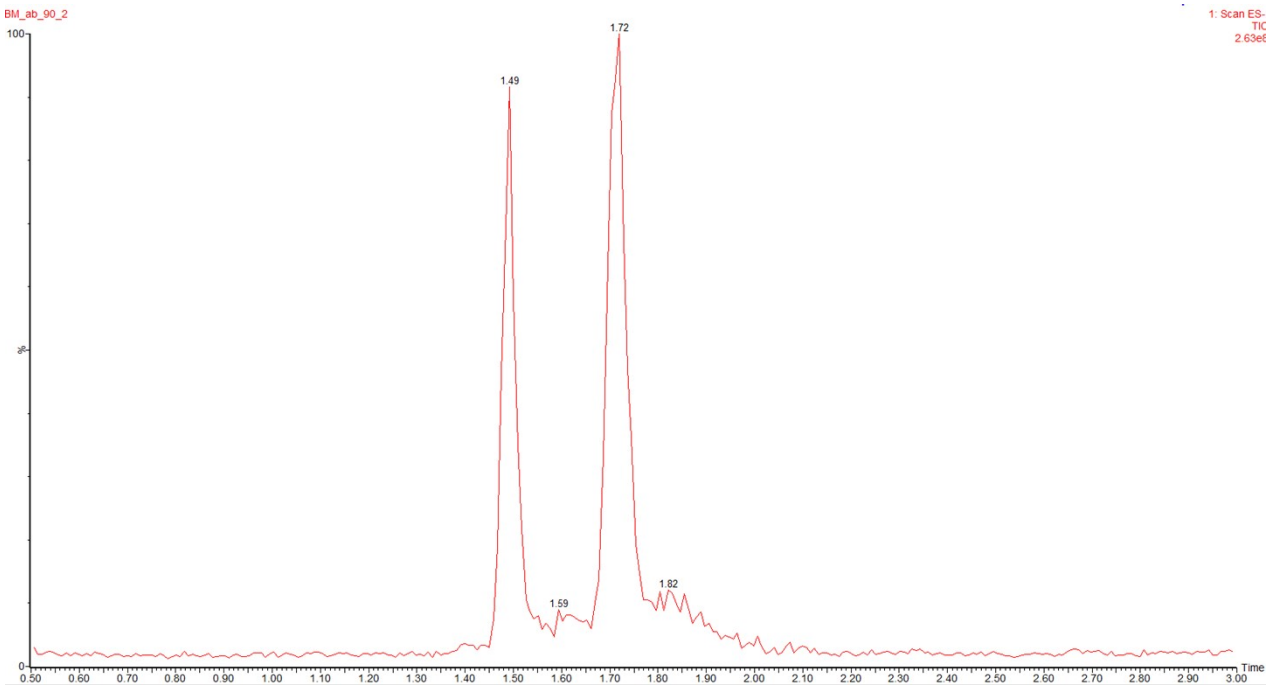
Raw MS of compound 2.



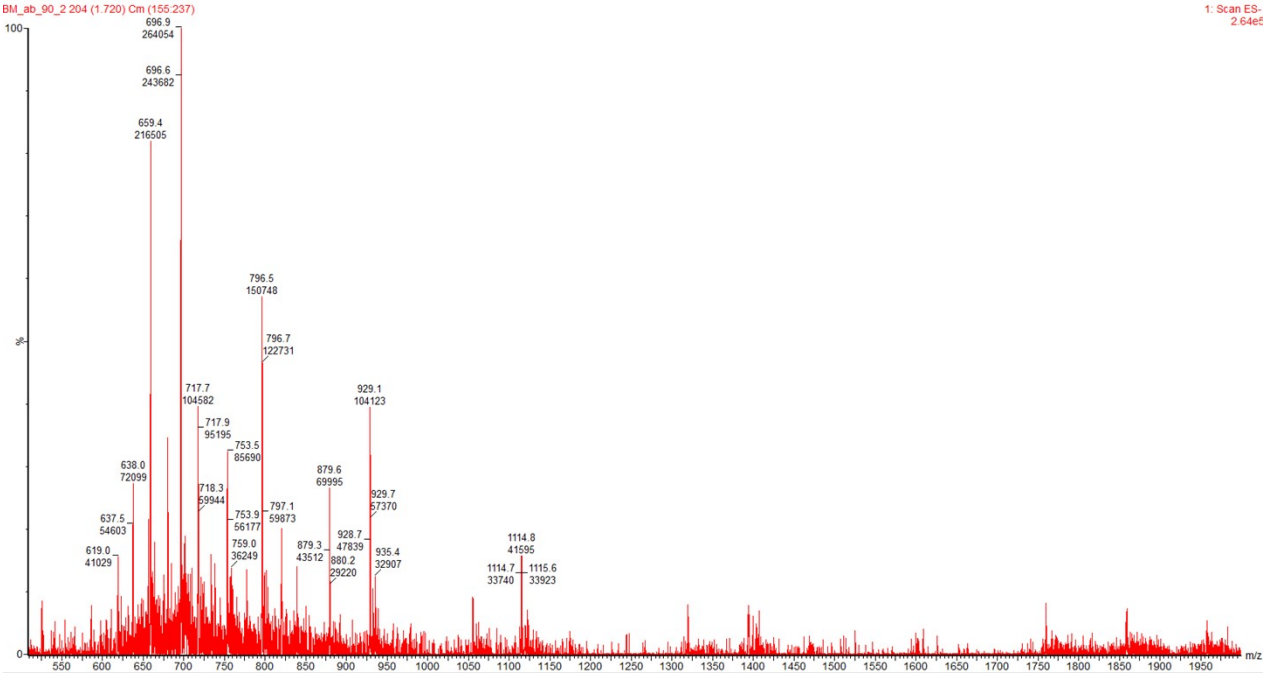
Deconvoluted MS of 2 (mass signals >10% of peak height).



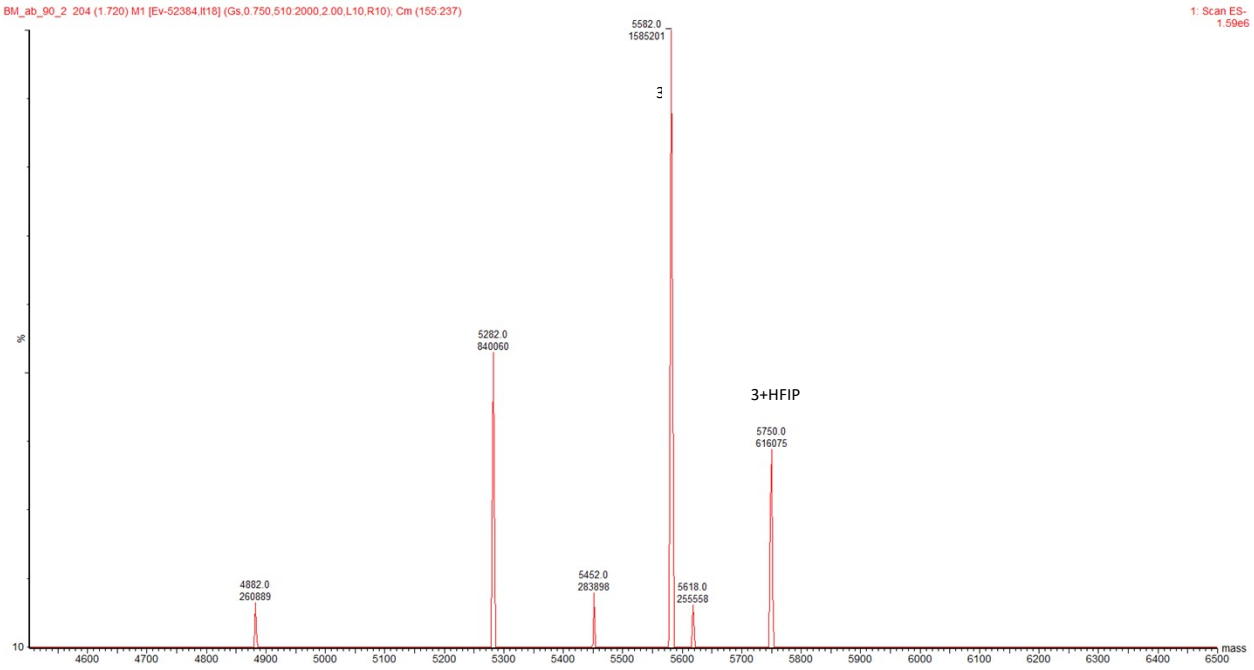
The title compound **3** was synthesized according to **GP-IV** from ethyl 5-(4-bromophenyl)-4,5-dihydroisoxazole-3-carboxylate and the DNA-tagged alkene **HP-1**. The product was formed with 57% conversion (36% recovered **HP-1**).



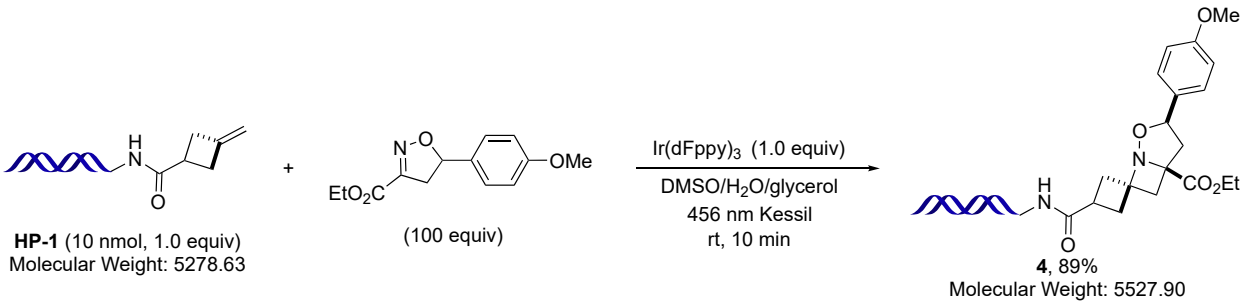
TIC of compound 3.



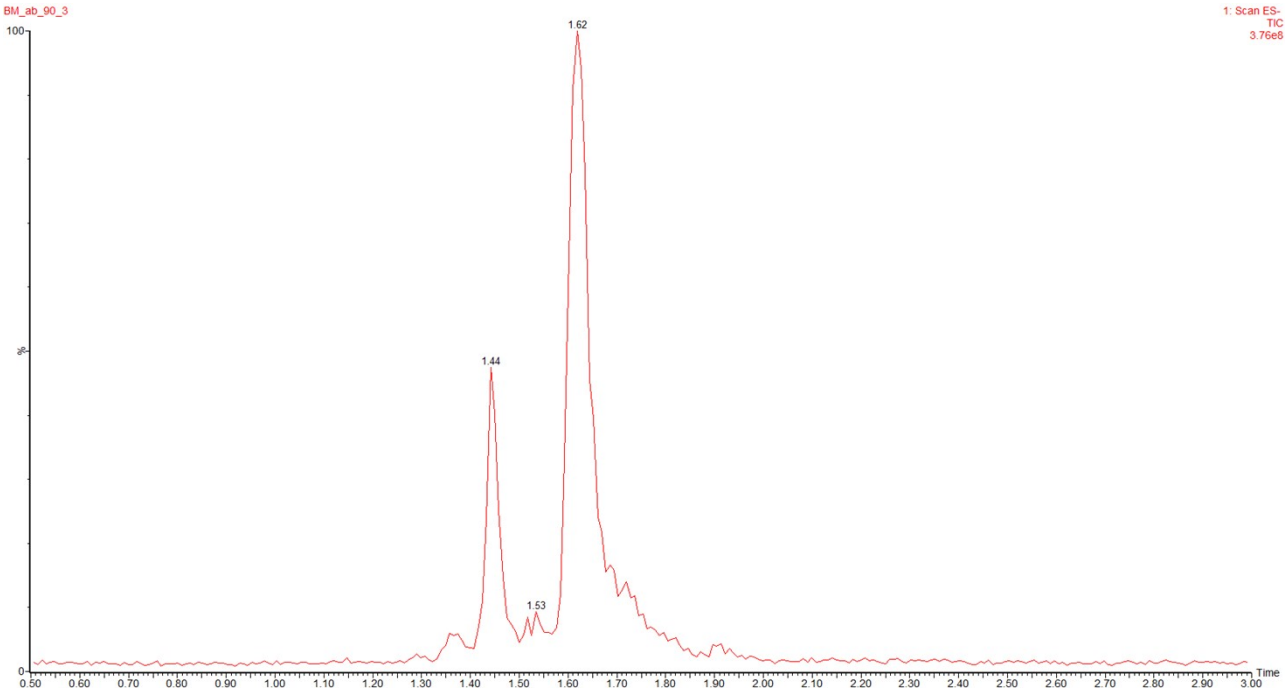
Raw MS of compound 3.



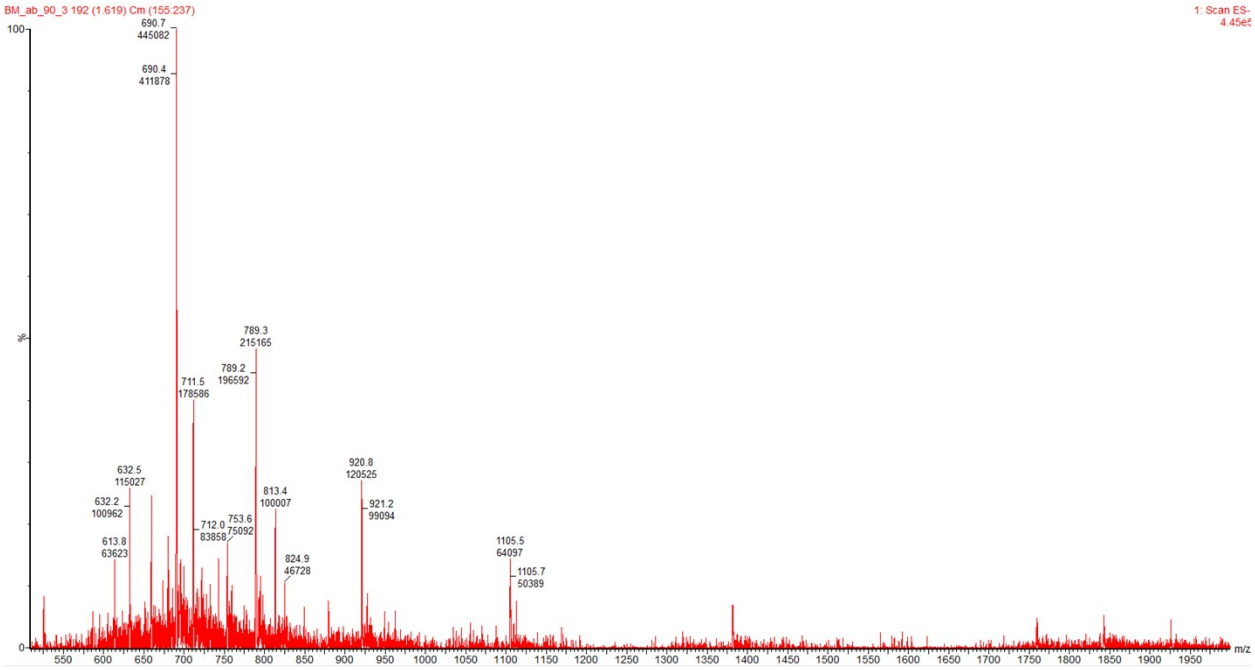
Deconvoluted MS of 3 (mass signals >10% of peak height).



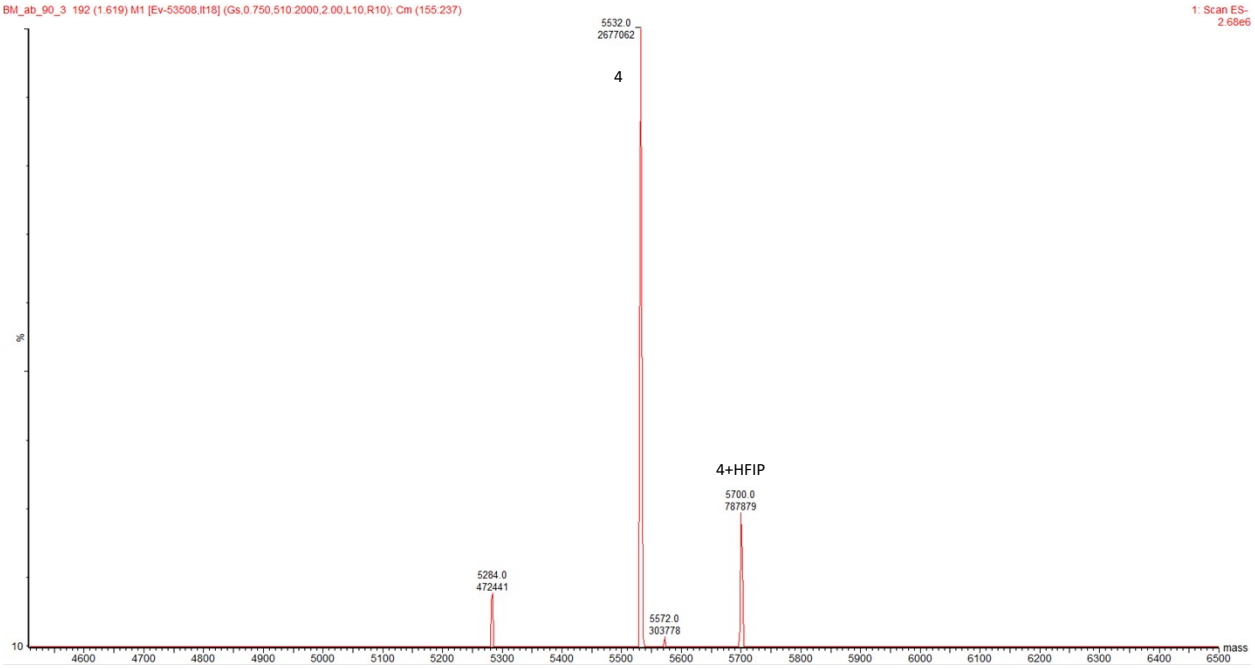
The title compound **4** was synthesized according to **GP-IV** from ethyl 5-(4-methoxyphenyl)-4,5-dihydroisoxazole-3-carboxylate and the DNA-tagged alkene **HP-1**. The product was formed with 89% conversion (11% recovered **HP-1**).



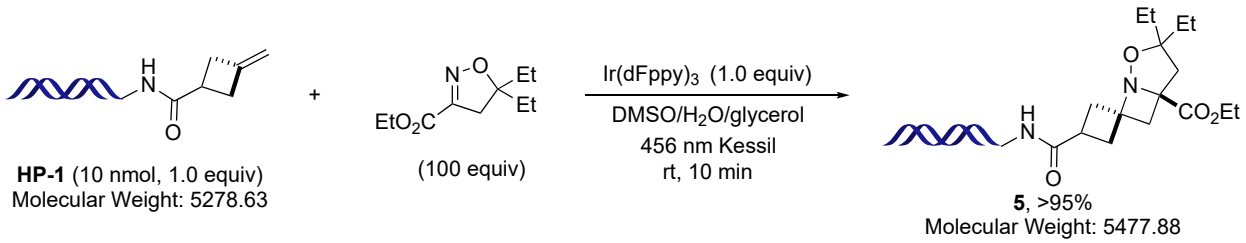
TIC of compound 4.



Raw MS of compound 4.



Deconvoluted MS of 4 (mass signals >10% of peak height).

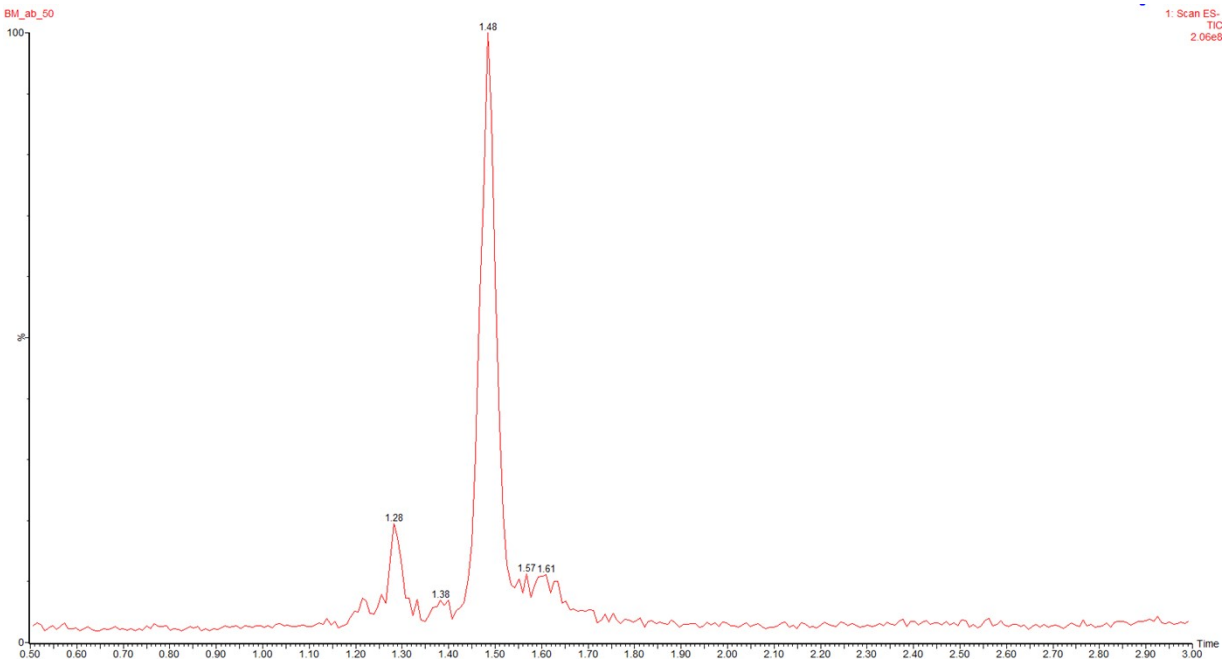


HP-1 (10 nmol, 1.0 equiv)
Molecular Weight: 5278.63

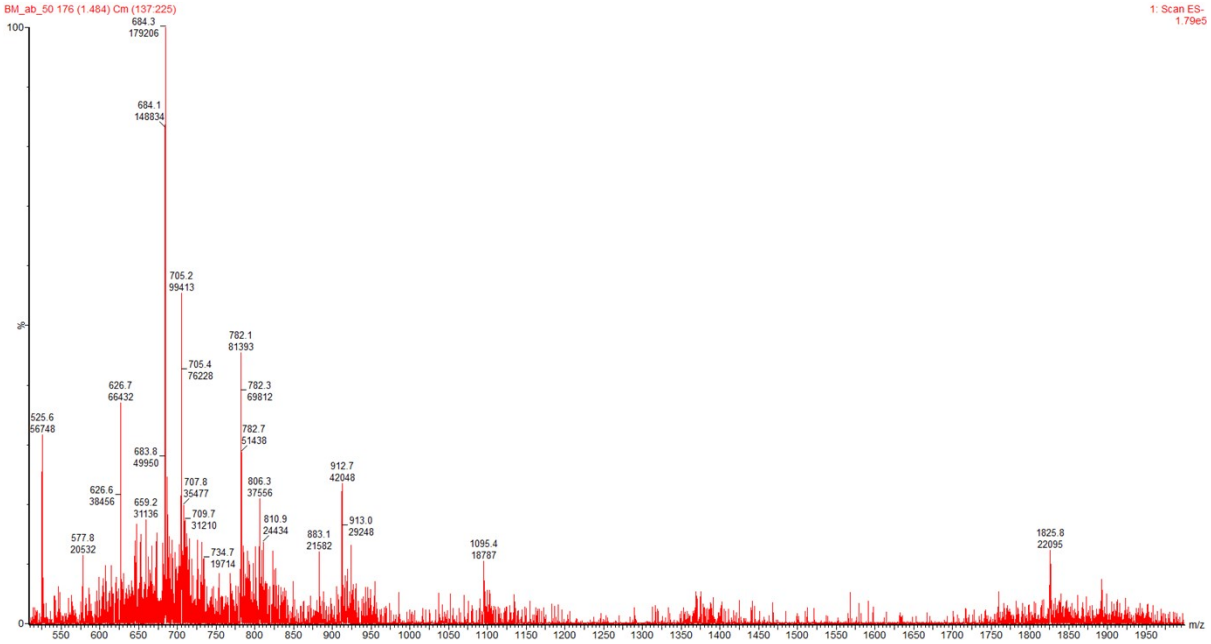
(100 equiv)

5, >95%
Molecular Weight: 5477.88

The title compound **5** was synthesized according to **GP-IV** from ethyl 5,5-diethyl-4,5-dihydroisoxazole-3-carboxylate and the DNA-tagged alkene **HP-1**. The product was formed with >95% conversion.

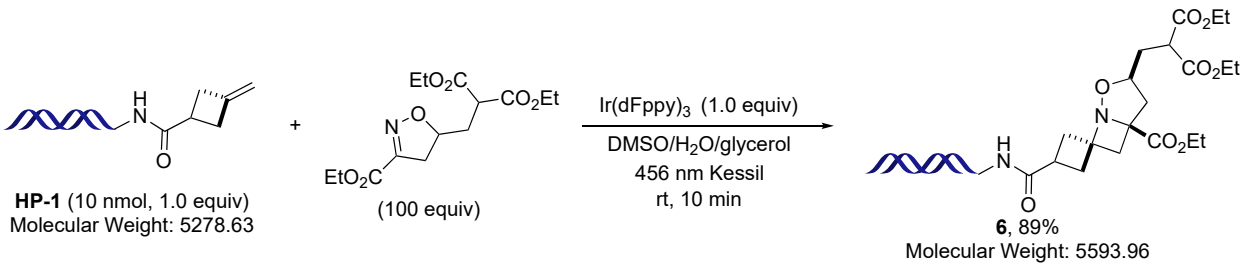


TIC of compound 5.

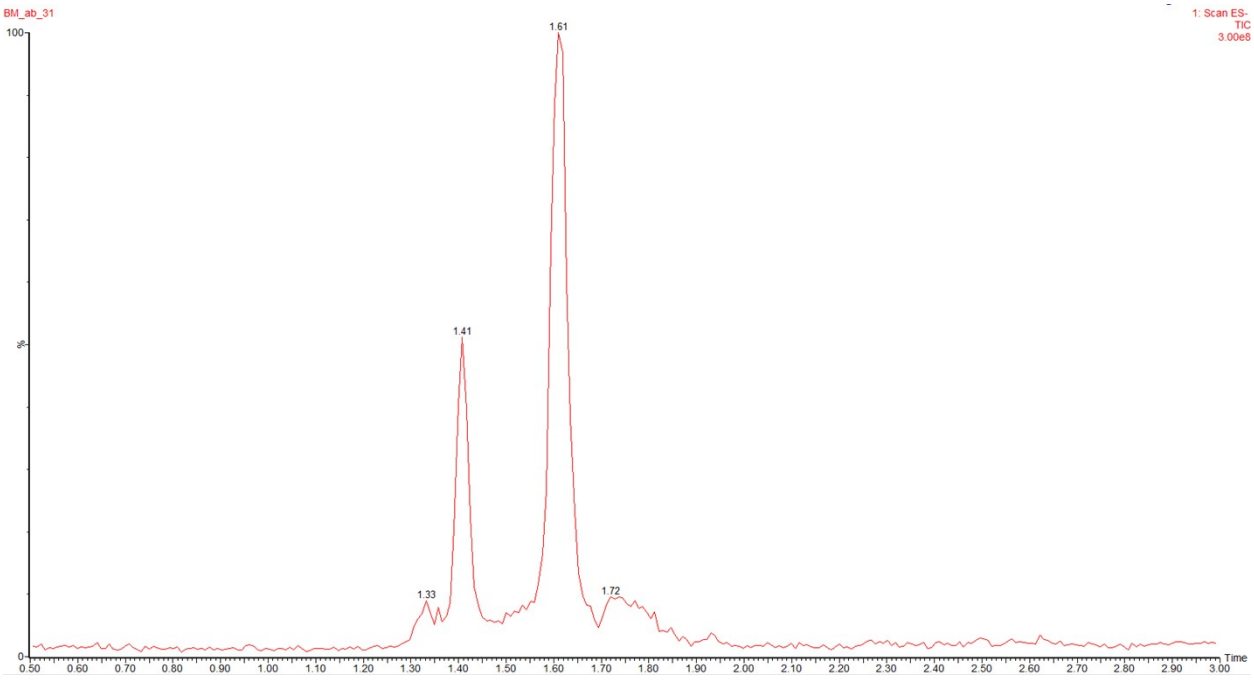


Raw MS of compound 5.

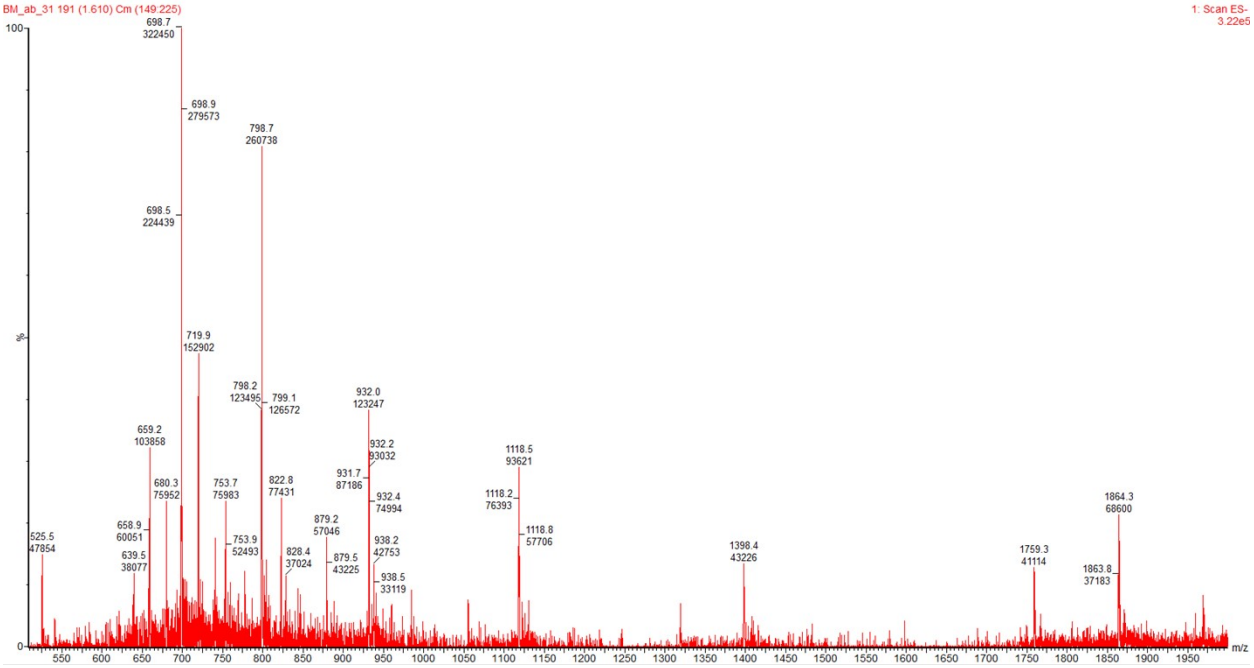
Deconvoluted MS of 5 (mass signals >10% of peak height).



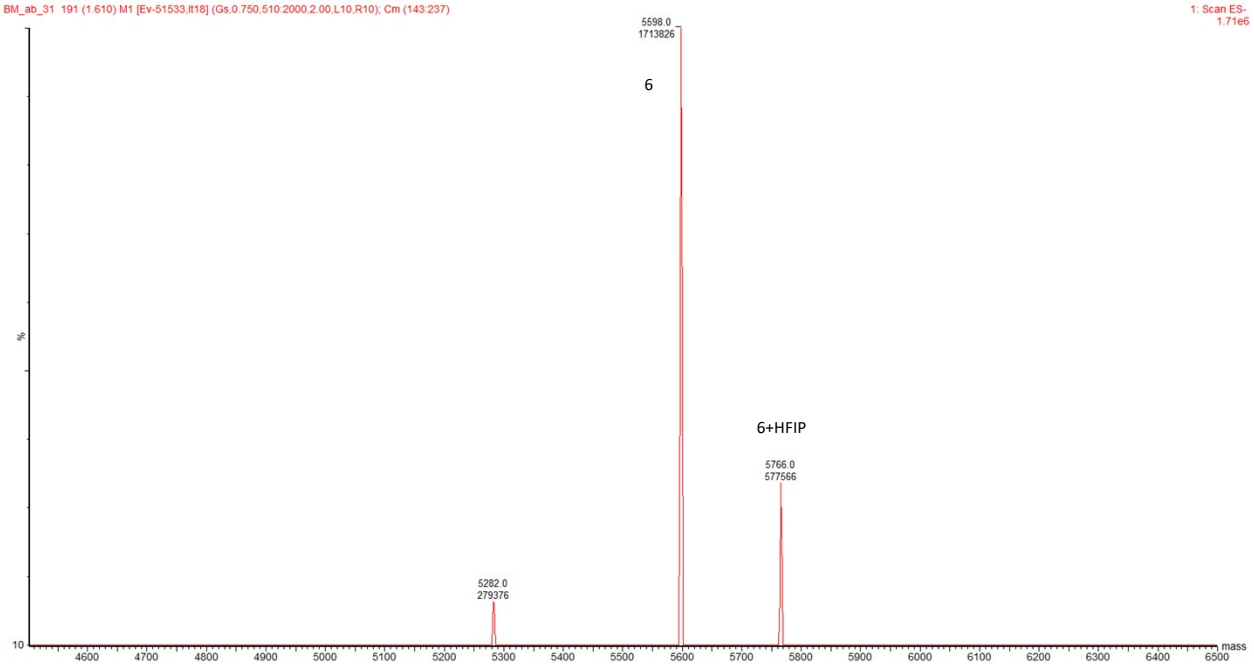
The title compound **6** was synthesized according to **GP-IV** from diethyl 2-((3-(ethoxycarbonyl)-4,5-dihydroisoxazol-5-yl)methyl)malonate and the DNA-tagged alkene **HP-1**. The product was formed with 89% conversion (11% recovered **HP-1**).



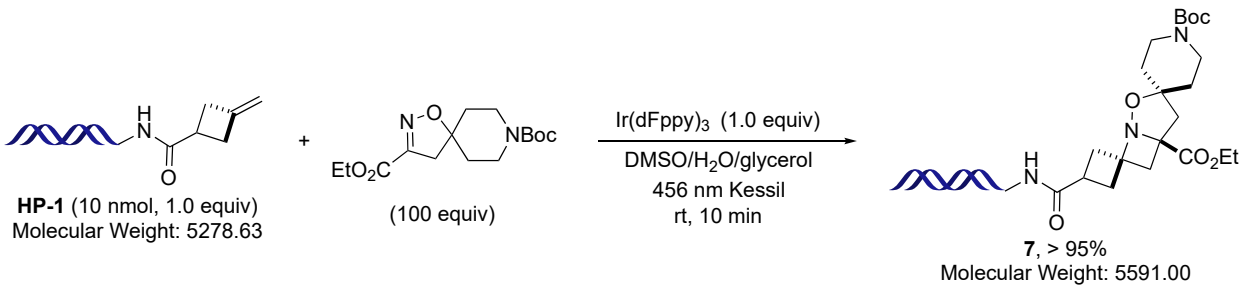
TIC of compound 6.



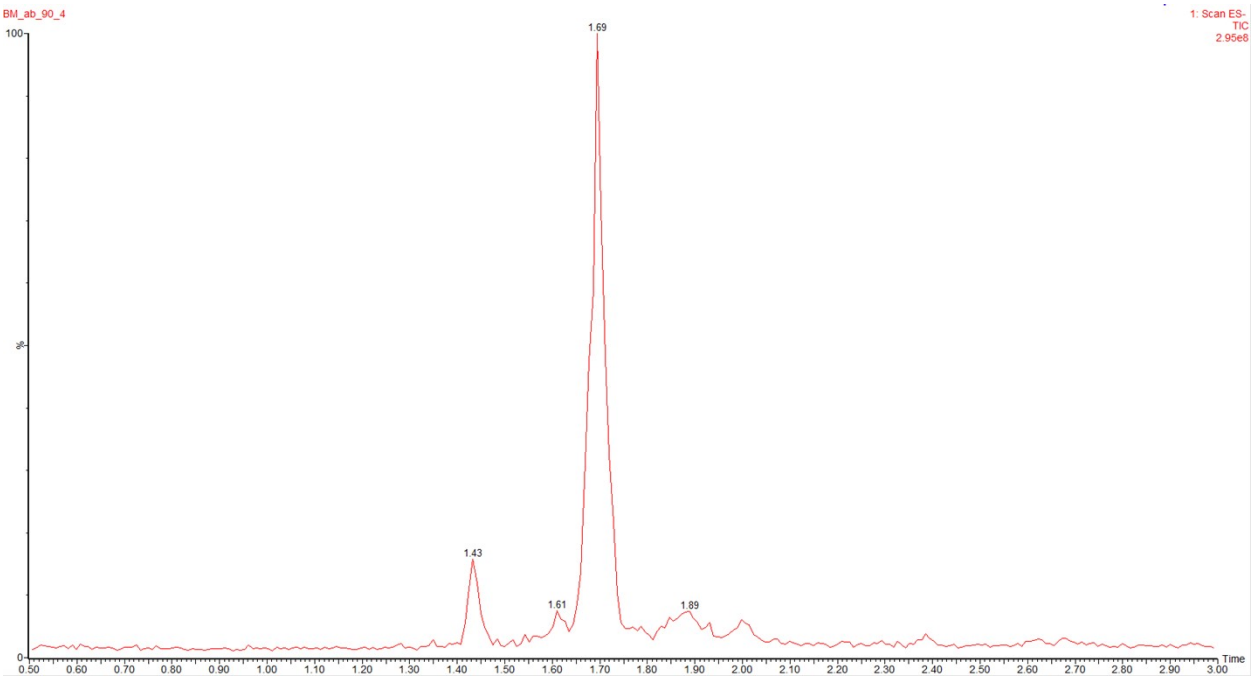
Raw MS of compound 6.



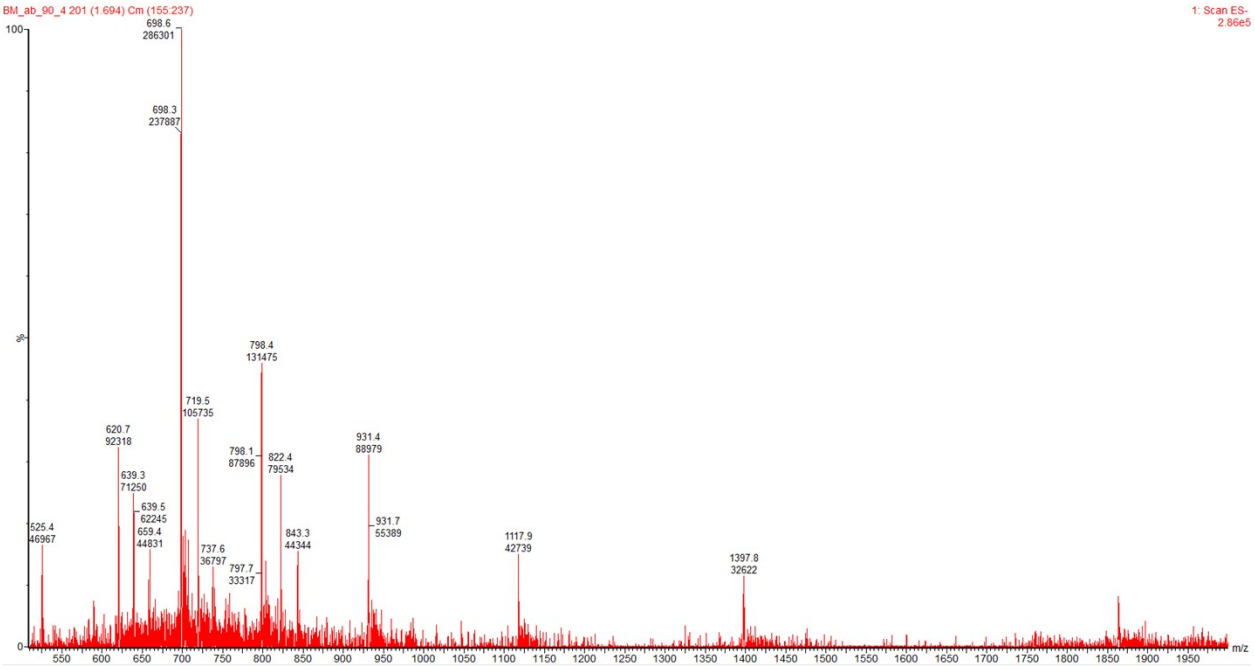
Deconvoluted MS of 6 (mass signals >10% of peak height).



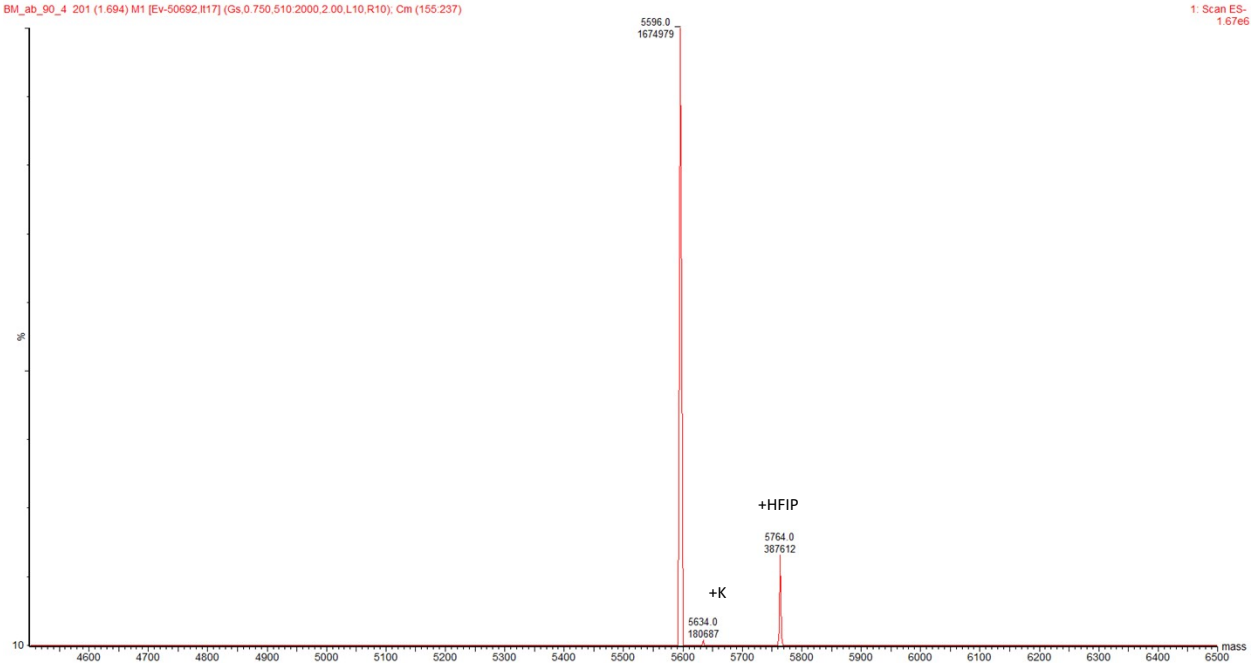
The title compound **7** was synthesized according to **GP-IV** from 8-(*tert*-butyl) 3-ethyl 1-oxa-2,8-diazaspiro[4.5]dec-2-ene-3,8-dicarboxylate and the DNA-tagged alkene **HP-1**. The product was formed with > 95% conversion.



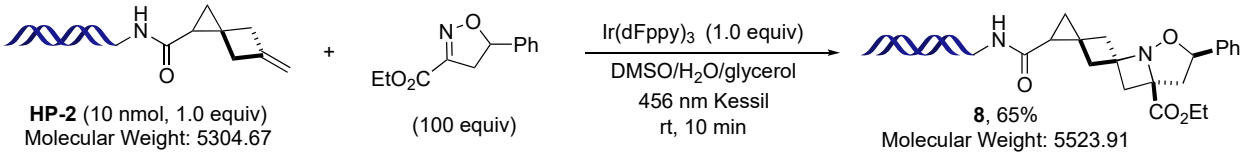
TIC of compound 7.



Raw MS of compound 7.



Deconvoluted MS of 7 (mass signals >10% of peak height).

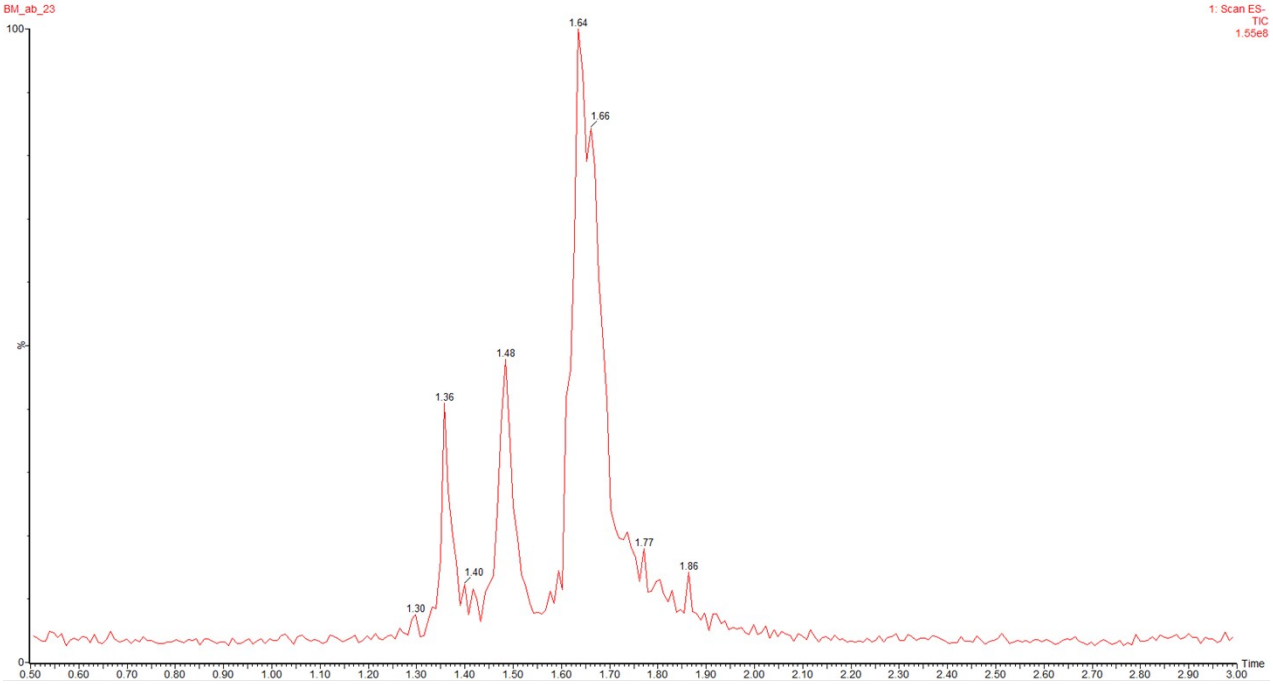


HP-2 (10 nmol, 1.0 equiv)
Molecular Weight: 5304.67

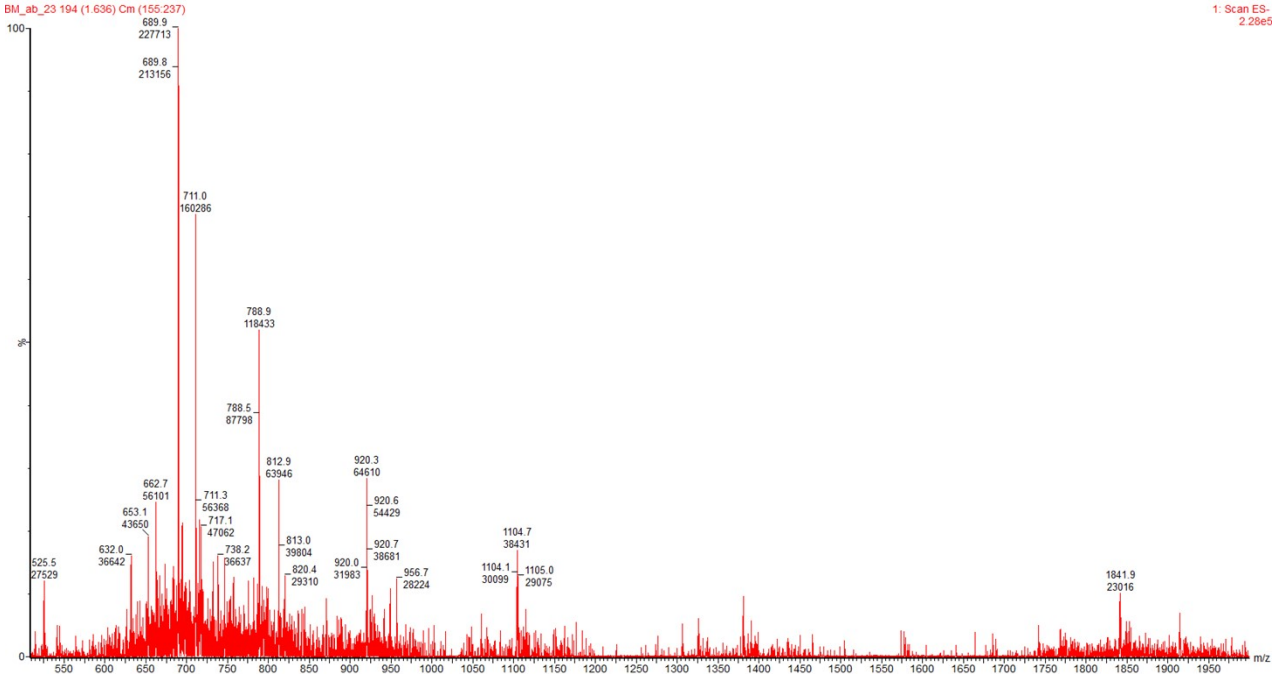
(100 equiv)

8, 65%
Molecular Weight: 5523.91

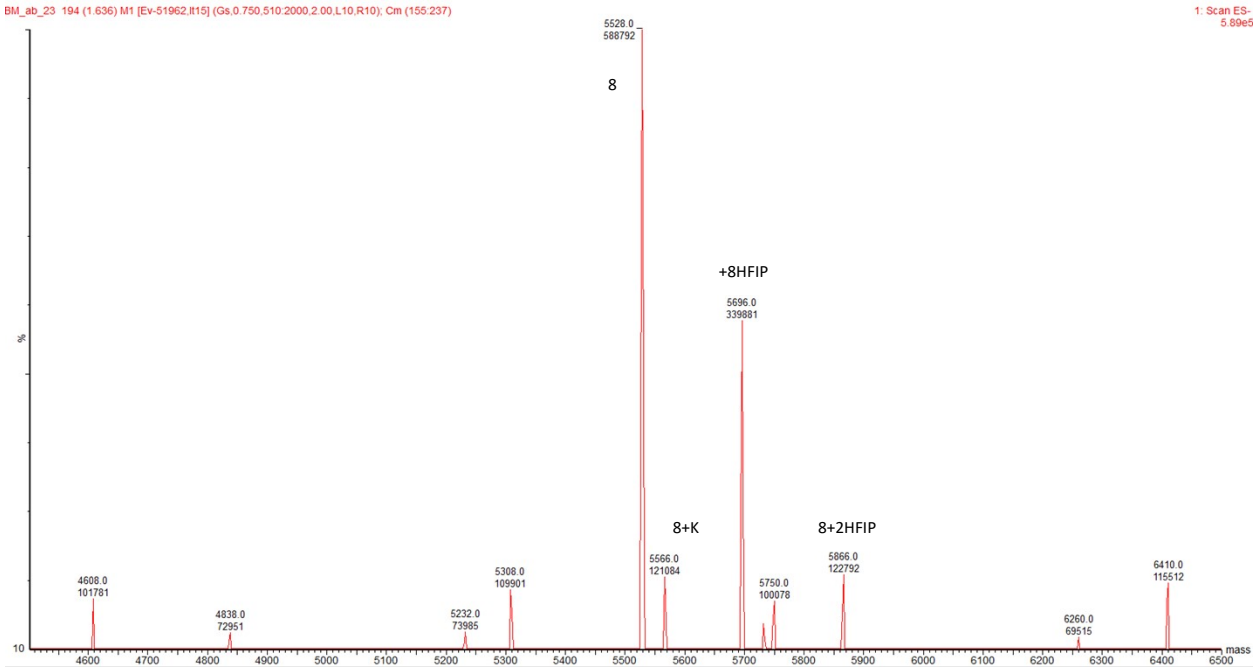
The title compound **8** was synthesized according to **GP-IV** from ethyl 5-phenyl-4,5-dihydroisoxazole-3-carboxylate and the DNA-tagged alkene **HP-2**. The product was formed with 65% conversion (6% recovered **HP-2**).



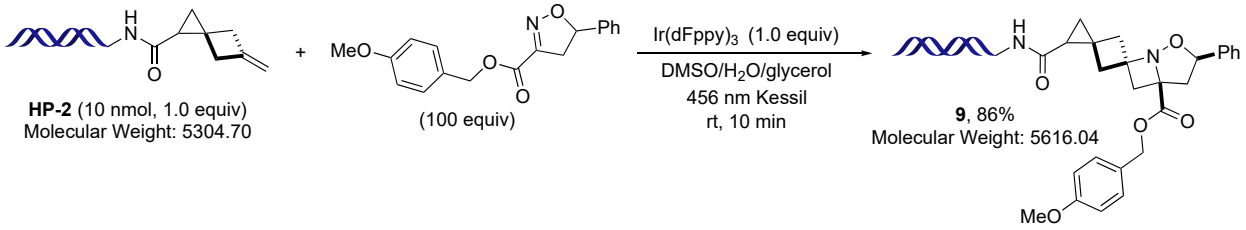
TIC of compound 8.



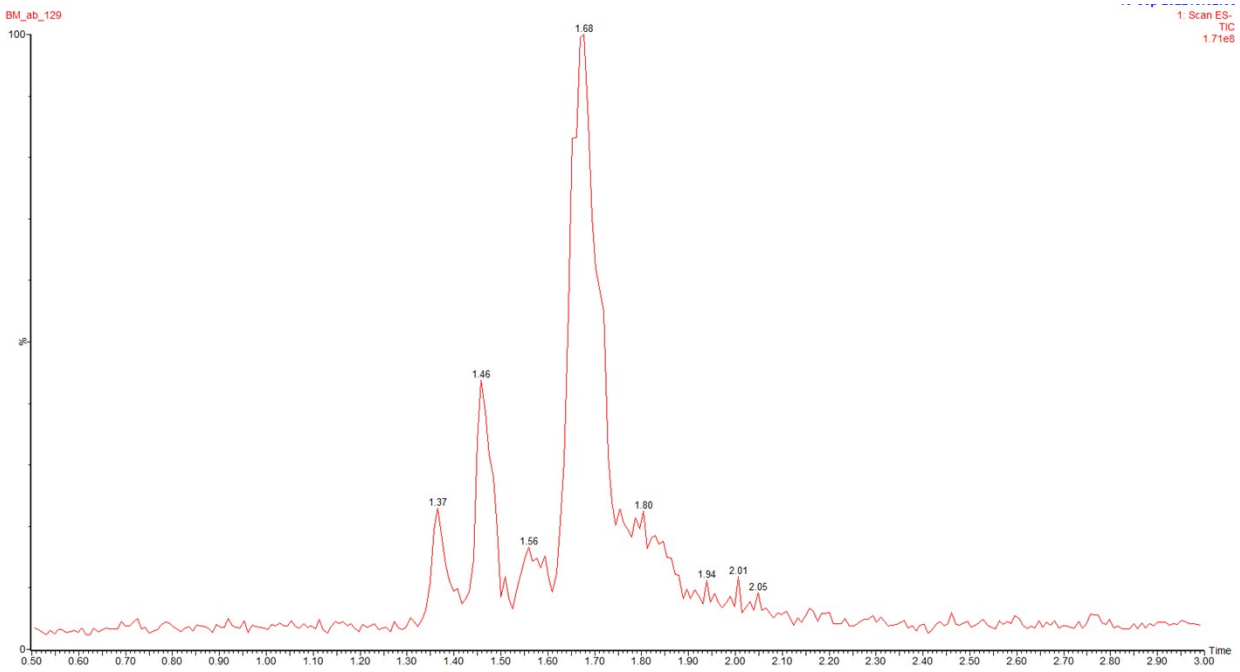
Raw MS of compound 8.



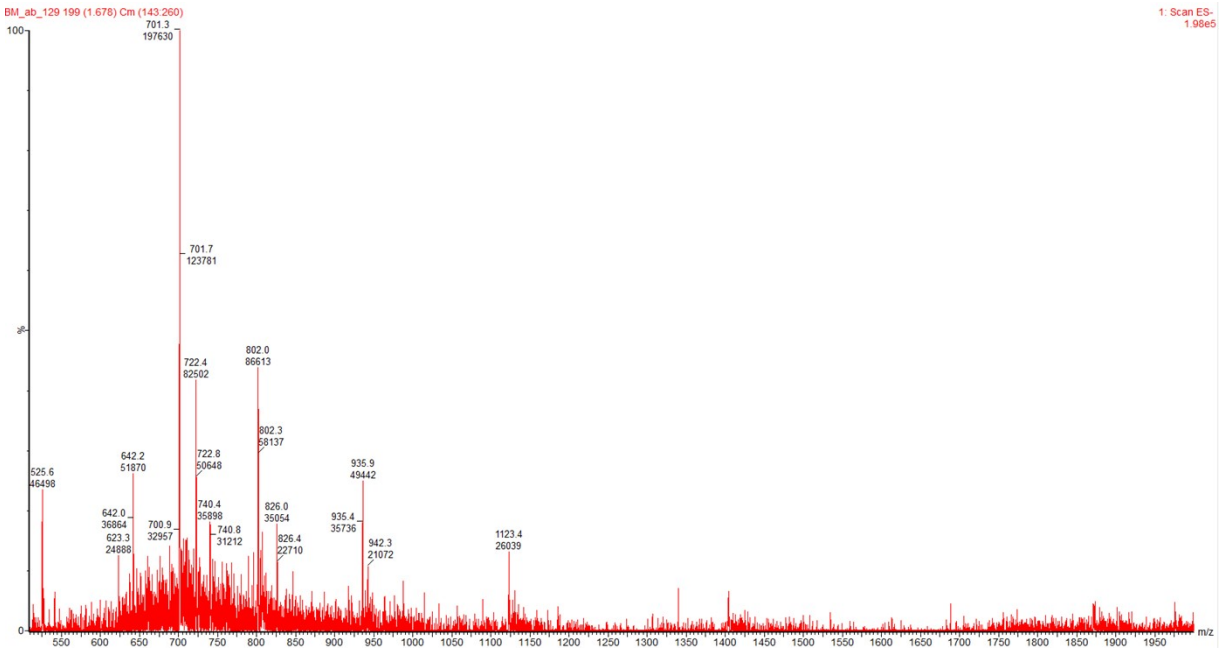
Deconvoluted MS of 8 (mass signals >10% of peak height).



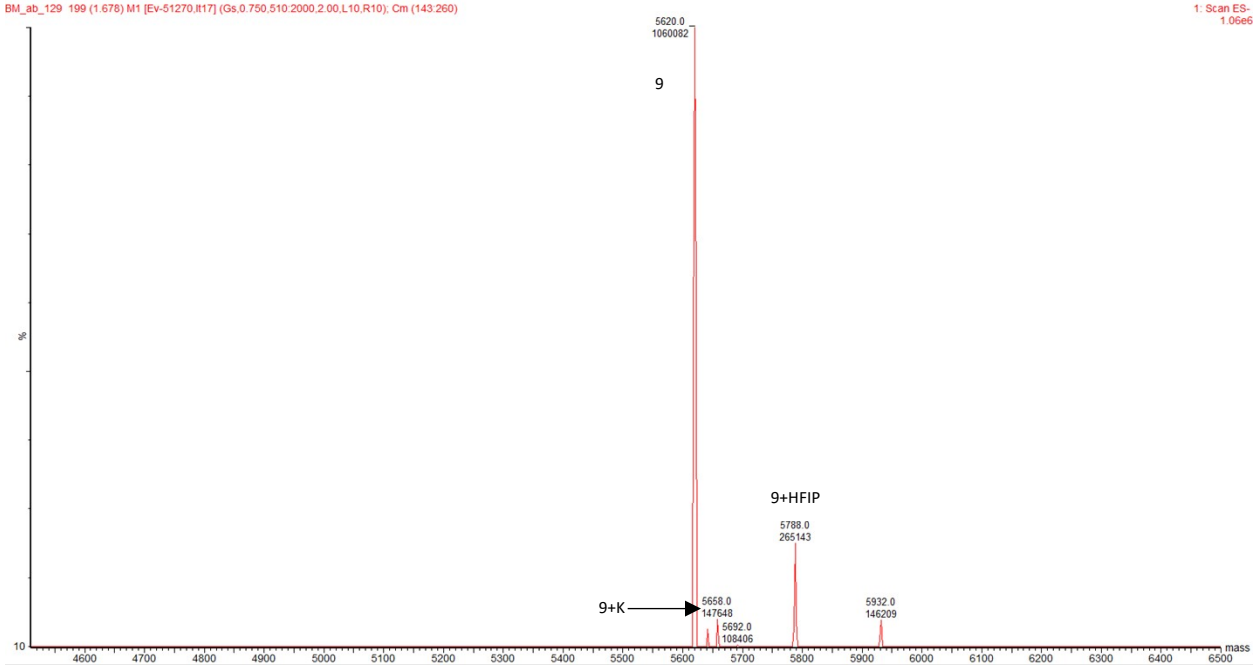
The title compound **9** was synthesized according to **GP-IV** from 4-methoxybenzyl 5-phenyl-4,5-dihydroisoxazole-3-carboxylate and the DNA-tagged alkene **HP-2**. The product was formed with 86% conversion (8% double addition product).



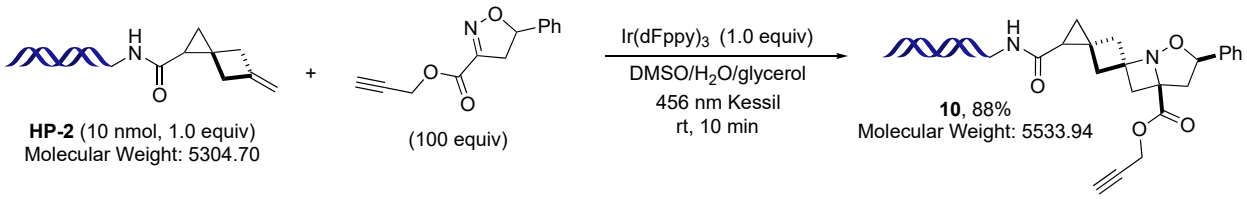
TIC of compound 9.



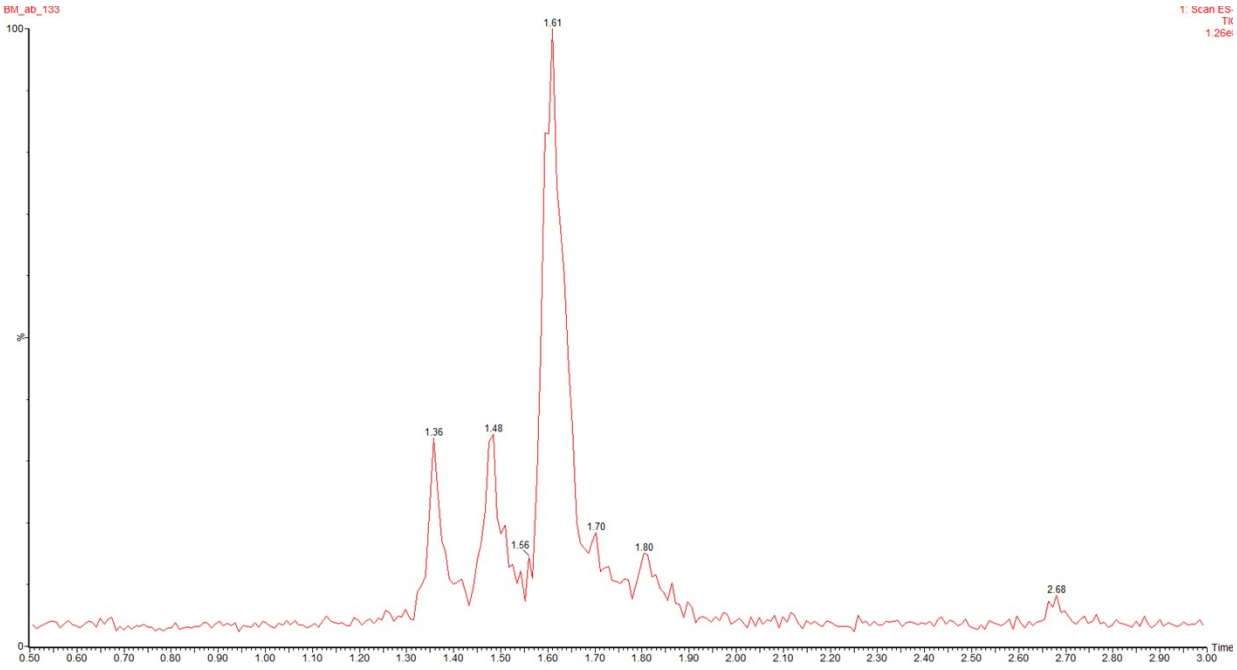
Raw MS of compound 9.



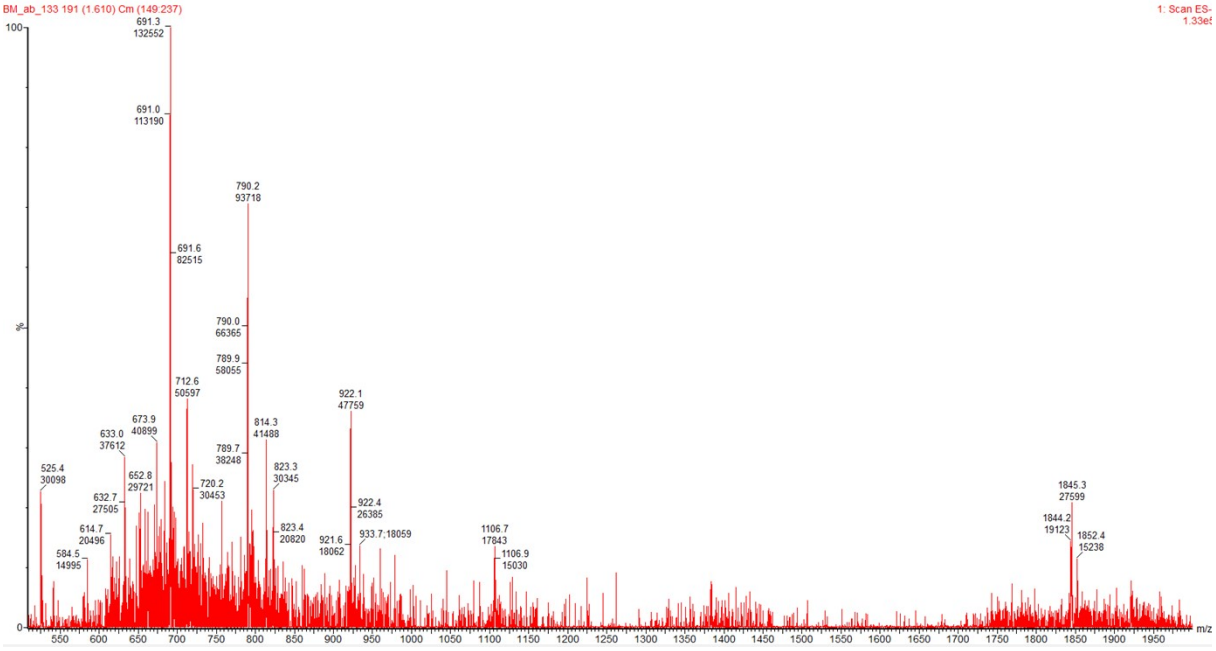
Deconvoluted MS of 9 (mass signals >10% of peak height).



The title compound **10** was synthesized according to **GP-IV** from prop-2-yn-1-yl 5-phenyl-4,5-dihydroisoxazole-3-carboxylate and the DNA-tagged alkene **HP-2**. The product was formed with 88% conversion (6% recovered **HP-2**).

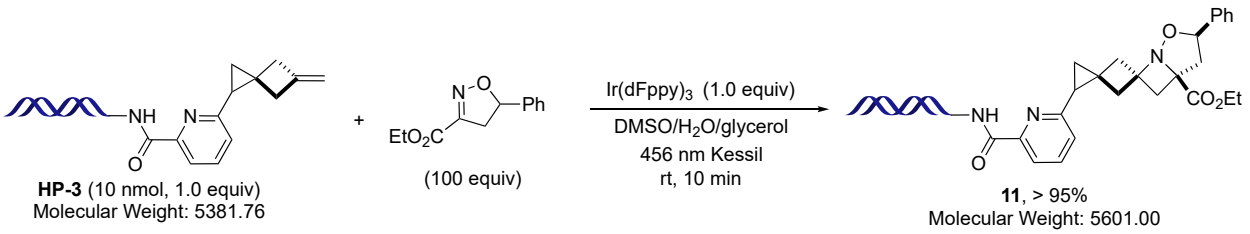


TIC of compound 10.

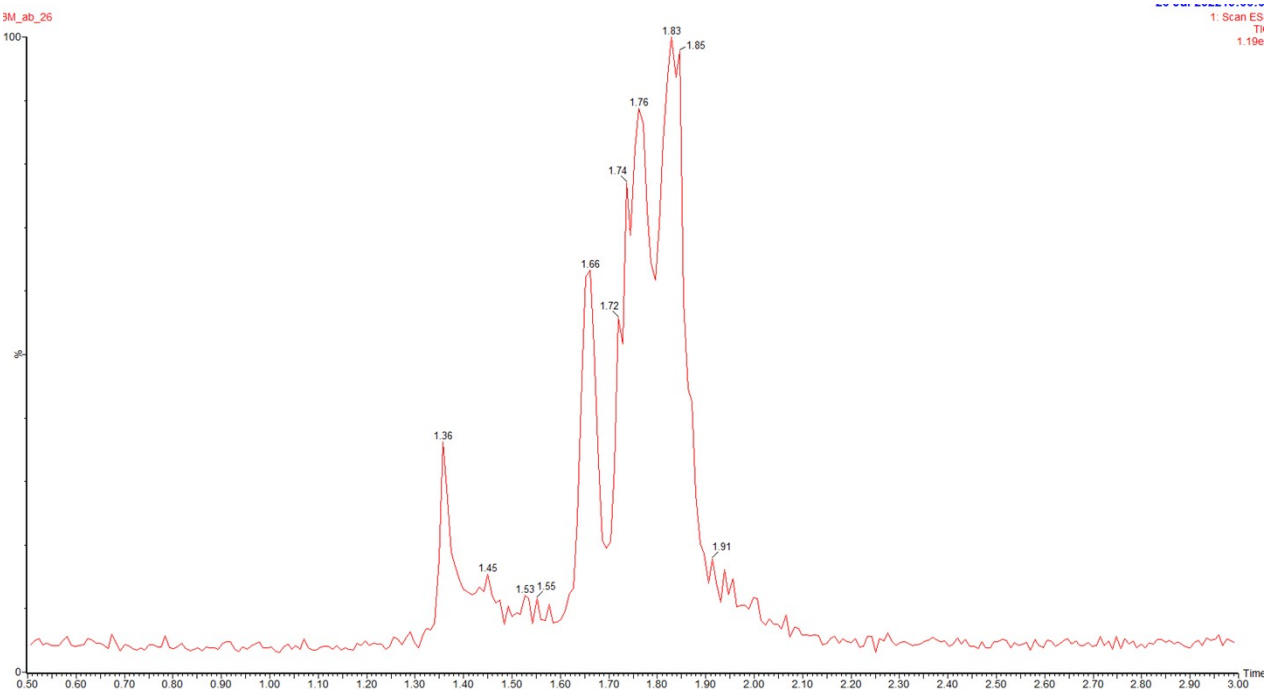


Raw MS of compound 10.

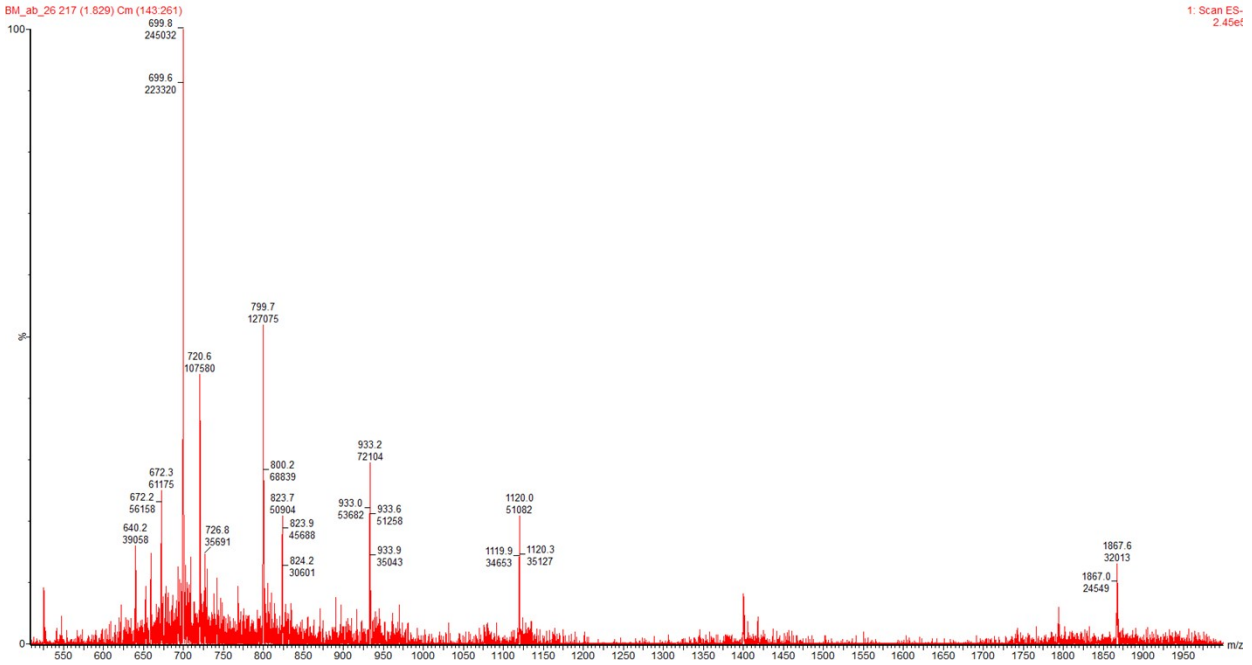
Deconvoluted MS of 10 (mass signals >10% of peak height).



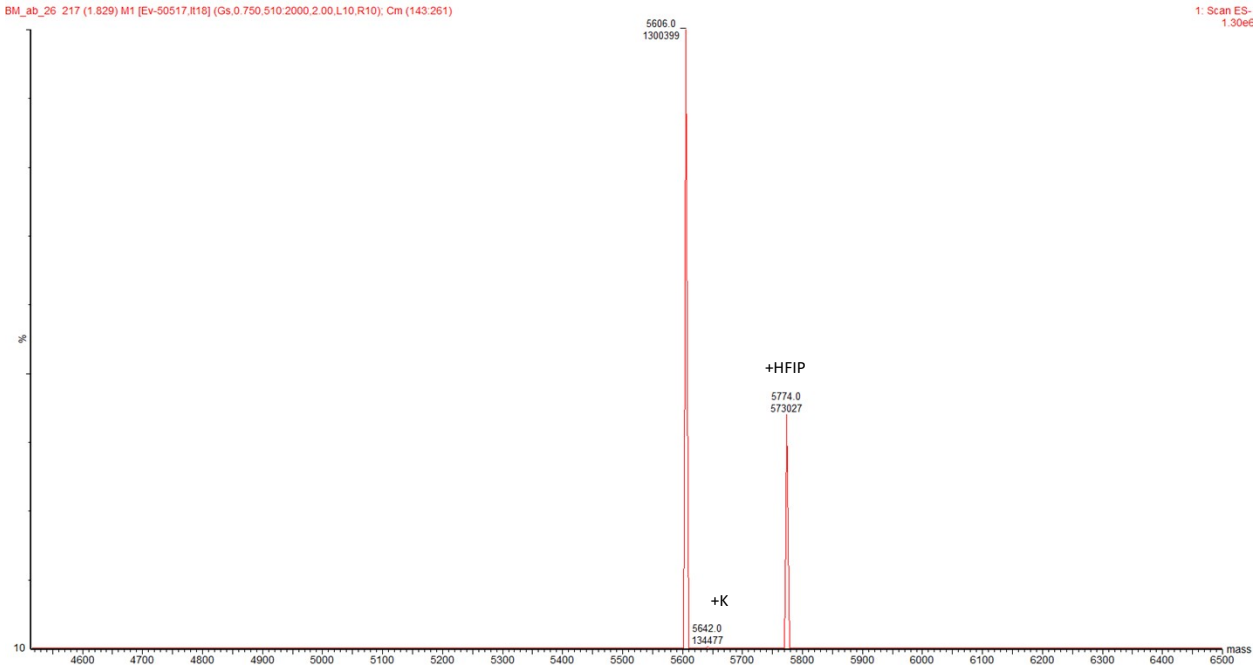
The title compound **11** was synthesized according to **GP-IV** from ethyl 5-phenyl-4,5-dihydroisoxazole-3-carboxylate and the DNA-tagged alkene **HP-3**. The product was formed with >95% conversion.



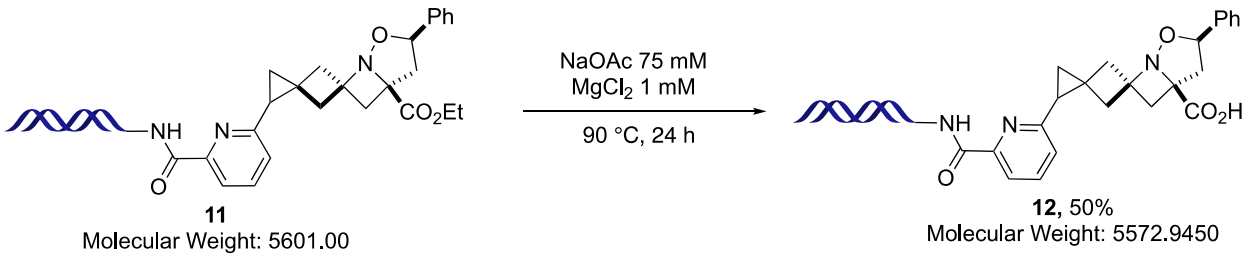
TIC of compound 11.



Raw MS of compound 11.

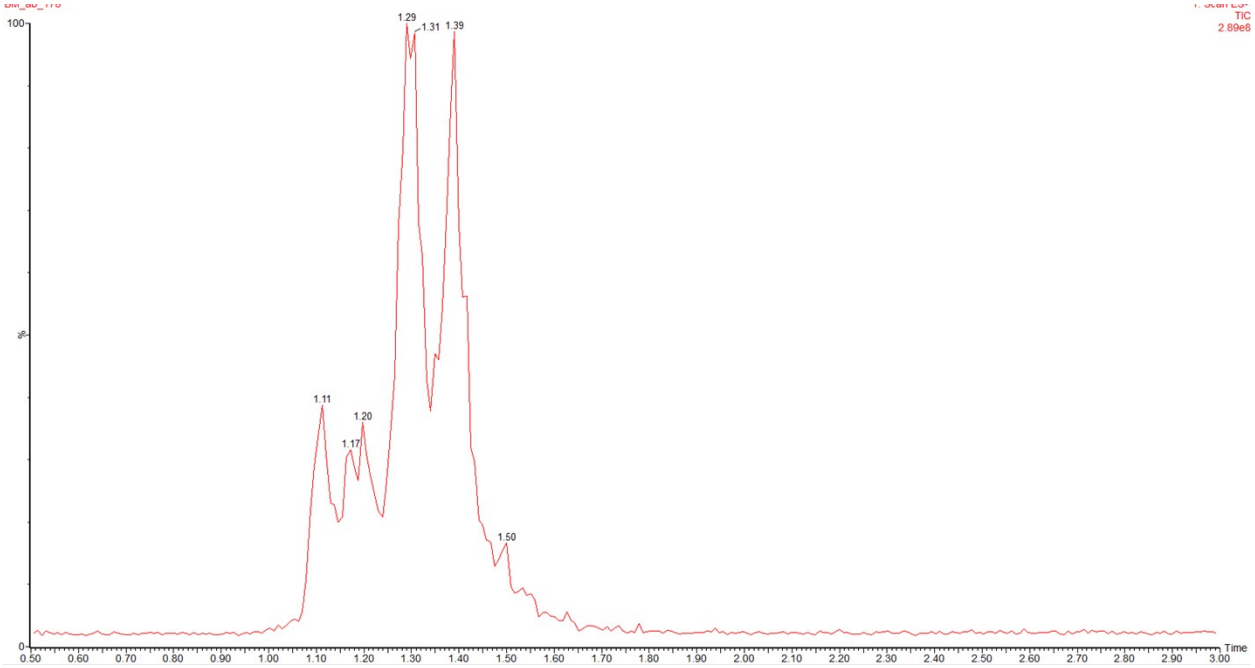


Deconvoluted MS of 11 (mass signals >10% of peak height).

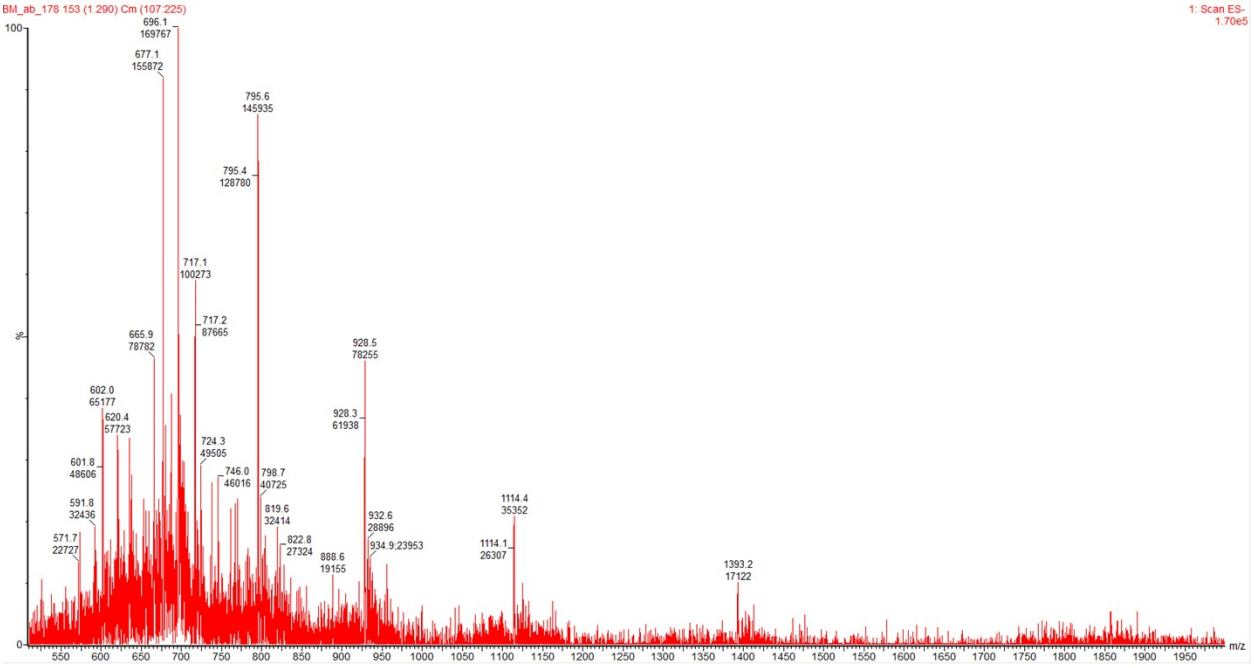


11
Molecular Weight: 5601.00

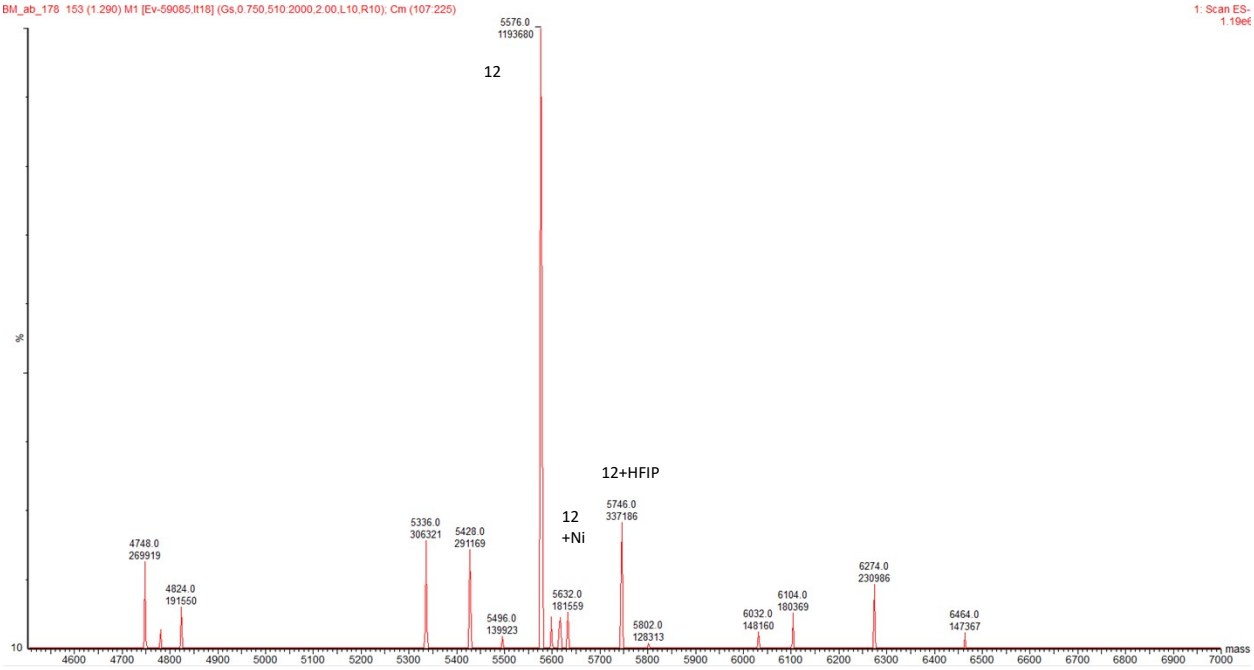
12, 50%
Molecular Weight: 5572.9450



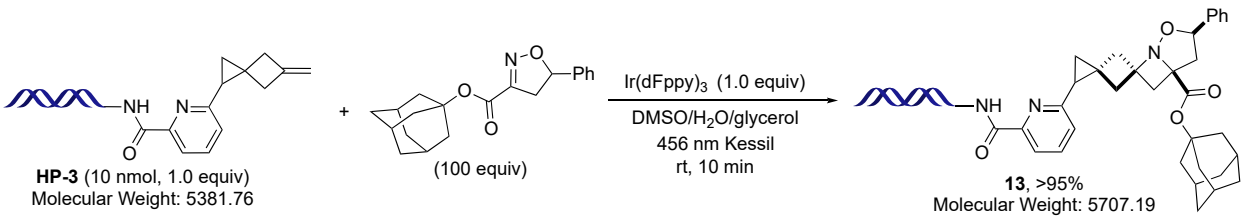
TIC of compound 12.



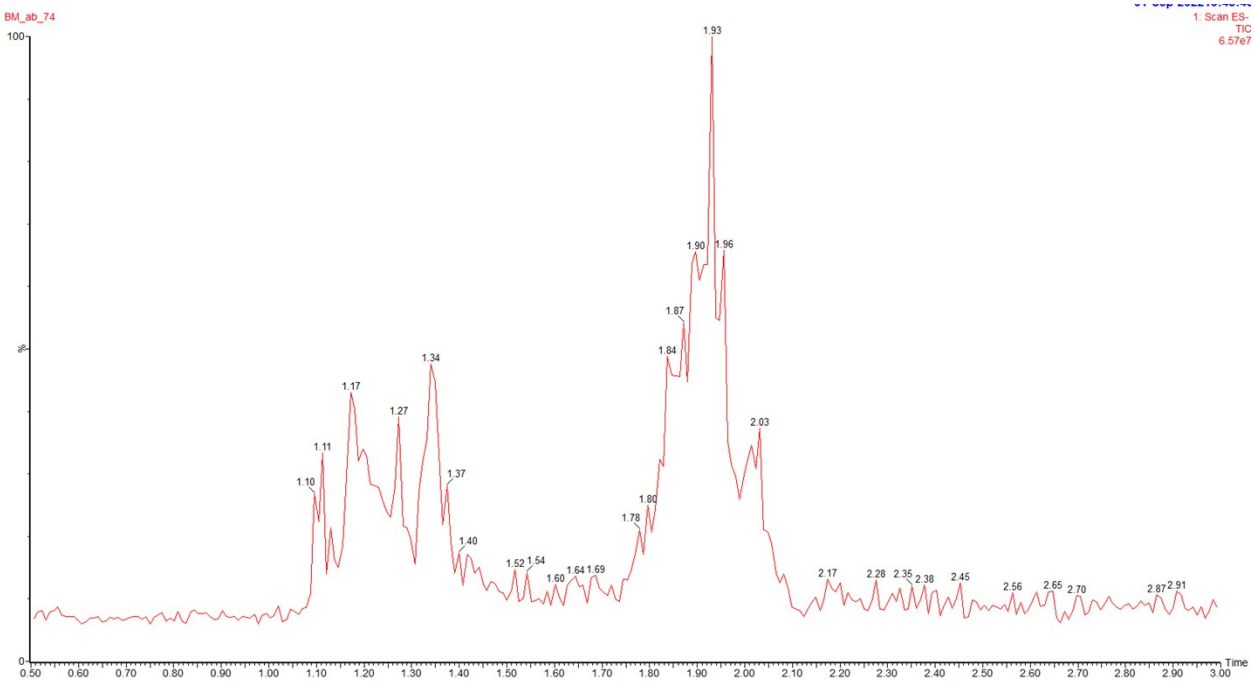
Raw MS of compound 12.



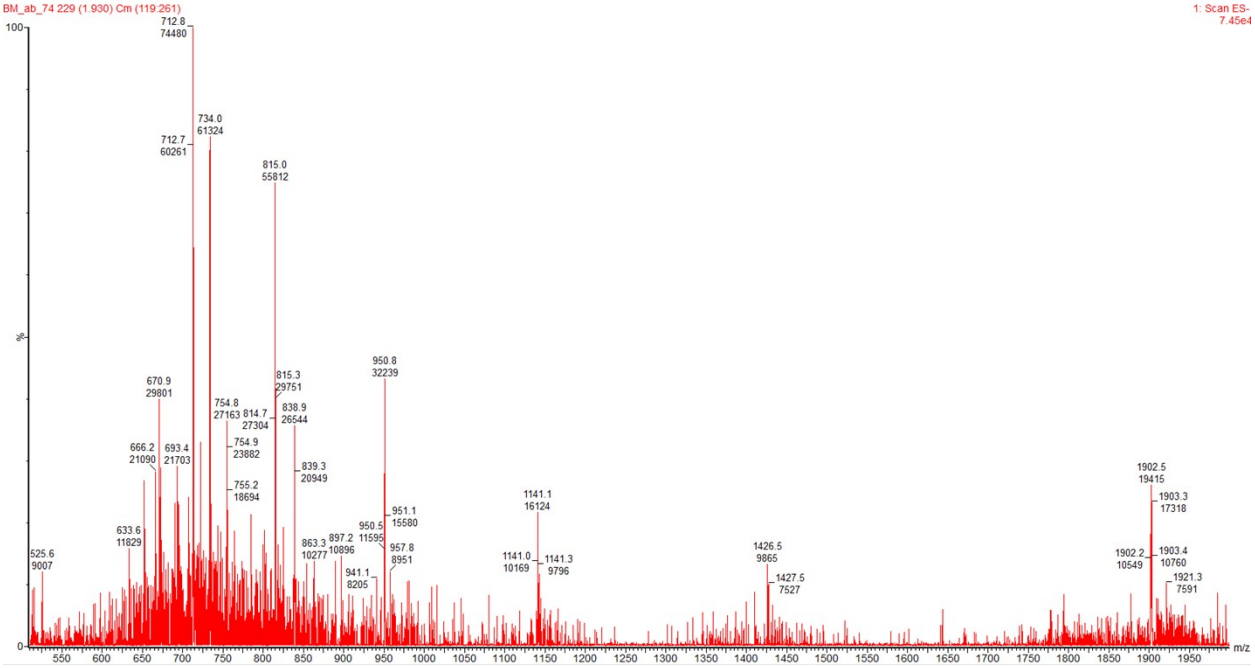
Deconvoluted MS of 12 (mass signals >10% of peak height).



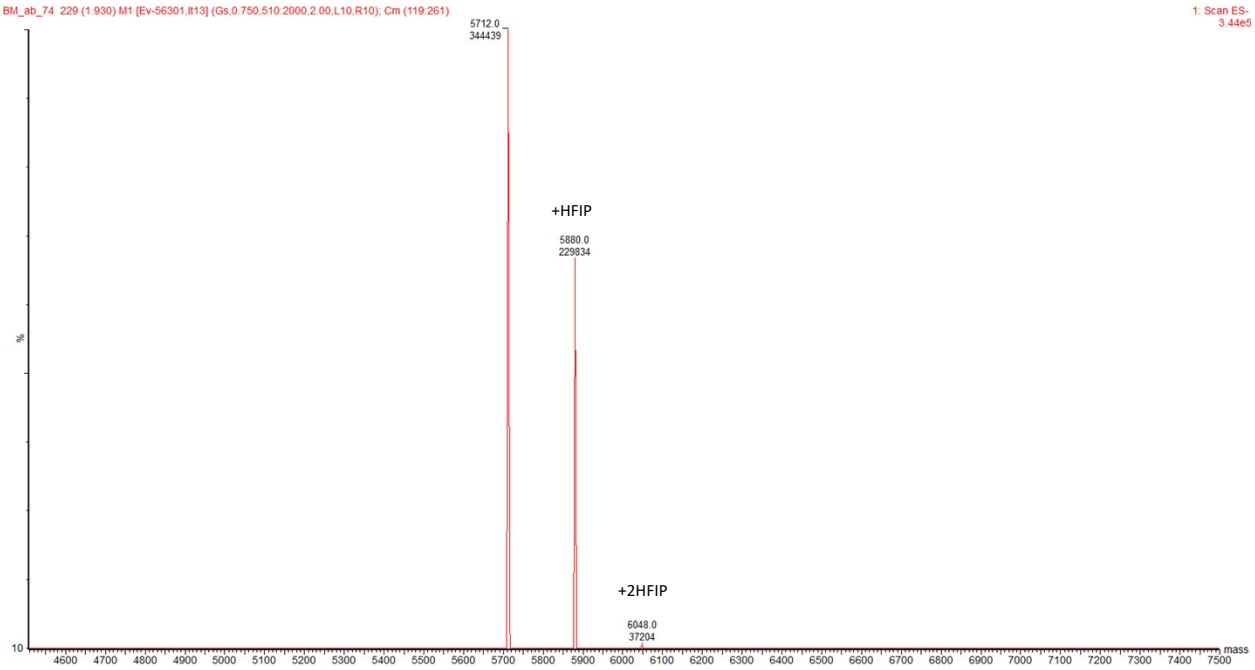
The title compound **13** was synthesized according to **GP-IV** from (3s,5s,7s)-adamantan-1-yl 5-phenyl-4,5-dihydroisoxazole-3-carboxylate and the DNA-tagged alkene **HP-3**. The product was formed with > 95% conversion.



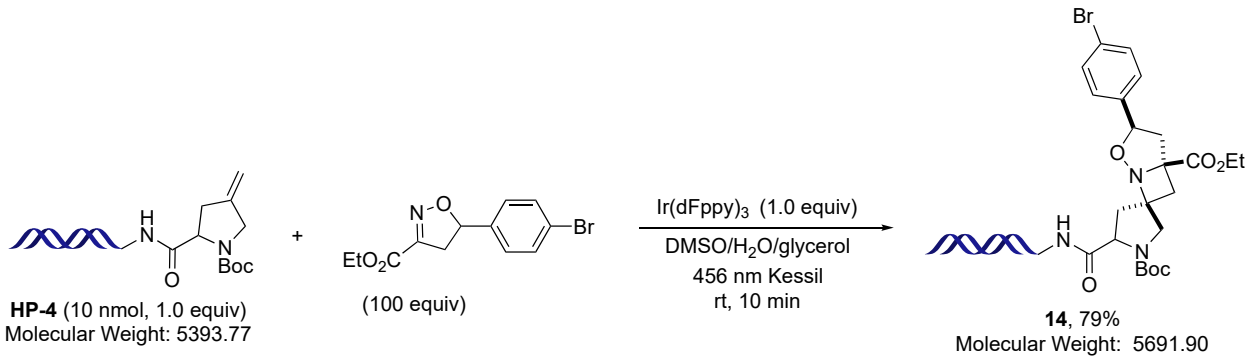
TIC of compound 13.



Raw MS of compound 13.



Deconvoluted MS of 13 (mass signals >10% of peak height).

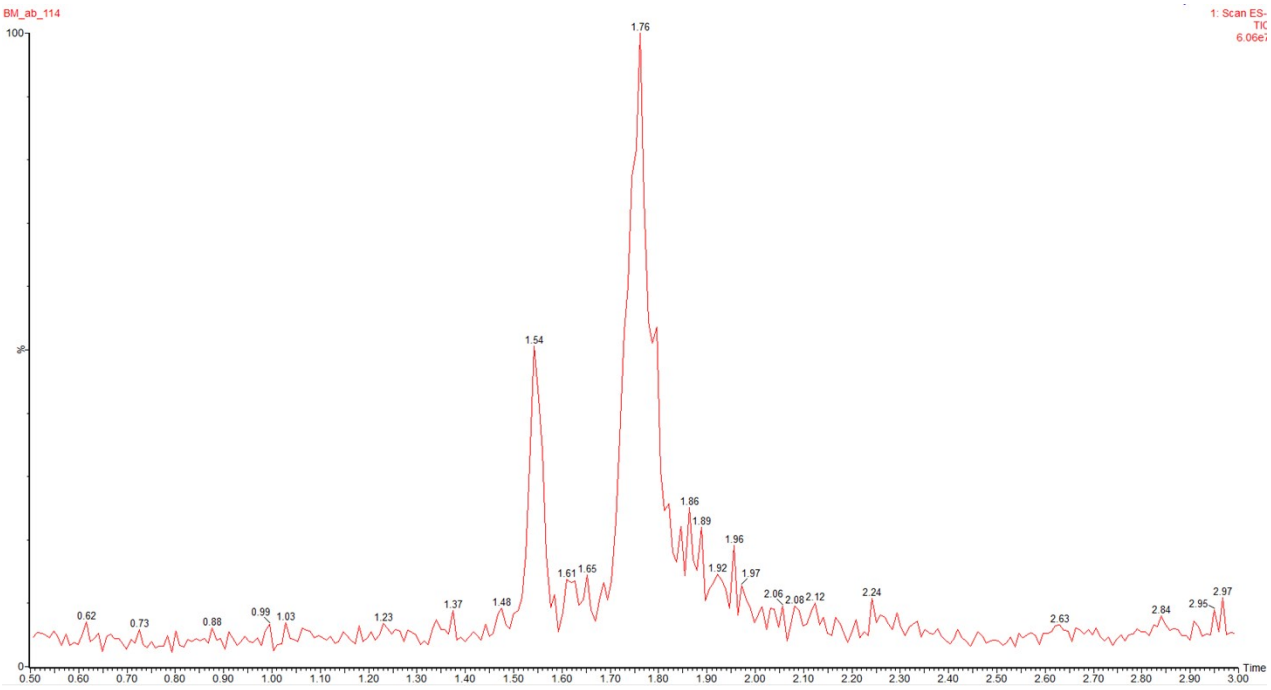


HP-4 (10 nmol, 1.0 equiv)
Molecular Weight: 5393.77

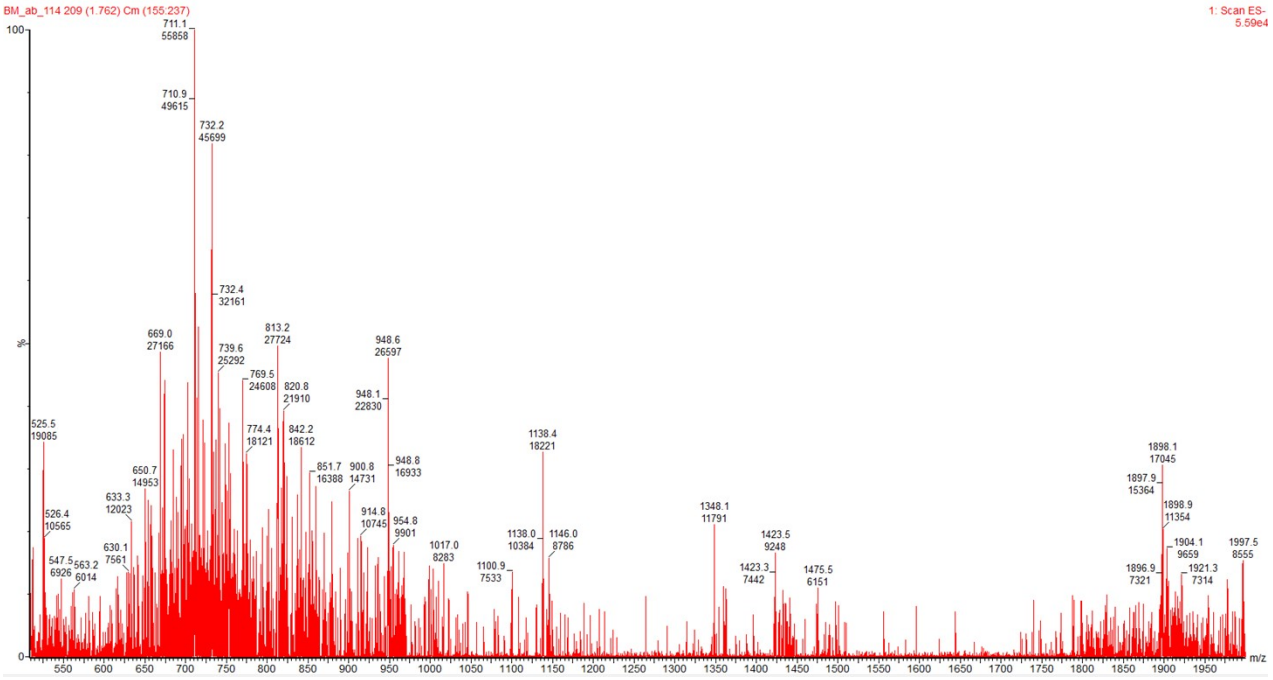
(100 equiv)

14, 79%
Molecular Weight: 5691.90

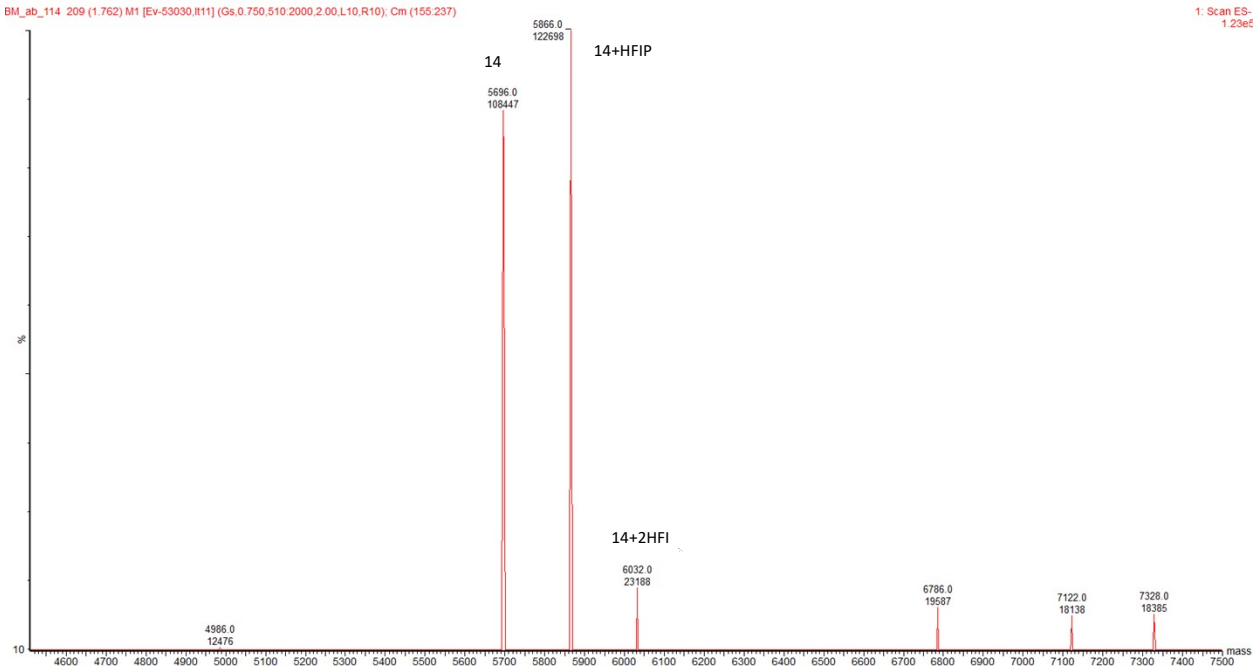
The title compound **14** was synthesized according to **GP-IV** from ethyl 5-(4-bromophenyl)-4,5-dihydroisoxazole-3-carboxylate and the DNA-tagged alkene **HP-4**. The product was formed with 79% conversion.



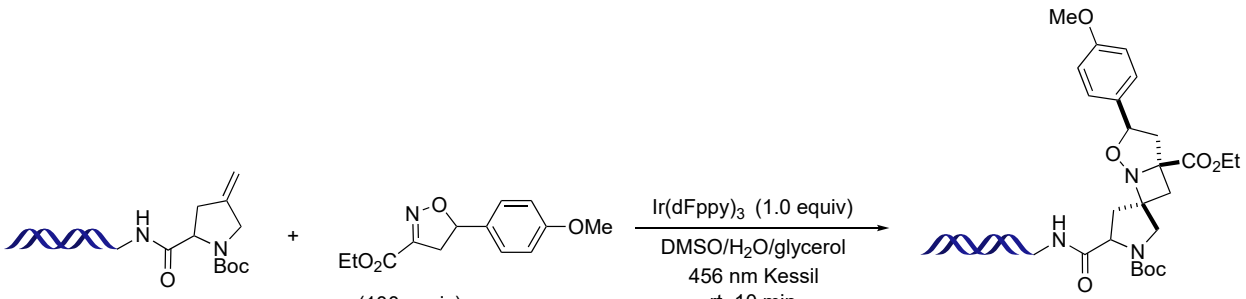
TIC of compound 14.



Raw MS of compound 14.



Deconvoluted MS of 14 (mass signals >10% of peak height).

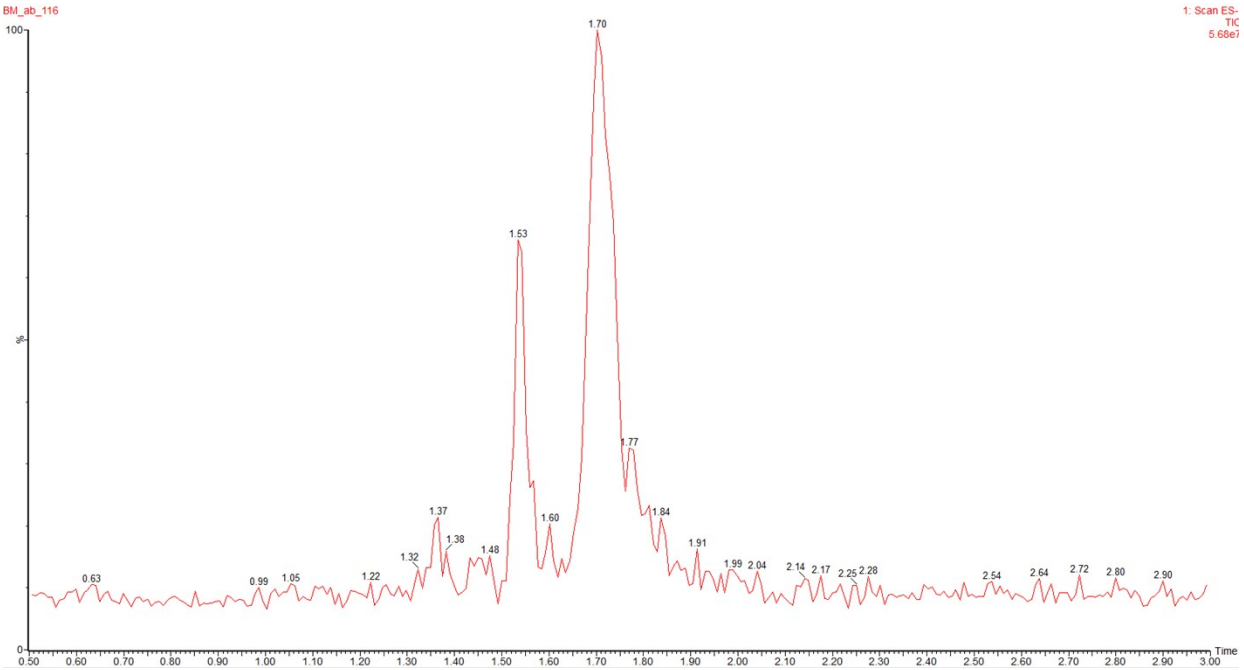


HP-4 (10 nmol, 1.0 equiv)
Molecular Weight: 5393.77

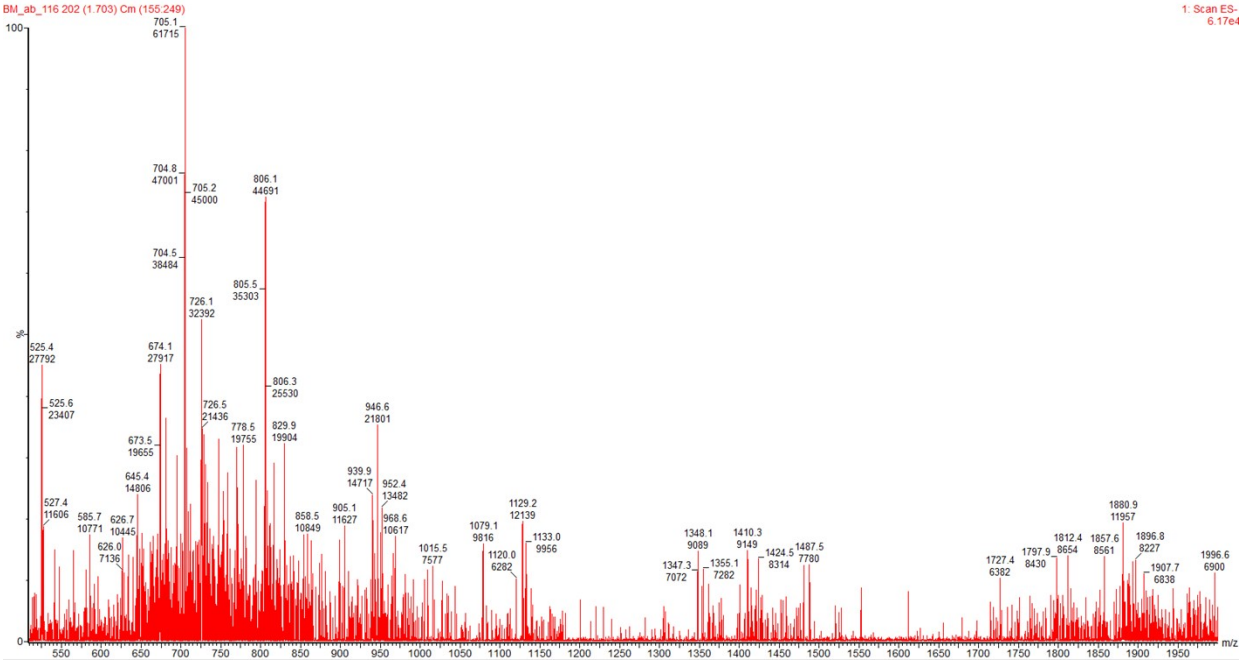
(100 equiv)

15, 66%
Molecular Weight: 5643.03

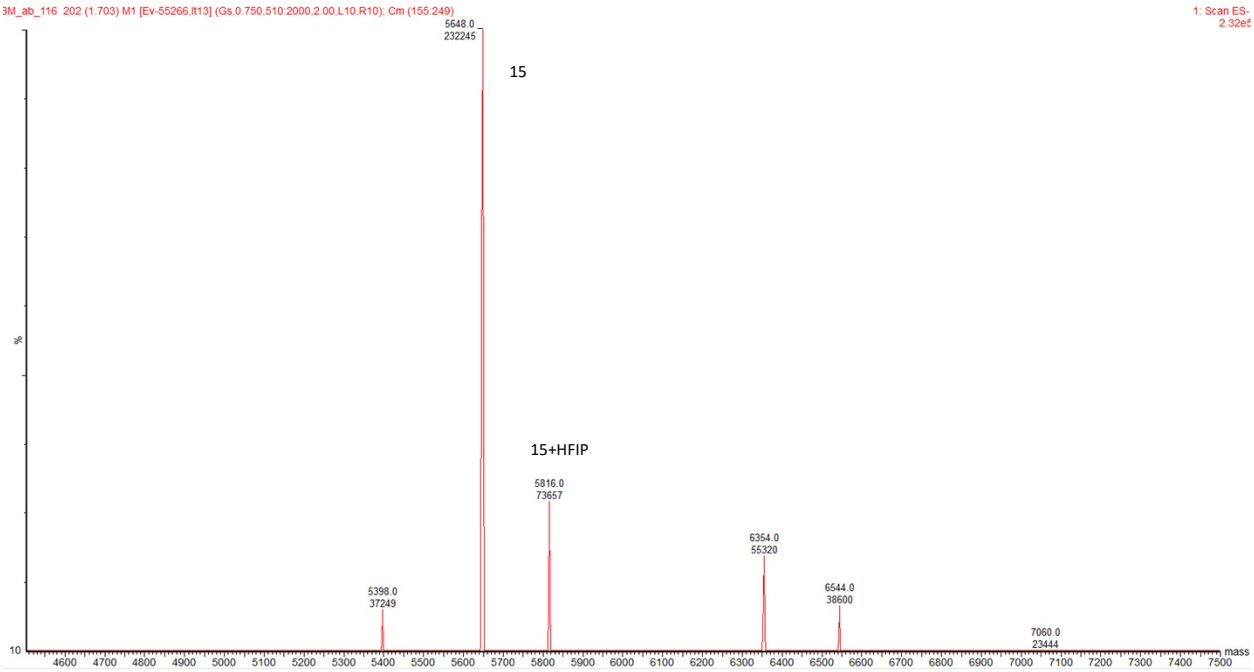
The title compound **15** was synthesized according to **GP-IV** from ethyl 5-(4-methoxyphenyl)-4,5-dihydroisoxazole-3-carboxylate and the DNA-tagged alkene **HP-4**. The product was formed with 66% conversion (8% recovered **HP-4**).



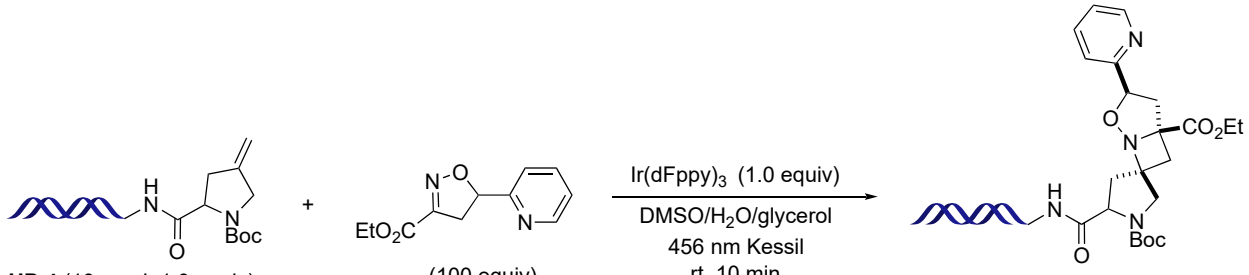
TIC of compound 15.



Raw MS of compound 15.



Deconvoluted MS of 15 (mass signals >10% of peak height).

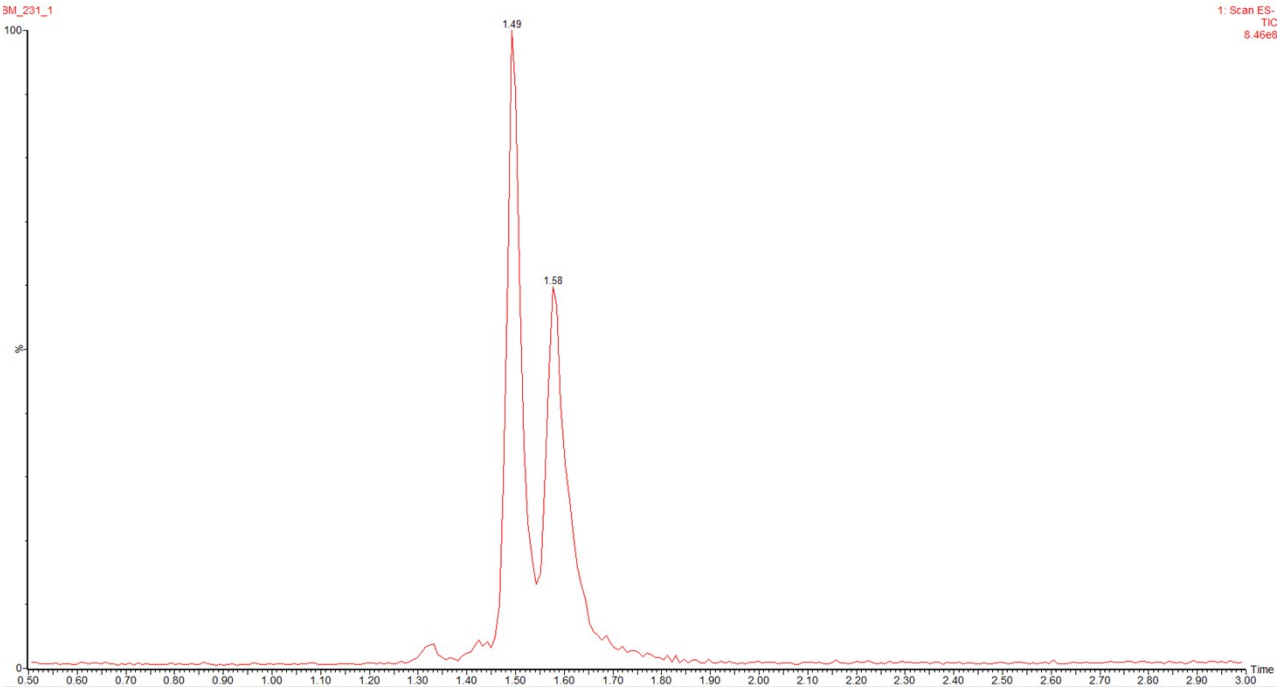


HP-4 (10 nmol, 1.0 equiv)
Molecular Weight: 5393.77

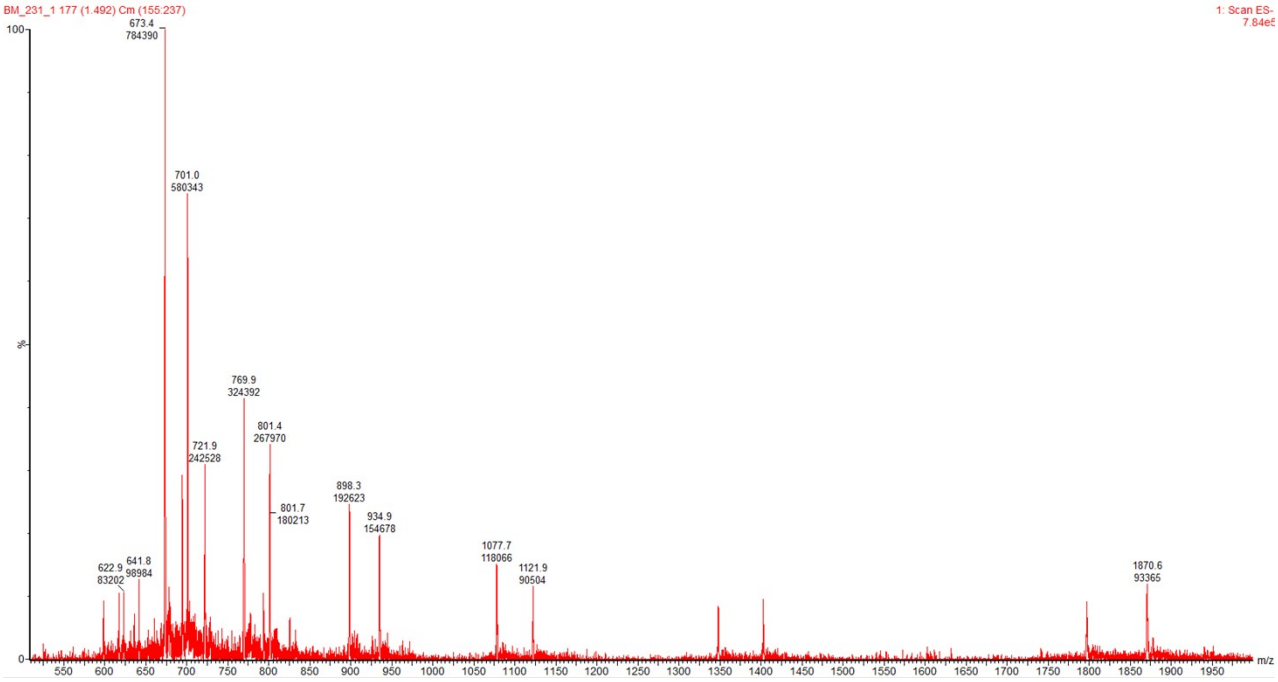
(100 equiv)

16, 43%
Molecular Weight: 5613.99

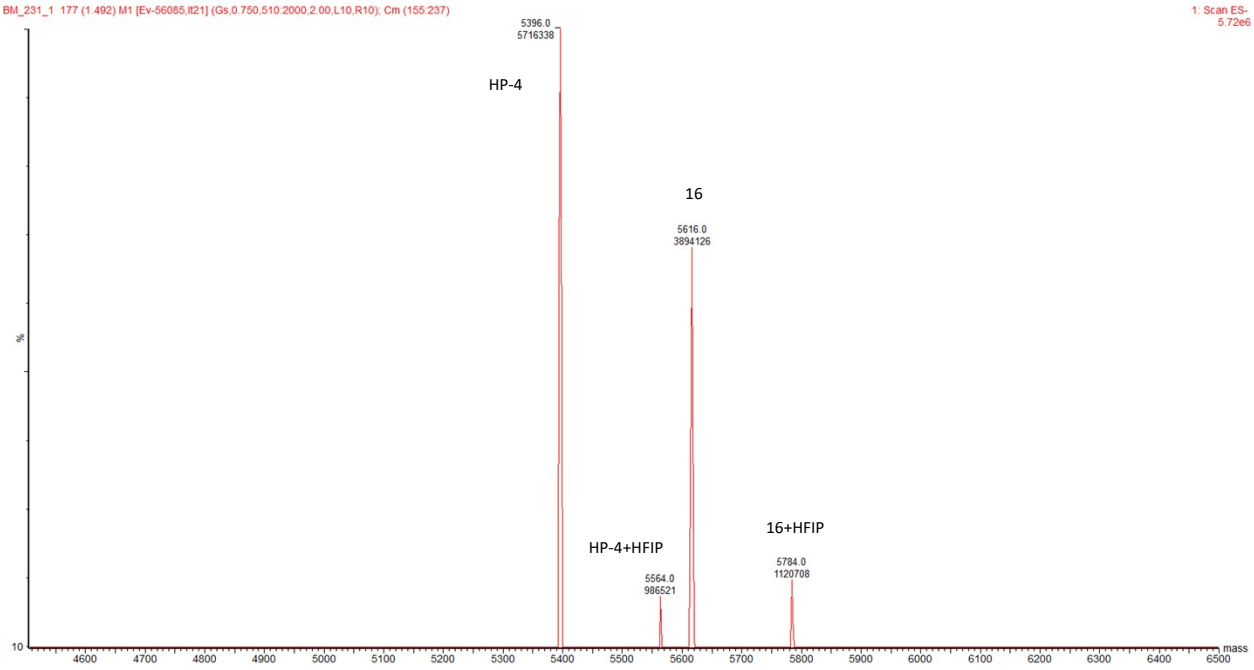
The title compound **16** was synthesized according to **GP-IV** from ethyl 5-(pyridin-2-yl)-4,5-dihydroisoxazole-3-carboxylate and the DNA-tagged alkene **HP-4**. The product was formed with 43% conversion (57% recovered **HP-4**).



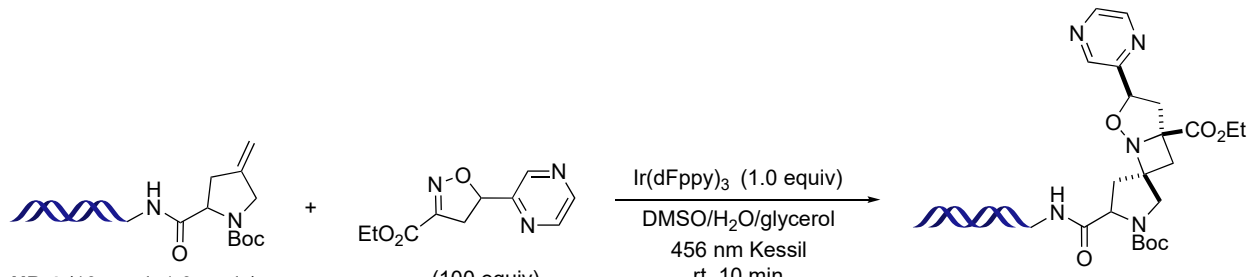
TIC of compound 16.



Raw MS of compound 16.



Deconvoluted MS of 16 (mass signals >10% of peak height).

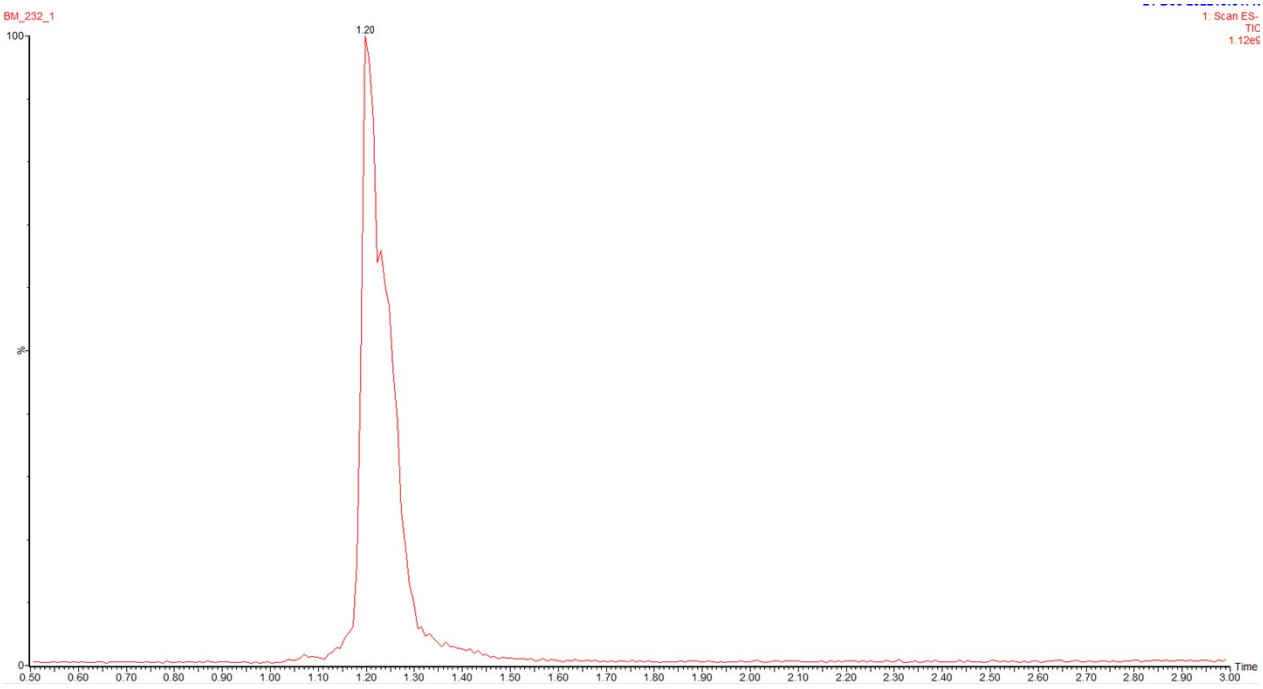


HP-4 (10 nmol, 1.0 equiv)
Molecular Weight: 5393.77

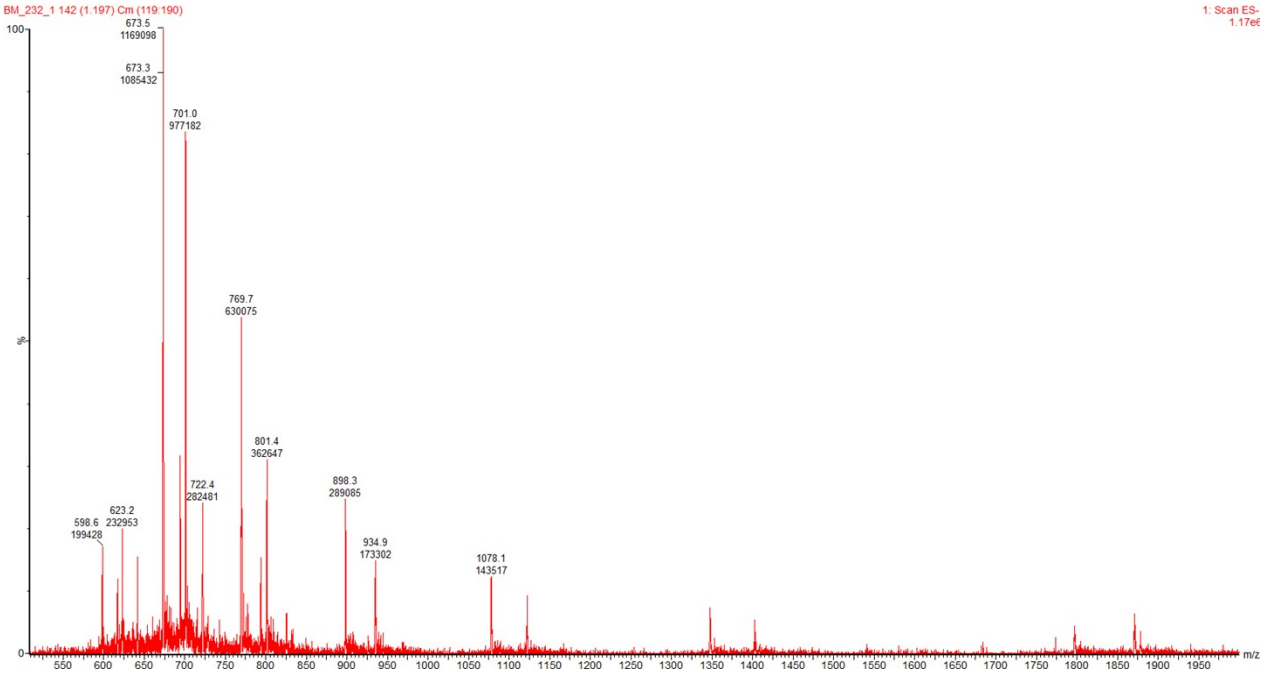
(100 equiv)

17, 35%
Molecular Weight: 5614.98

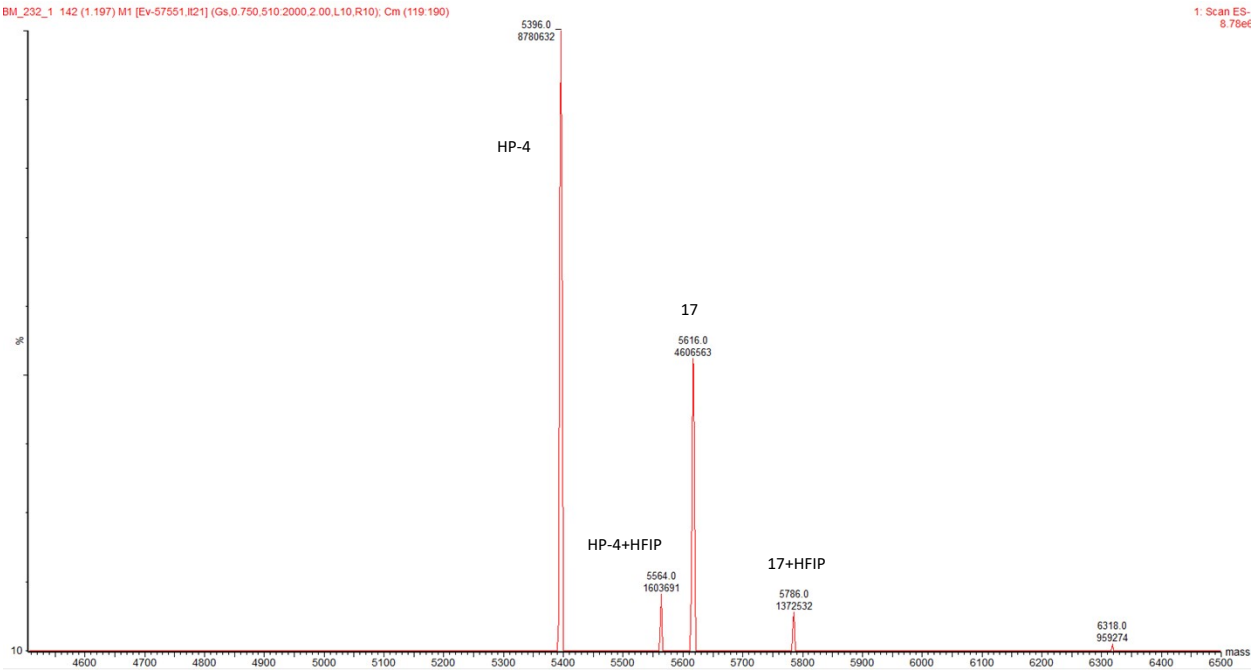
The title compound **17** was synthesized according to **GP-IV** from ethyl 5-(pyrazin-2-yl)-4,5-dihydroisoxazole-3-carboxylate and the DNA-tagged alkene **HP-4**. The product was formed with 35% conversion (60% recovered **HP-4**).



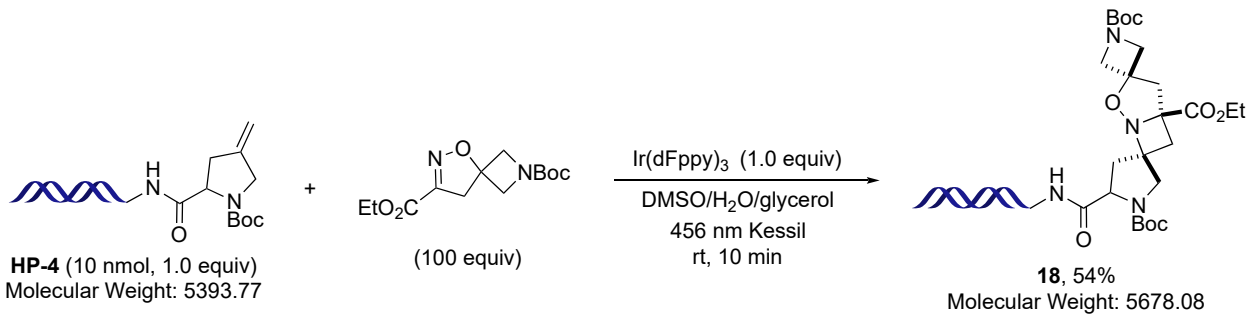
TIC of compound 17.



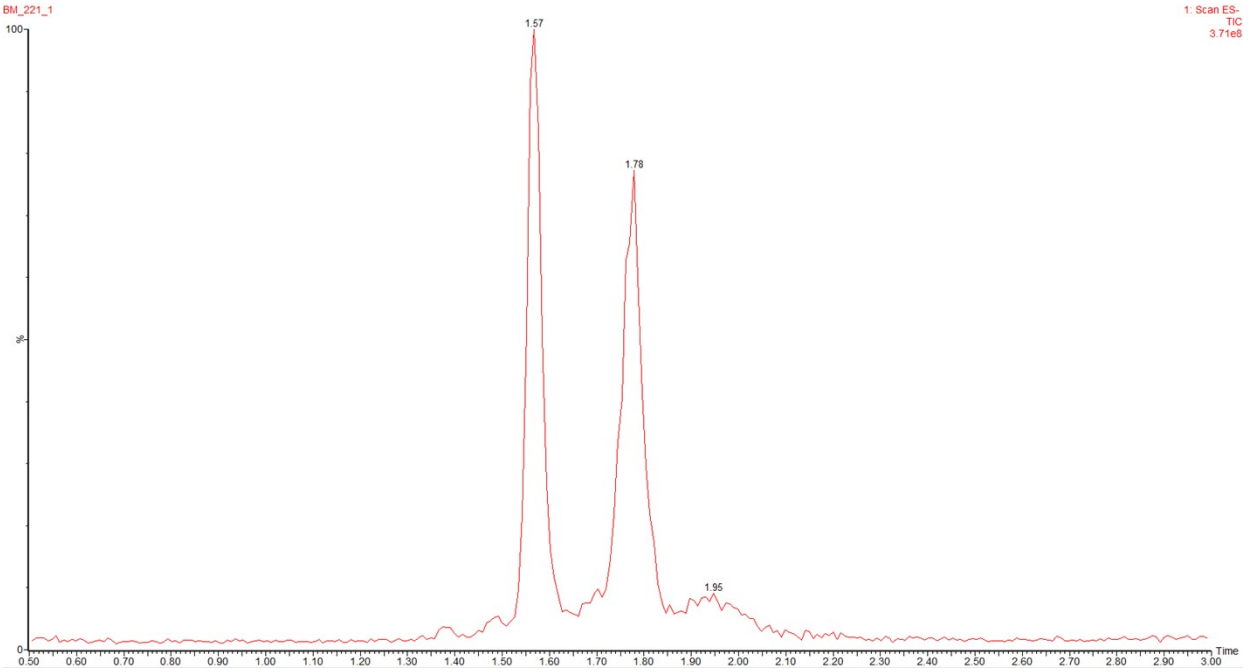
Raw MS of compound 17.



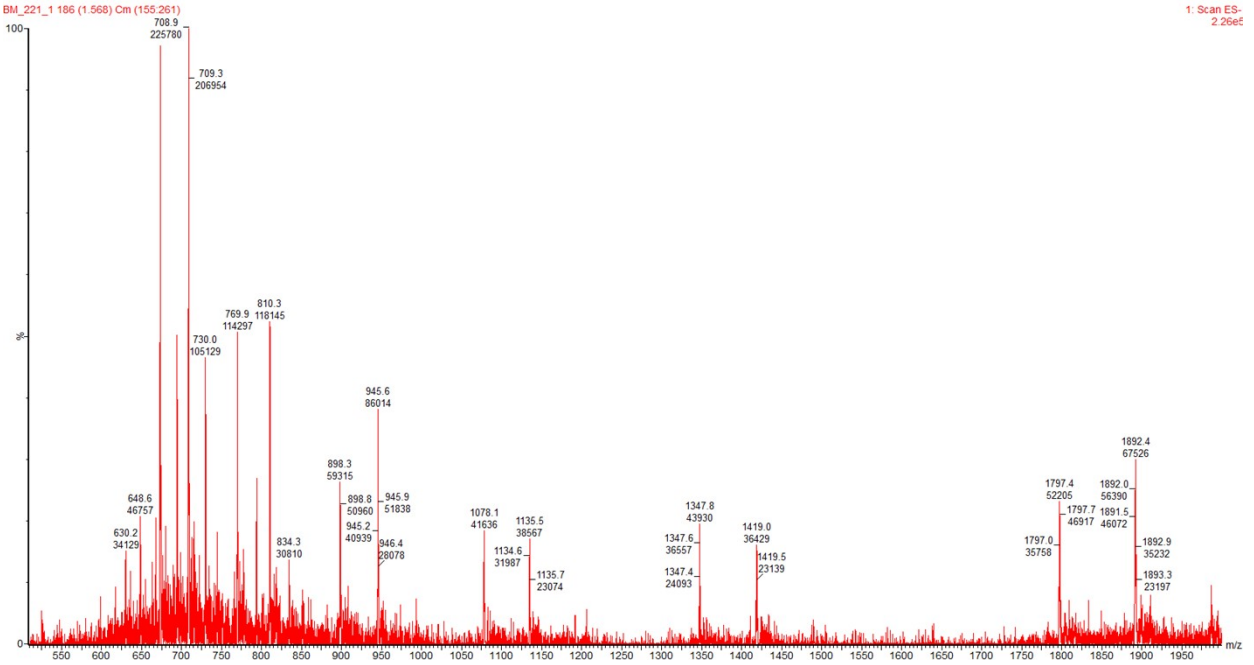
Deconvoluted MS of 17 (mass signals >10% of peak height).



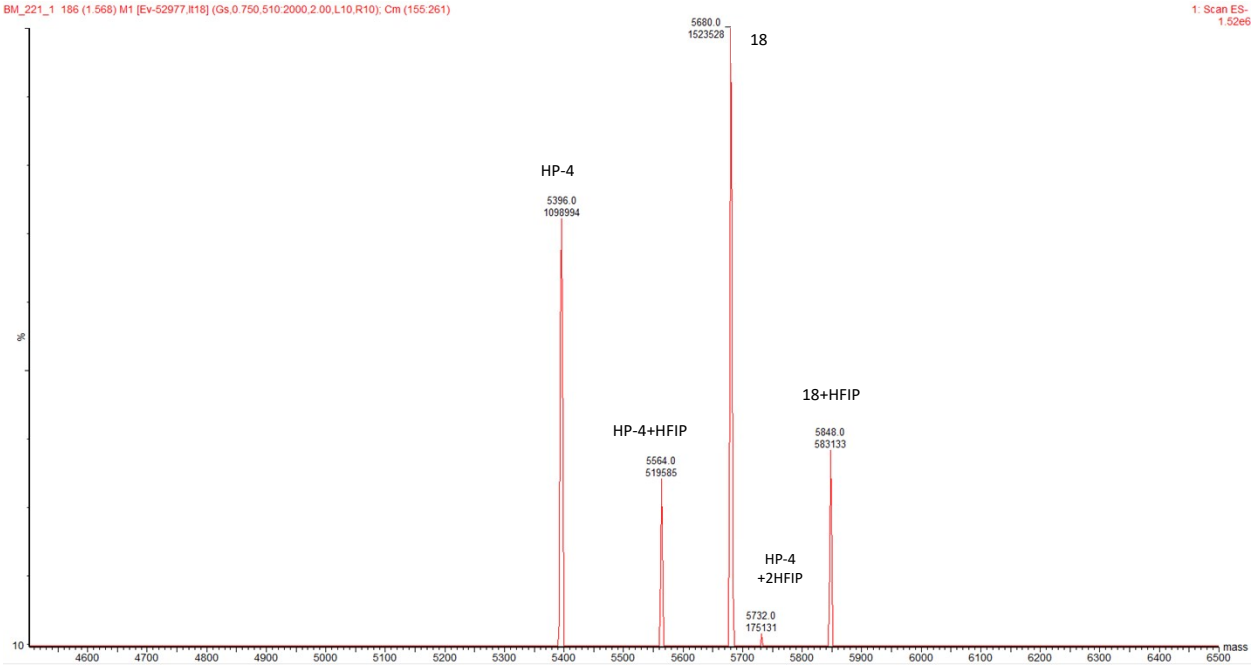
The title compound **18** was synthesized according to **GP-IV** from 8-(*tert*-butyl) 3-ethyl 1-oxa-2,8-diazaspiro[4.5]dec-2-ene-3,8-dicarboxylate and the DNA-tagged alkene **HP-4**. The product was formed with 54% conversion (46% recovered **HP-4**).



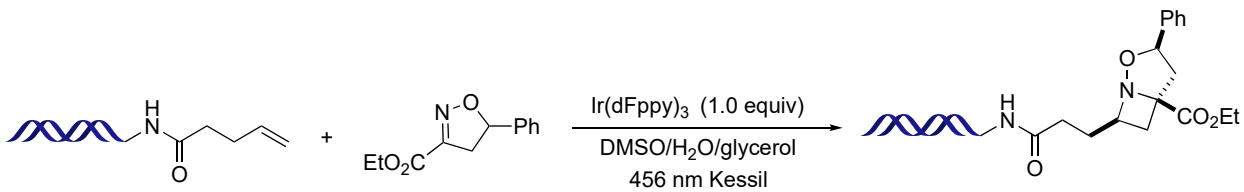
TIC of compound 18.



Raw MS of compound 18.



Deconvoluted MS of 18 (mass signals >10% of peak height).

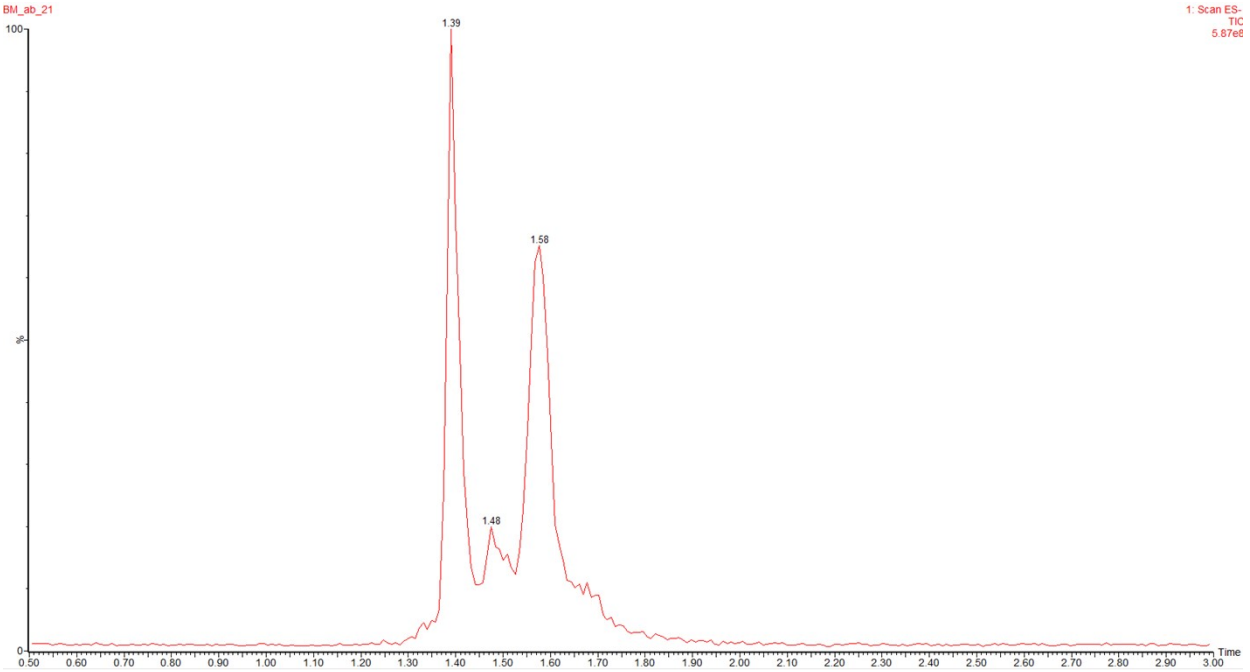


HP-5 (10 nmol, 1.0 equiv)
Molecular Weight: 5266.62

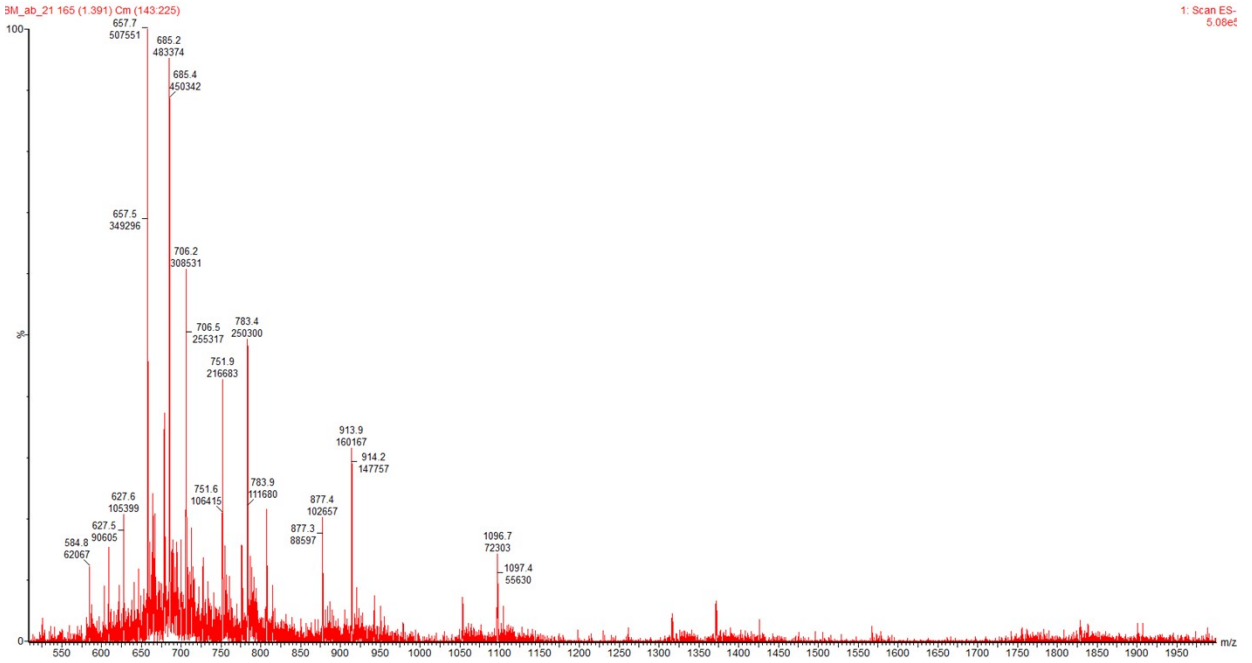
(100 equiv)

19, 61%
Molecular Weight: 5485.86

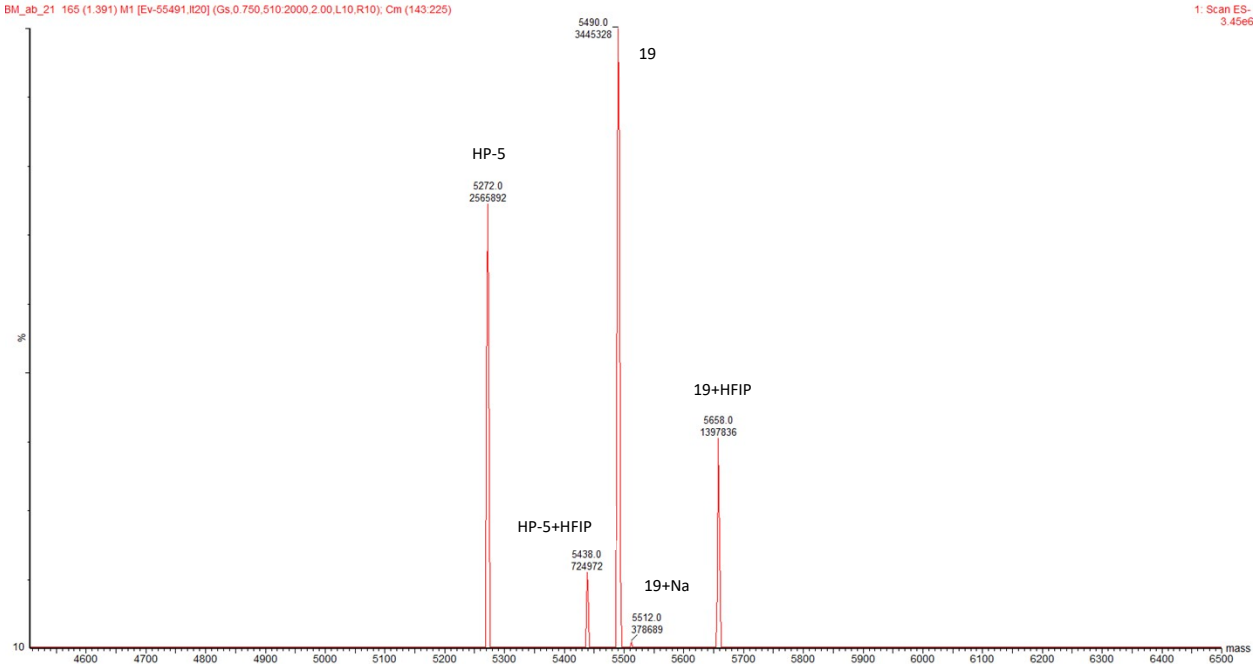
The title compound **19** was synthesized according to **GP-IV** from ethyl 5-phenyl-4,5-dihydroisoxazole-3-carboxylate and the DNA-tagged alkene **HP-5**. The product was formed with 61% conversion (39% recovered **HP-5**).



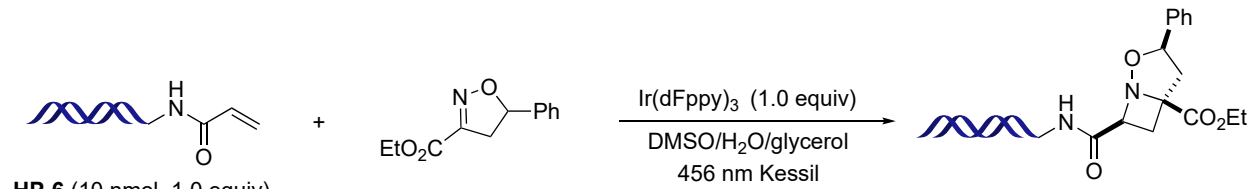
TIC of compound 19.



Raw MS of compound 19.



Deconvoluted MS of 19 (mass signals >10% of peak height).

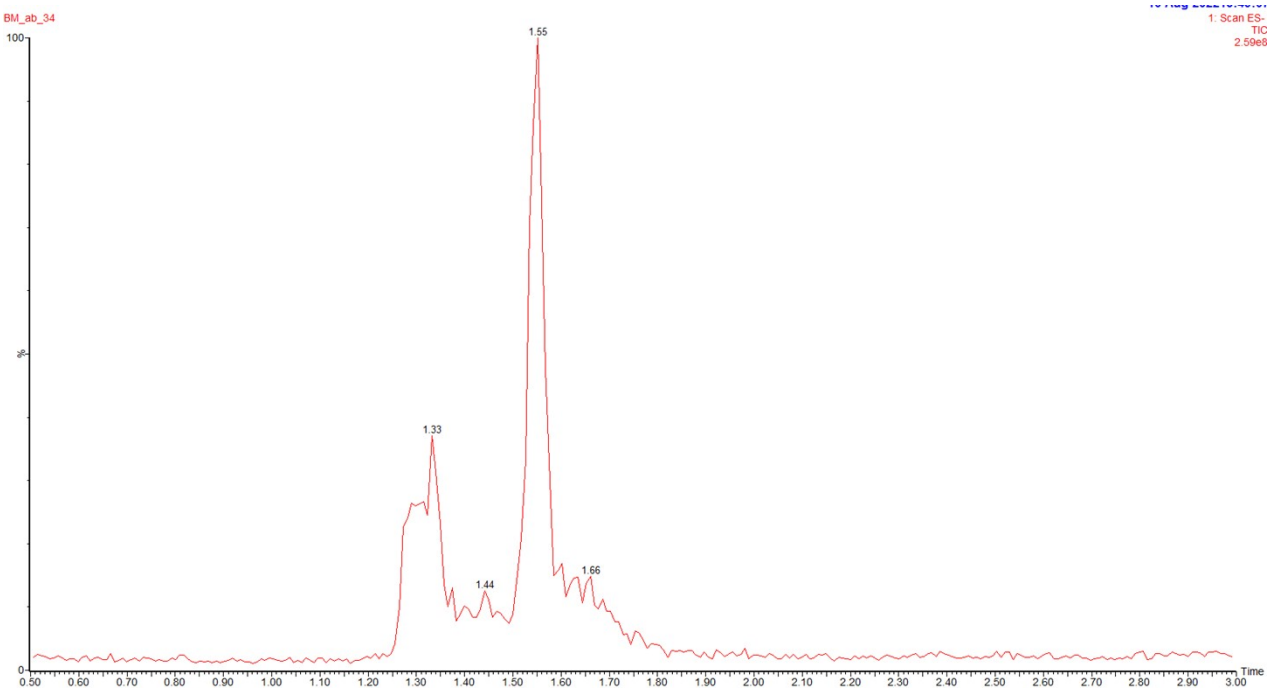


HP-6 (10 nmol, 1.0 equiv)
Molecular Weight: 5238.57

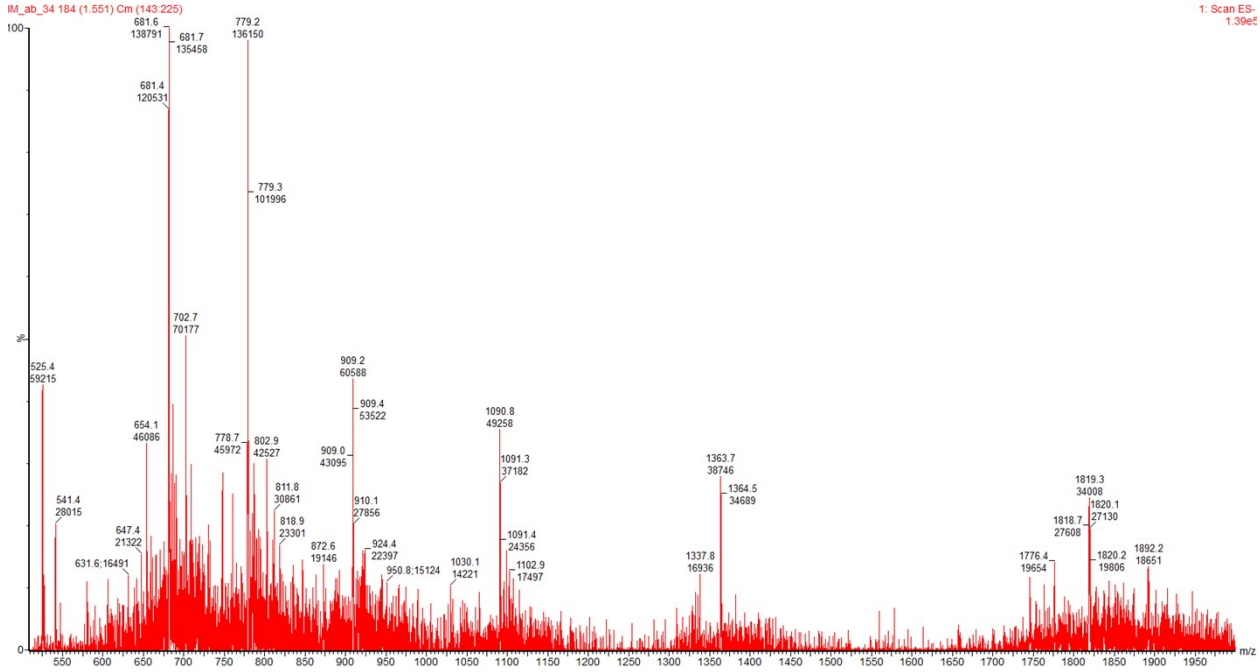
(100 equiv)

20, 64%
Molecular Weight: 5457.81

The title compound **20** was synthesized according to **GP-IV** from ethyl 5-phenyl-4,5-dihydroisoxazole-3-carboxylate and the DNA-tagged alkene **HP-6**. The product was formed with 64% conversion (9% recovered **HP-6**; 8% double addition product).

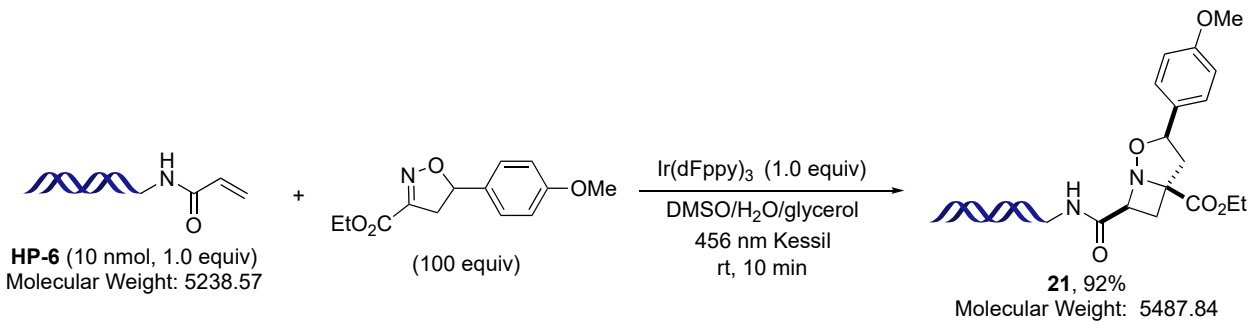


TIC of compound 20.

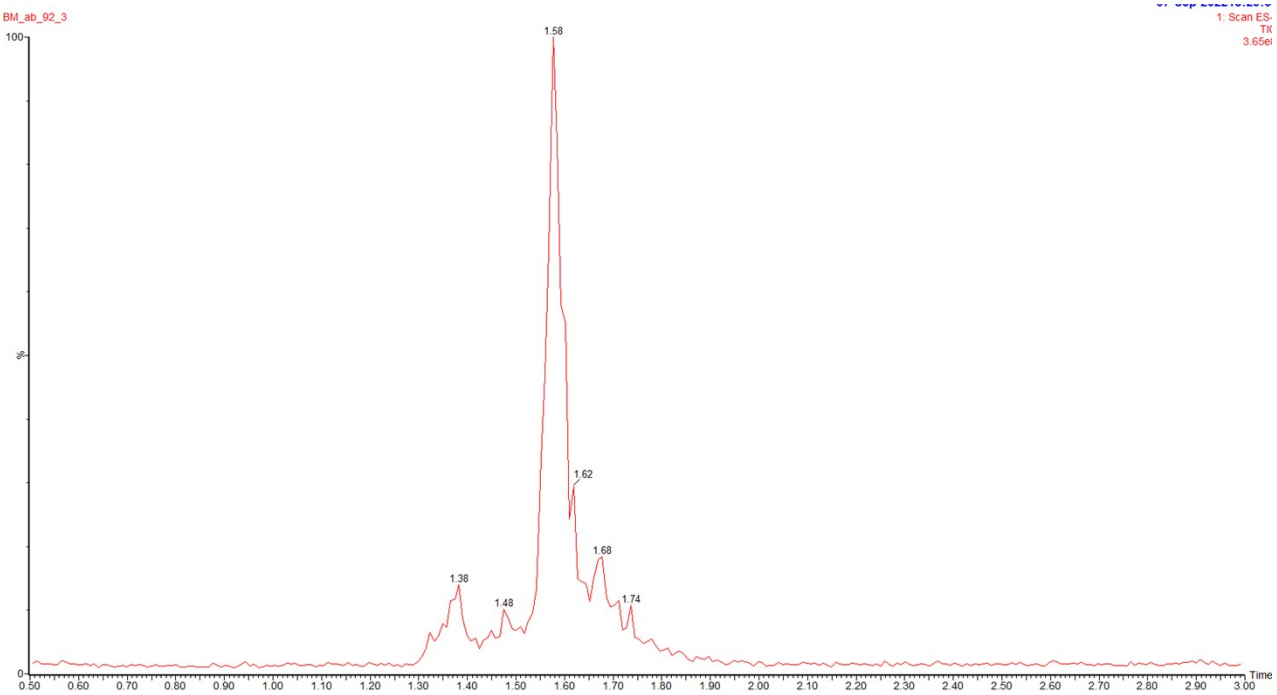


Raw MS of compound 20.

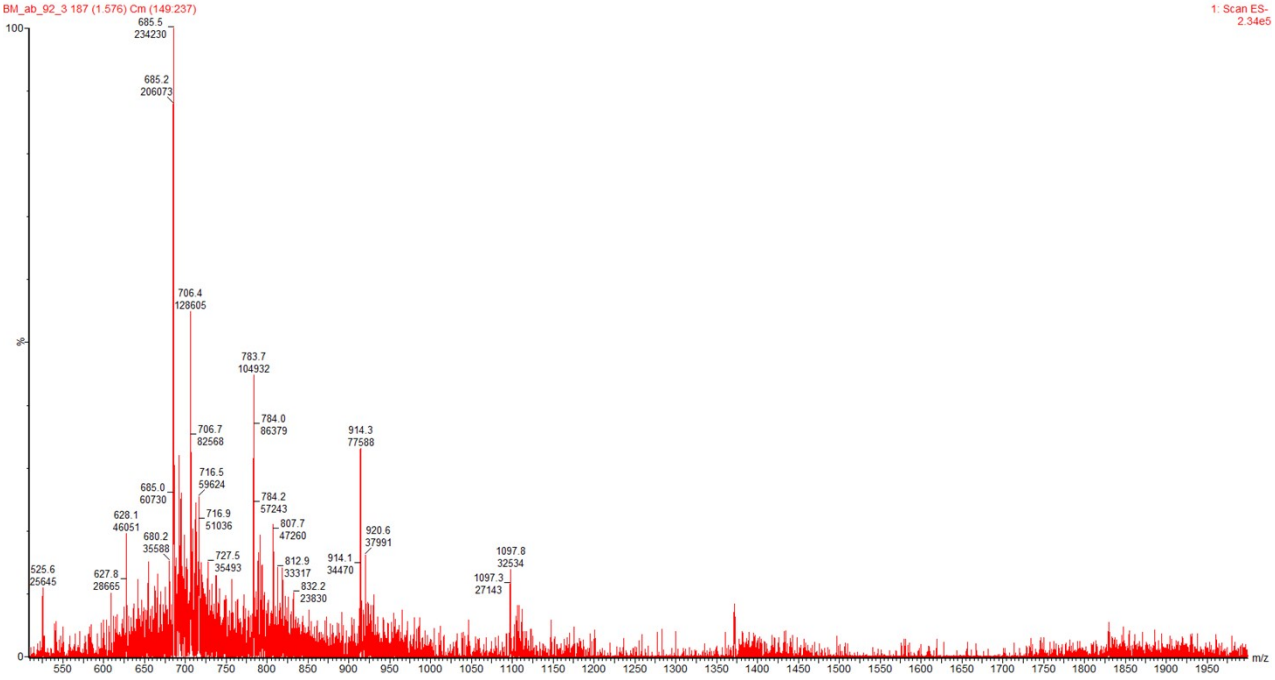
Deconvoluted MS of 20 (mass signals >10% of peak height).



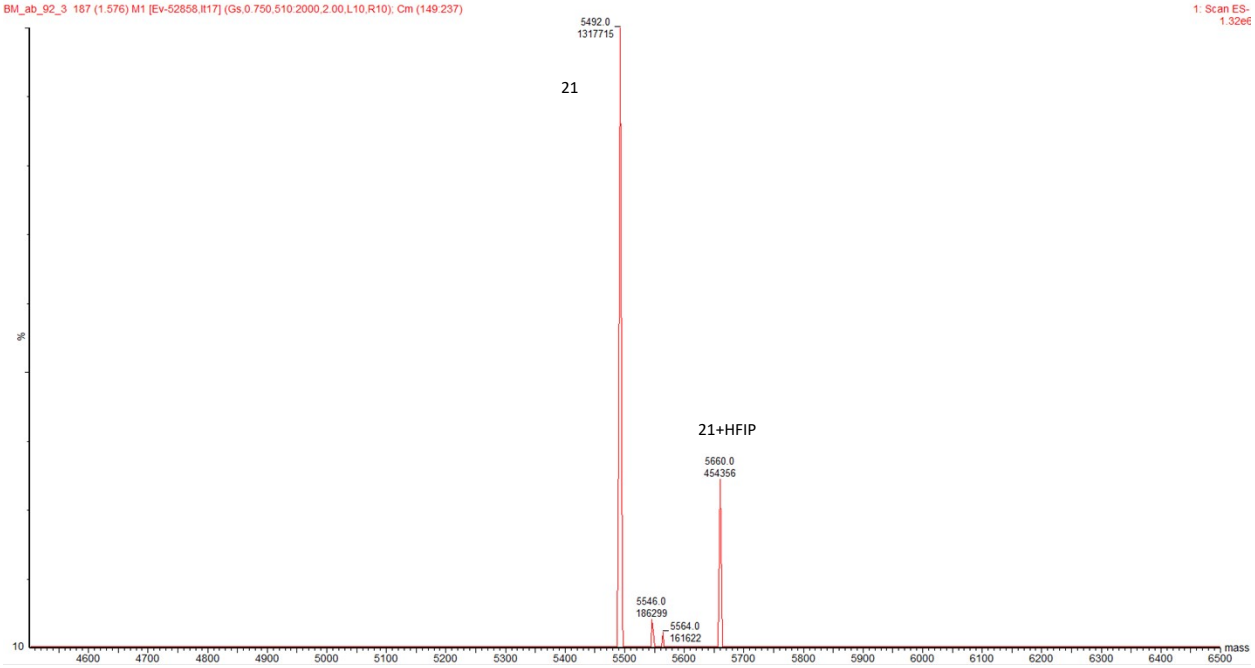
The title compound **21** was synthesized according to **GP-IV** from ethyl 5-(4-methoxyphenyl)-4,5-dihydroisoxazole-3-carboxylate and the DNA-tagged alkene **HP-6**. The product was formed with 92% conversion.



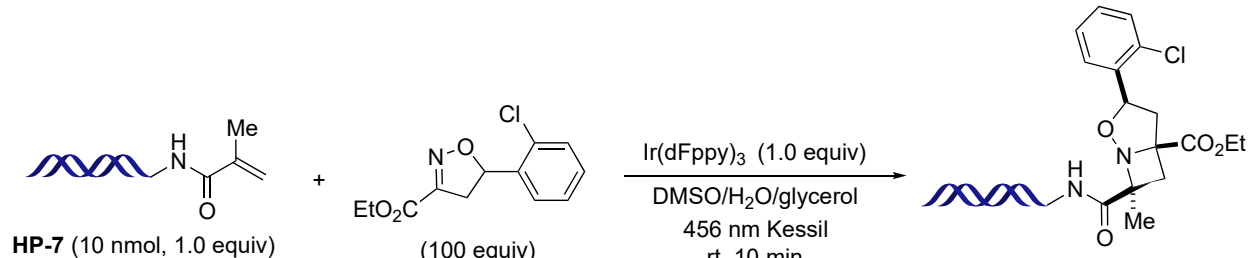
TIC of compound 21.



Raw MS of compound 21.



Deconvoluted MS of 21 (mass signals >10% of peak height).

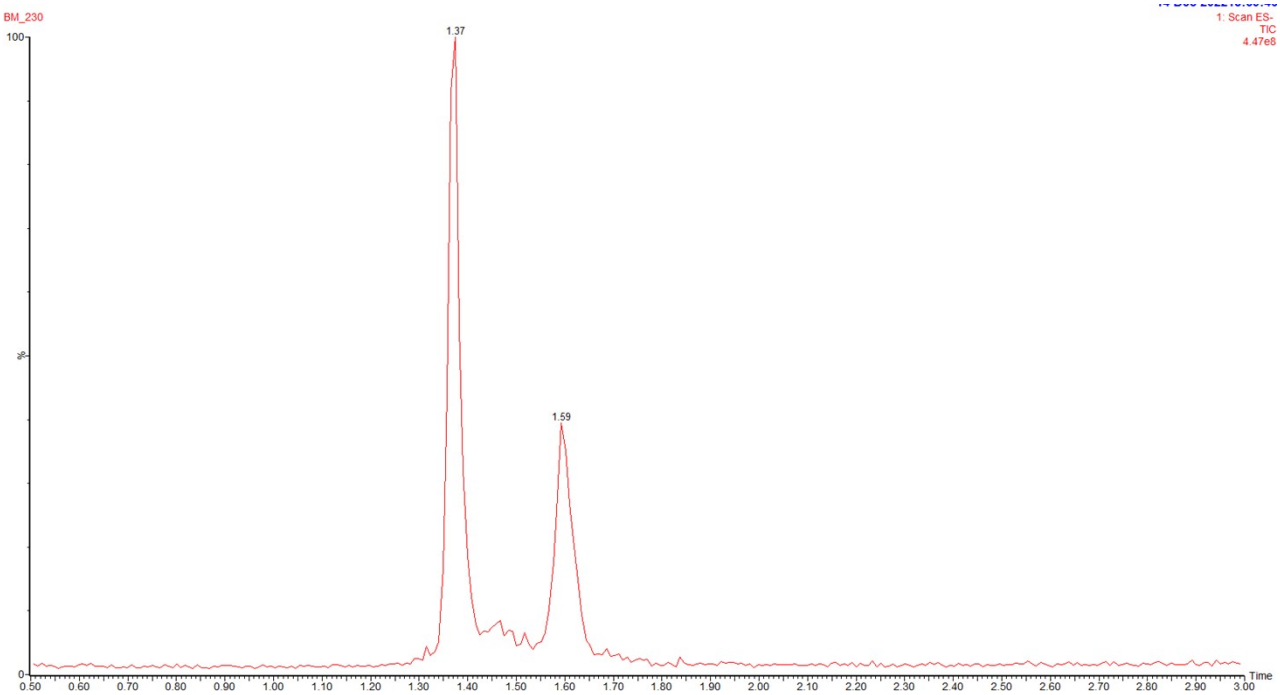


HP-7 (10 nmol, 1.0 equiv)
Molecular Weight: 5252.60

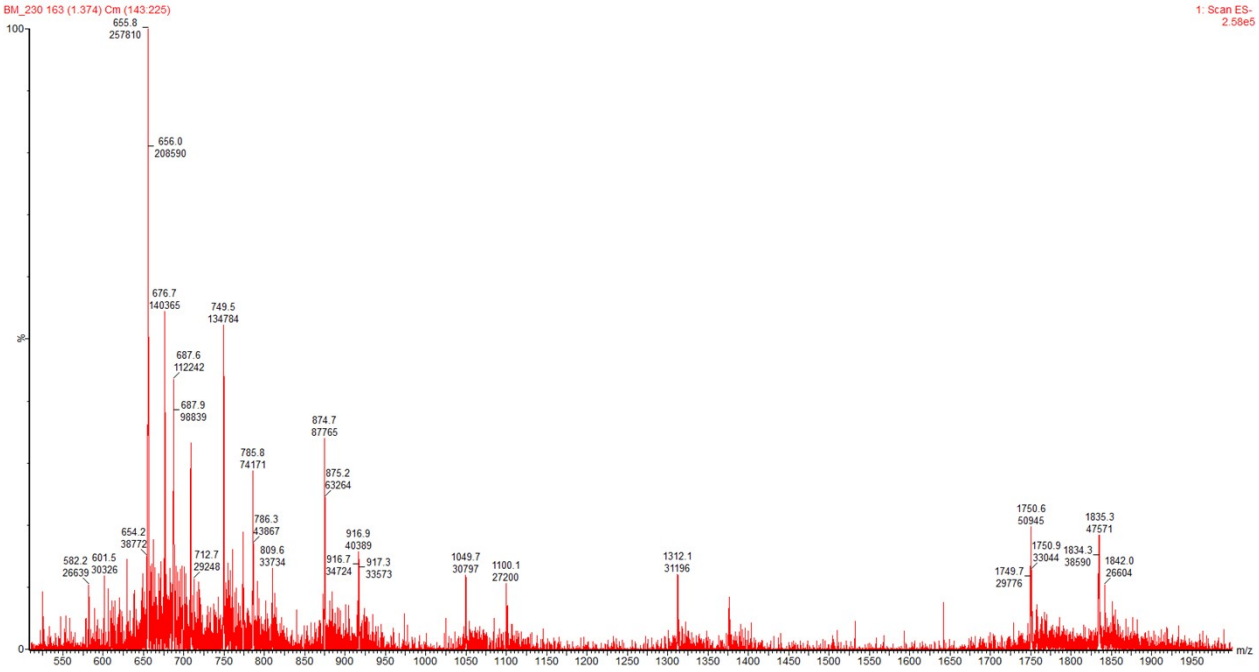
(100 equiv)

22, 39%
Molecular Weight: 5506.28

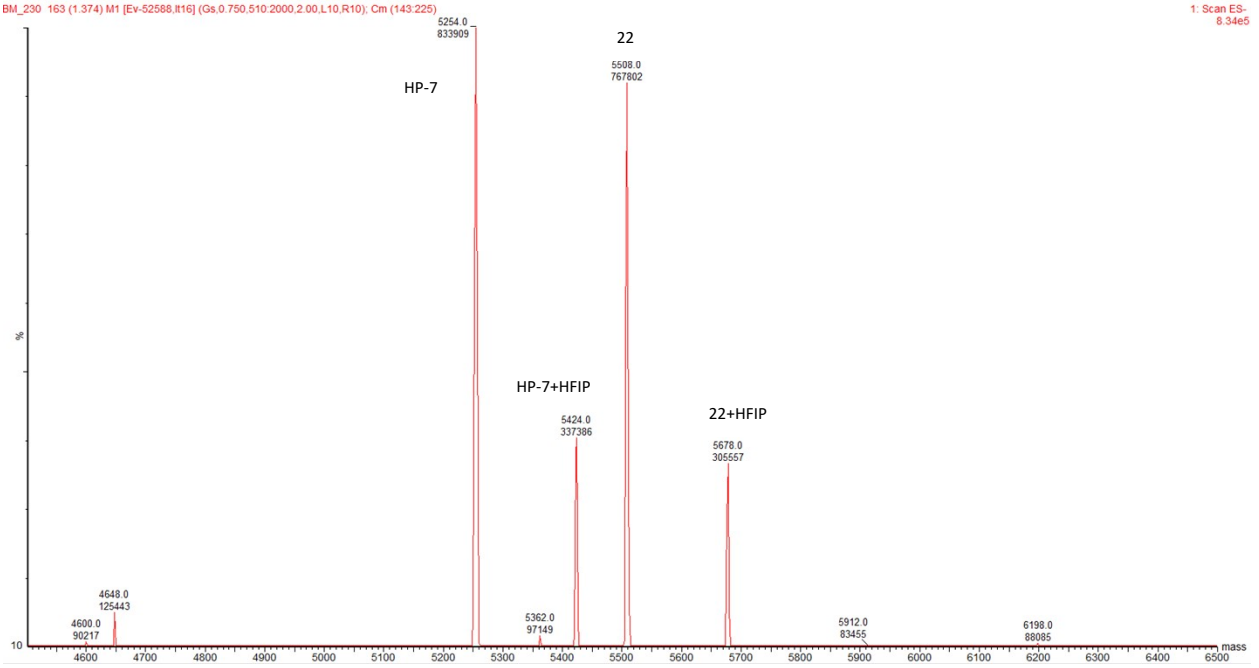
The title compound **22** was synthesized according to **GP-IV** from ethyl 5-(2-chlorophenyl)-4,5-dihydroisoxazole-3-carboxylate and the DNA-tagged alkene **HP-7**. The product was formed with 39% conversion (43% recovered **HP-7**).



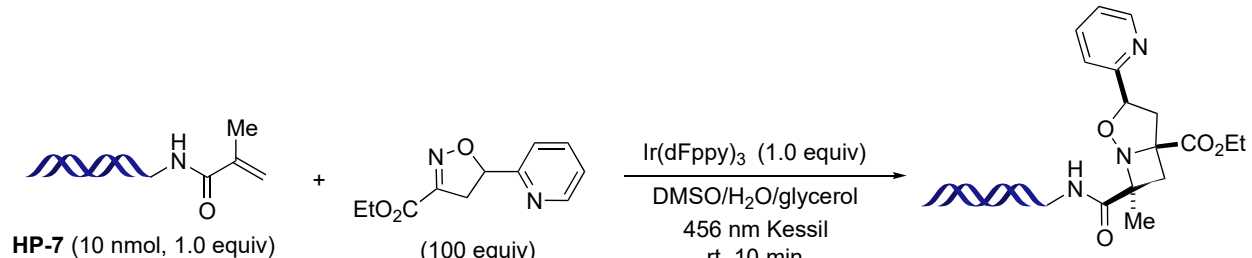
TIC of compound 22.



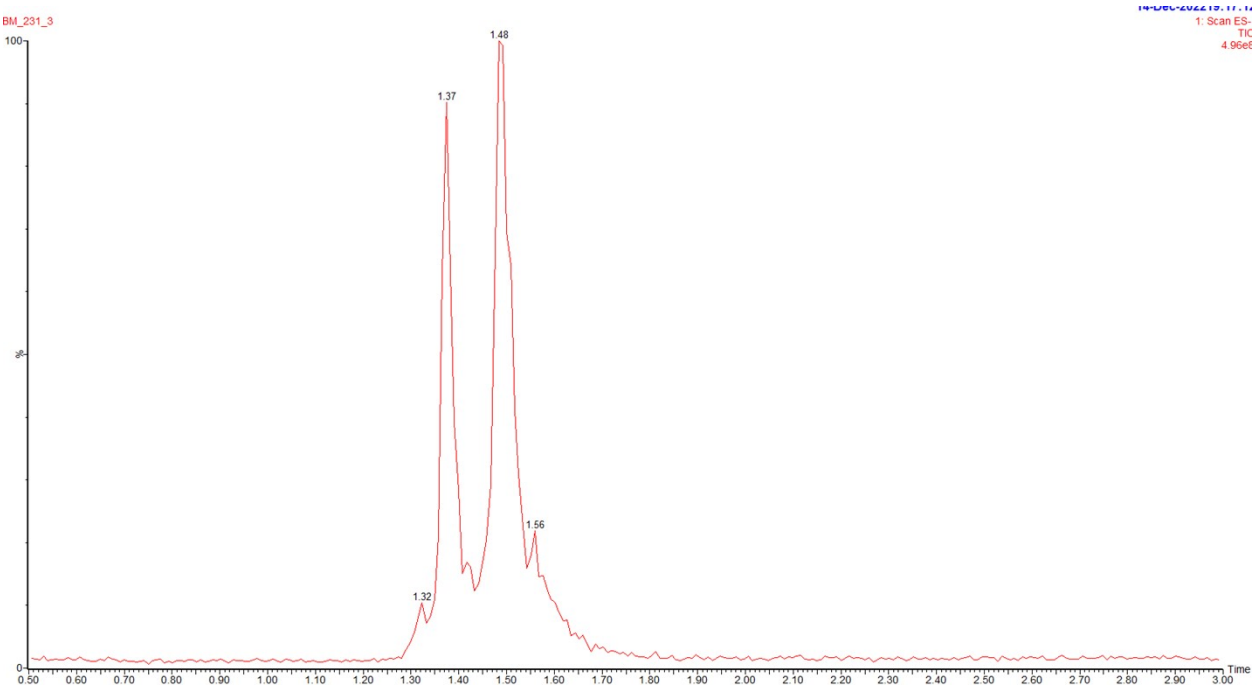
Raw MS of compound 22.



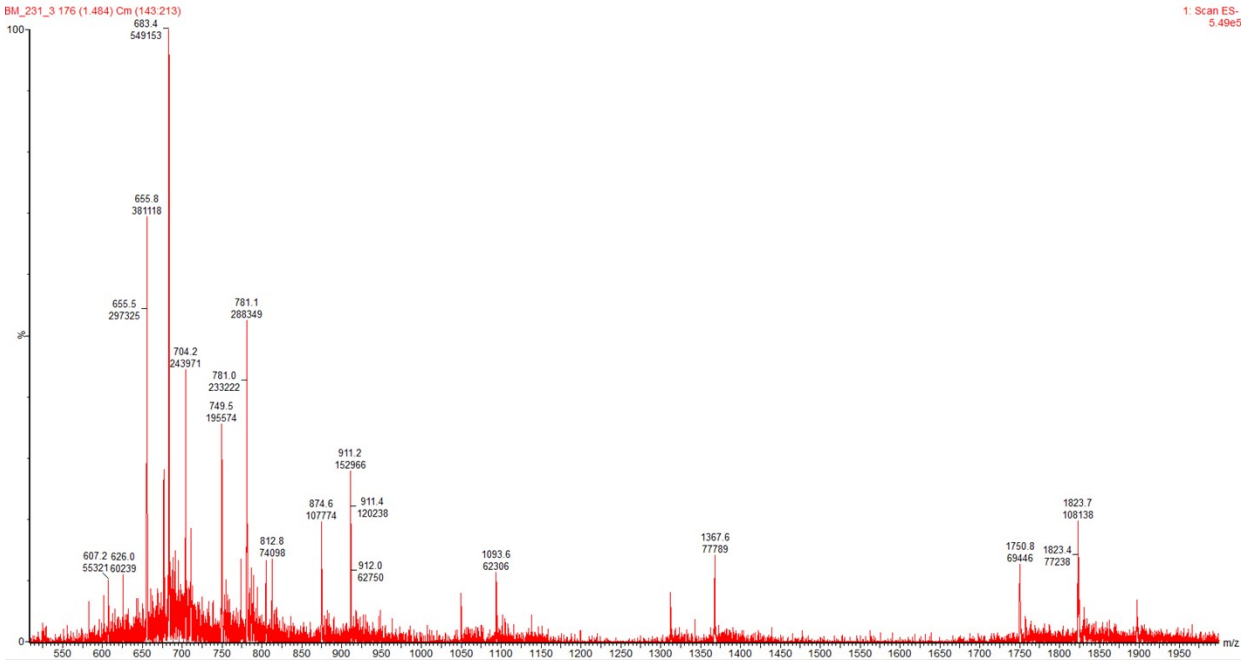
Deconvoluted MS of 22 (mass signals >10% of peak height).



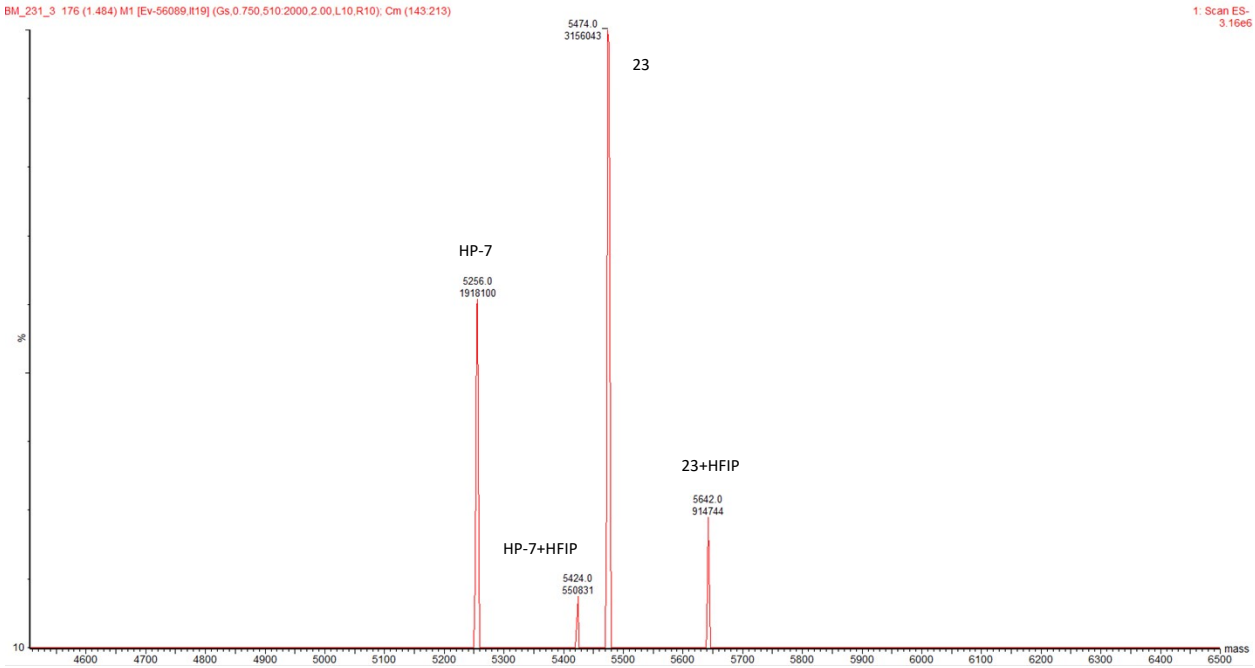
The title compound **23** was synthesized according to **GP-IV** from ethyl 5-(pyridin-2-yl)-4,5-dihydroisoxazole-3-carboxylate and the DNA-tagged alkene **HP-7**. The product was formed with 62% conversion (38% recovered **HP-7**).



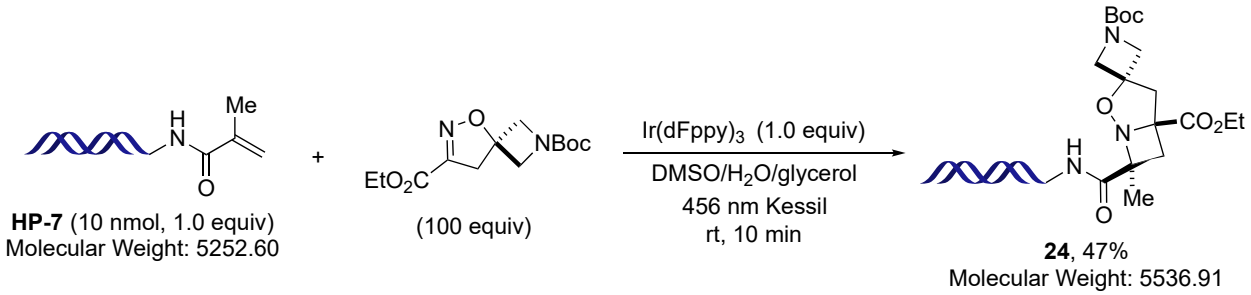
TIC of compound 23.



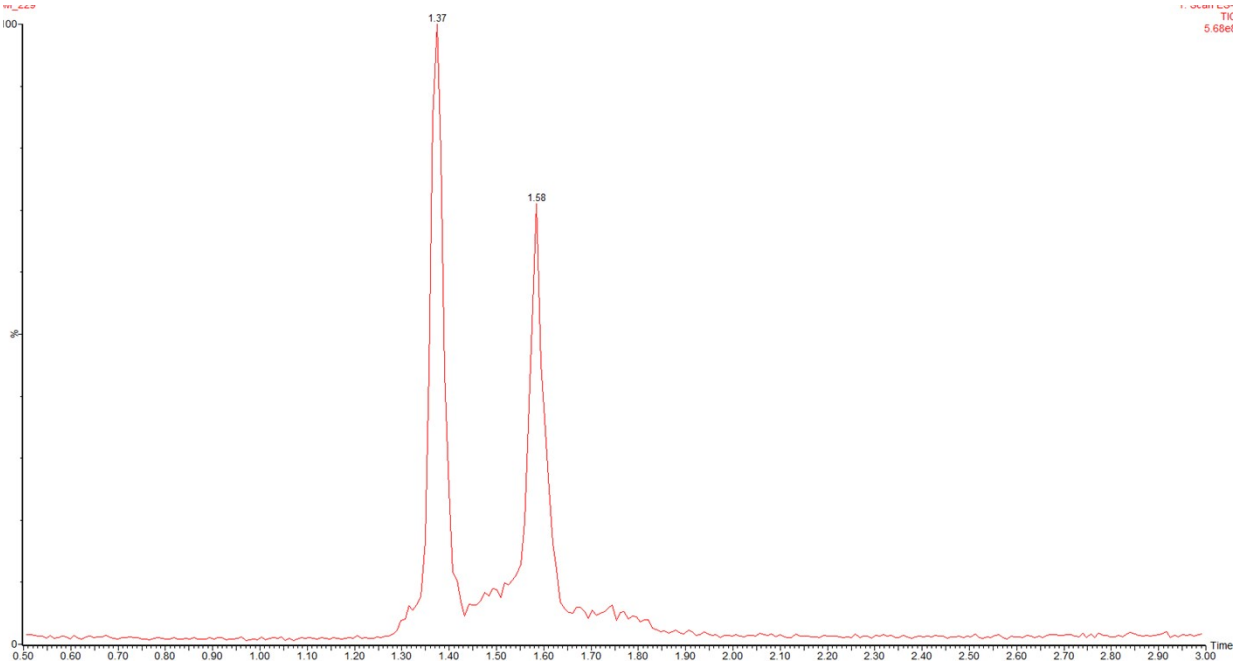
Raw MS of compound 23.



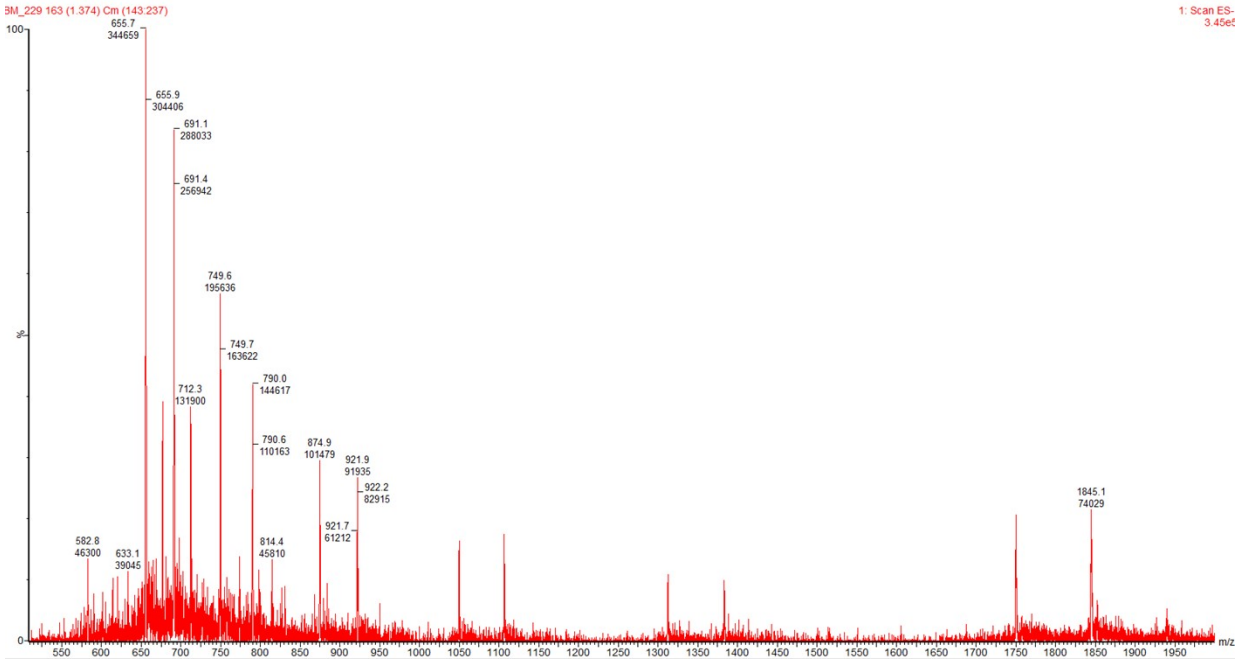
Deconvoluted MS of 23 (mass signals >10% of peak height).



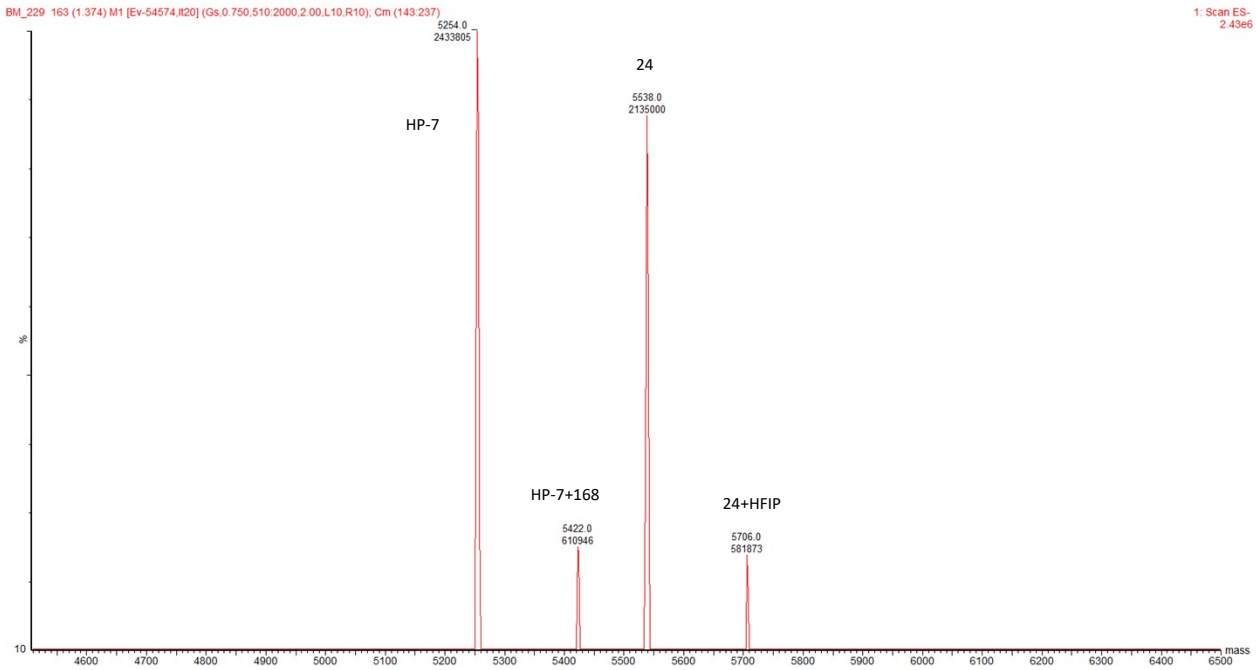
The title compound **24** was synthesized according to **GP-IV** from 2-(*tert*-butyl) 7-ethyl 5-oxa-2,6-diazaspiro[3.4]oct-6-ene-2,7-dicarboxylate and the DNA-tagged alkene **HP-7**. The product was formed with 47% conversion (53% recovered **HP-7**).



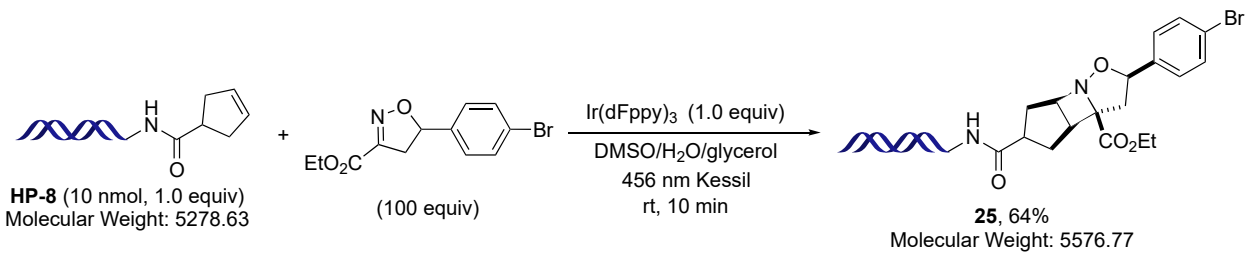
TIC of compound 24.



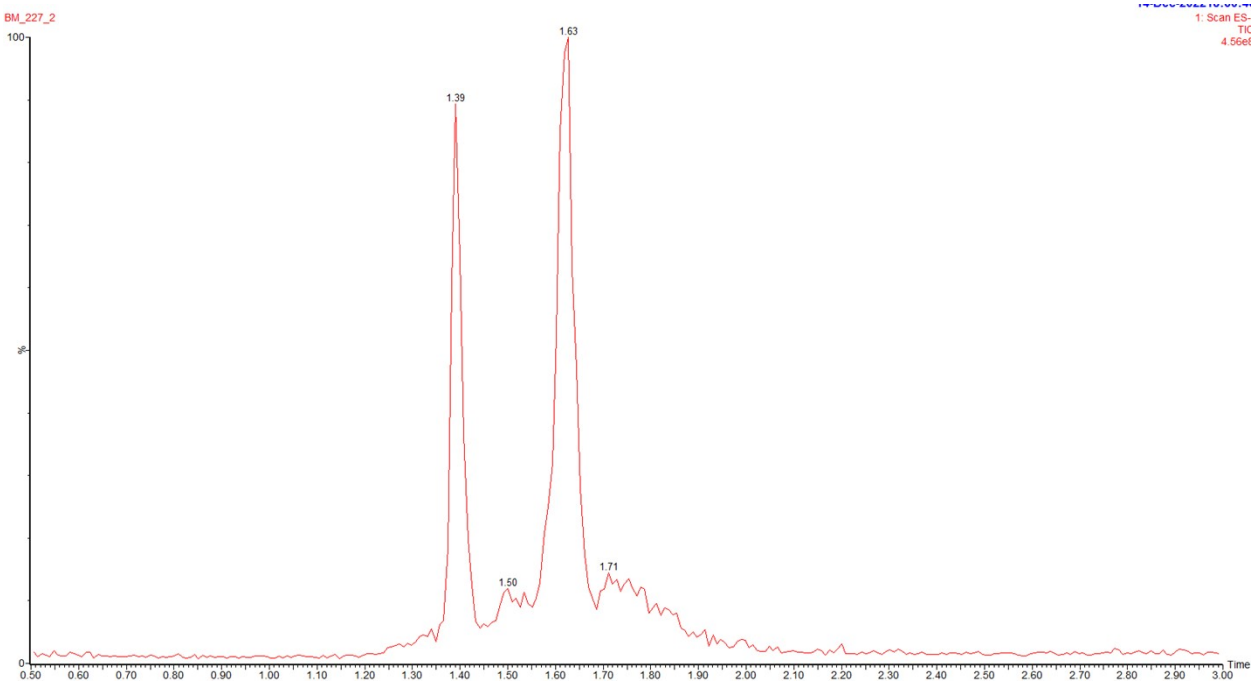
Raw MS of compound 24.



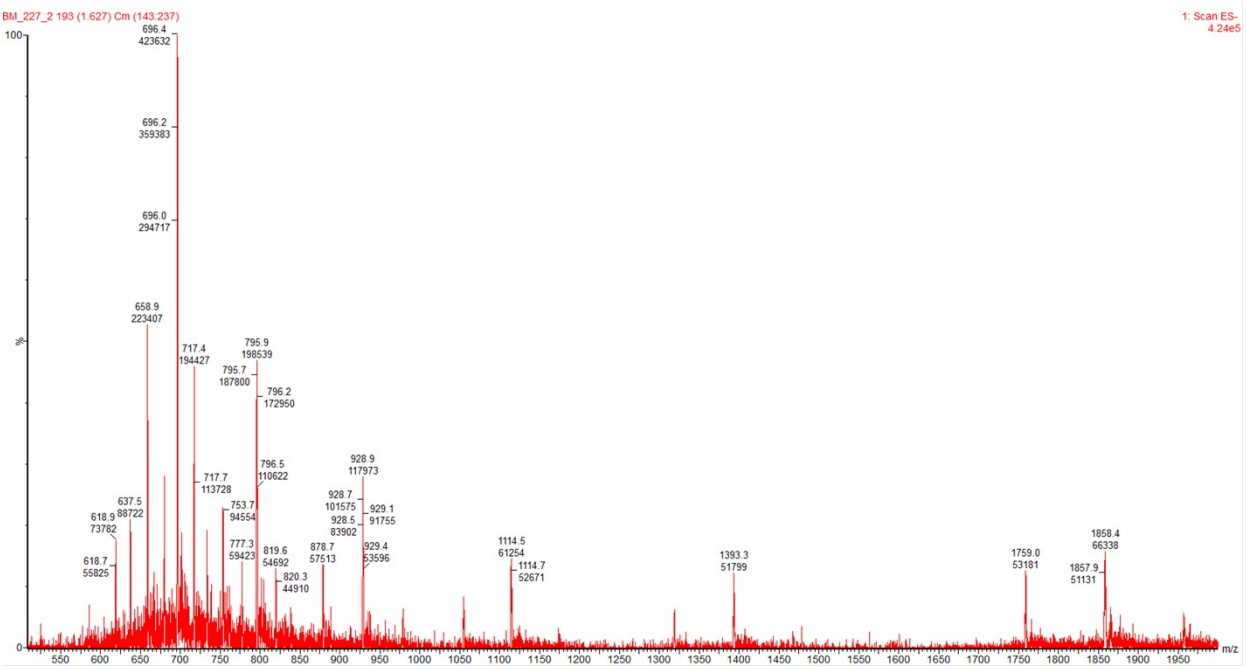
Deconvoluted MS of 24 (mass signals >10% of peak height).



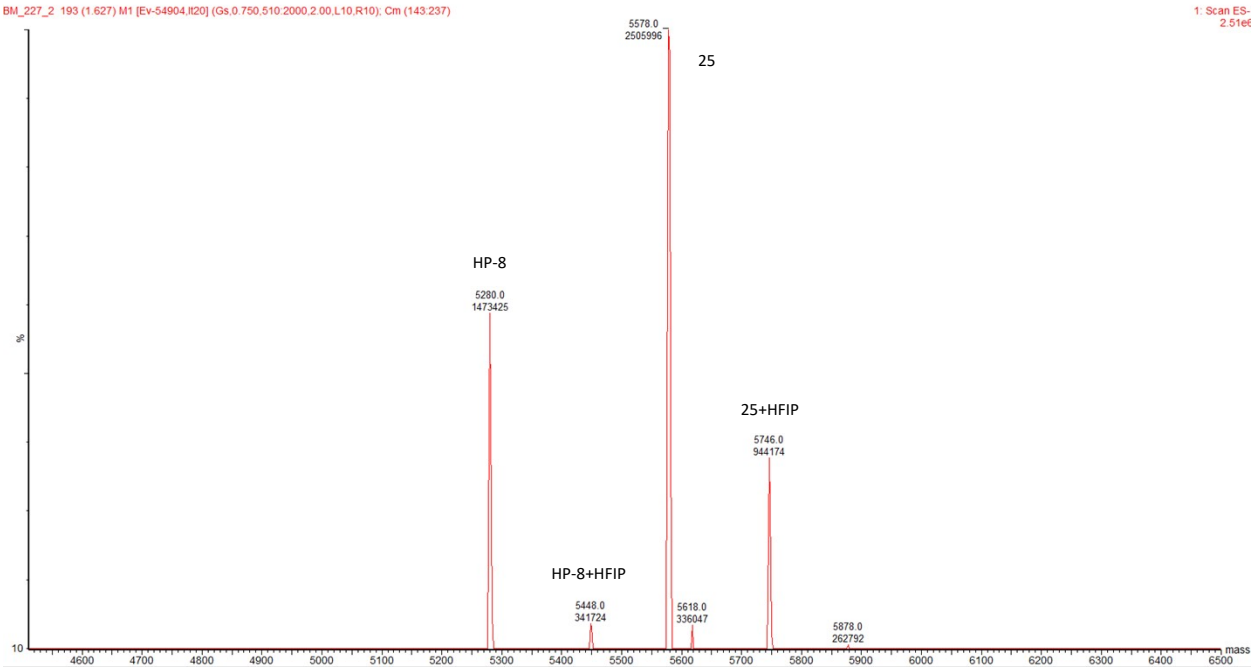
The title compound **25** was synthesized according to **GP-IV** from ethyl 5-(4-bromophenyl)-4,5-dihydroisoxazole-3-carboxylate and the DNA-tagged alkene **HP-8**. The product was formed with 64% conversion (31% recovered **HP-8**; 5% double addition product).



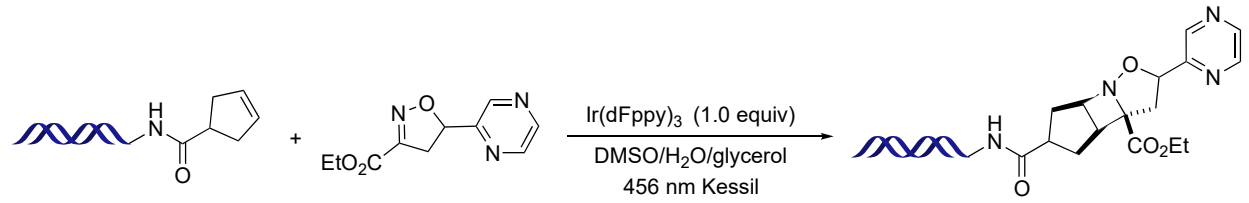
TIC of compound 25.



Raw MS of compound 25.



Deconvoluted MS of 25 (mass signals >10% of peak height).

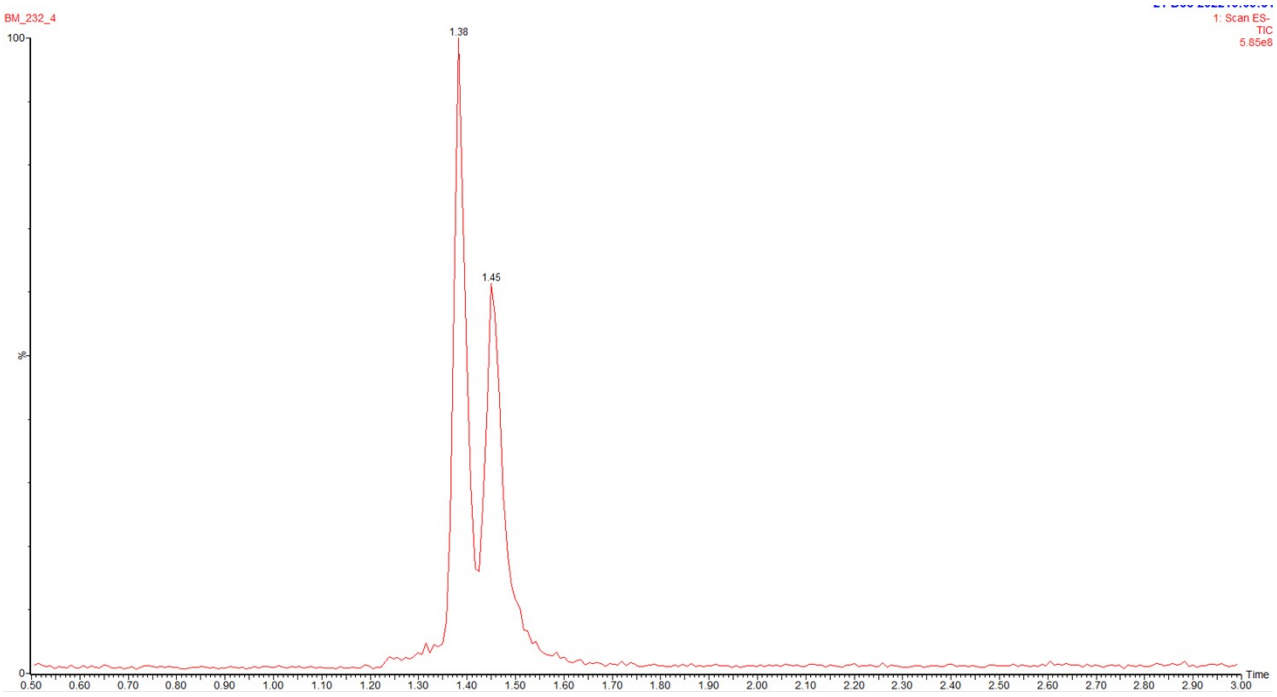


HP-8 (10 nmol, 1.0 equiv)
Molecular Weight: 5278.63

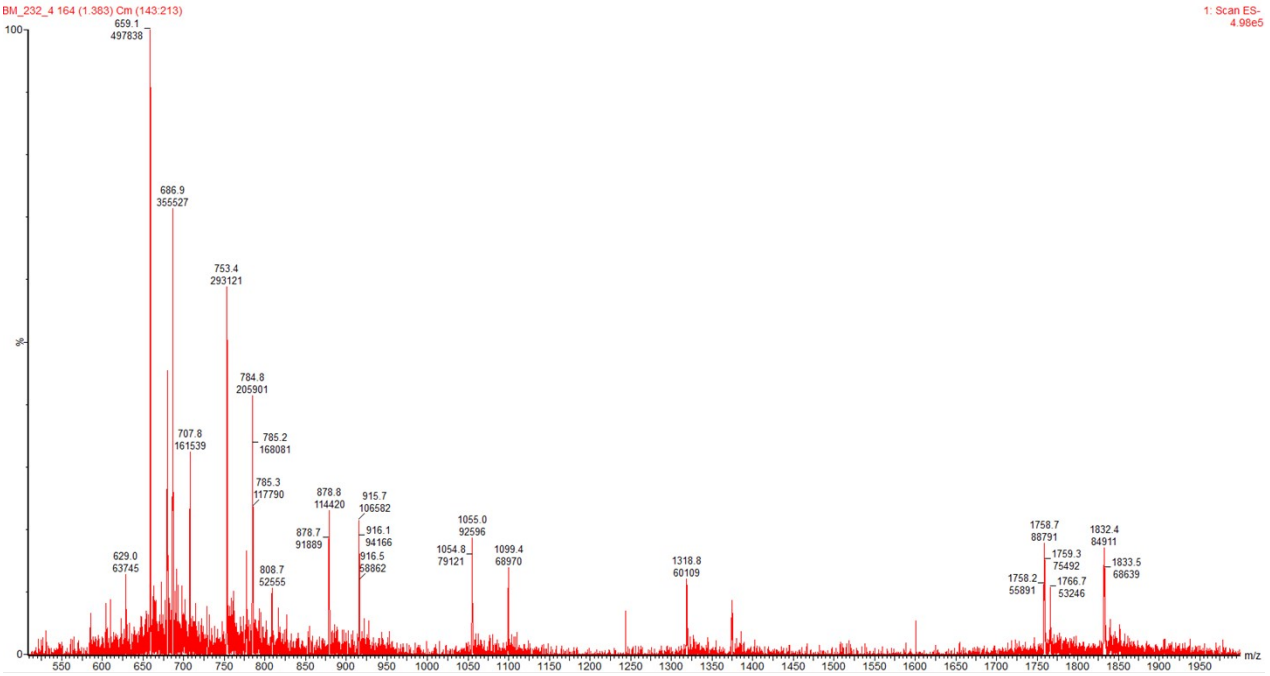
(100 equiv)

26, 46%
Molecular Weight: 5499.85

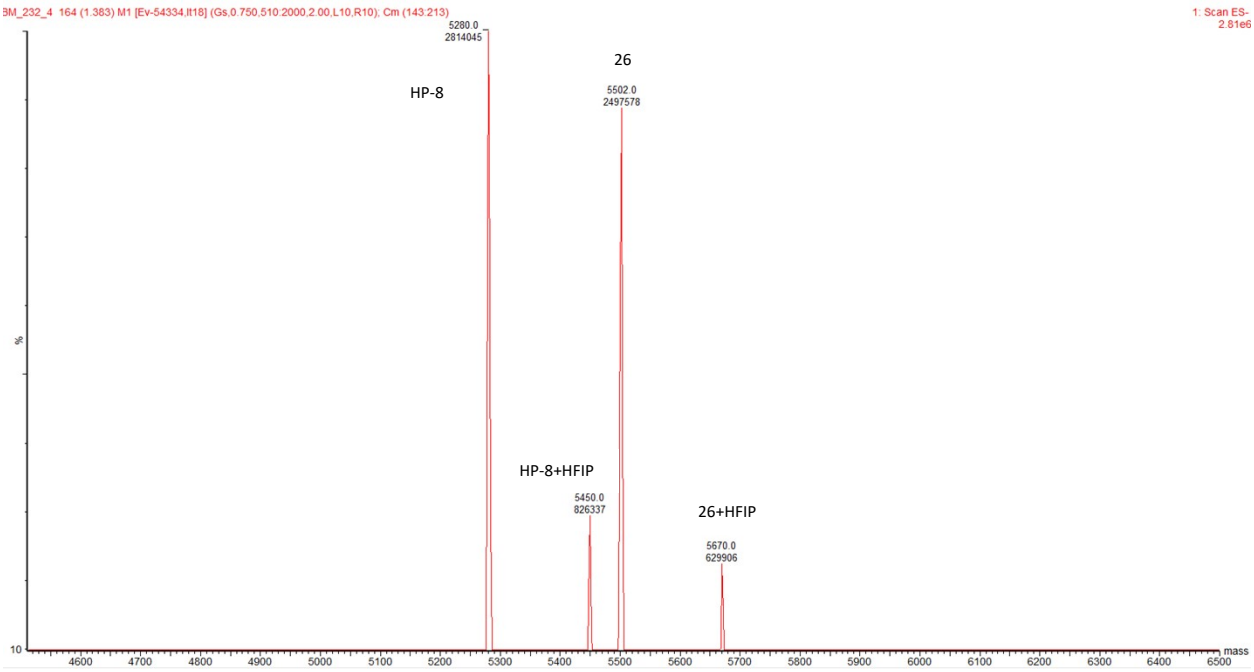
The title compound **26** was synthesized according to **GP-IV** from ethyl 5-(pyrazin-2-yl)-4,5-dihydroisoxazole-3-carboxylate and the DNA-tagged alkene **HP-8**. The product was formed with 46% yield (54% recovered **HP-8**).



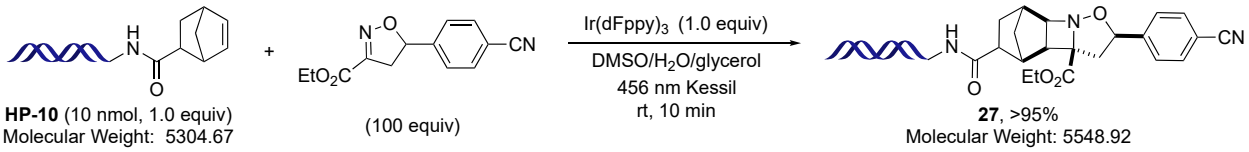
TIC of compound 26.



Raw MS of compound 26.



Deconvoluted MS of 26 (mass signals >10% of peak height).

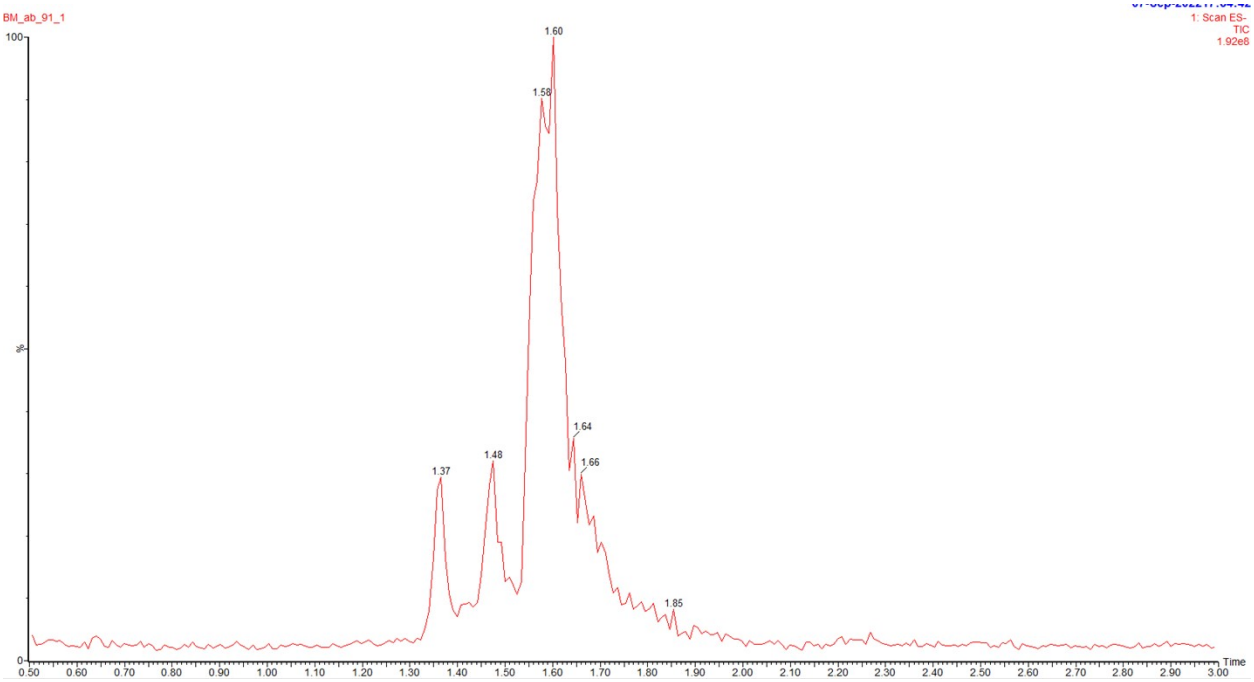


HP-10 (10 nmol, 1.0 equiv)
Molecular Weight: 5304.67

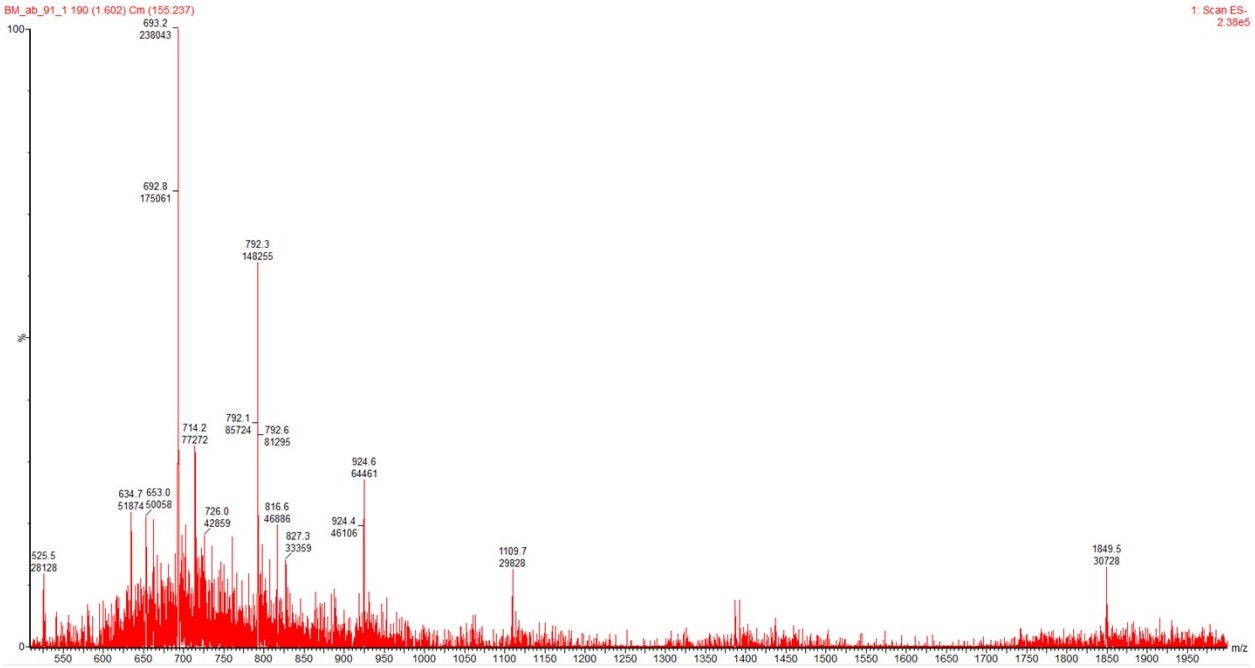
(100 equiv)

27, >95%
Molecular Weight: 5548.92

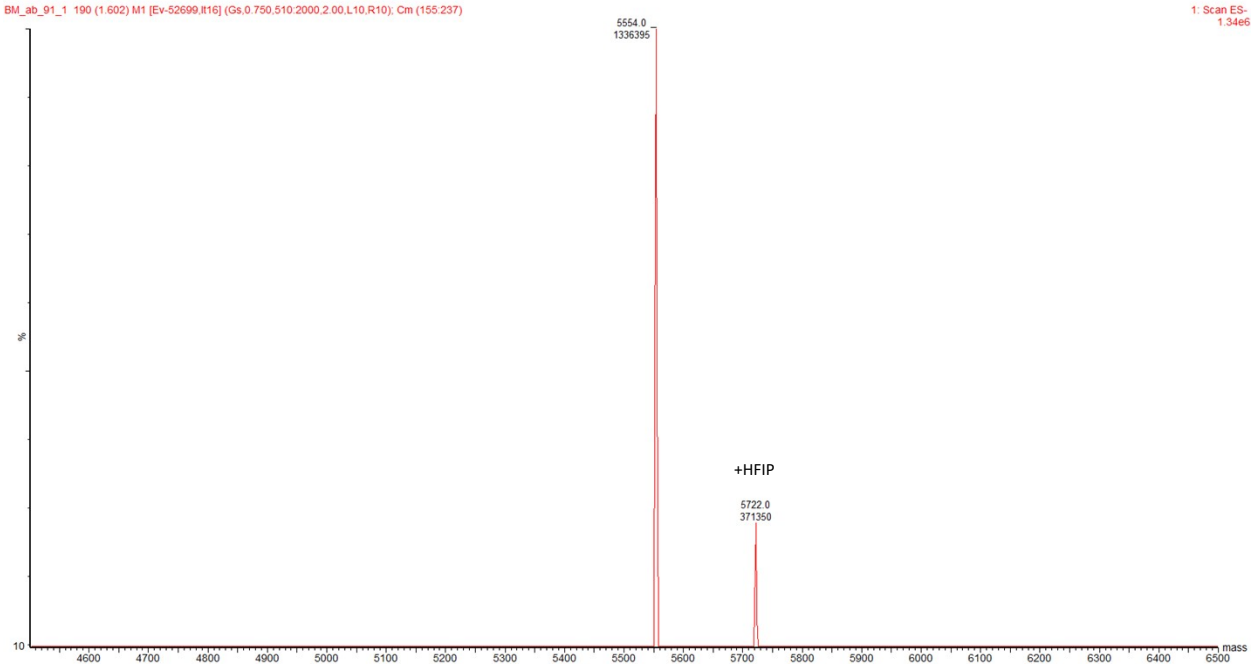
The title compound **27** was synthesized according to **GP-IV** from ethyl 5-(4-cyanophenyl)-4,5-dihydroisoxazole-3-carboxylate and the DNA-tagged alkene **HP-10**. The product was formed with > 95% conversion.



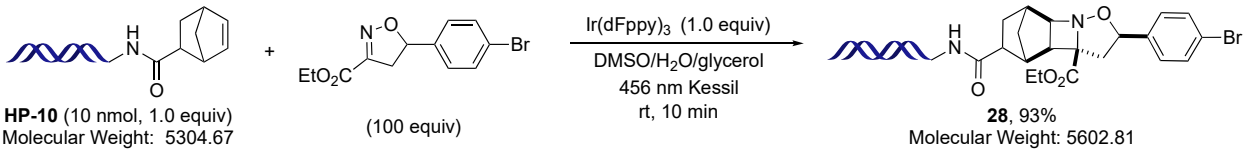
TIC of compound 27.



Raw MS of compound 27.



Deconvoluted MS of 27 (mass signals >10% of peak height).

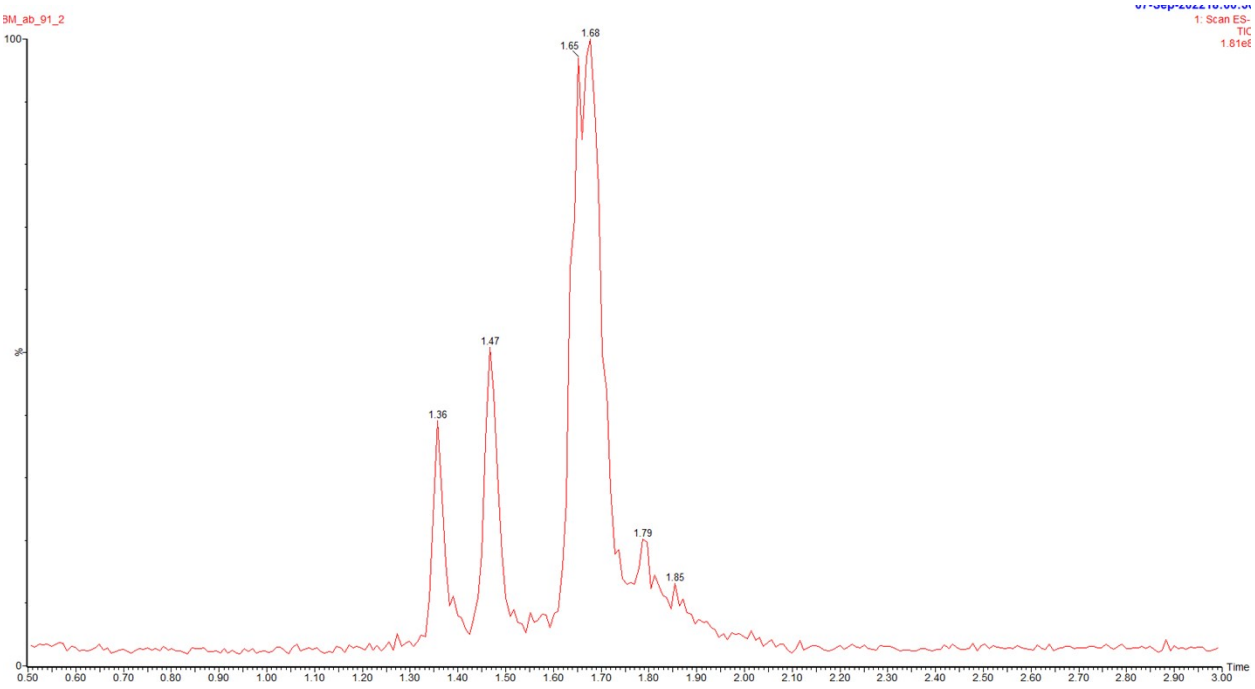


HP-10 (10 nmol, 1.0 equiv)
Molecular Weight: 5304.67

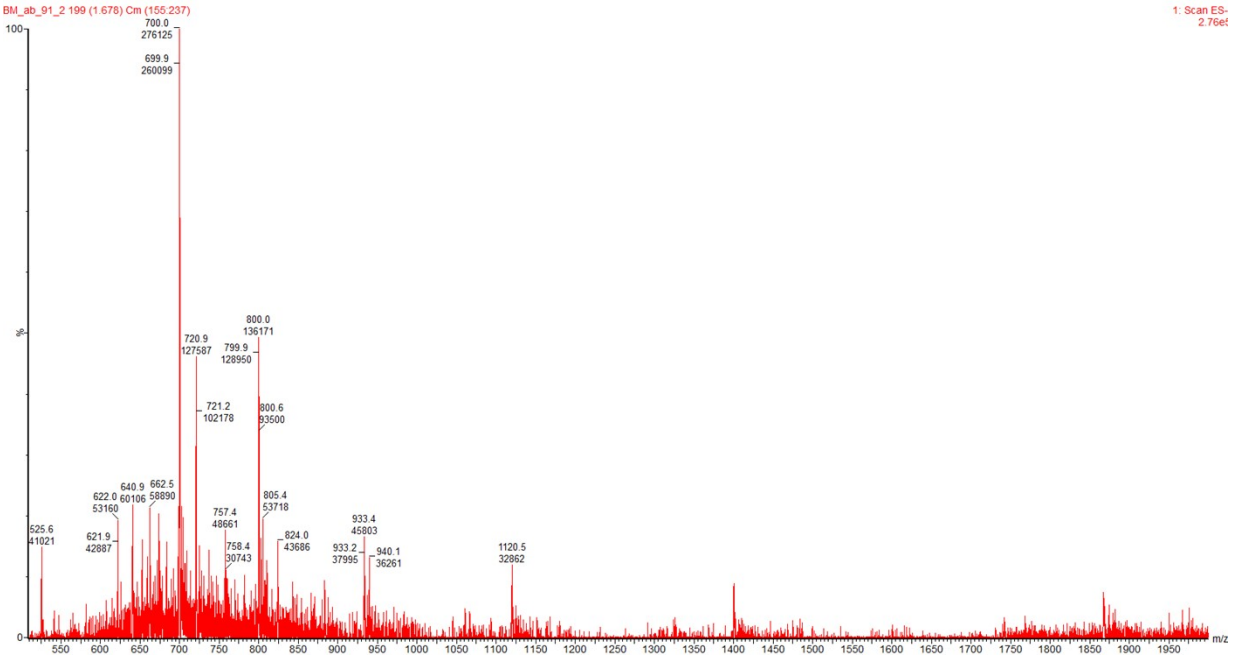
(100 equiv)

28, 93%
Molecular Weight: 5602.81

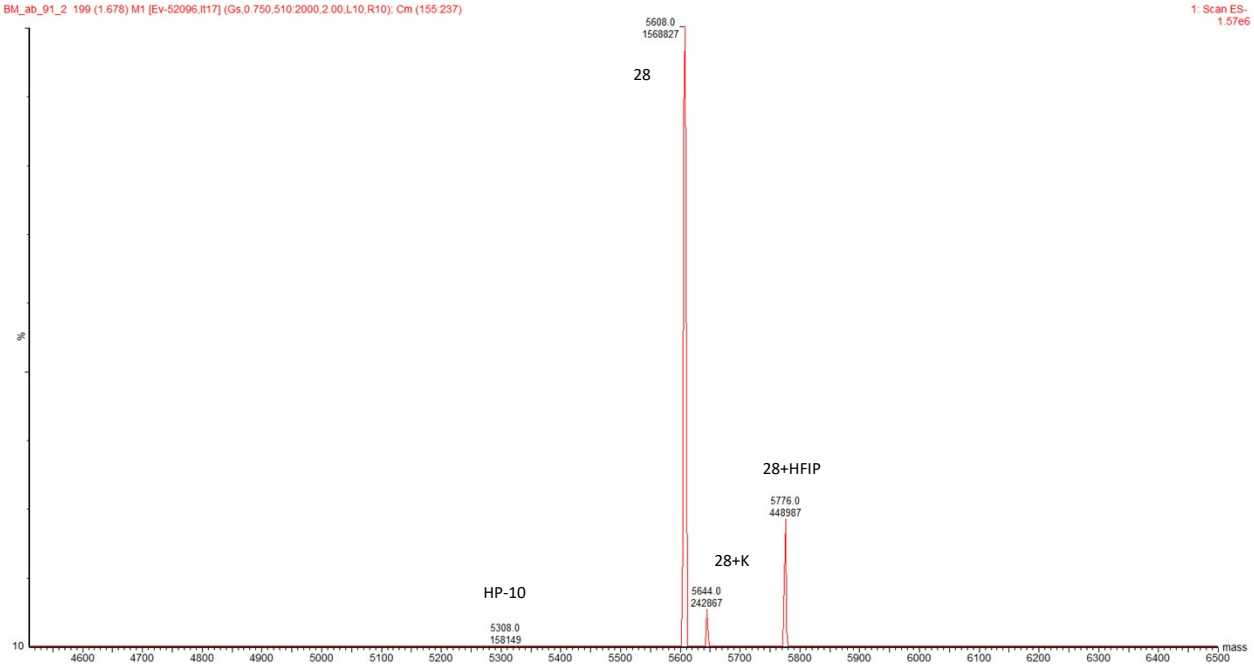
The title compound **28** was synthesized according to **GP-IV** from ethyl 5-(4-bromophenyl)-4,5-dihydroisoxazole-3-carboxylate and the DNA-tagged alkene **HP-10**. The product was formed with 93% conversion (6.5% recovered **HP-10**).



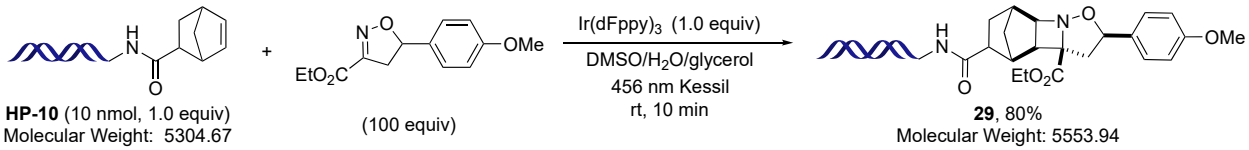
TIC of compound 28.



Raw MS of compound 28.



Deconvoluted MS of 28 (mass signals >10% of peak height).

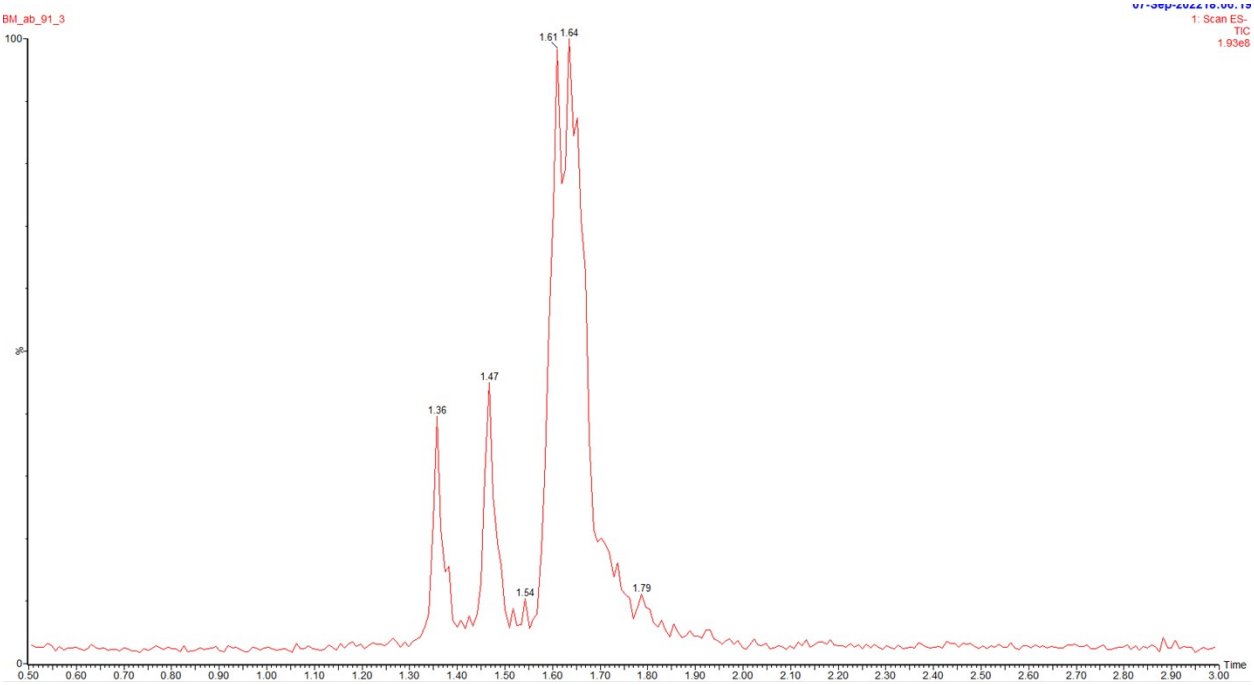


HP-10 (10 nmol, 1.0 equiv)
Molecular Weight: 5304.67

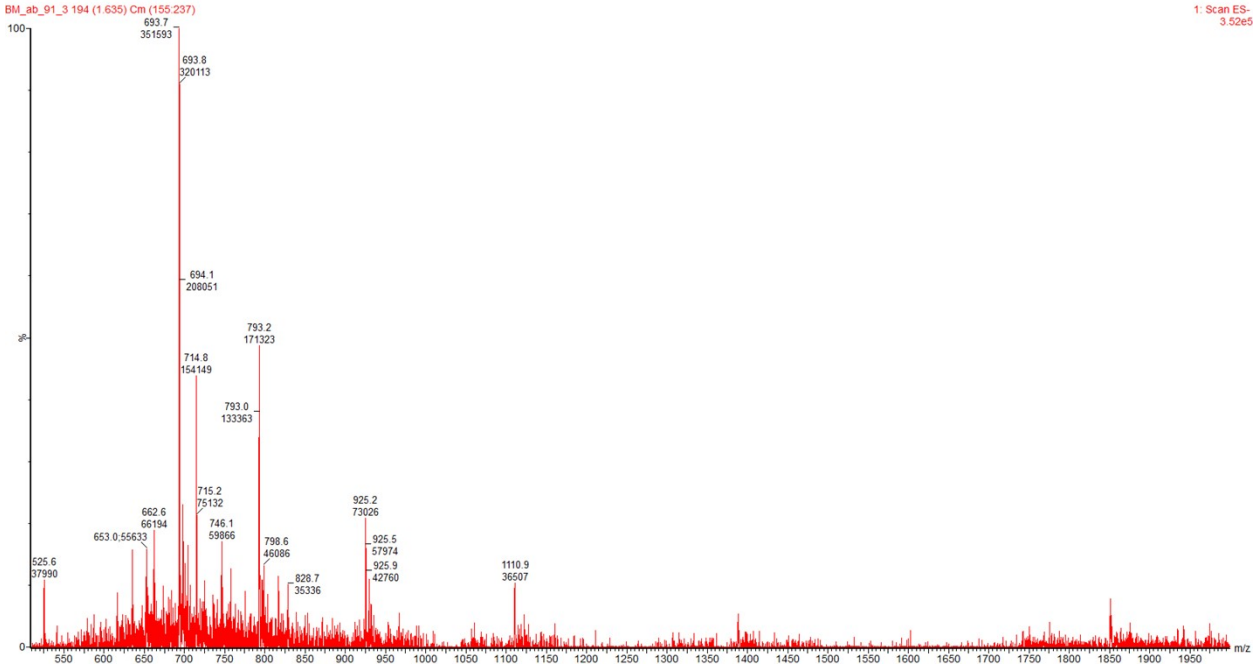
(100 equiv)

29, 80%
Molecular Weight: 5553.94

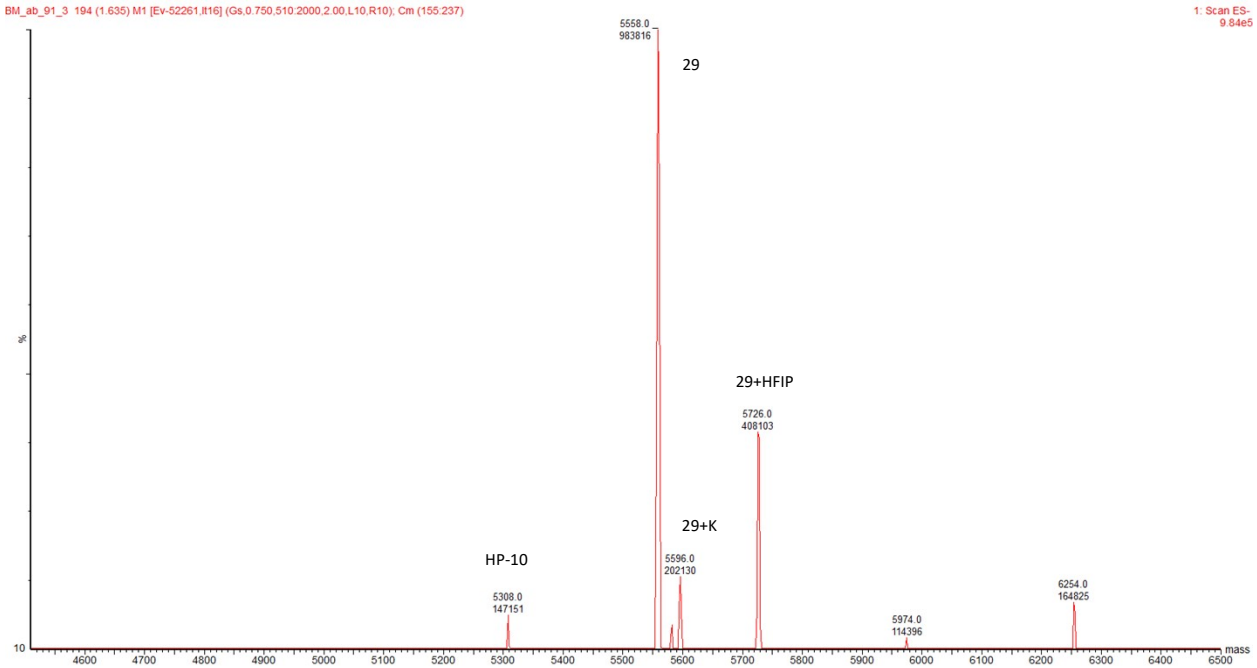
The title compound **29** was synthesized according to **GP-III** from ethyl 5-(4-methoxyphenyl)-4,5-dihydroisoxazole-3-carboxylate and the DNA-tagged alkene **HP-10**. The product was formed with 80% yield (7% recovered **HP-10**).



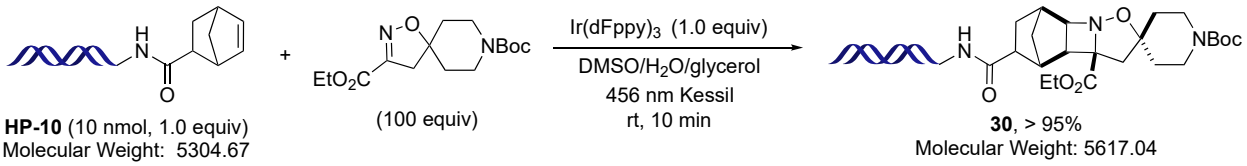
TIC of compound 29.



Raw MS of compound 29.



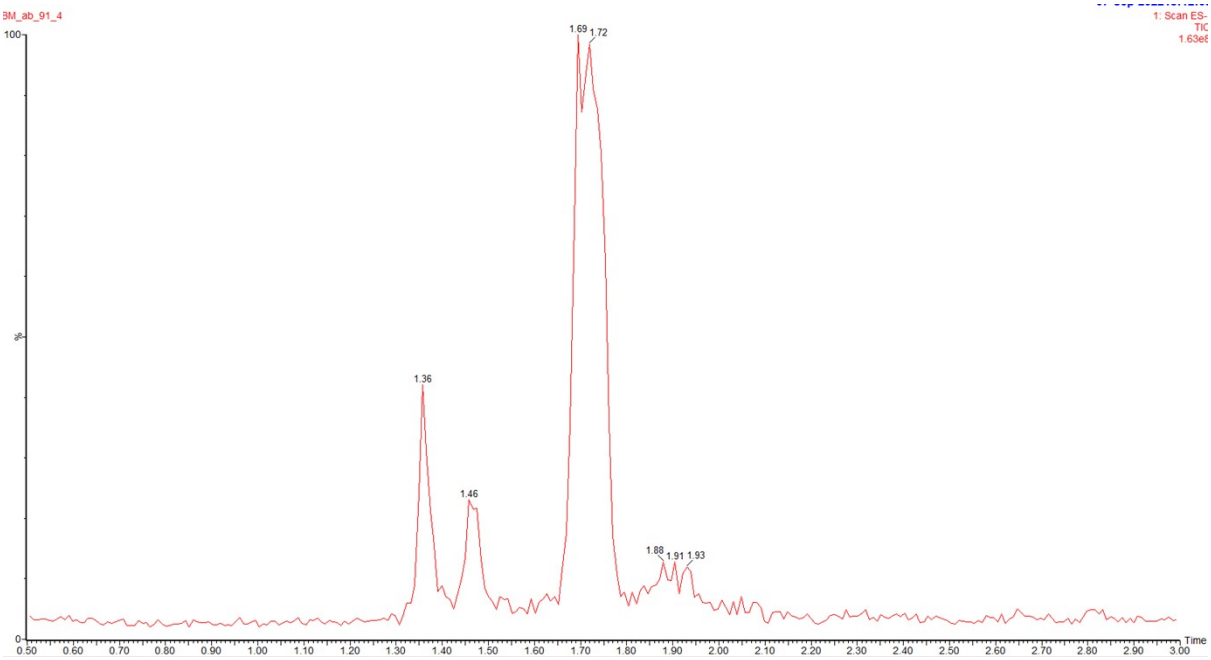
Deconvoluted MS of 29 (mass signals >10% of peak height).



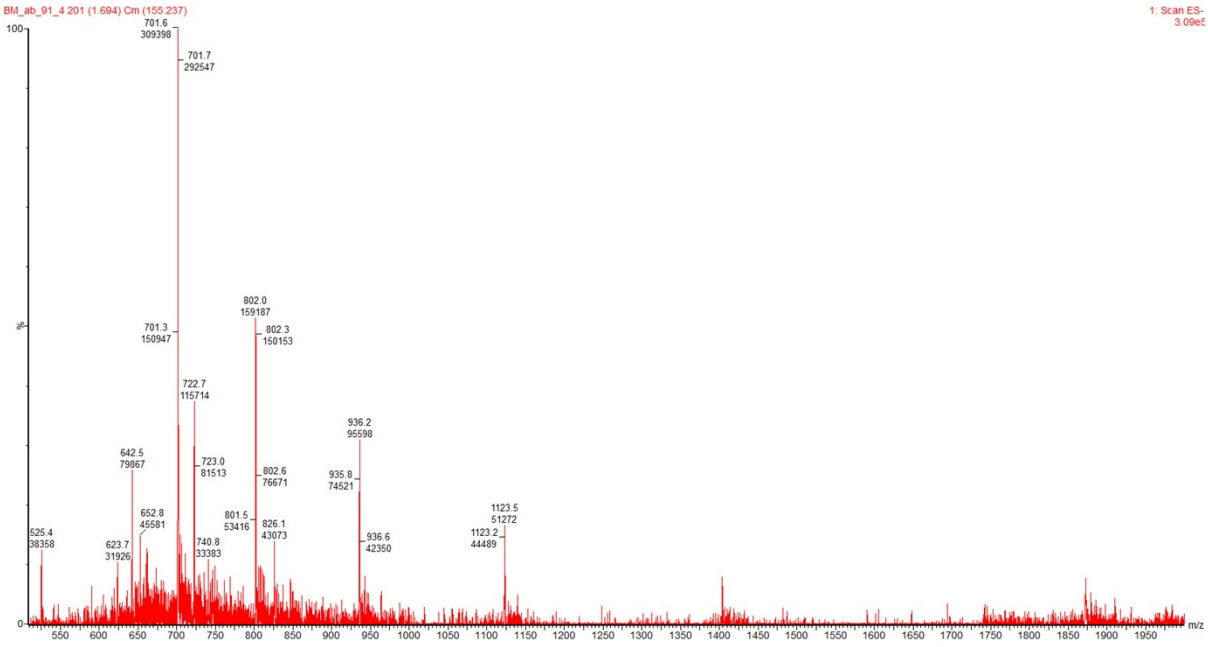
HP-10 (10 nmol, 1.0 equiv)
Molecular Weight: 5304.67

30, > 95%
Molecular Weight: 5617.04

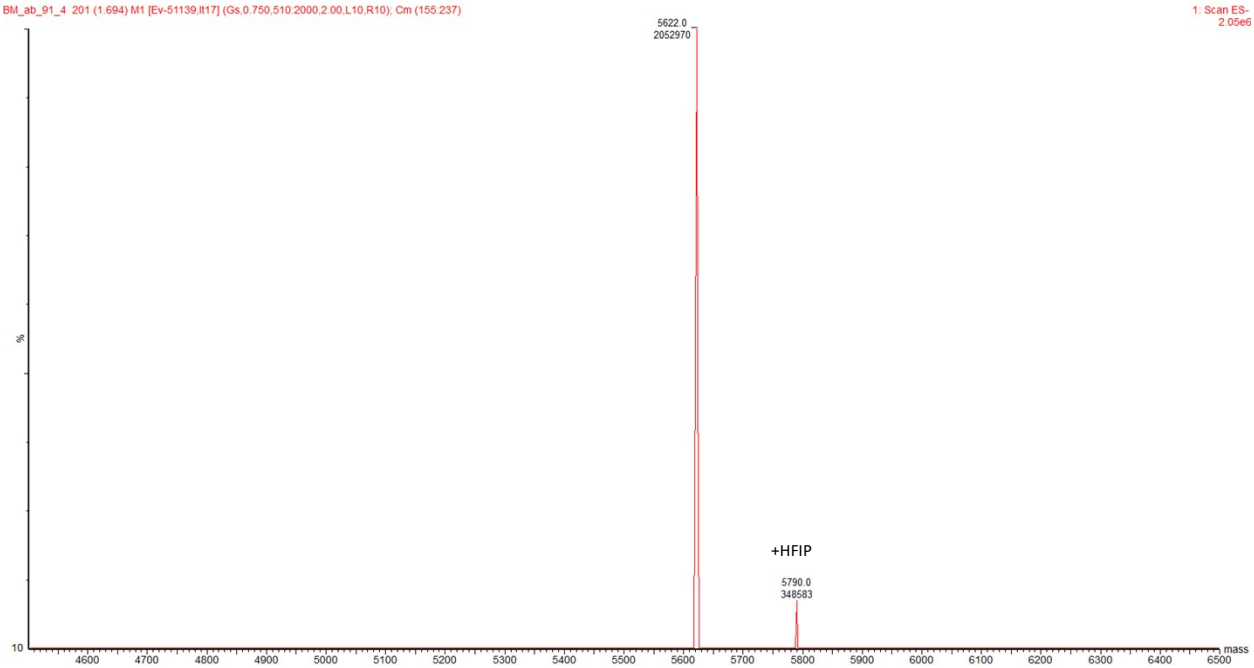
The title compound **30** was synthesized according to **GP-IV** from 8-(*tert*-butyl) 3-ethyl 1-oxa-2,8-diazaspiro[4.5]dec-2-ene-3,8-dicarboxylate and the DNA-tagged alkene **HP-10**. The product was formed with > 95% conversion.



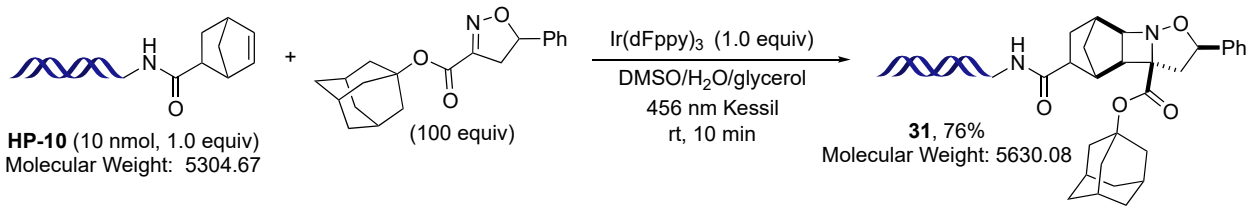
TIC of compound 30.



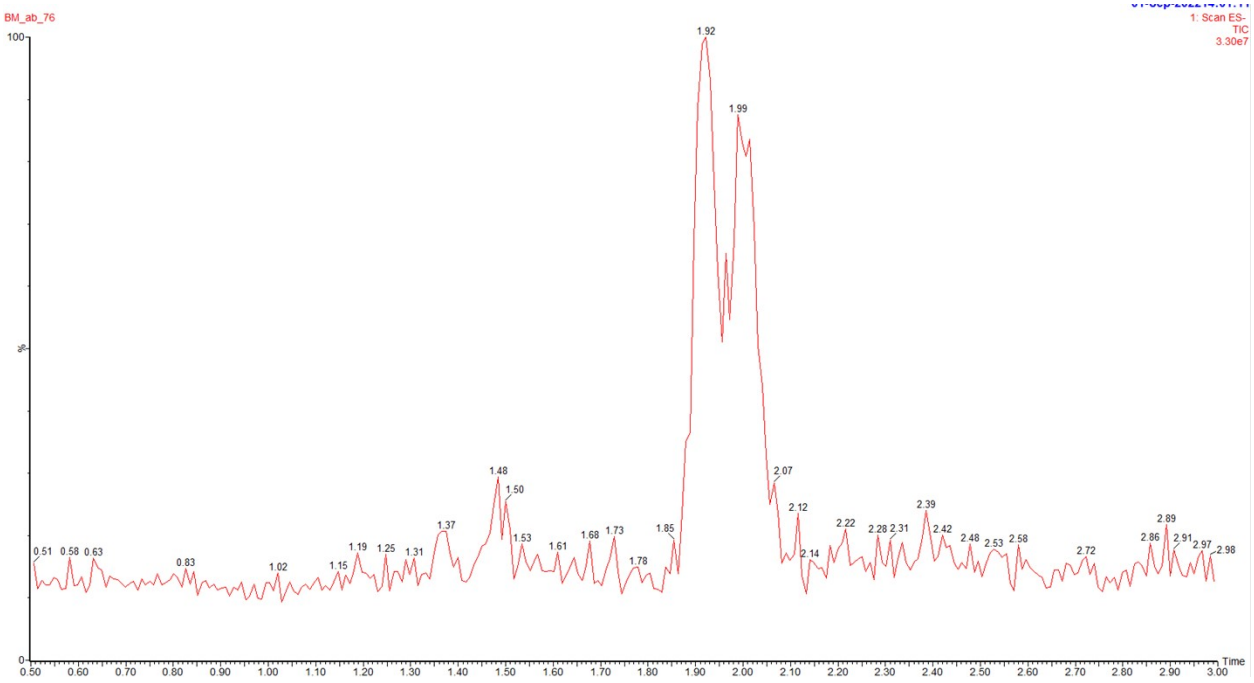
Raw MS of compound 30.



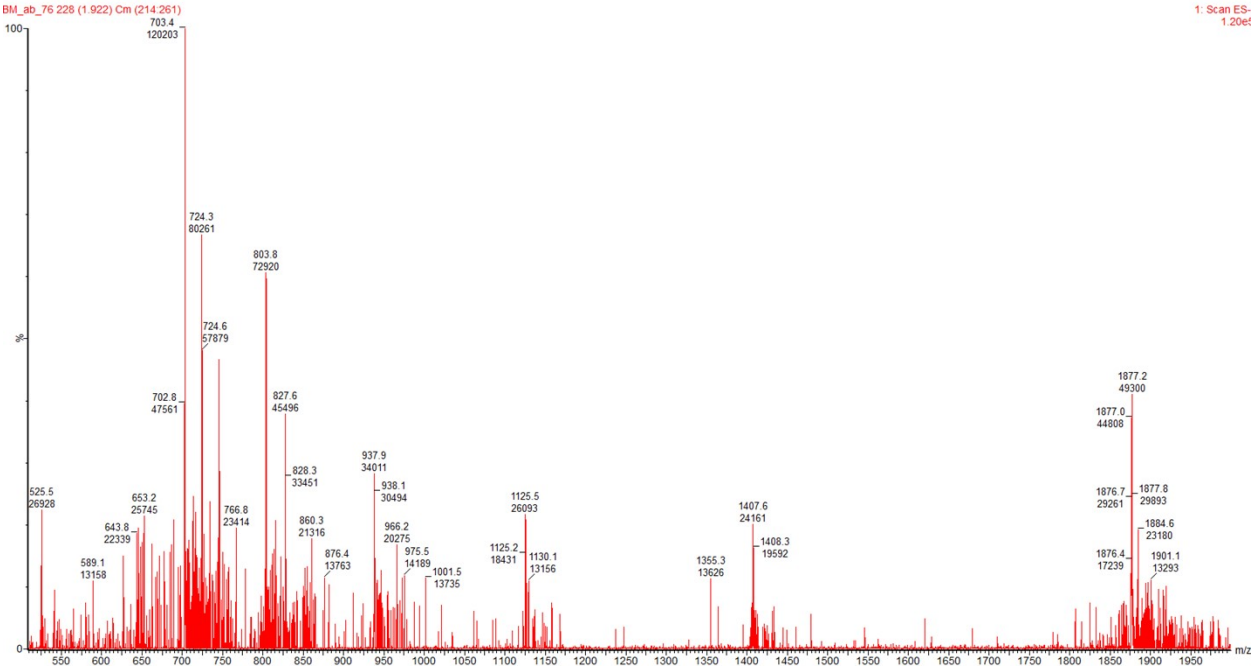
Deconvoluted MS of 30 (mass signals >10% of peak height).



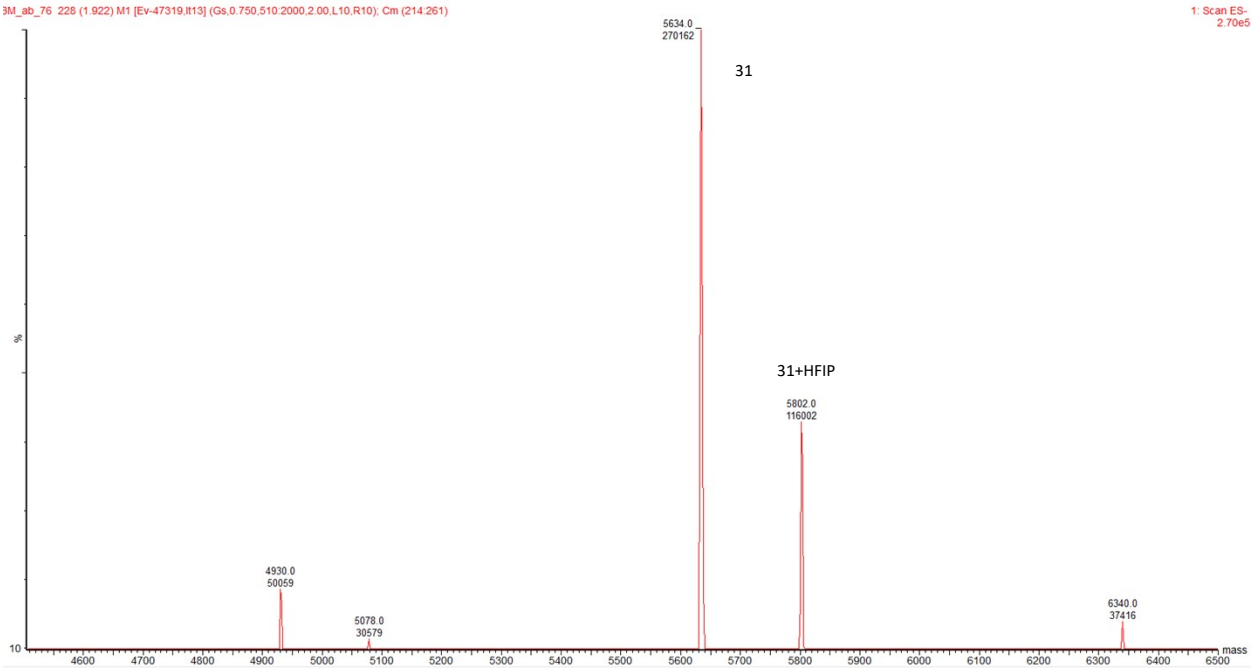
The title compound **31** was synthesized according to **GP-IV** from (3s,5s,7s)-adamantan-1-yl 5-phenyl-4,5-dihydroisoxazole-3-carboxylate and the DNA-tagged alkene **HP-10**. The product was formed with 76% conversion.



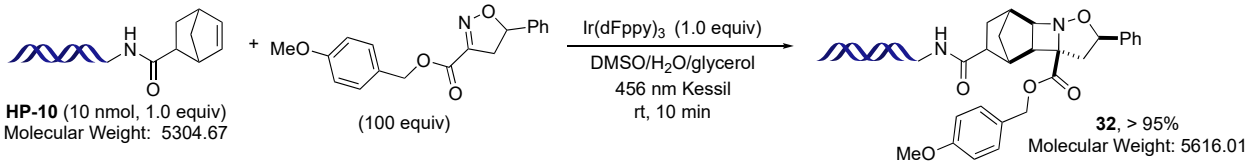
TIC of compound 31.



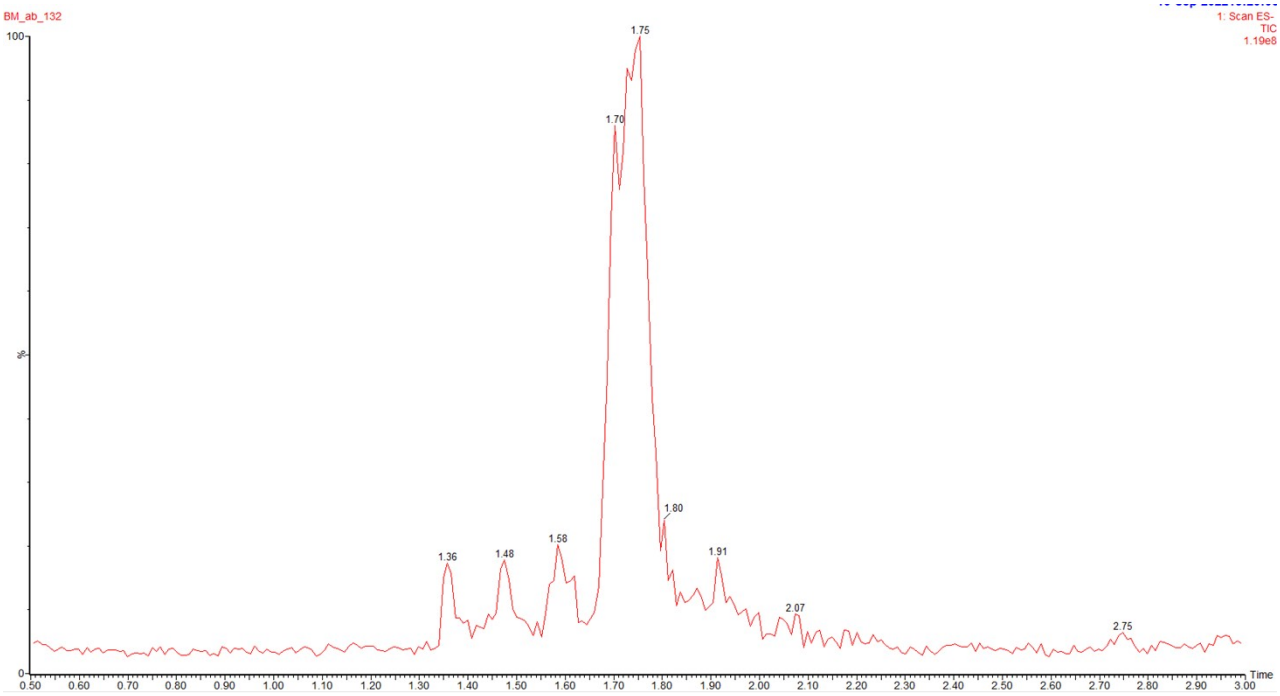
Raw MS of compound 31.



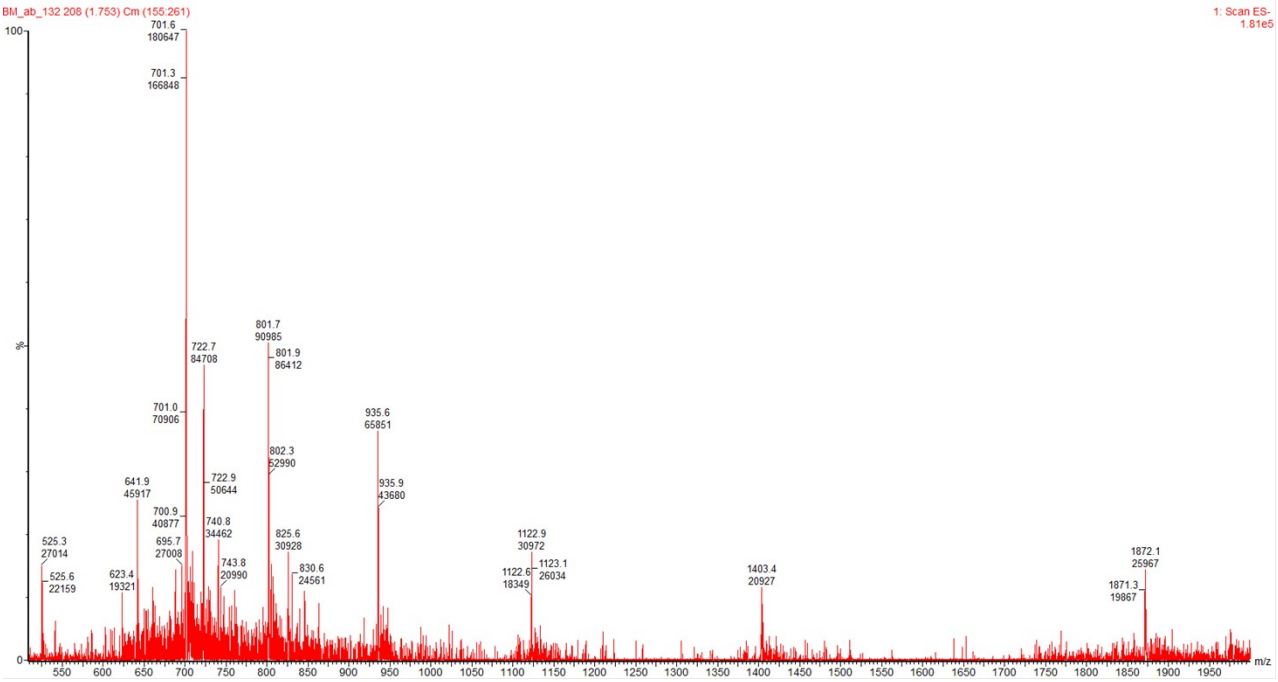
Deconvoluted MS of 31 (mass signals >10% of peak height).



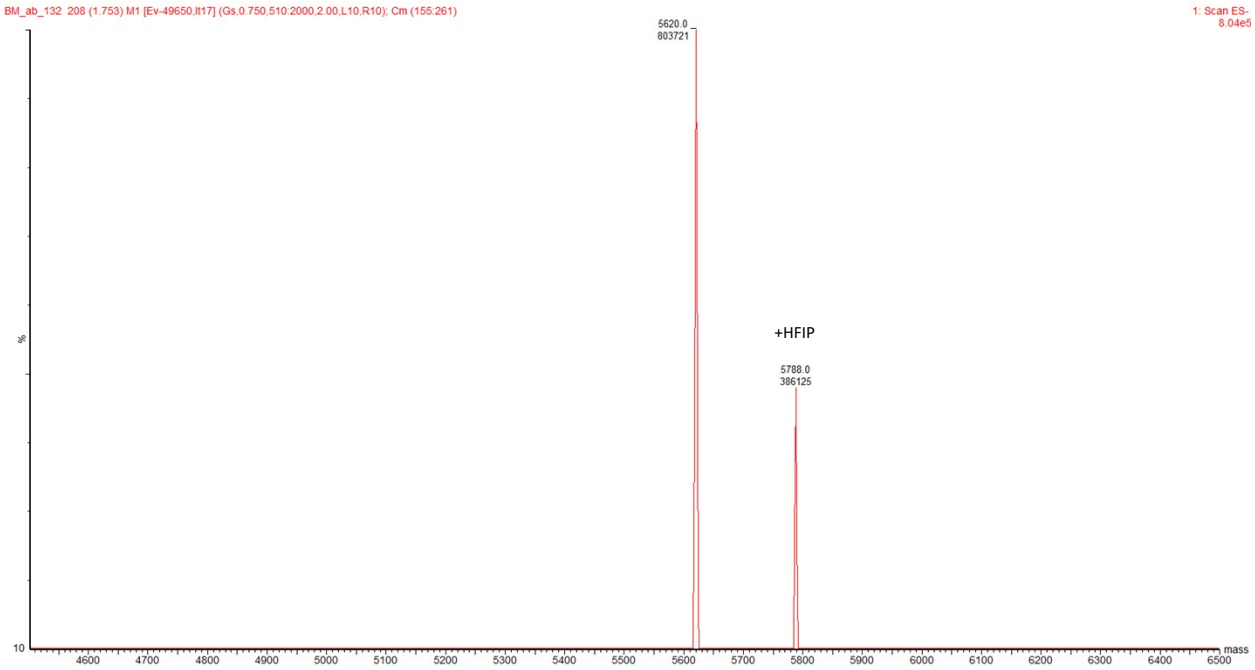
The title compound **32** was synthesized according to **GP-IV** from 4-methoxybenzyl 5-phenyl-4,5-dihydroisoxazole-3-carboxylate and the DNA-tagged alkene **HP-10**. The product was formed with >95% conversion.



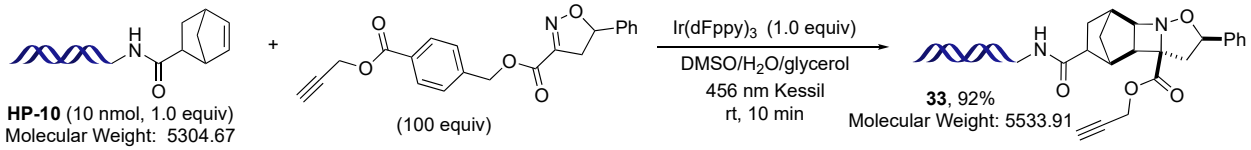
TIC of compound 32.



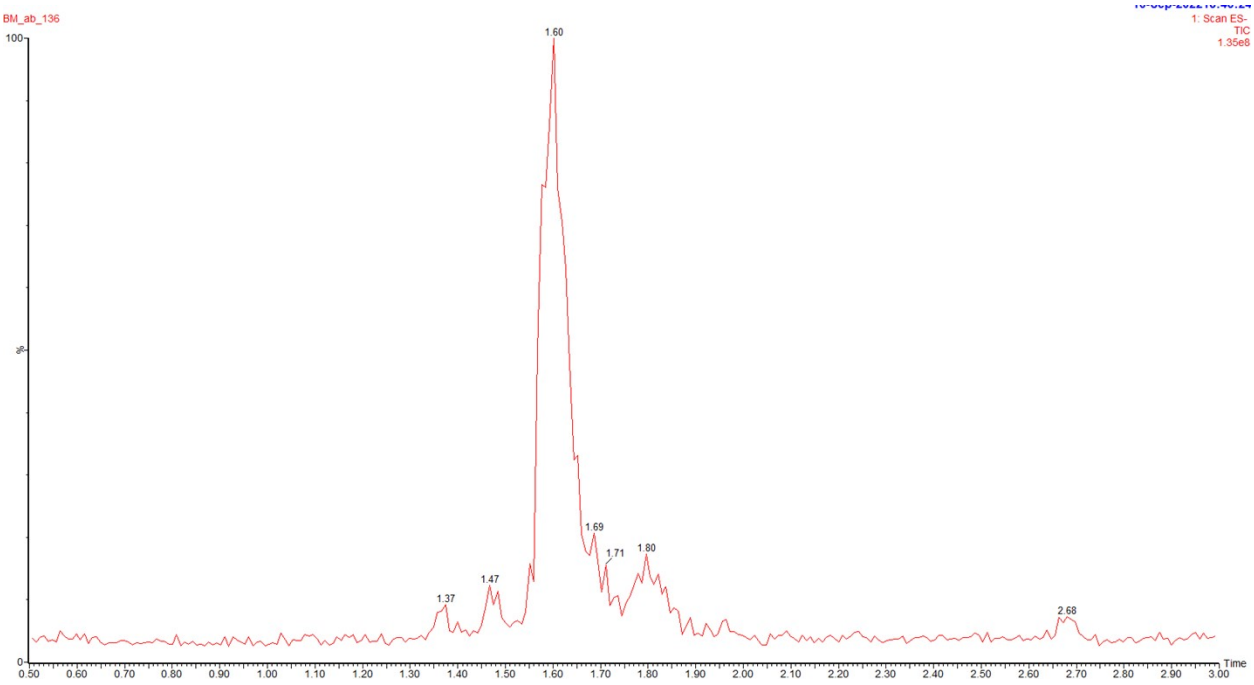
Raw MS of compound 32.



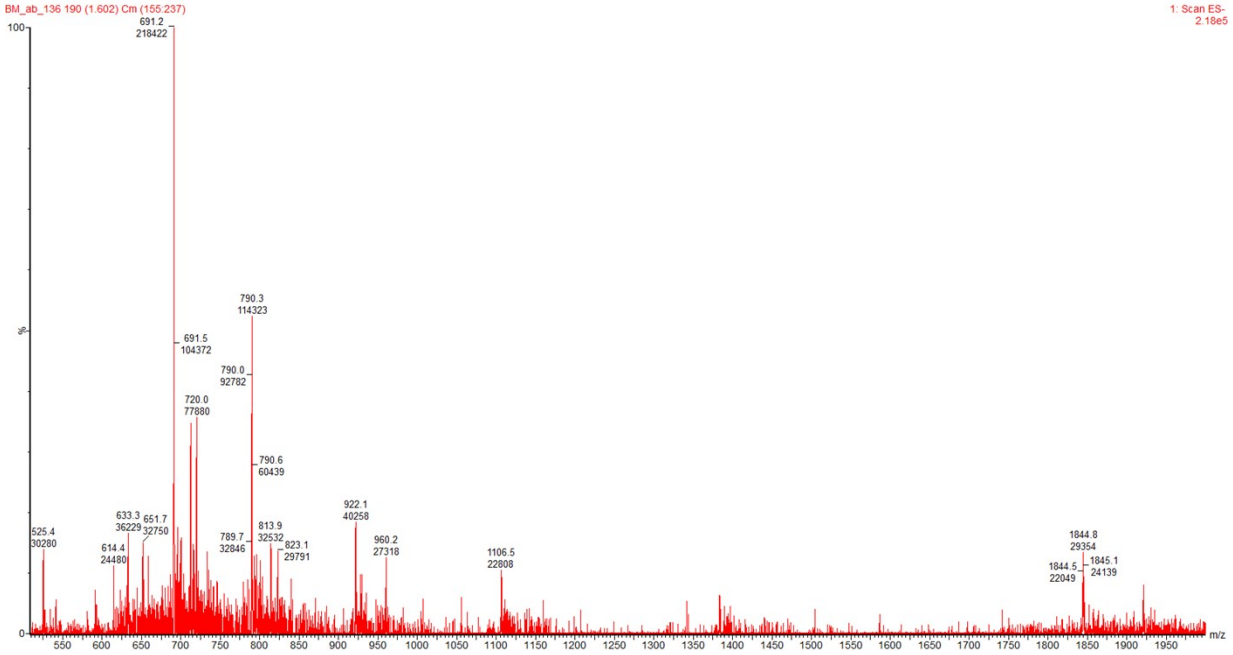
Deconvoluted MS of 32 (mass signals >10% of peak height).



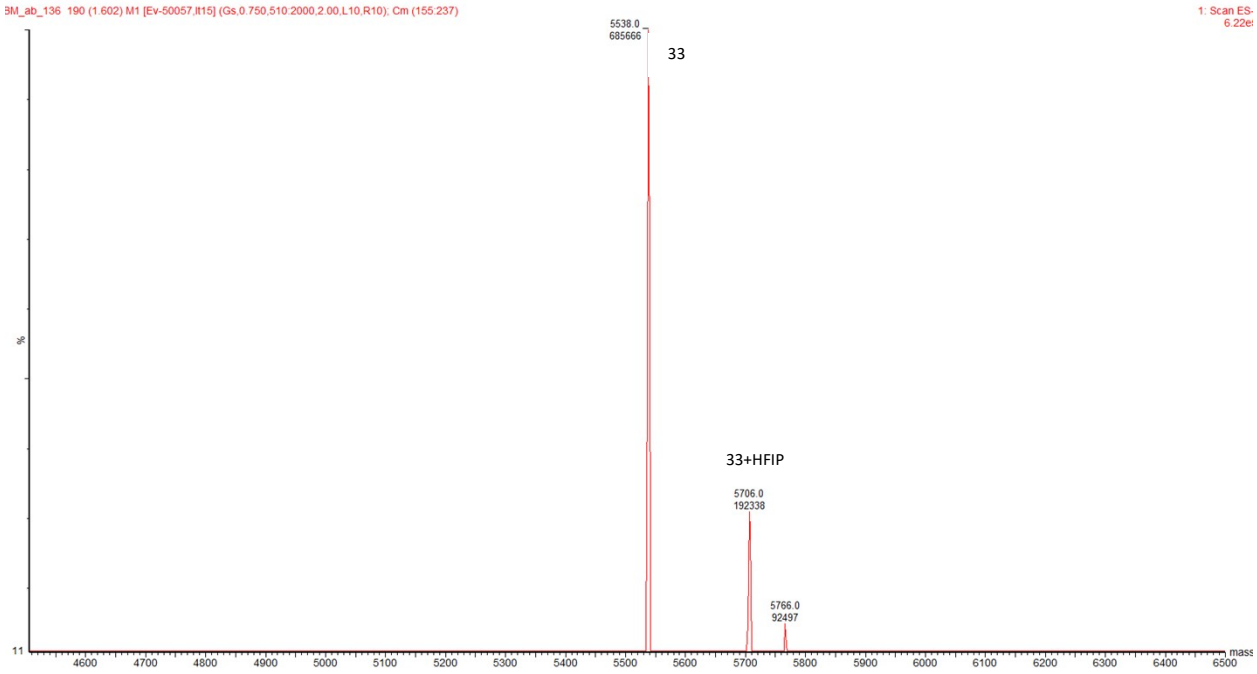
The title compound **33** was synthesized according to **GP-I**V from prop-2-yn-1-yl 5-phenyl-4,5-dihydroisoxazole-3-carboxylate and the DNA-tagged alkene **HP-10**. The product was formed with 92% conversion.



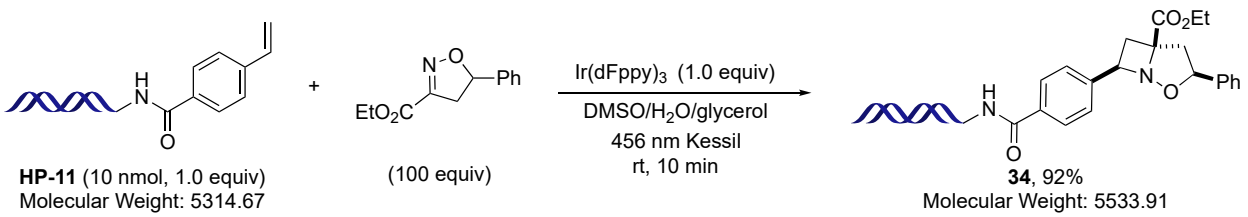
TIC of compound 33.



Raw MS of compound 33.



Deconvoluted MS of 33 (mass signals >10% of peak height).

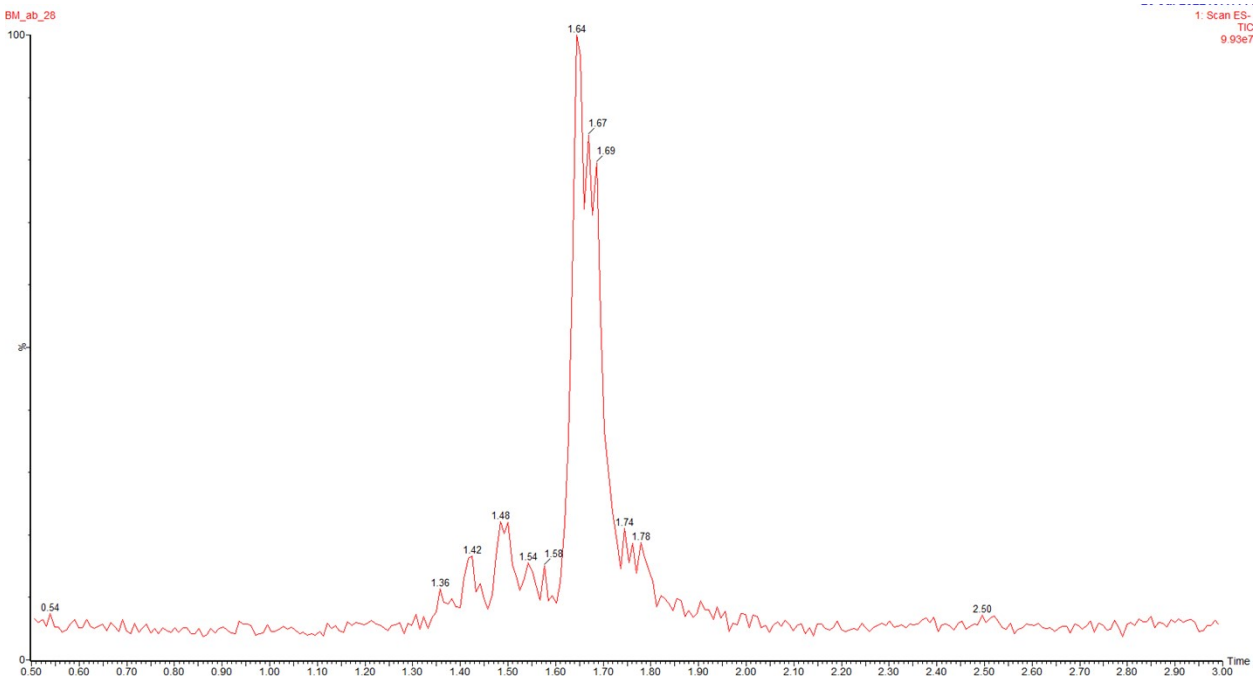


HP-11 (10 nmol, 1.0 equiv)
Molecular Weight: 5314.67

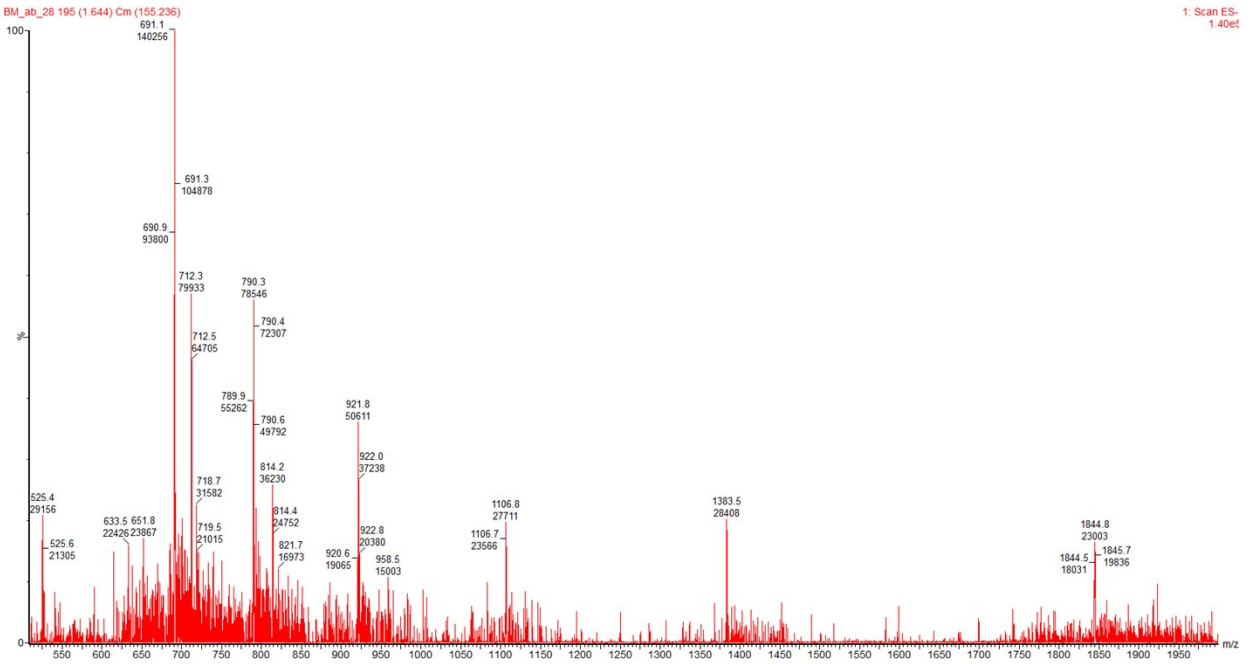
(100 equiv)

34, 92%
Molecular Weight: 5533.91

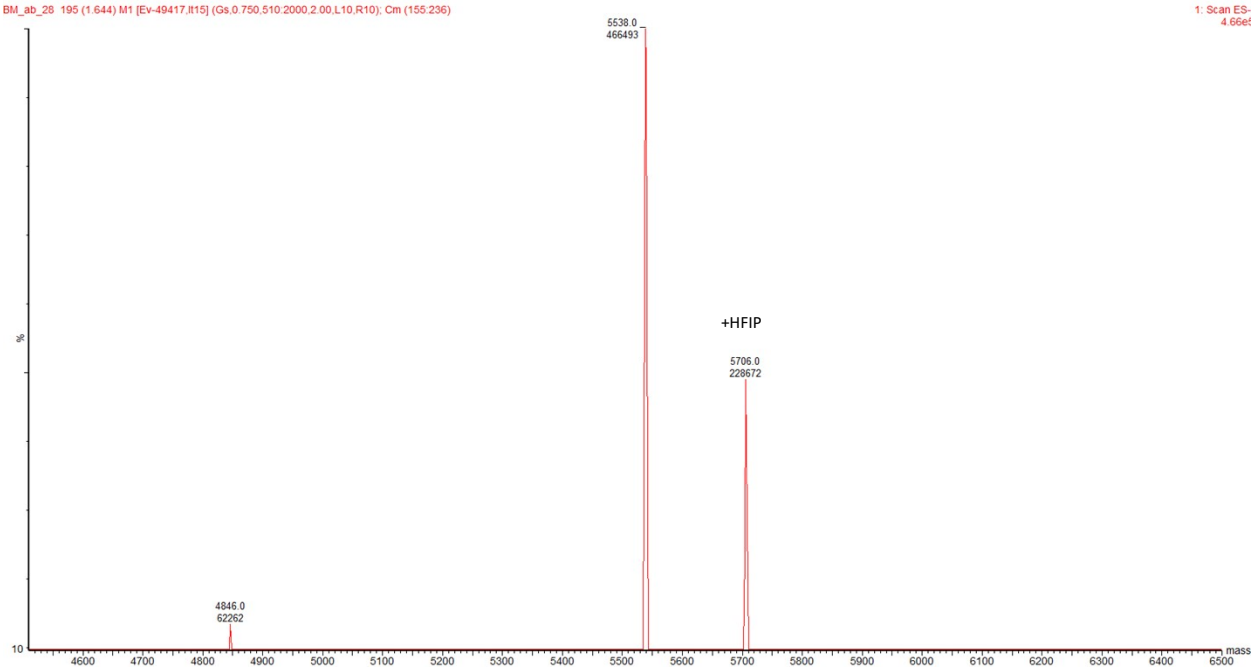
The title compound **34** was synthesized according to **GP-IV** from ethyl 5-phenyl-4,5-dihydroisoxazole-3-carboxylate and the DNA-tagged alkene **HP-11**. The product was formed with 92% conversion.



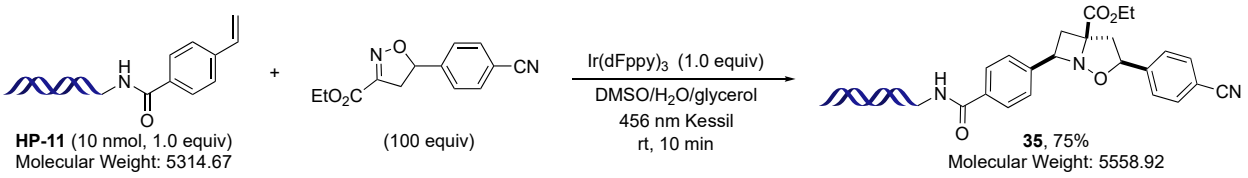
TIC of compound 34.



Raw MS of compound 34.



Deconvoluted MS of 34 (mass signals >10% of peak height).

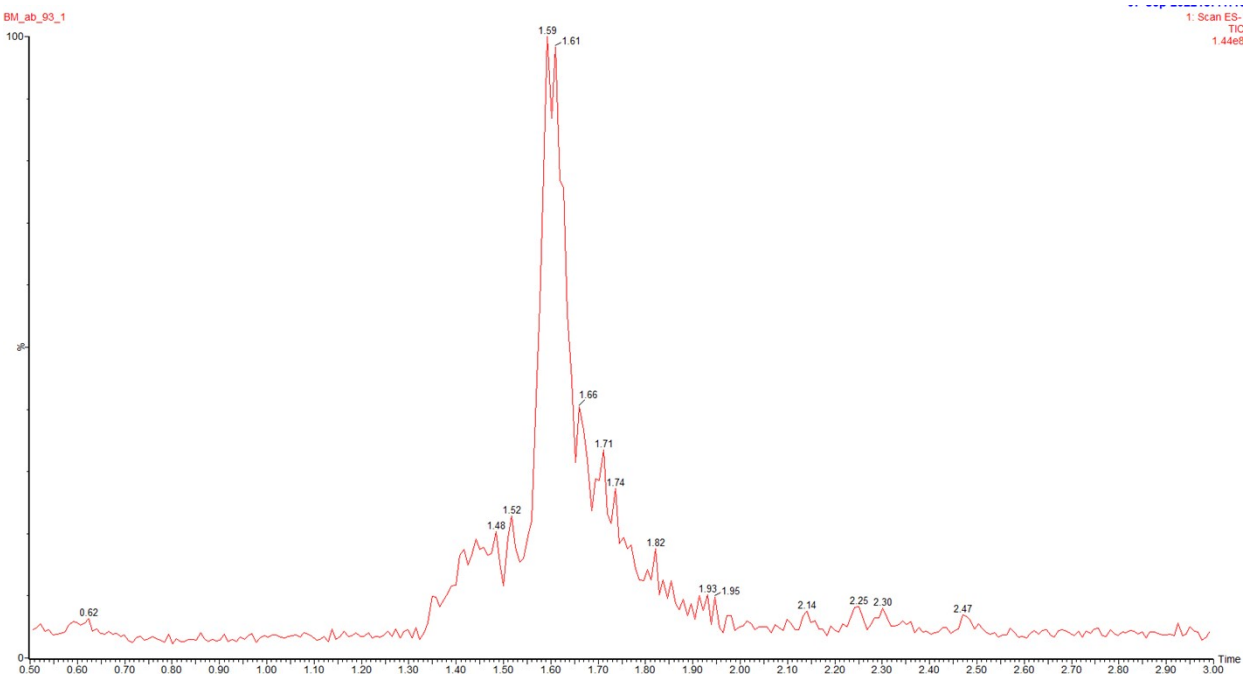


HP-11 (10 nmol, 1.0 equiv)
Molecular Weight: 5314.67

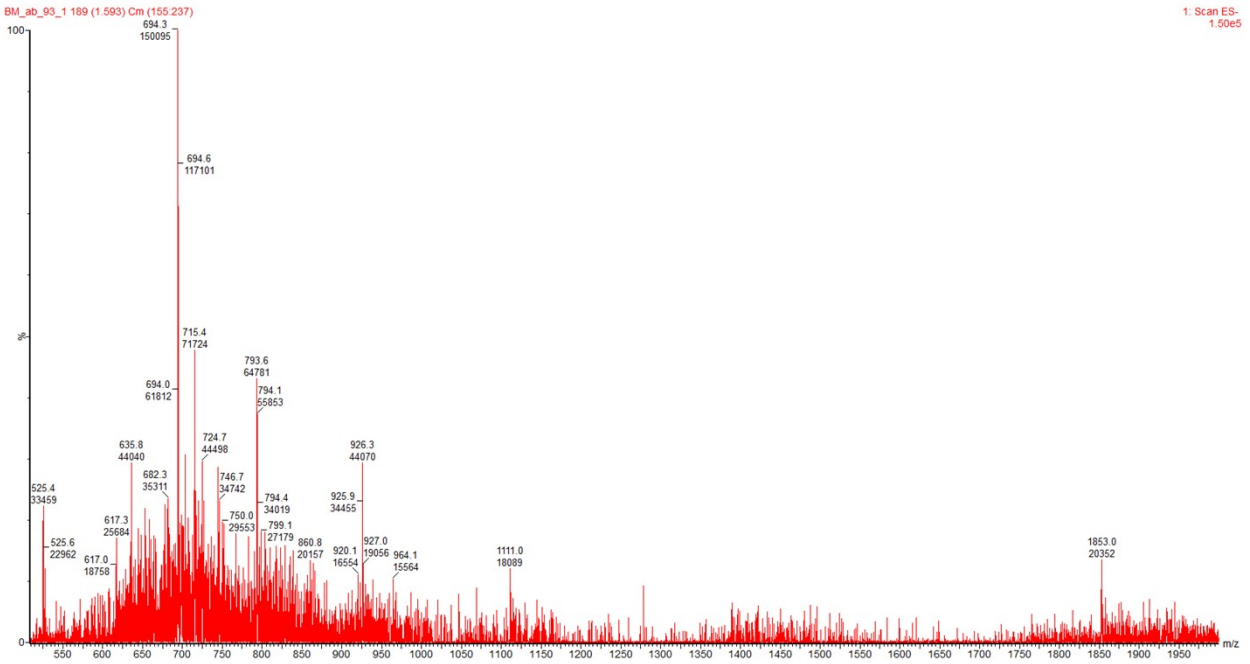
(100 equiv)

35, 75%
Molecular Weight: 5558.92

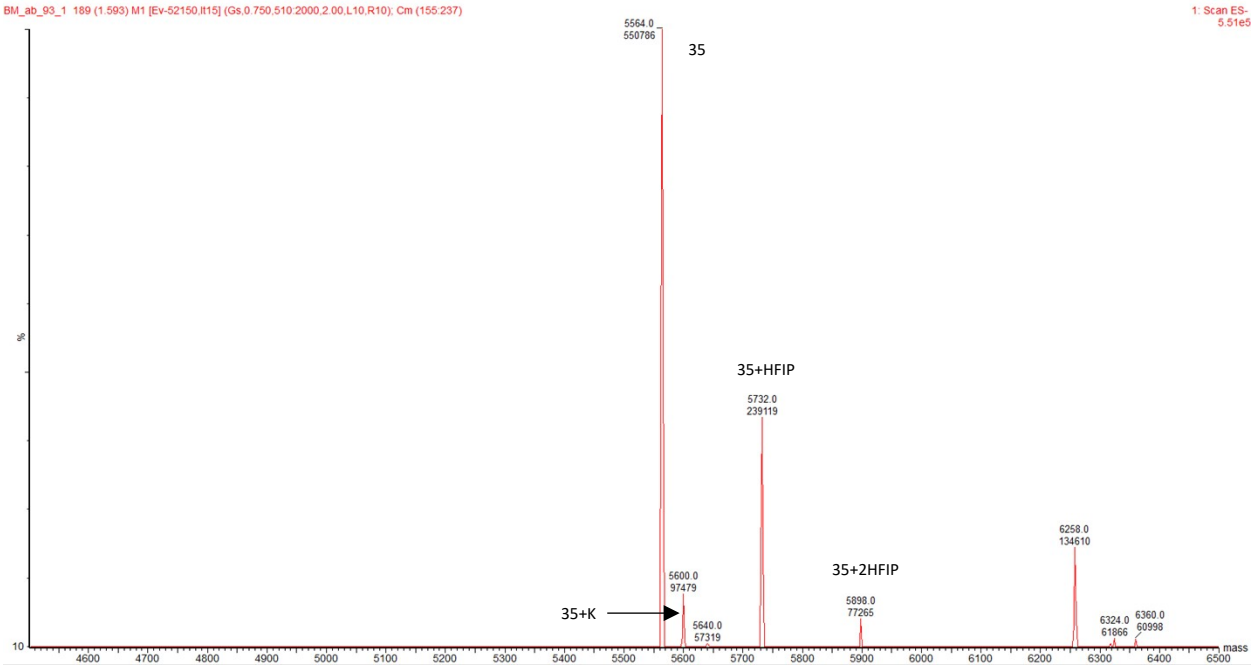
The title compound **35** was synthesized according to **GP-IV** from ethyl 5-(4-cyanophenyl)-4,5-dihydroisoxazole-3-carboxylate and the DNA-tagged alkene **HP-11**. The product was formed with 75% conversion.



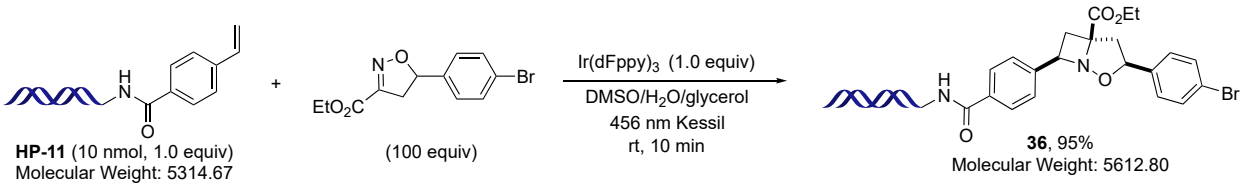
TIC of compound 35.



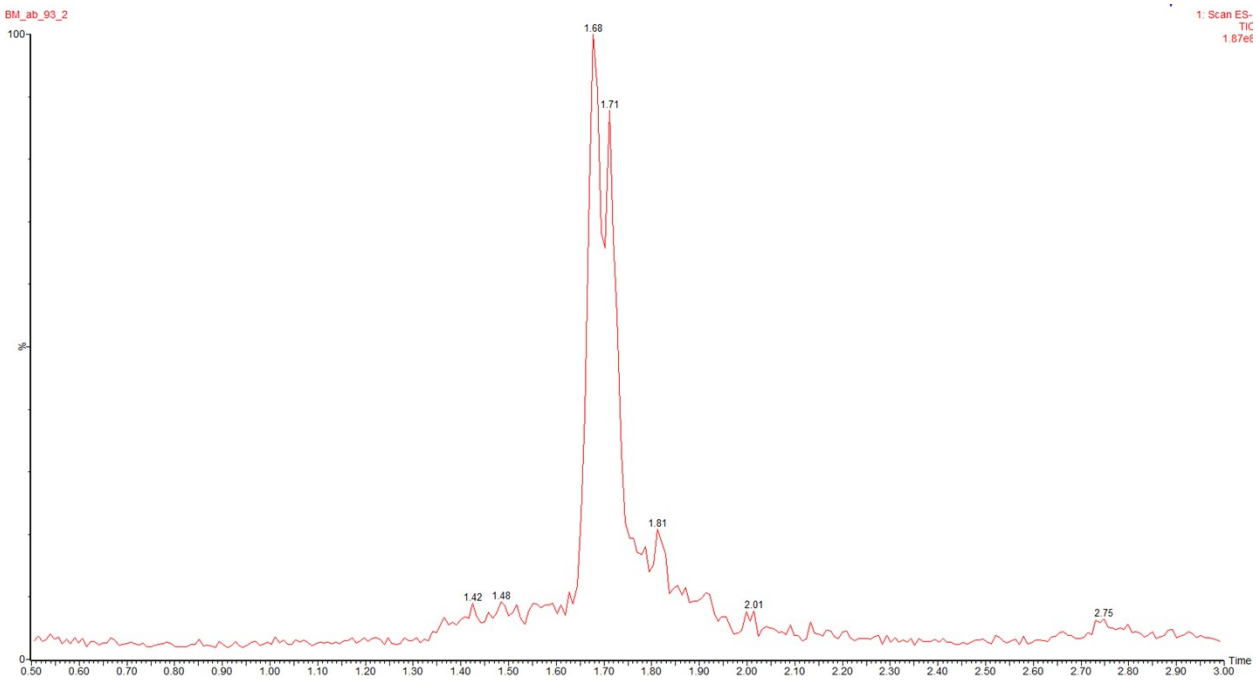
Raw MS of compound 35.



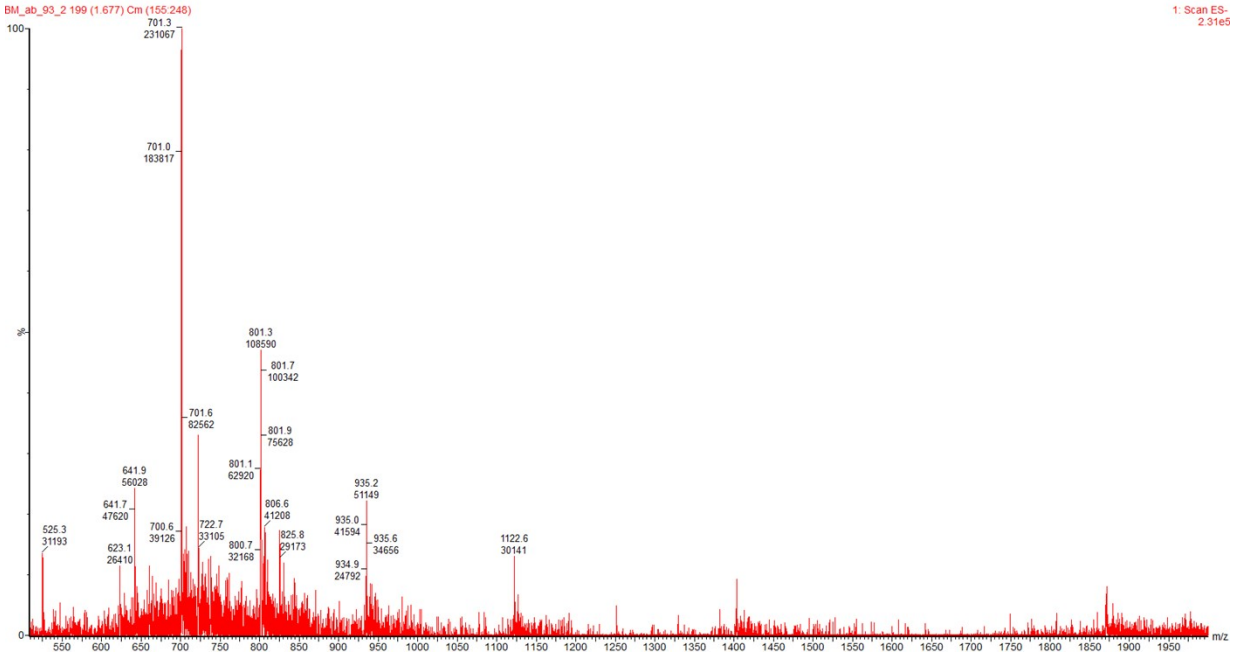
Deconvoluted MS of 35 (mass signals >10% of peak height).



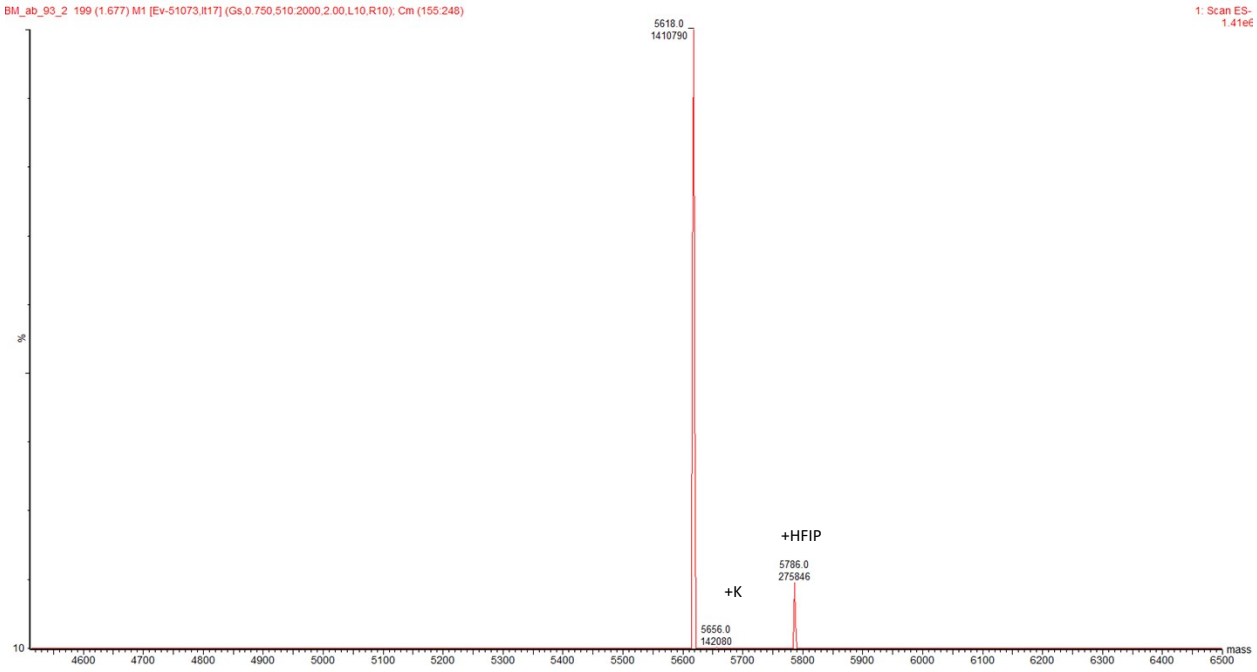
The title compound **36** was synthesized according to **GP-IV** from ethyl 5-(4-bromophenyl)-4,5-dihydroisoxazole-3-carboxylate and the DNA-tagged alkene **HP-11**. The product was formed with 95% conversion.



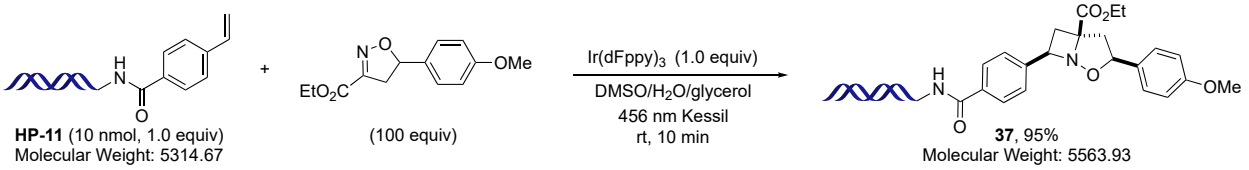
TIC of compound 36.



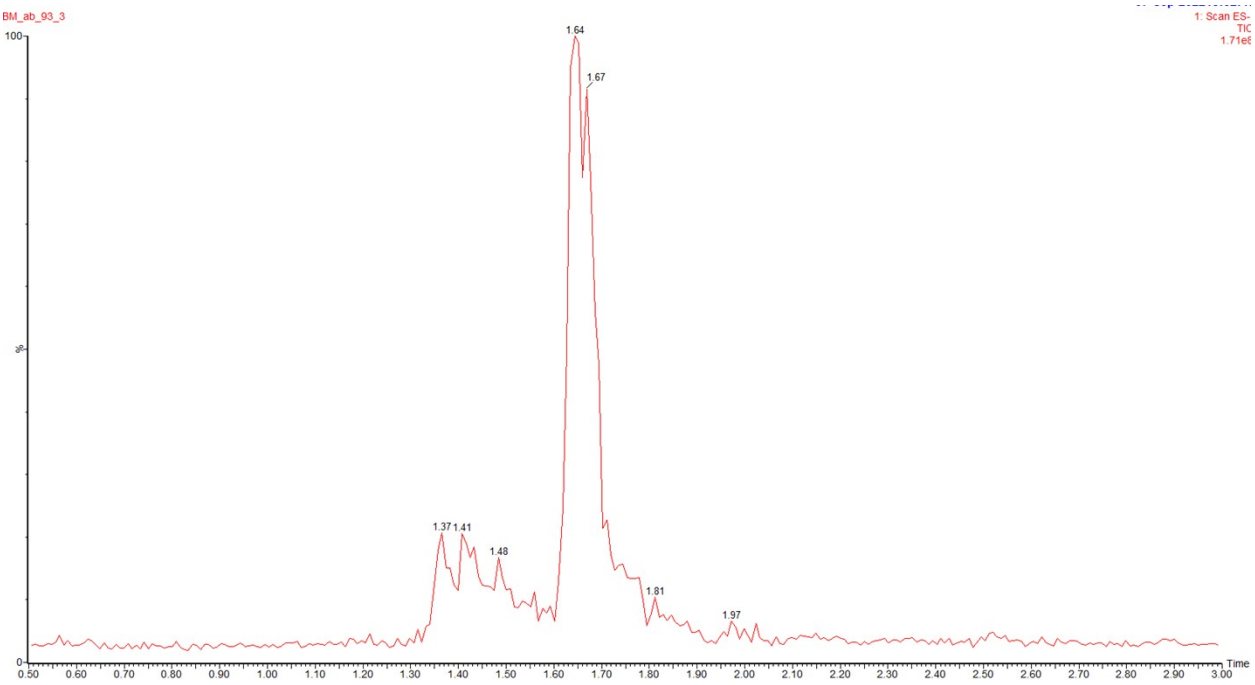
Raw MS of compound 36.



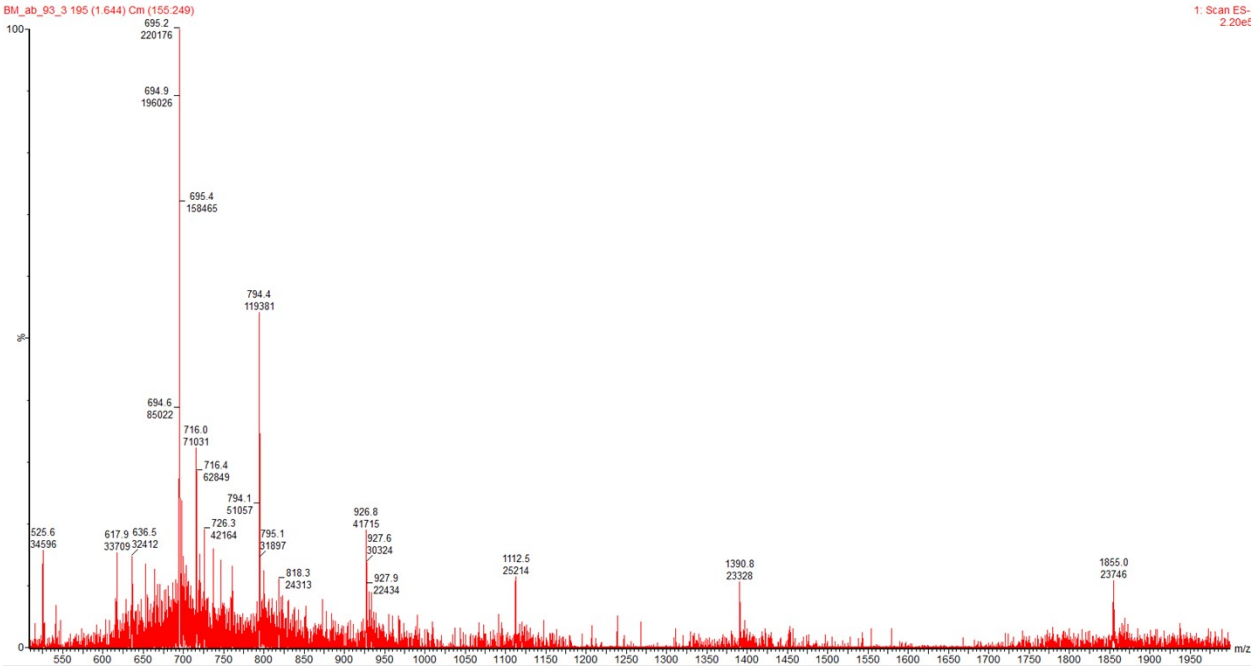
Deconvoluted MS of 36 (mass signals >10% of peak height).



The title compound **37** was synthesized according to **GP-IV** from ethyl 5-(4-bromophenyl)-4,5-dihydroisoxazole-3-carboxylate and the DNA-tagged alkene **HP-11**. The product was formed with 95% conversion.

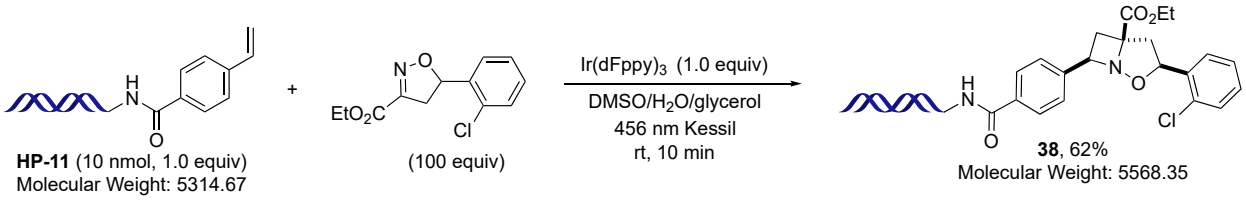


TIC of compound 37.



Raw MS of compound 37.

Deconvoluted MS of 37 (mass signals >10% of peak height).

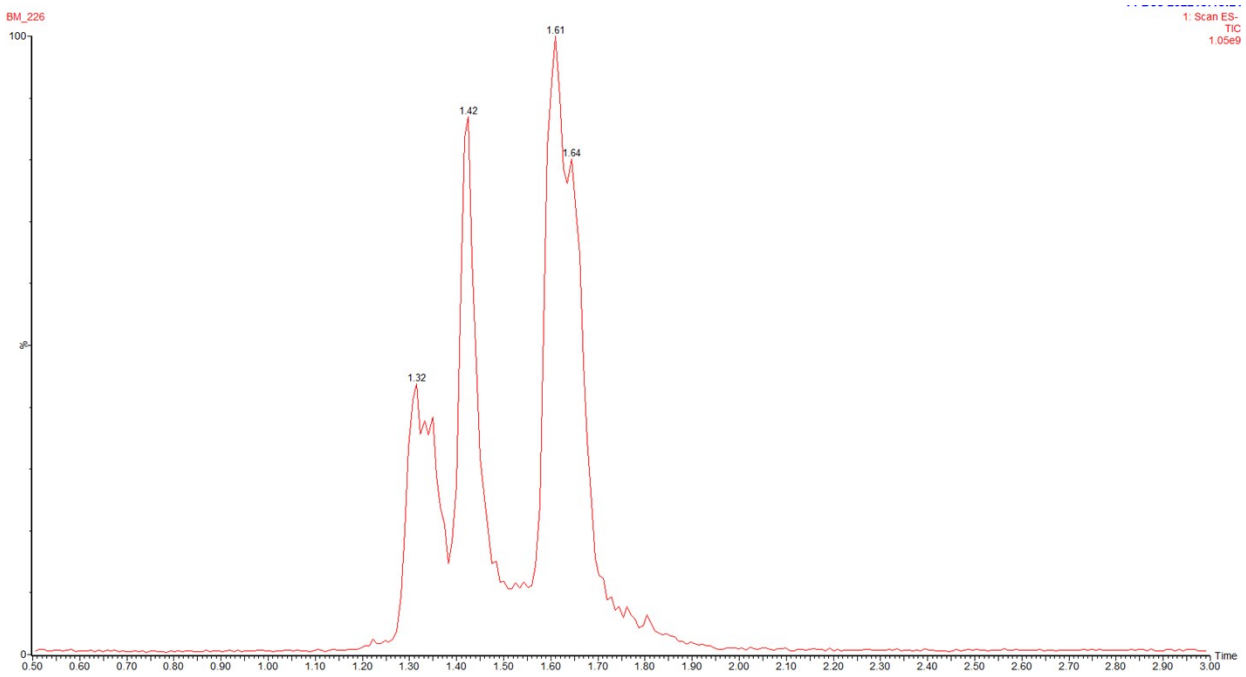


HP-11 (10 nmol, 1.0 equiv)
Molecular Weight: 5314.67

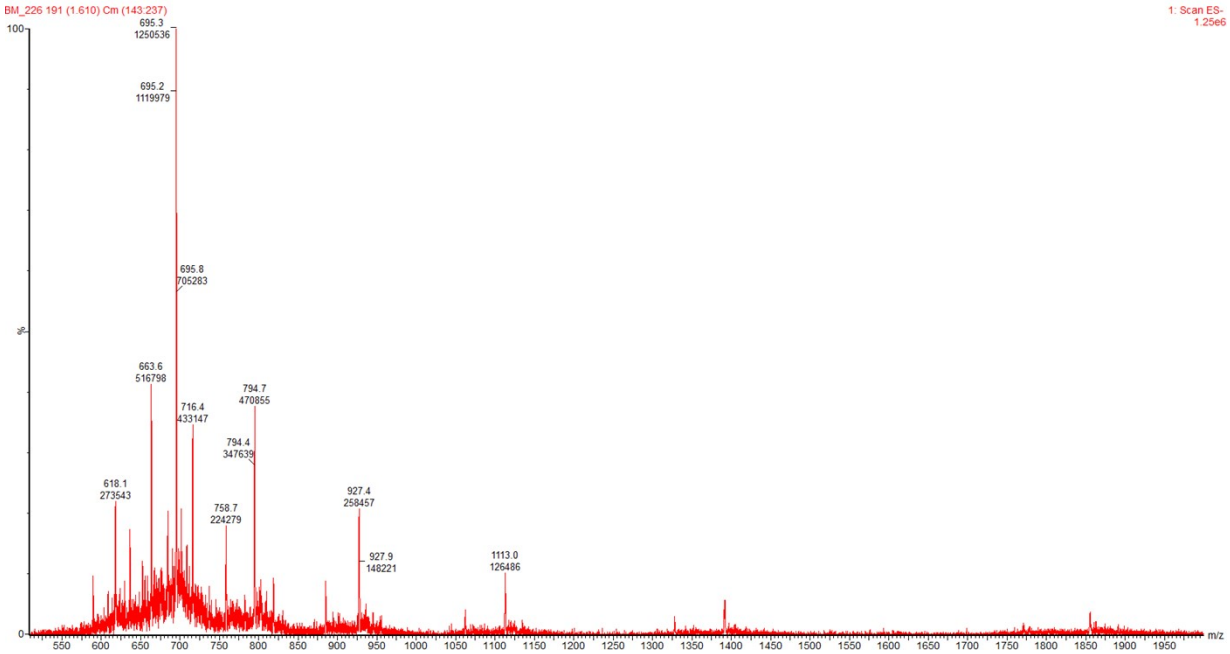
(100 equiv)

38, 62%
Molecular Weight: 5568.35

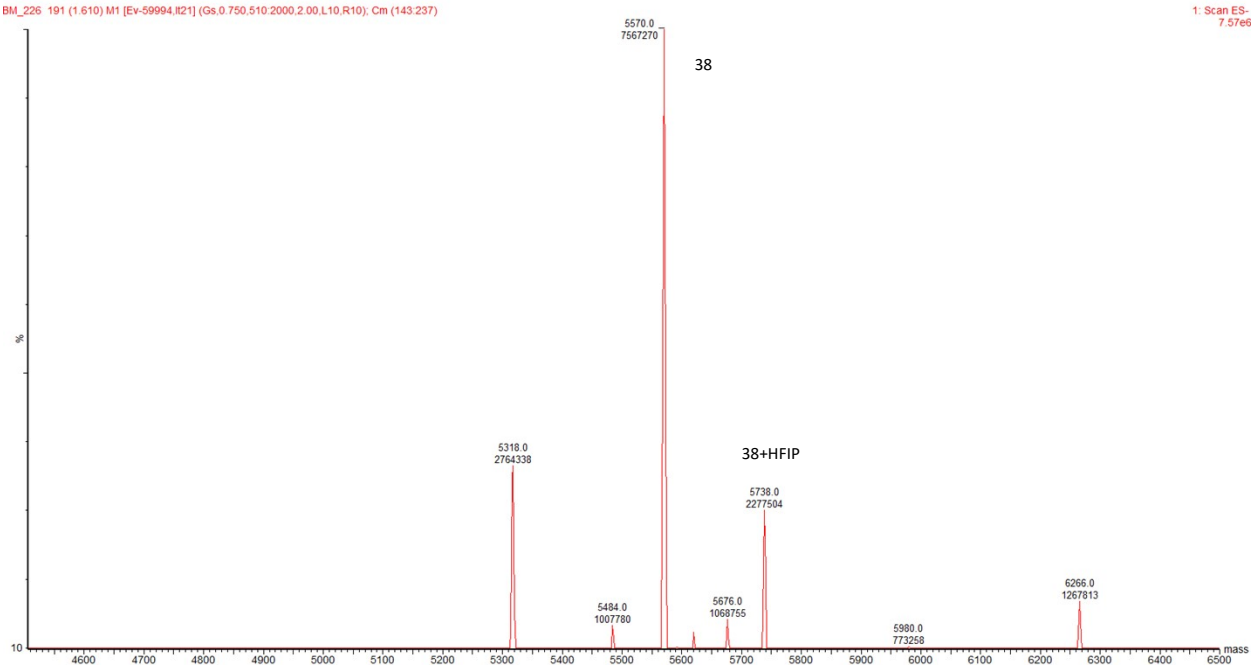
The title compound **38** was synthesized according to **GP-IV** from ethyl 5-(2-chlorophenyl)-4,5-dihydroisoxazole-3-carboxylate and the DNA-tagged alkene **HP-11**. The product was formed with 62% conversion.



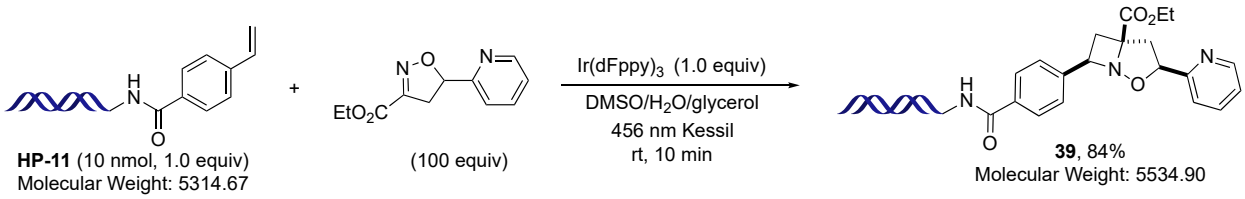
TIC of compound 38.



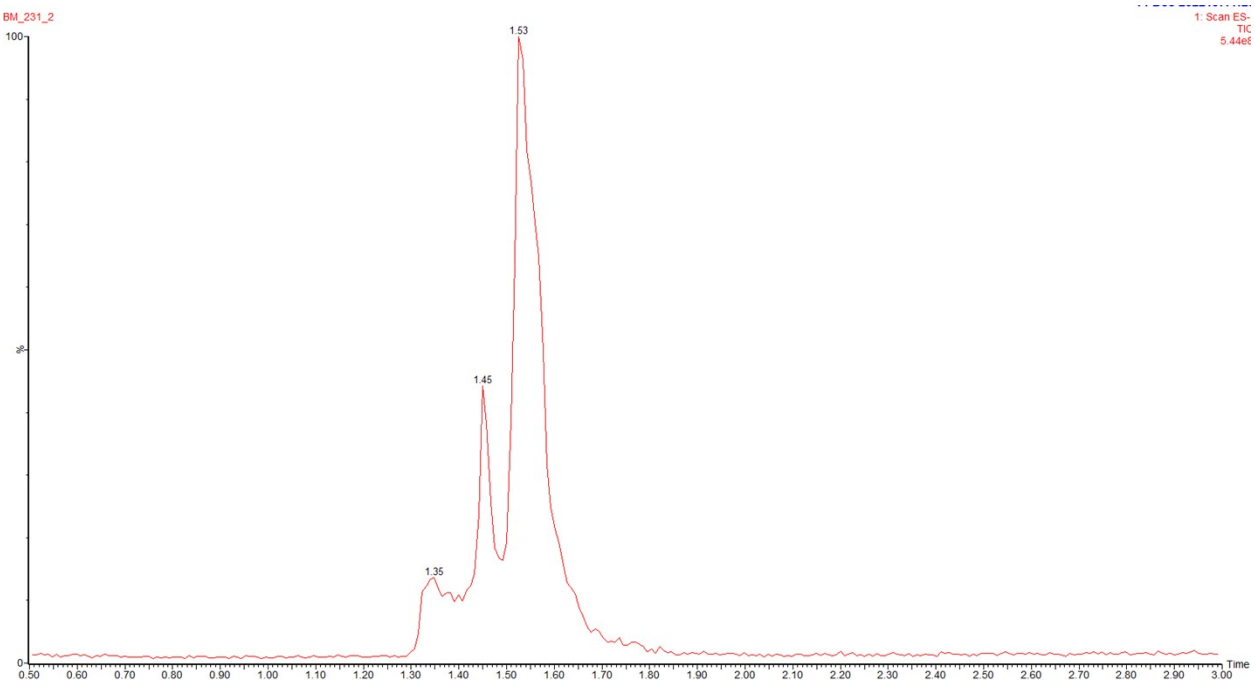
Raw MS of compound 38.



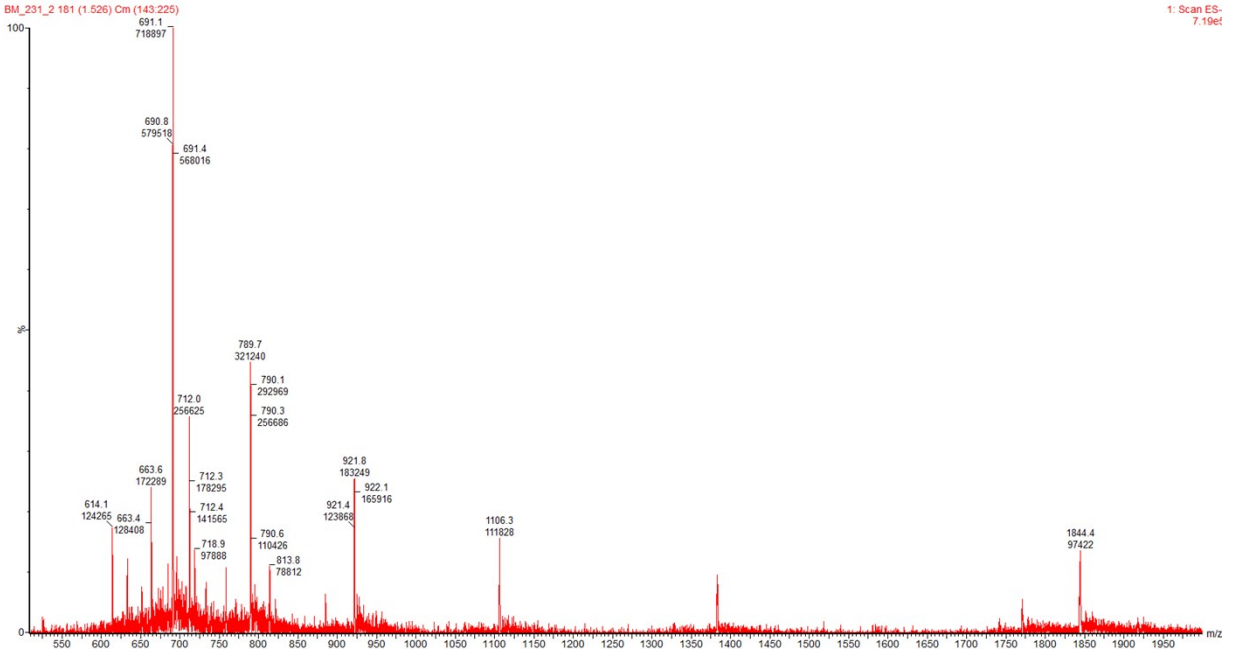
Deconvoluted MS of 38 (mass signals >10% of peak height).



The title compound **39** was synthesized according to **GP-III** from ethyl 5-(pyridin-2-yl)-4,5-dihydroisoxazole-3-carboxylate and the DNA-tagged alkene **HP-11**. The product was formed with 84% yield (8% recovered **HP-11**).

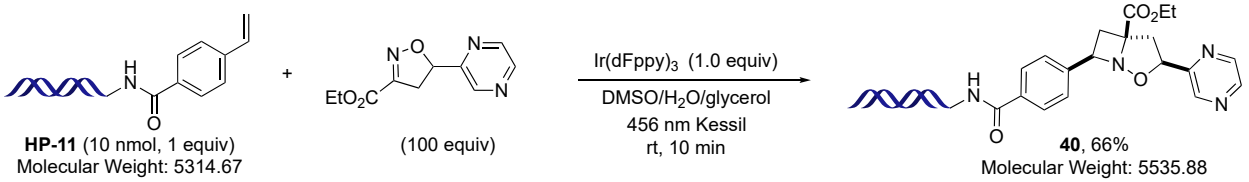


TIC of compound 39.

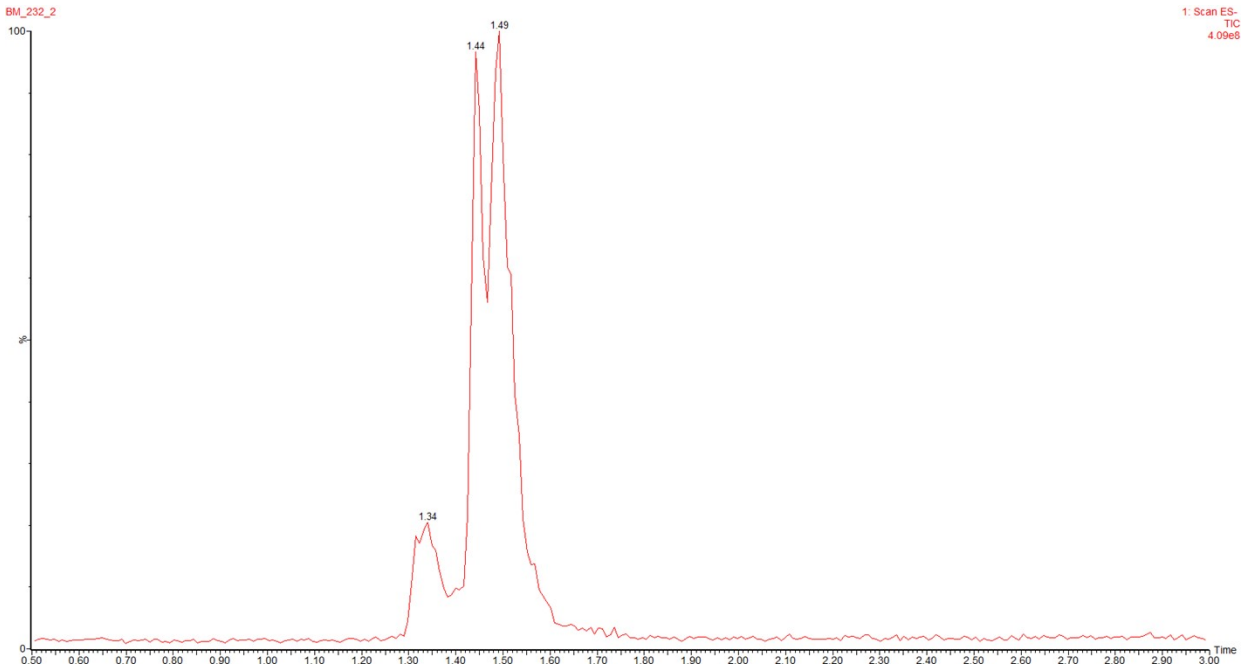


Raw MS of compound 39.

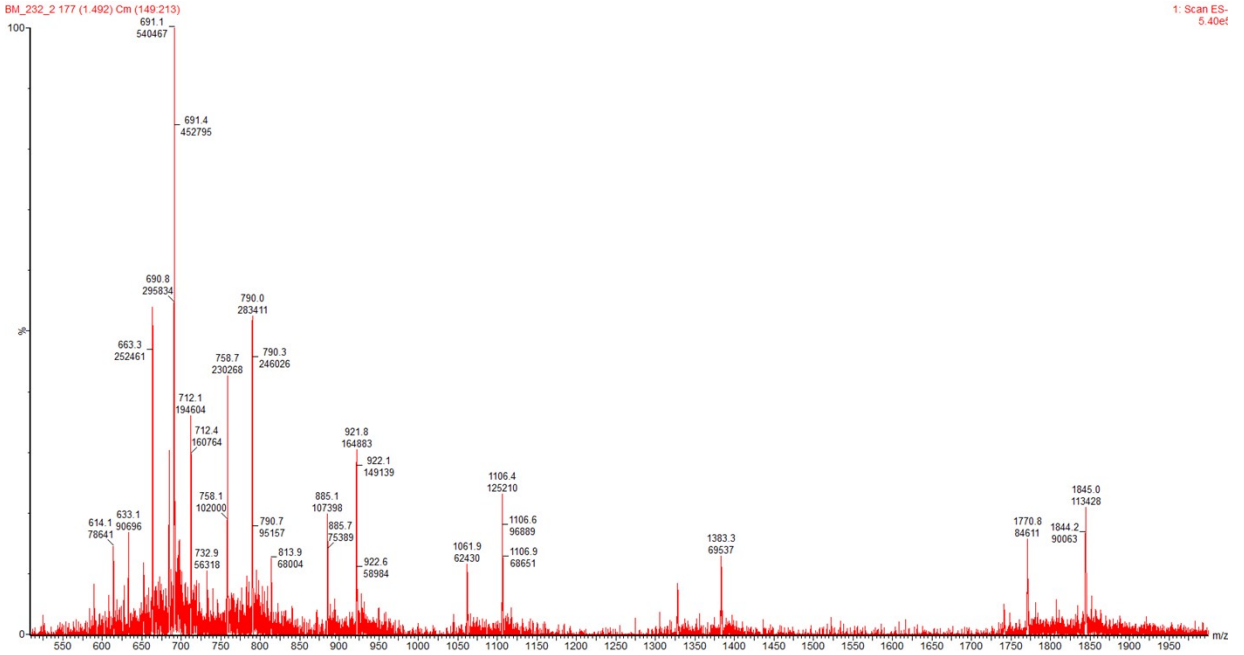
Deconvoluted MS of 39 (mass signals >10% of peak height).



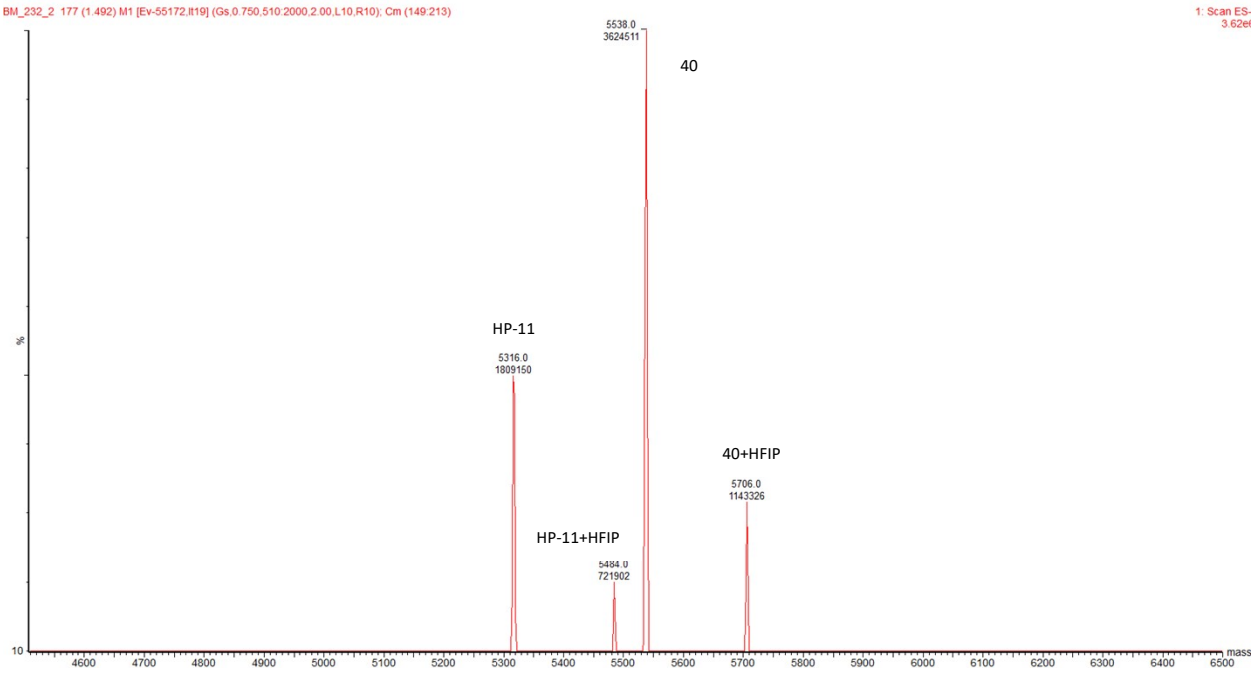
The title compound **40** was synthesized according to **GP-III** from ethyl 5-(pyridin-2-yl)-4,5-dihydroisoxazole-3-carboxylate and the DNA-tagged alkene **HP-11**. The product was formed with 66% yield.



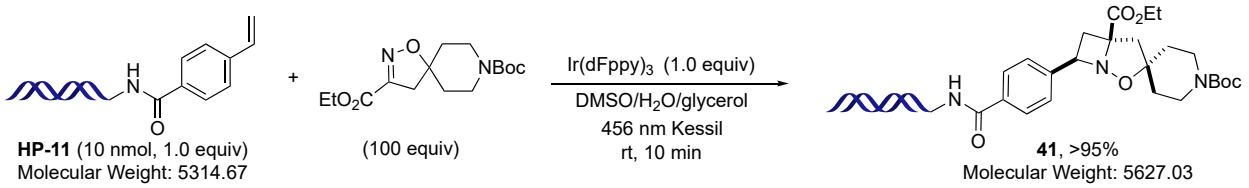
TIC of compound 40.



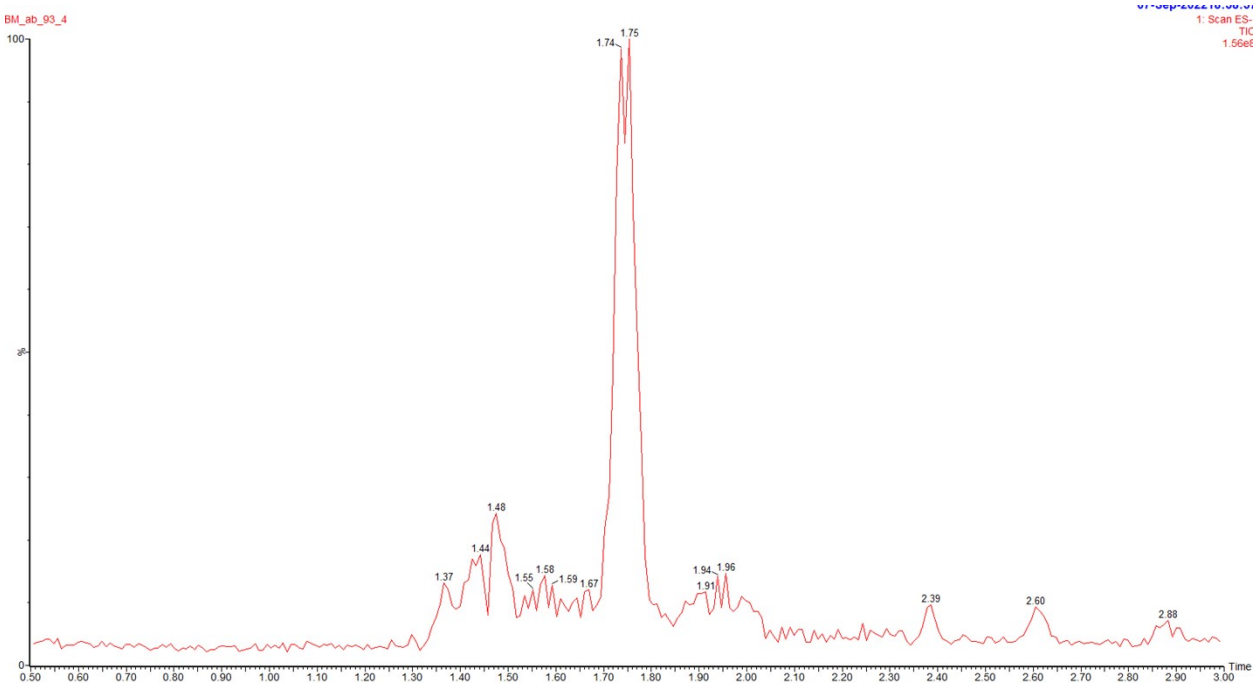
Raw MS of compound 40.



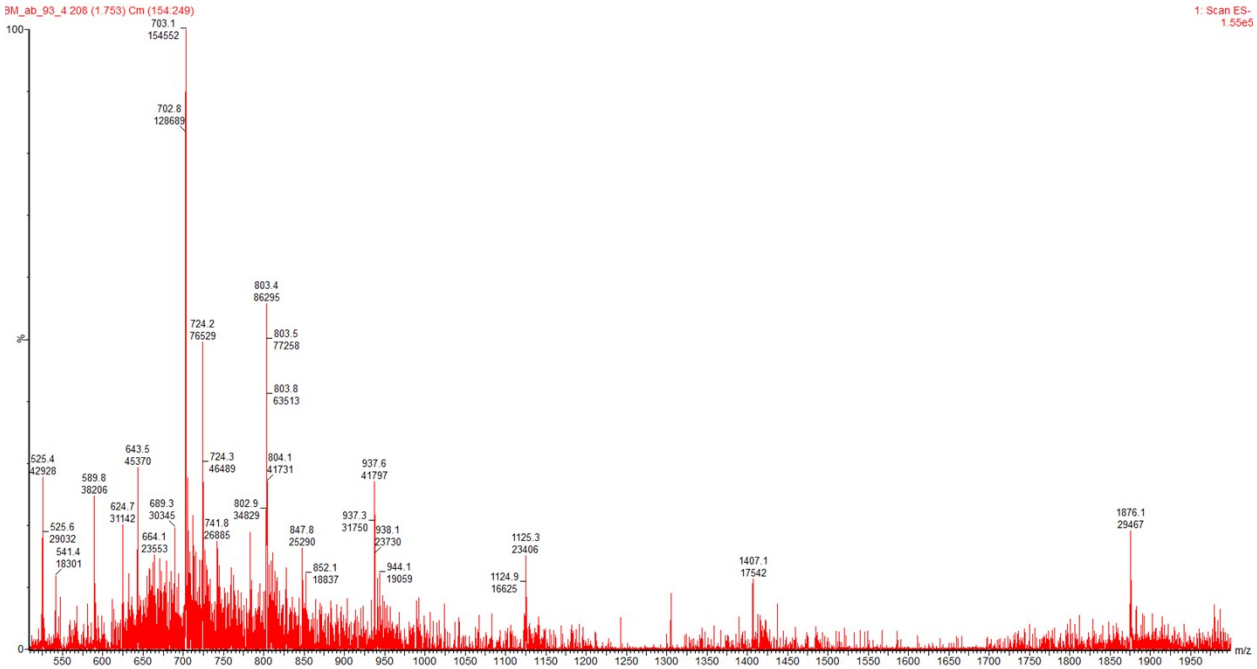
Deconvoluted MS of 40 (mass signals >10% of peak height).



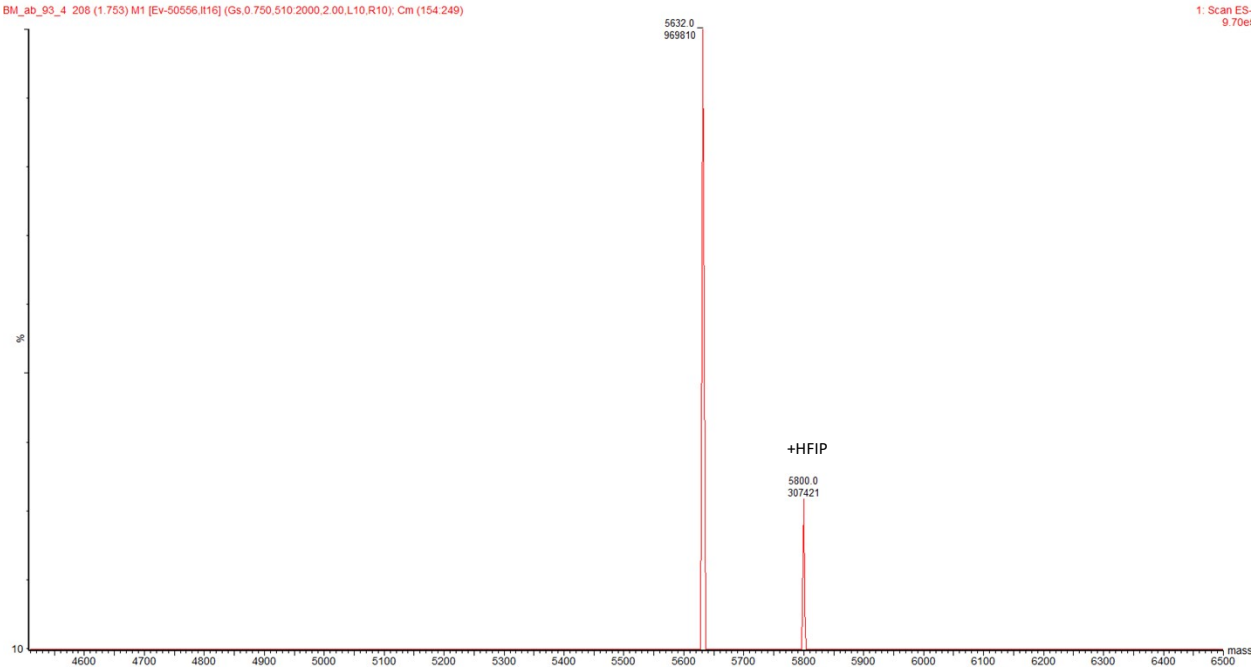
The title compound **41** was synthesized according to **GP-IV** from 8-(*tert*-butyl) 3-ethyl 1-oxa-2,8-diazaspiro[4.5]dec-2-ene-3,8-dicarboxylate and the DNA-tagged alkene **HP-11**. The product was formed with >95% conversion.



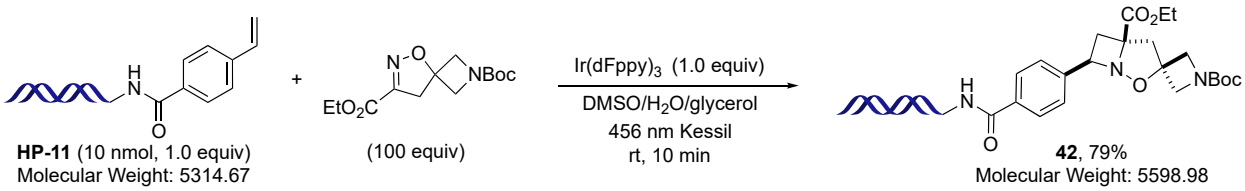
TIC of compound 41.



Raw MS of compound 41.



Deconvoluted MS of 41 (mass signals >10% of peak height).

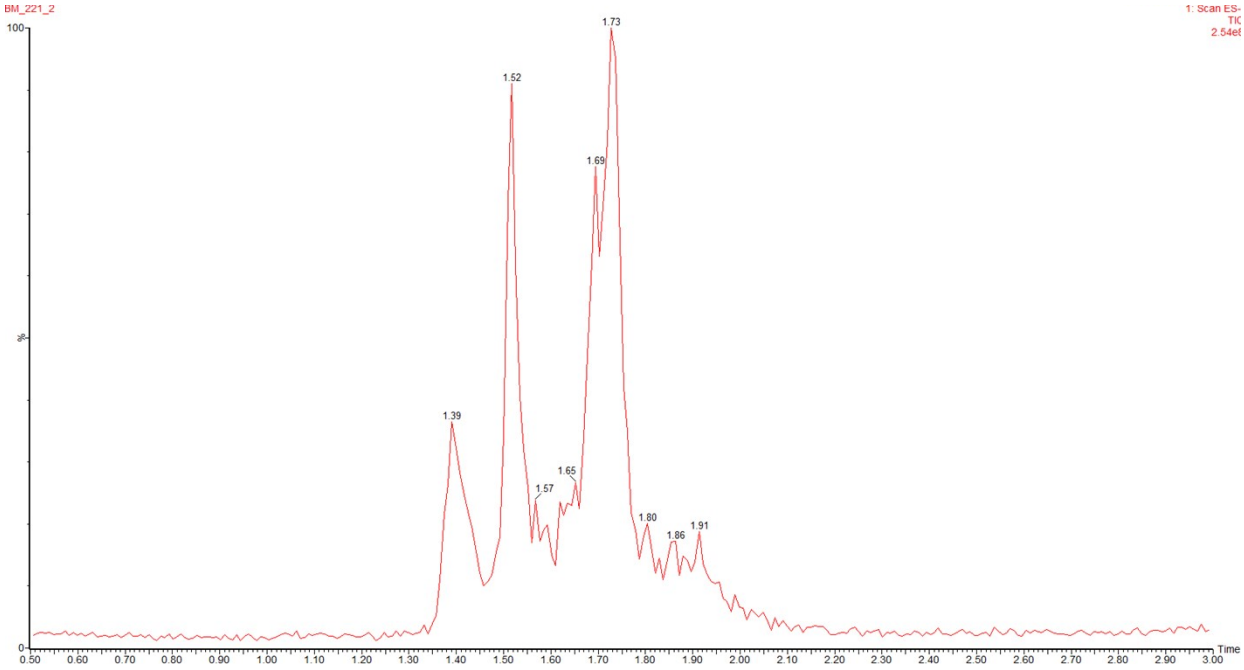


HP-11 (10 nmol, 1.0 equiv)
Molecular Weight: 5314.67

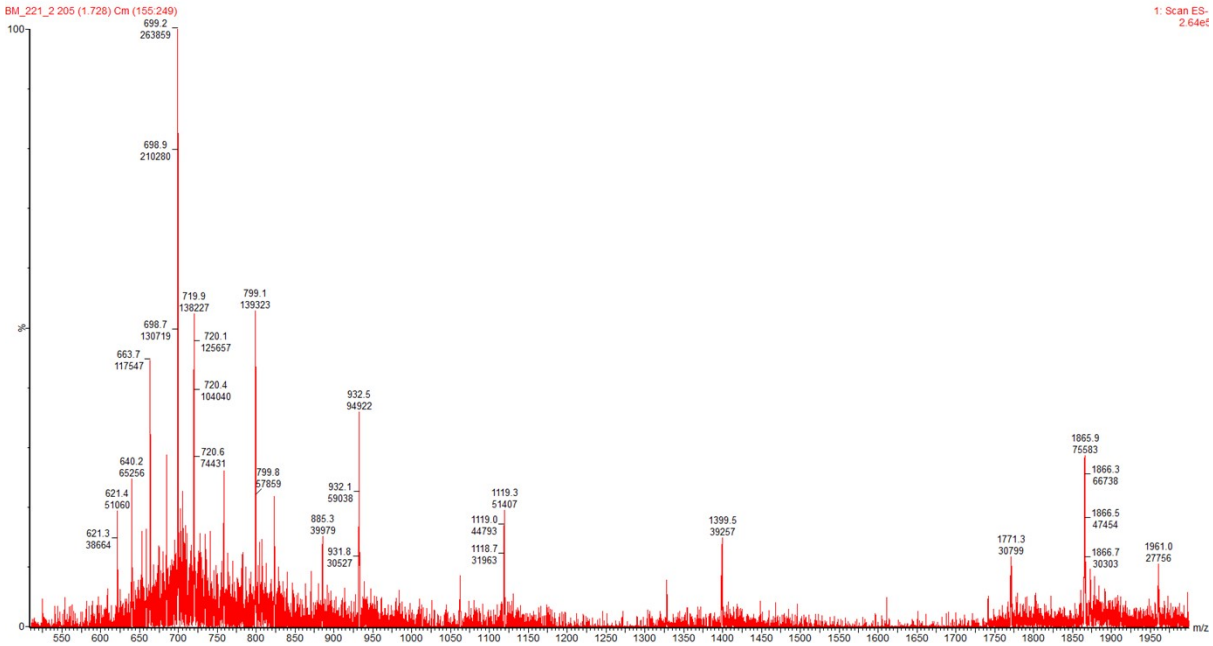
(100 equiv)

42, 79%
Molecular Weight: 5598.98

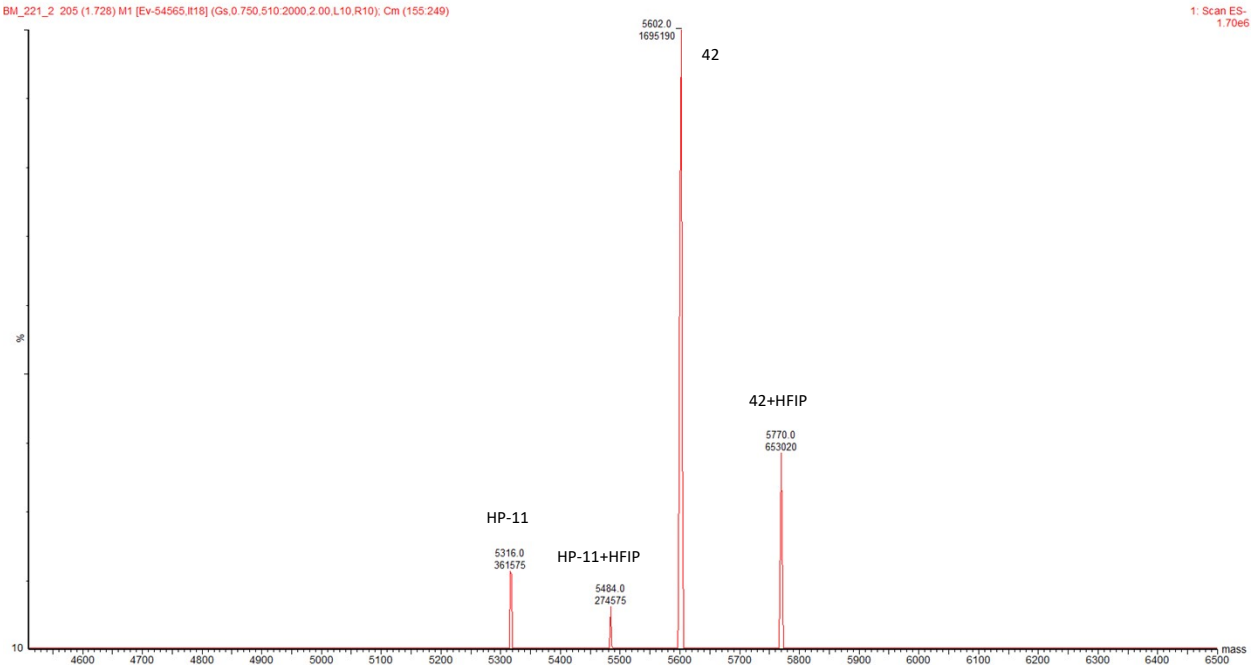
The title compound **42** was synthesized according to **GP-IV** from 2-(*tert*-butyl) 7-ethyl 5-oxa-2,6-diazaspiro[3.4]oct-6-ene-2,7-dicarboxylate and the DNA-tagged alkene **HP-11**. The product was formed with 79% conversion (21% recovered **HP-11**).



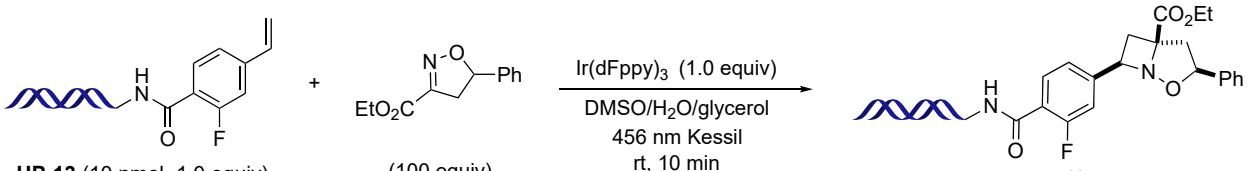
TIC of compound 42.



Raw MS of compound 42.



Deconvoluted MS of 42 (mass signals >10% of peak height).

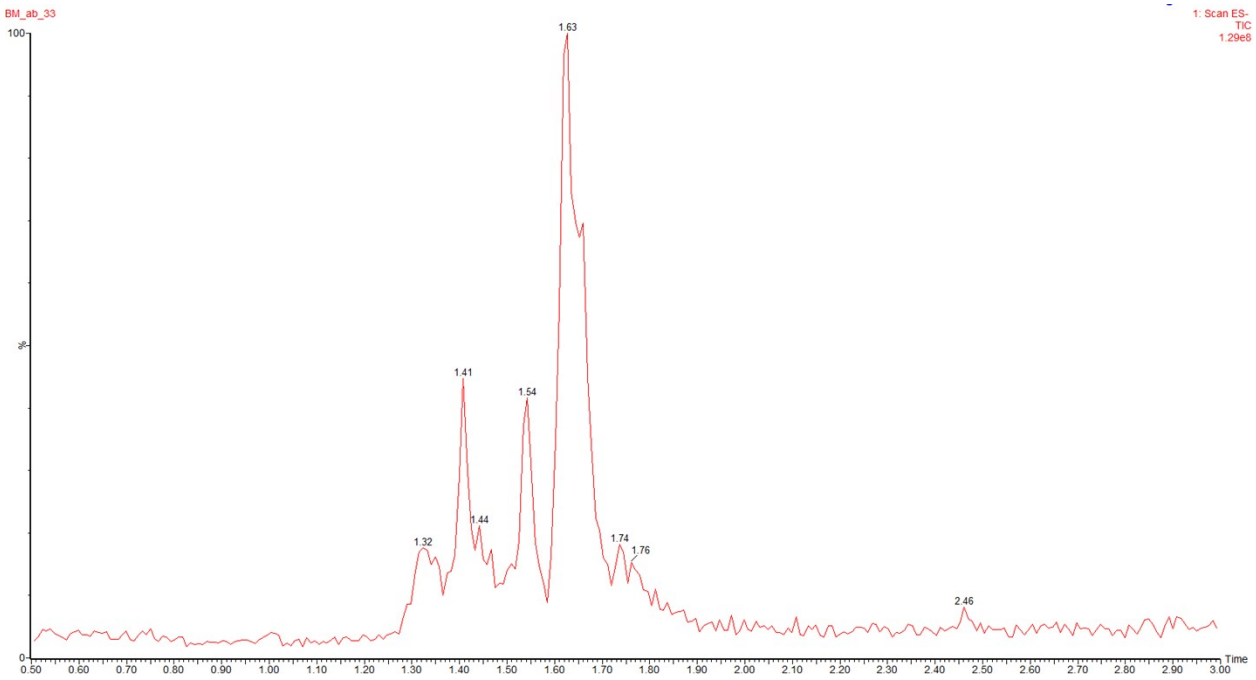


HP-13 (10 nmol, 1.0 equiv)
Molecular Weight: 5332.66

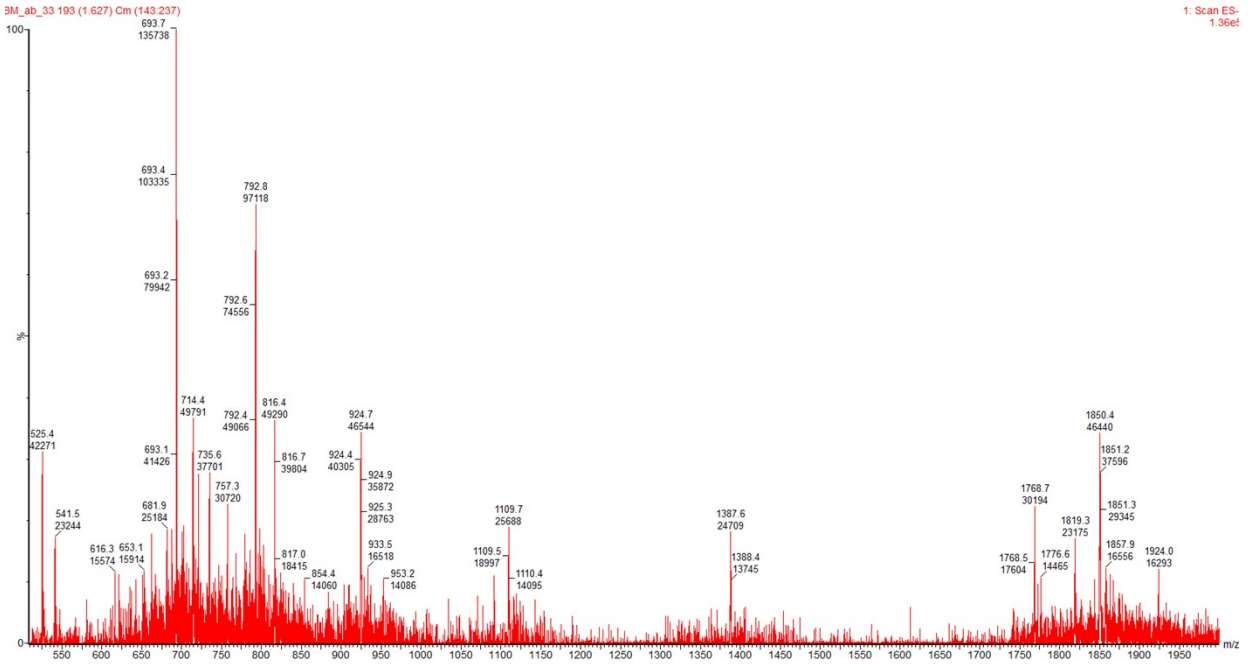
(100 equiv)

43, 34%
Molecular Weight: 5551.90

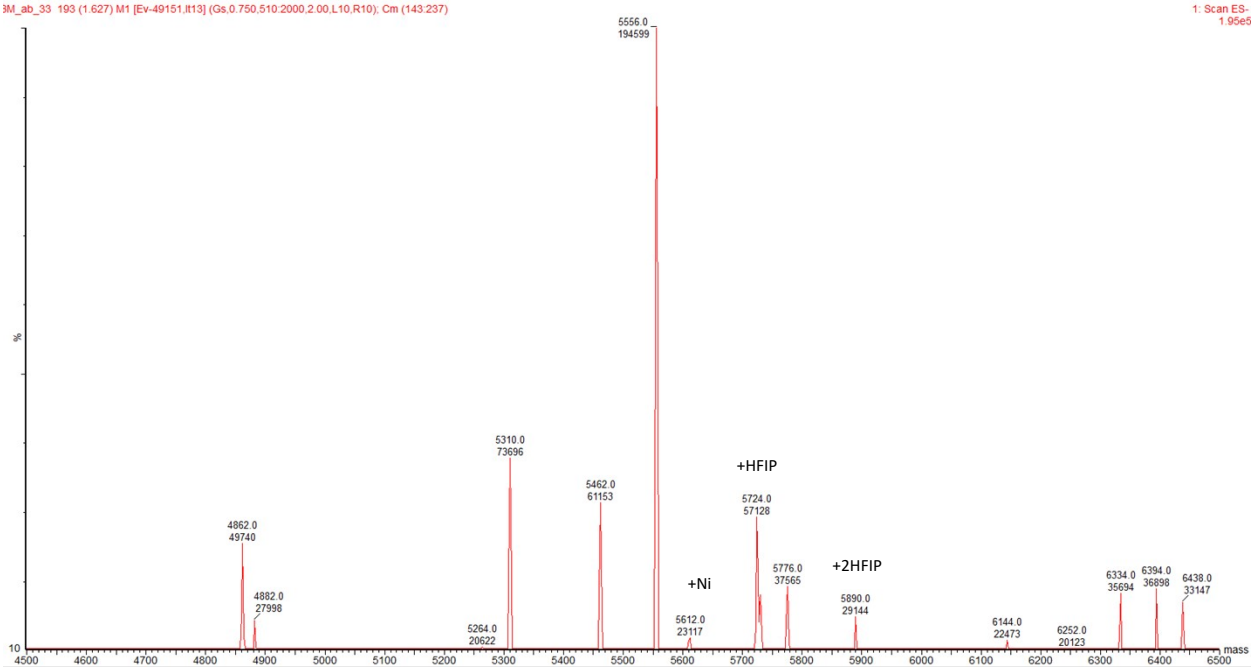
The title compound **43** was synthesized according to **GP-IV** from ethyl 5-phenyl-4,5-dihydroisoxazole-3-carboxylate and the DNA-tagged alkene **HP-13**. The product was formed with 34% yield.



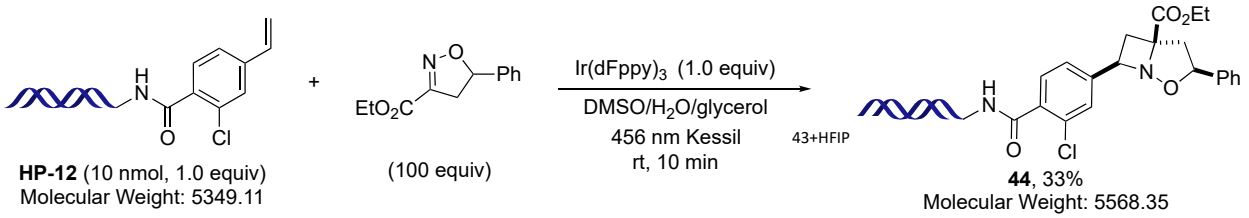
TIC of compound 43.



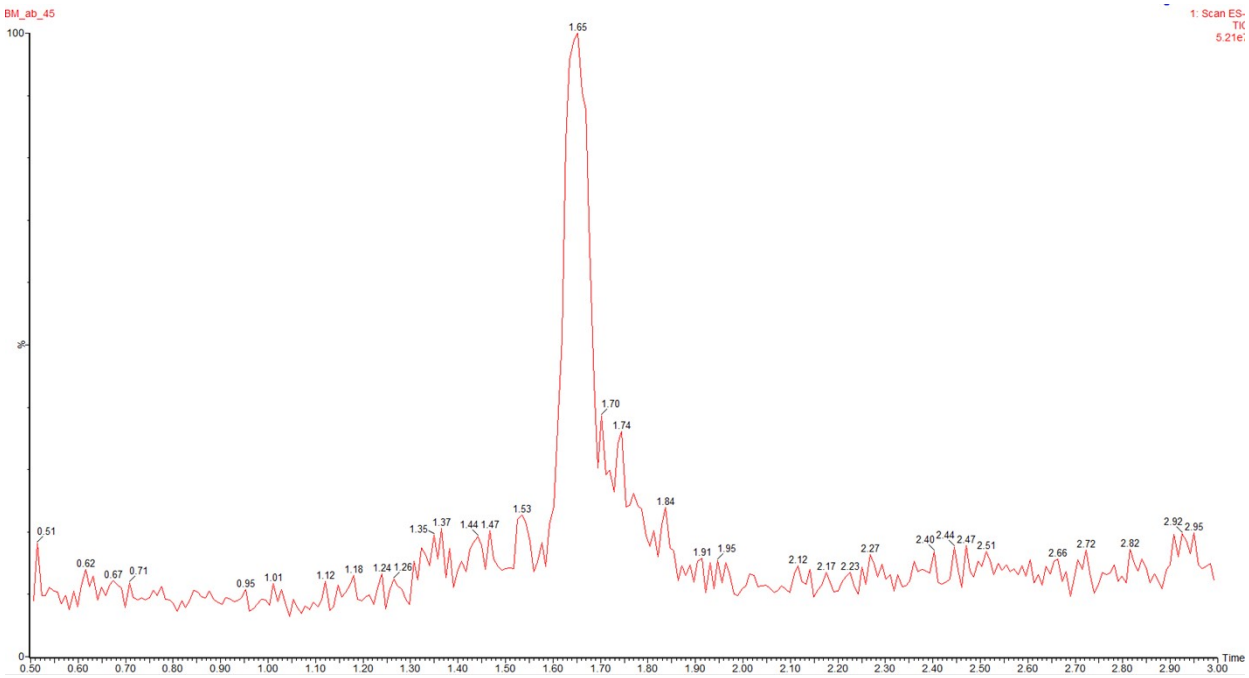
Raw MS of compound 43.



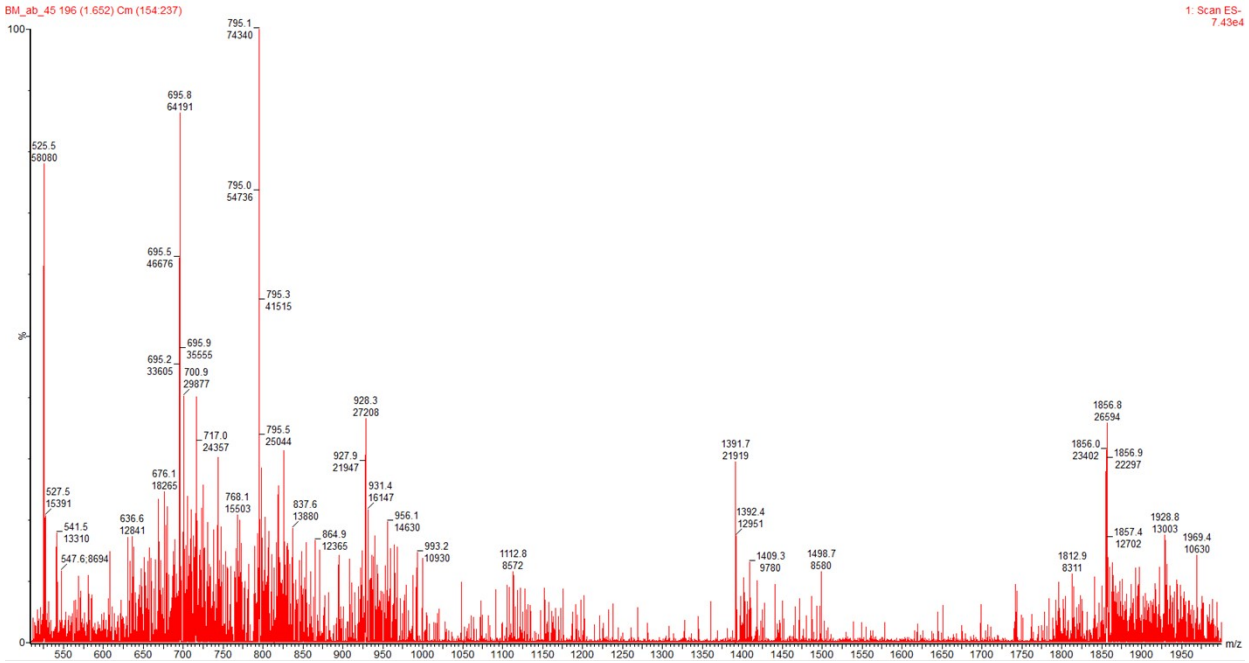
Deconvoluted MS of 43 (mass signals >10% of peak height).



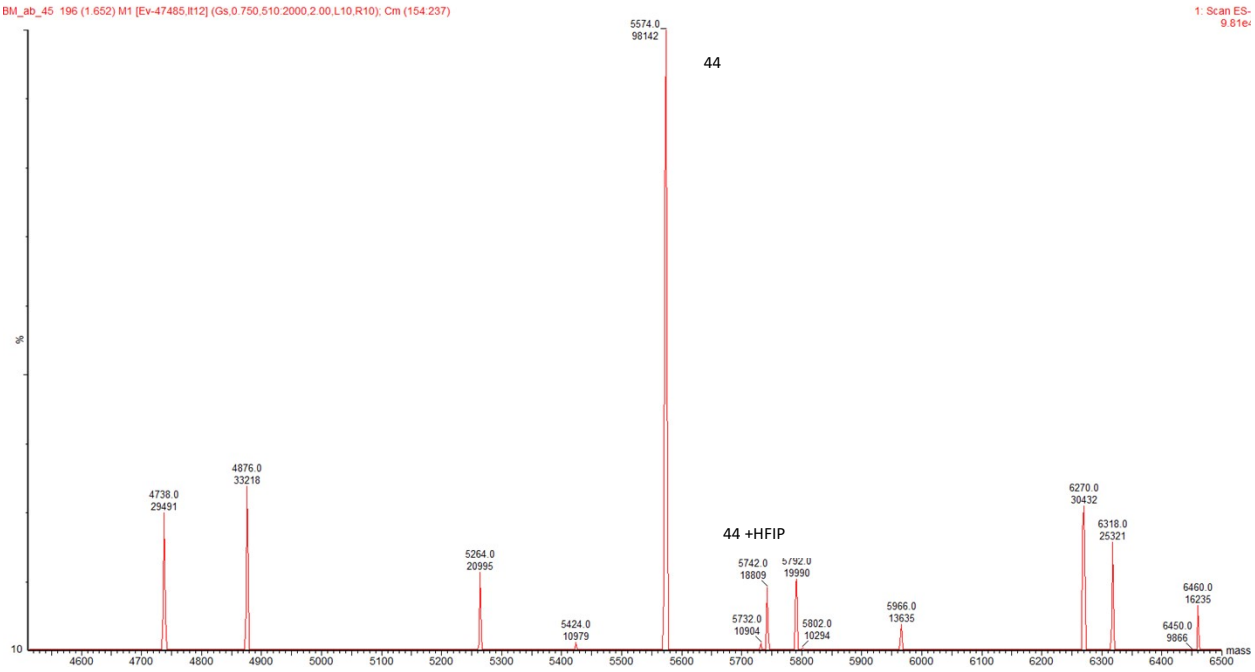
The title compound **44** was synthesized according to **GP-IV** from ethyl 5-phenyl-4,5-dihydroisoxazole-3-carboxylate and the DNA-tagged alkene **HP-12**. The product was formed with 33% conversion.



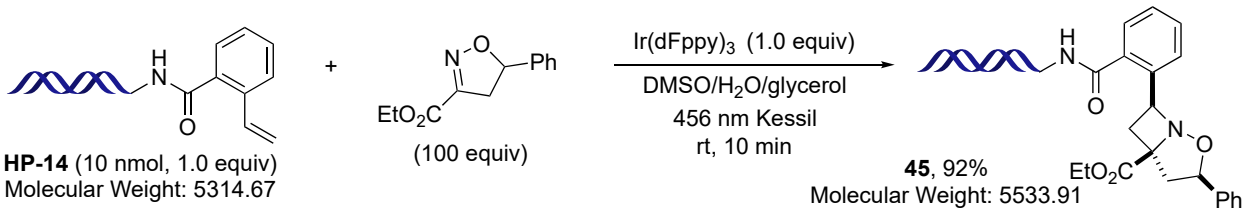
TIC of compound 44.



Raw MS of compound 44.



Deconvoluted MS of 44 (mass signals >10% of peak height).

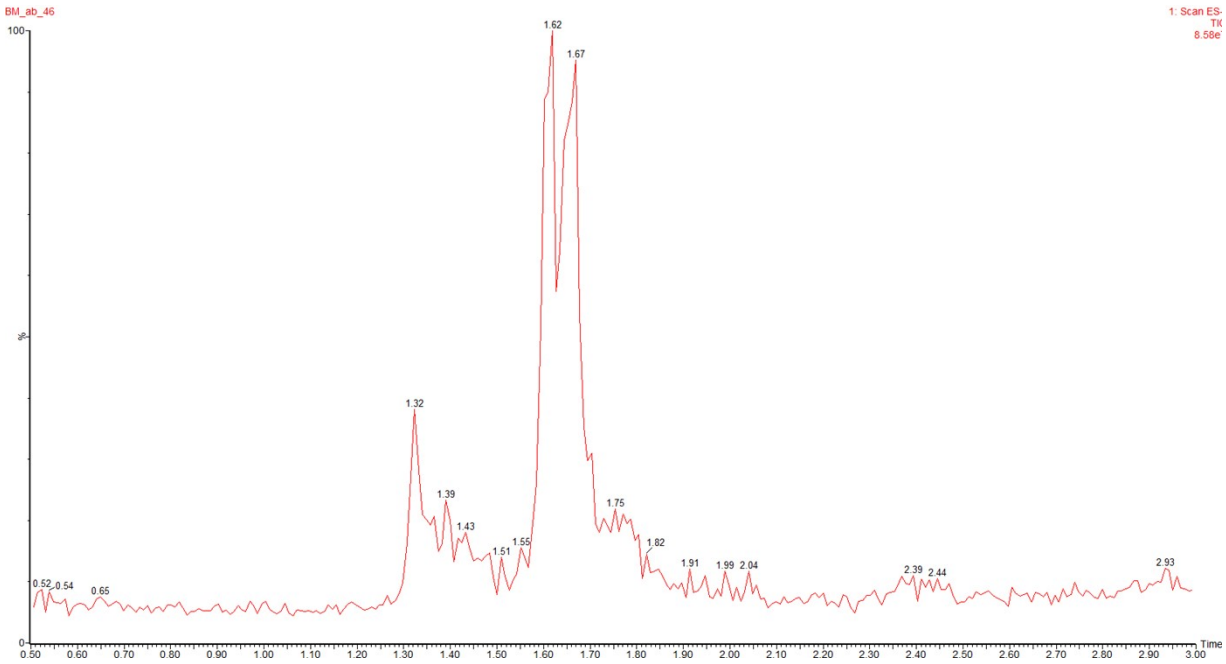


HP-14 (10 nmol, 1.0 equiv)
Molecular Weight: 5314.67

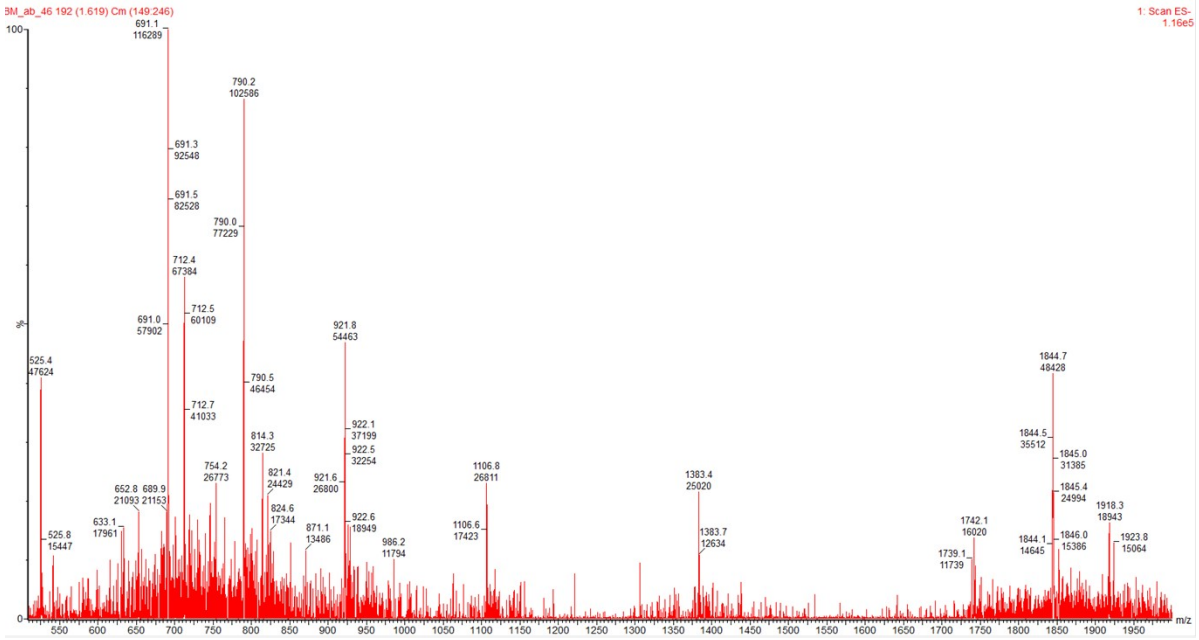
(100 equiv)

45, 92%
Molecular Weight: 5533.91

The title compound **45** was synthesized according to **GP-IV** from ethyl 5-phenyl-4,5-dihydroisoxazole-3-carboxylate and the DNA-tagged alkene **HP-14**. The product was formed with 92% yield.

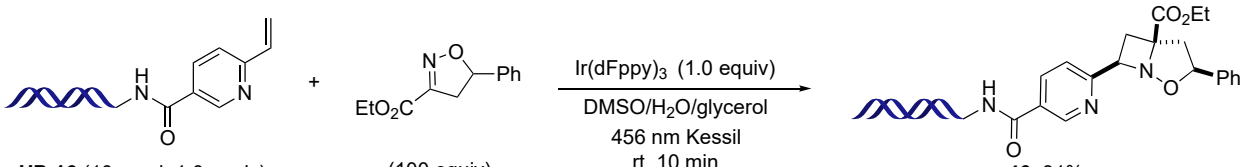


TIC of compound 45.



Raw MS of compound 45.

Deconvoluted MS of 45 (mass signals >10% of peak height).

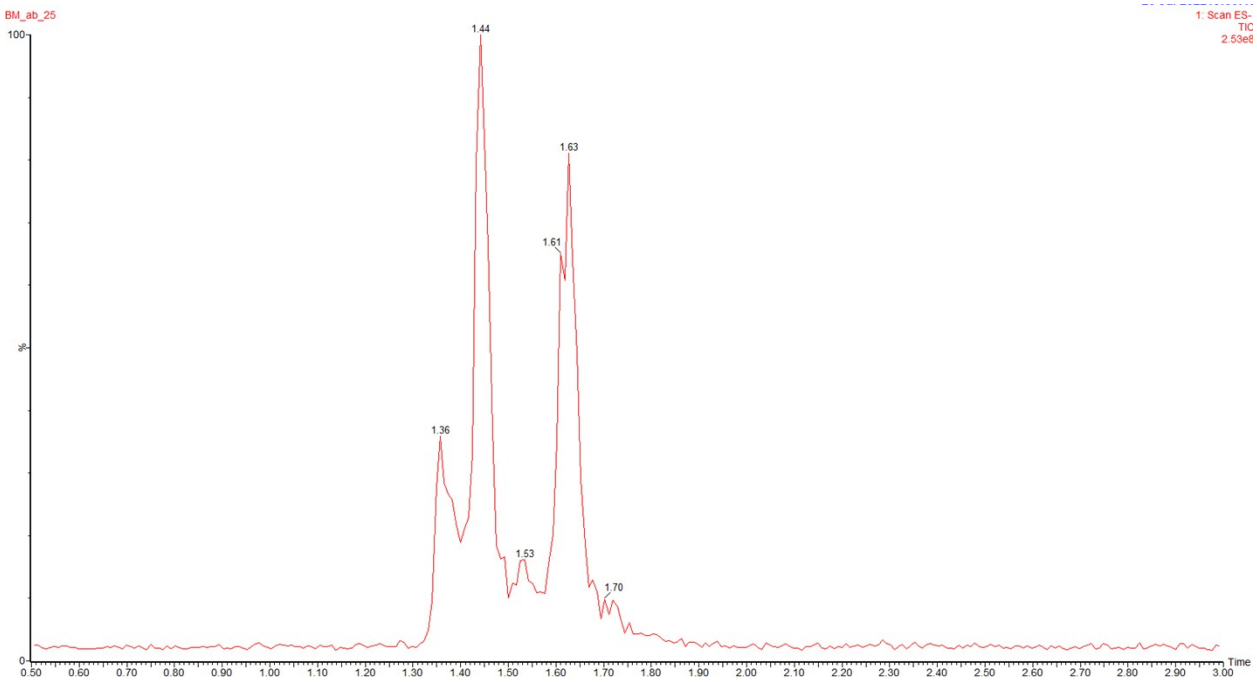


HP-16 (10 nmol, 1.0 equiv)
Molecular Weight: 5315.66

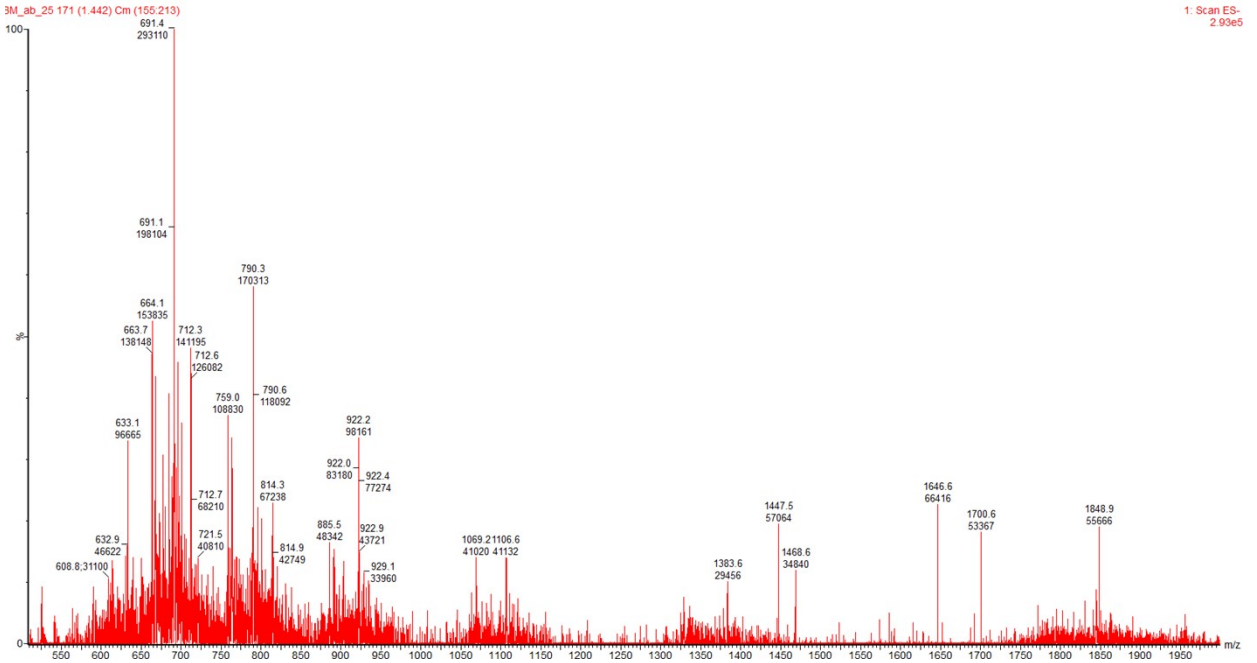
(100 equiv)

46, 31%
Molecular Weight: 5534.90

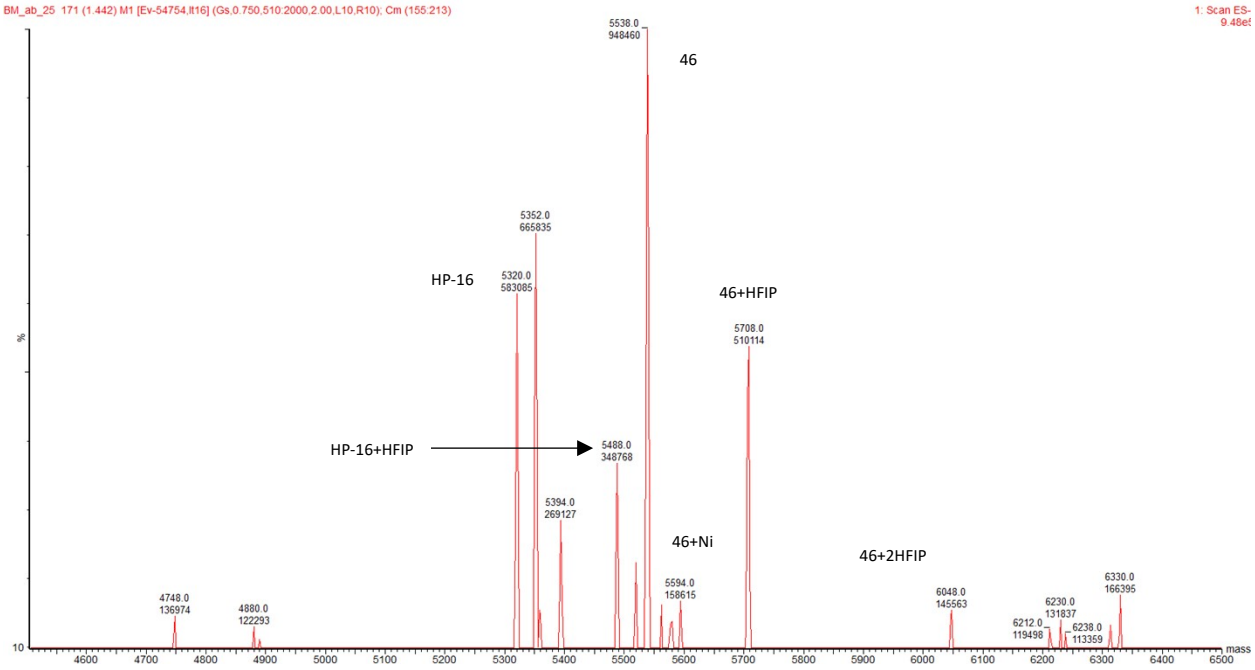
The title compound **46** was synthesized according to **GP-IV** from ethyl 5-phenyl-4,5-dihydroisoxazole-3-carboxylate and the DNA-tagged alkene **HP-16**. The product was formed with 31% conversion.



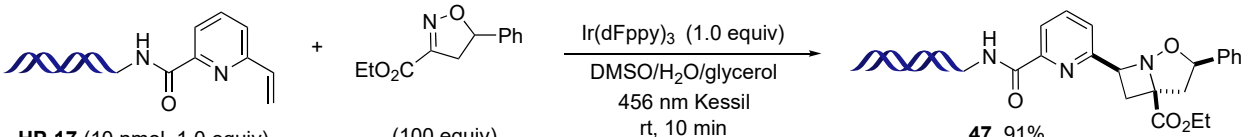
TIC of compound 46.



Raw MS of compound 46.



Deconvoluted MS of 46 (mass signals >10% of peak height).

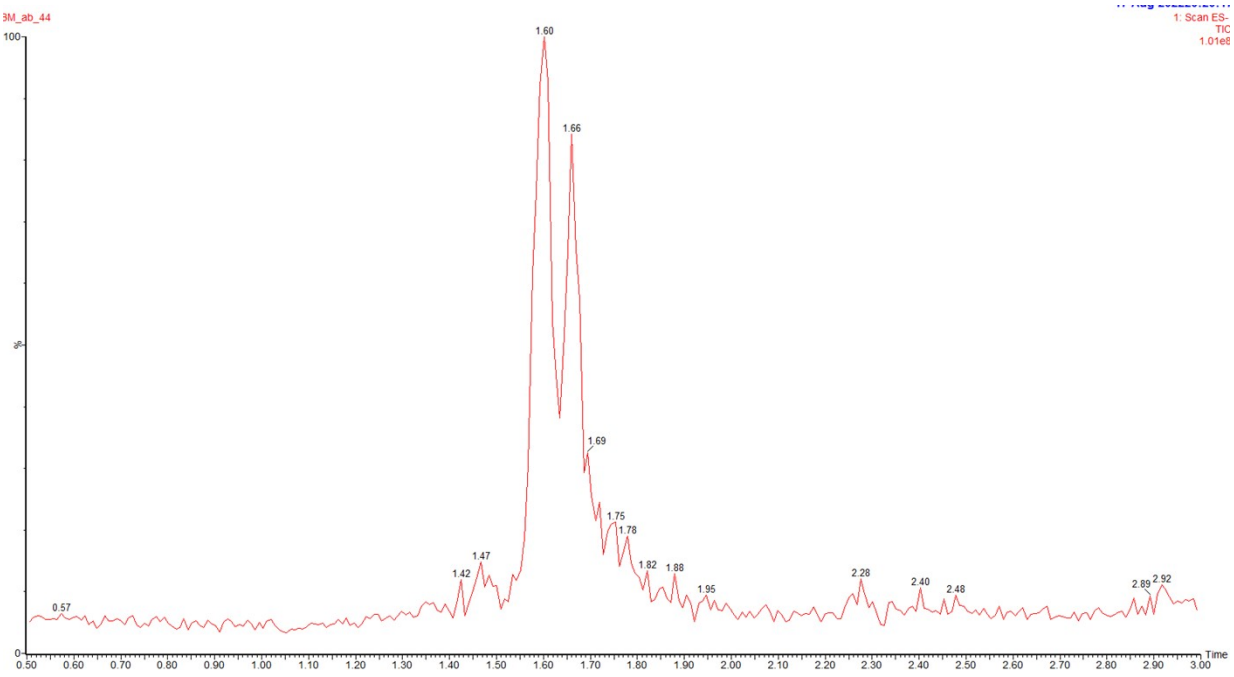


HP-17 (10 nmol, 1.0 equiv)
Molecular Weight: 5315.66

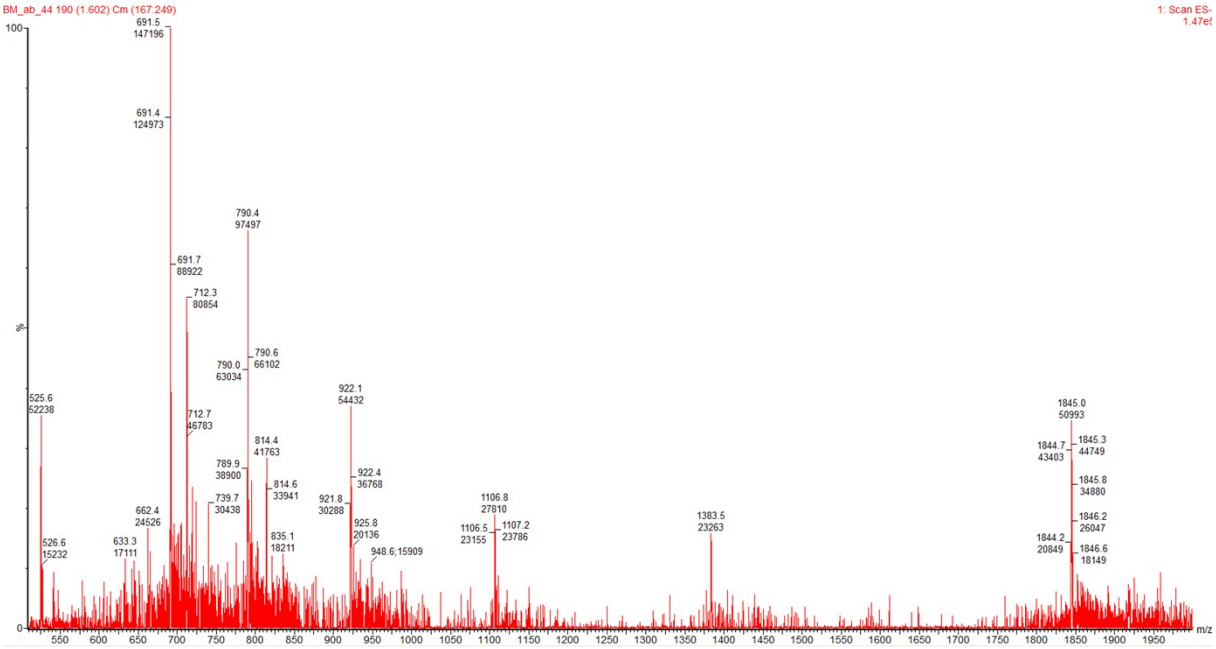
(100 equiv)

47, 91%
Molecular Weight: 5534.90

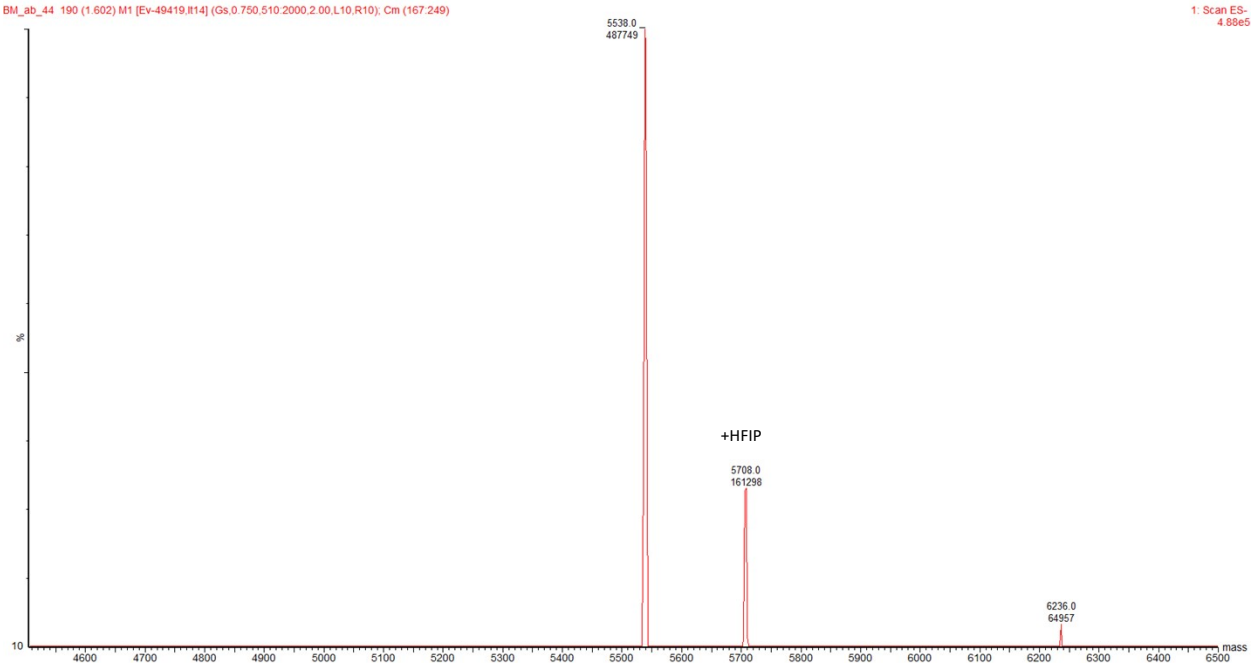
The title compound **47** was synthesized according to **GP-IV** from ethyl 5-phenyl-4,5-dihydroisoxazole-3-carboxylate and the DNA-tagged alkene **HP-17**. The product was formed with 91% conversion.



TIC of compound 47.

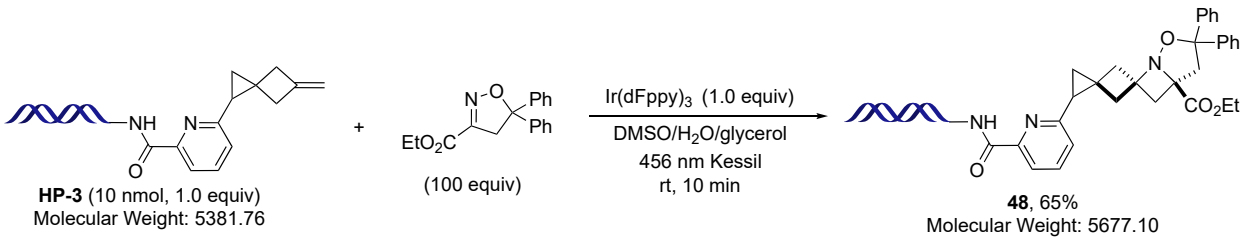


Raw MS of compound 47.

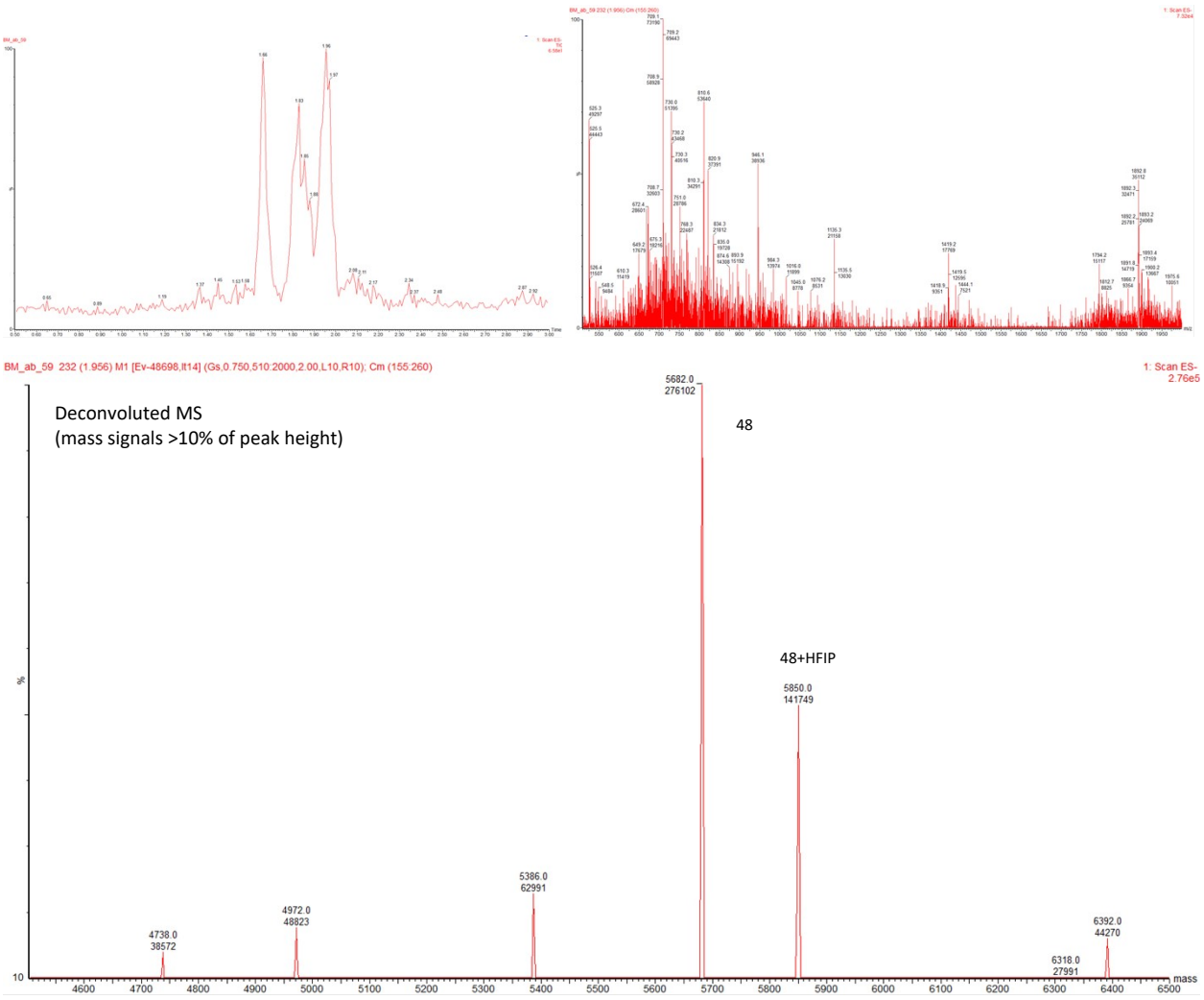


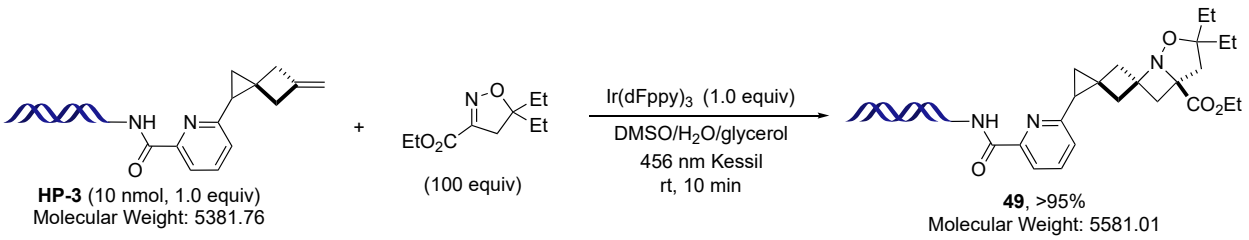
Deconvoluted MS of 47 (mass signals >10% of peak height).

UPLC/MS Spectra: Additional Scope of 2-Oxa-1-azaspiro(bicyclo[3.2.0])heptanes On-DNA



The title compound **48** was synthesized according to **GP-IV** from ethyl 5,5-diphenyl-4,5-dihydroisoxazole-3-carboxylate and the DNA-tagged alkene **HP-3**. The product was formed with 65% conversion (10% recovered **HP-3**).



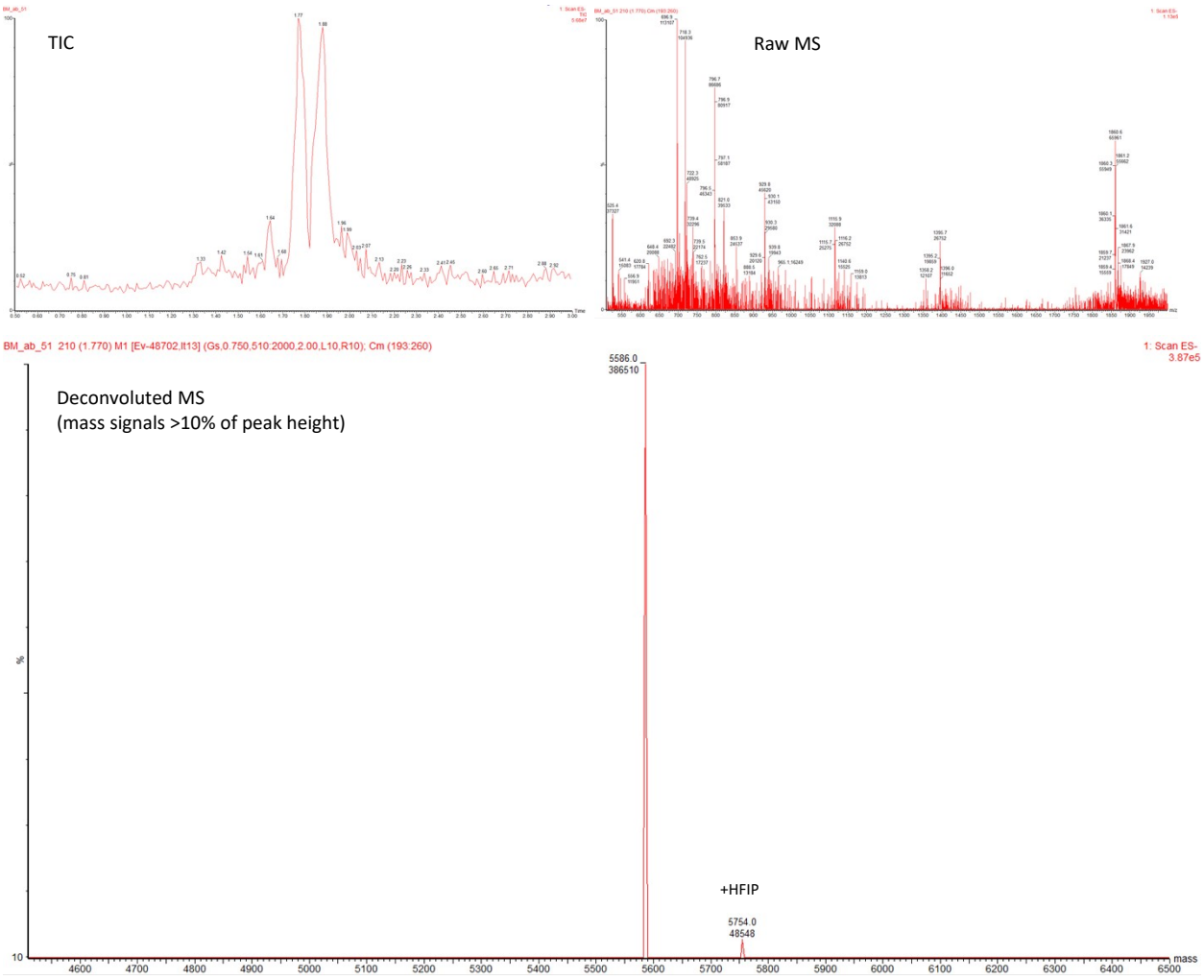


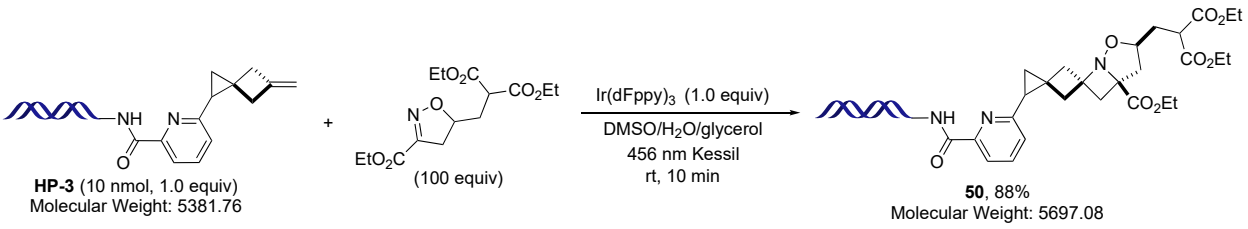
HP-3 (10 nmol, 1.0 equiv)
Molecular Weight: 5381.76

(100 equiv)

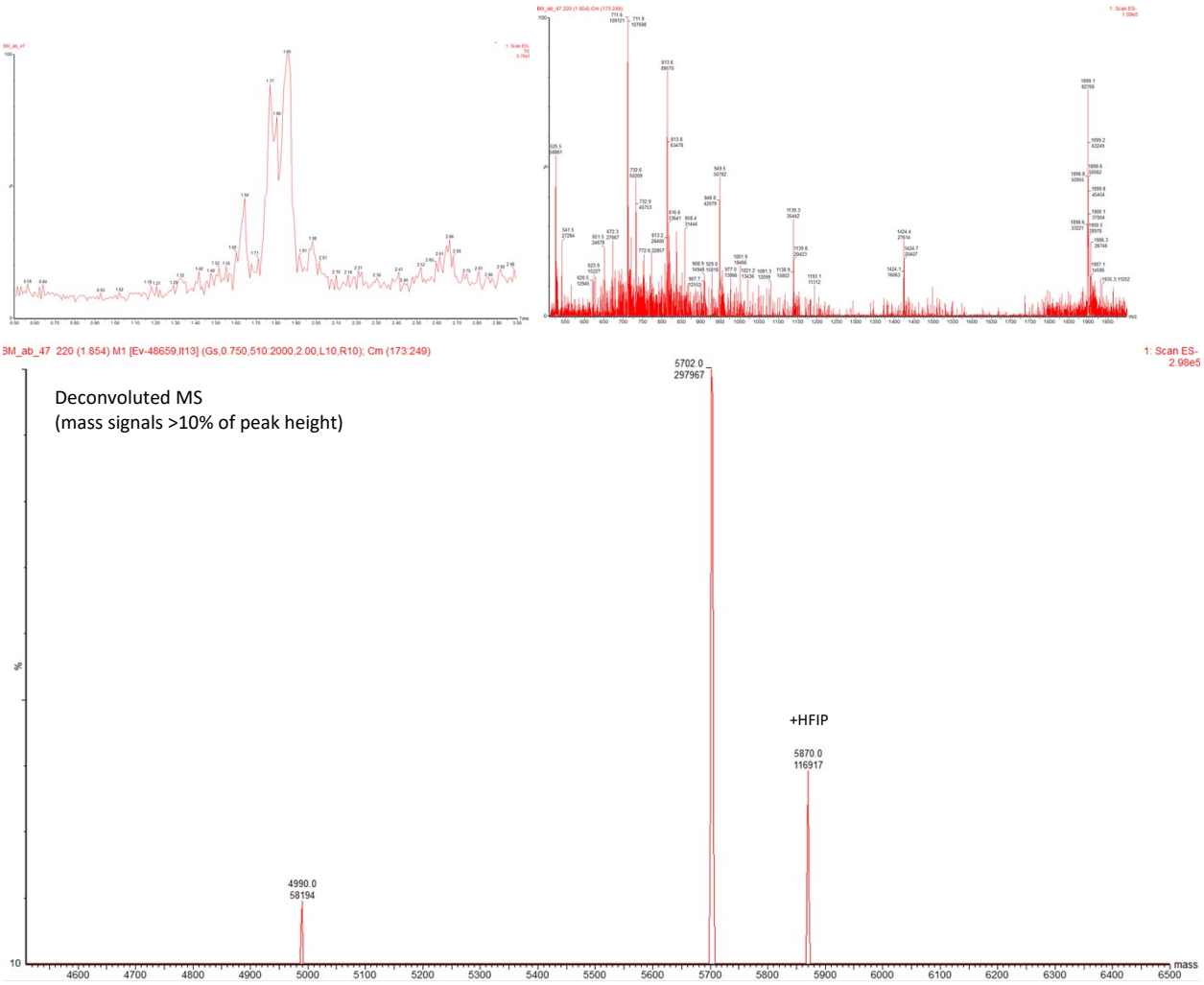
49, >95%
Molecular Weight: 5581.01

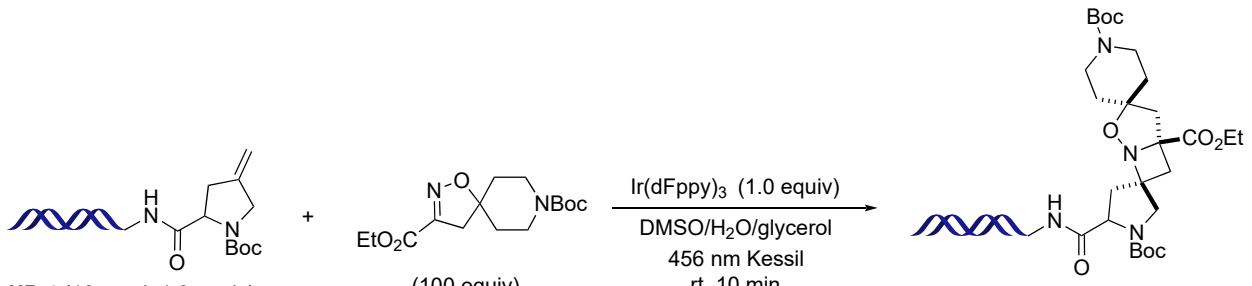
The title compound **49** was synthesized according to **GP-IV** from ethyl 5,5-diethyl-4,5-dihydroisoxazole-3-carboxylate and the DNA-tagged alkene **HP-3**. The product was formed with >95% conversion.





The title compound **50** was synthesized according to **GP-IV** from diethyl 2-((3-(ethoxycarbonyl)-4,5-dihydroisoxazol-5-yl)methyl)malonate and the DNA-tagged alkene **HP-3**. The product was formed with 88% conversion.





HP-4 (10 nmol, 1.0 equiv)
Molecular Weight: 5393.77

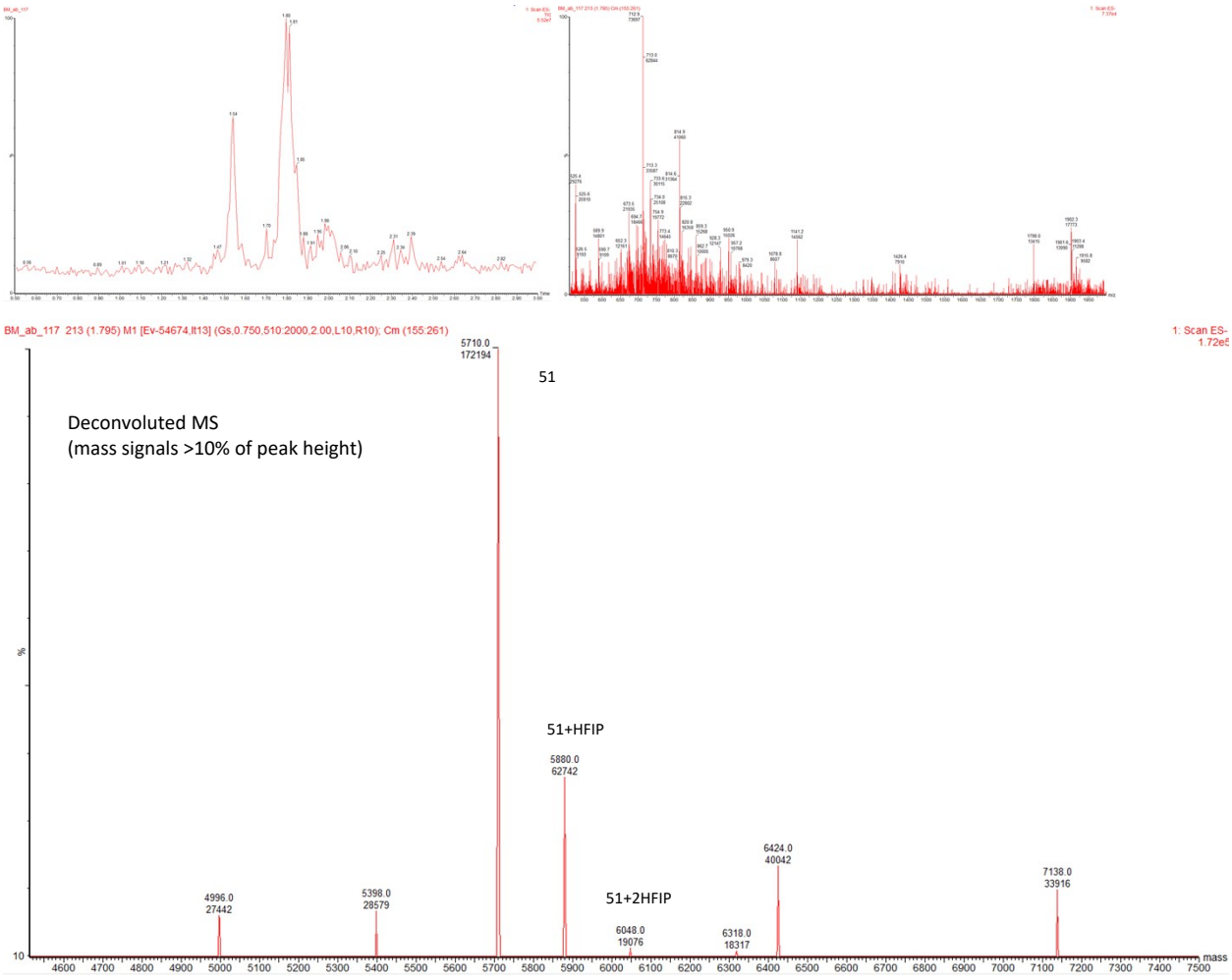
(100 equiv)

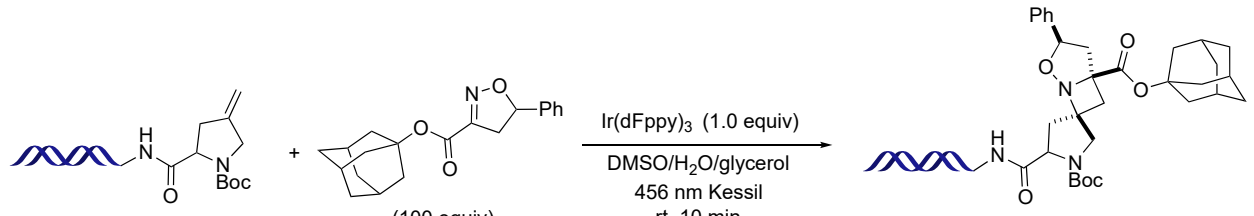
51, 63%
Molecular Weight: 5706.13

The title compound **51** was synthesized according to **GP-IV** from 8-(*tert*-butyl) 3-ethyl 1-oxa-2,8-diazaspiro[4.5]dec-2-ene-3,8-dicarboxylate and the DNA-tagged alkene **HP-4**. The product was formed with 63% conversion (7% recovered **HP-4**).

TIC

Raw MS



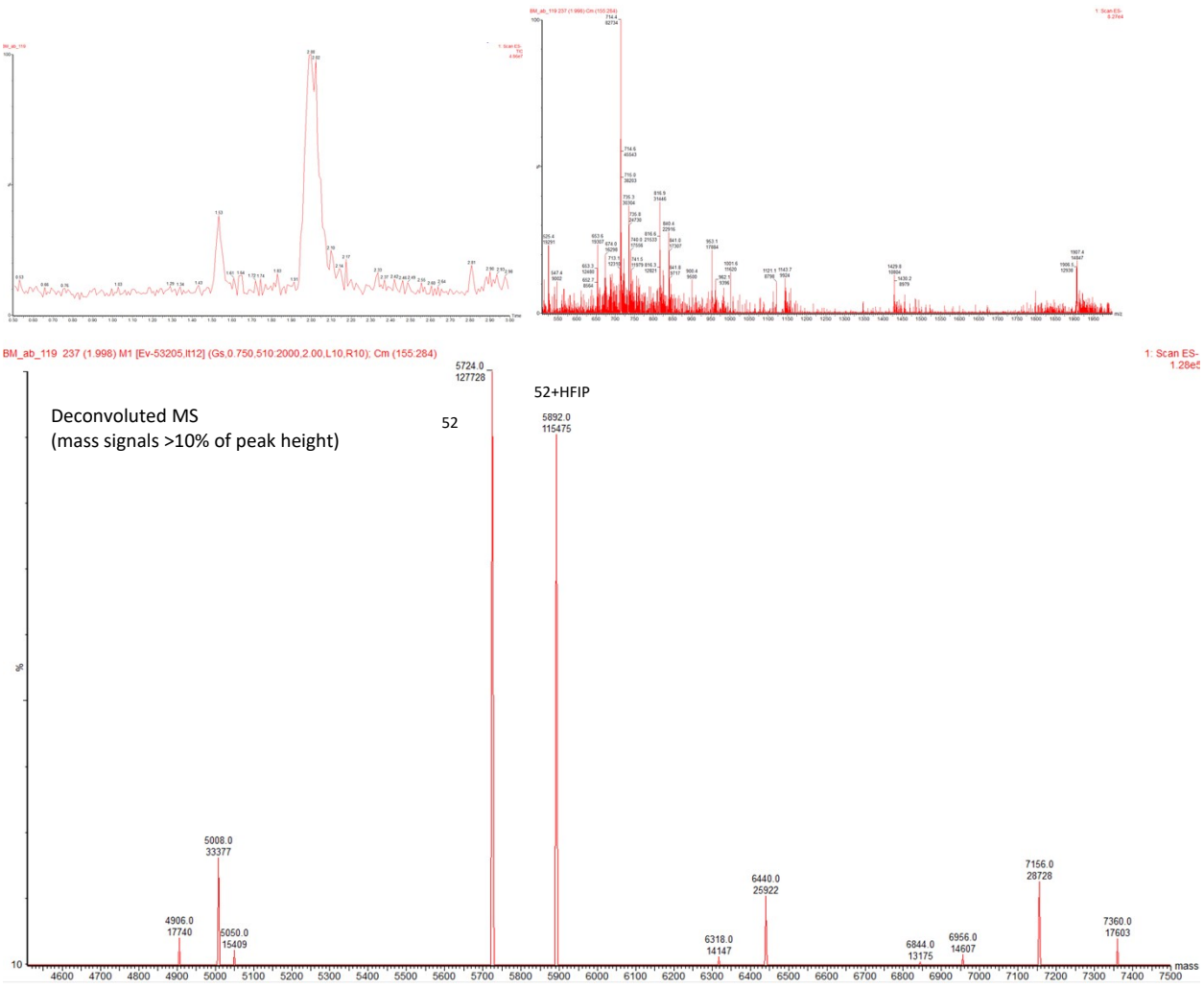


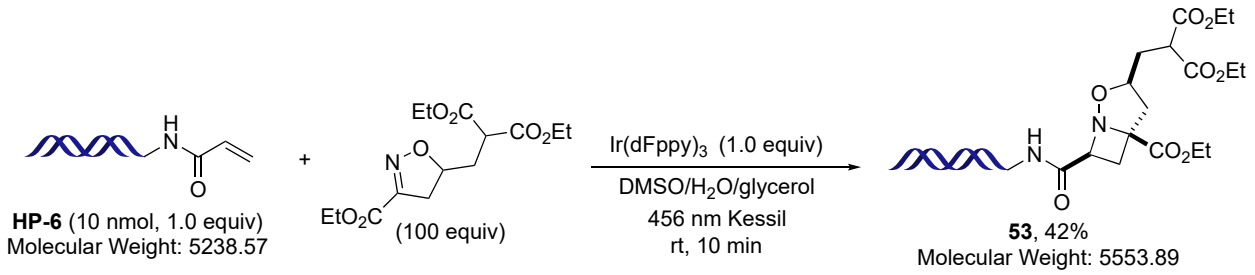
HP-4 (10 nmol, 1.0 equiv)
Molecular Weight: 5393.77

(100 equiv)

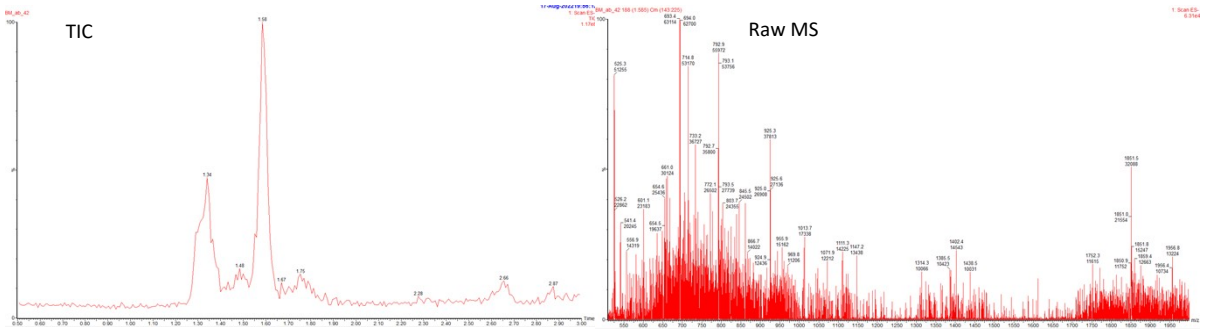
52, 57%
Molecular Weight: 5719.17

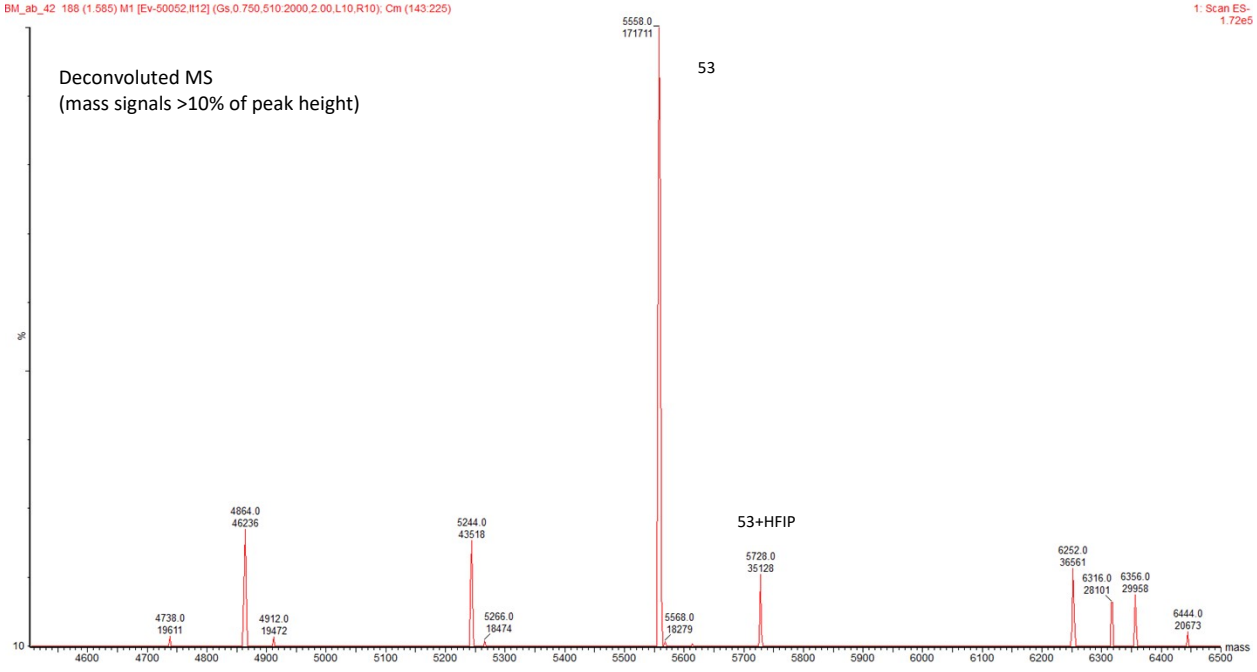
The title compound **52** was synthesized according to the **GP-IV** from (3s,5s,7s)-adamantan-1-yl 5-phenyl-4,5-dihydroisoxazole-3-carboxylate and the DNA-tagged alkene **HP-4**. The product was formed with 57% conversion.

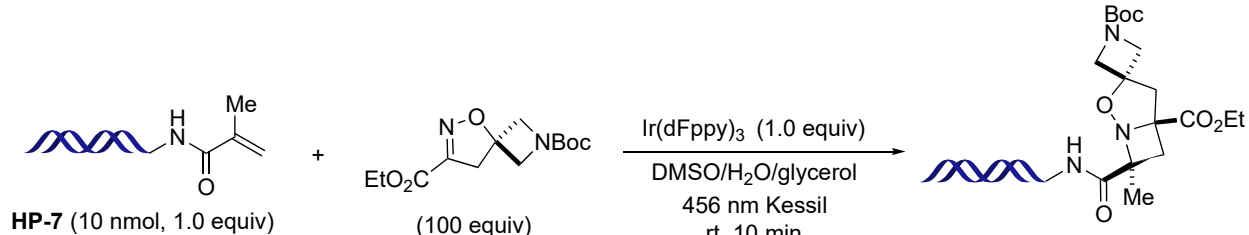




The title compound **37** was synthesized according to **GP-IV** from diethyl 2-((3-(ethoxycarbonyl)-4,5-dihydroisoxazol-5-yl)methyl)malonate and the DNA-tagged alkene **HP-6**. The product was formed with 42% conversion (13% recovered **HP-6**)





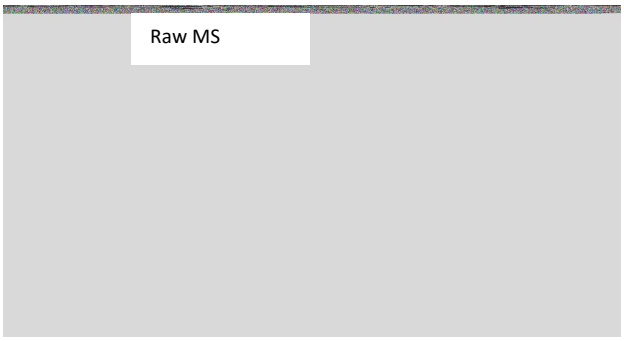


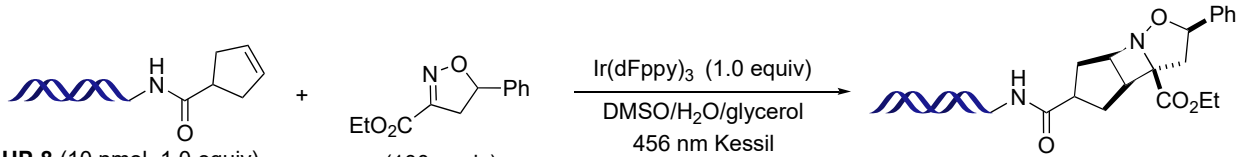
HP-7 (10 nmol, 1.0 equiv)
Molecular Weight: 5252.60

(100 equiv)

54, 47%
Molecular Weight: 5536.91

The title compound **41** was synthesized according to **GP-IV** from 2-(*tert*-butyl) 7-ethyl 5-oxa-2,6-diazaspiro[3.4]oct-6-ene-2,7-dicarboxylate and the DNA-tagged alkene **HP-7**. The product was formed with 47% conversion (53% recovered **HP-7**).



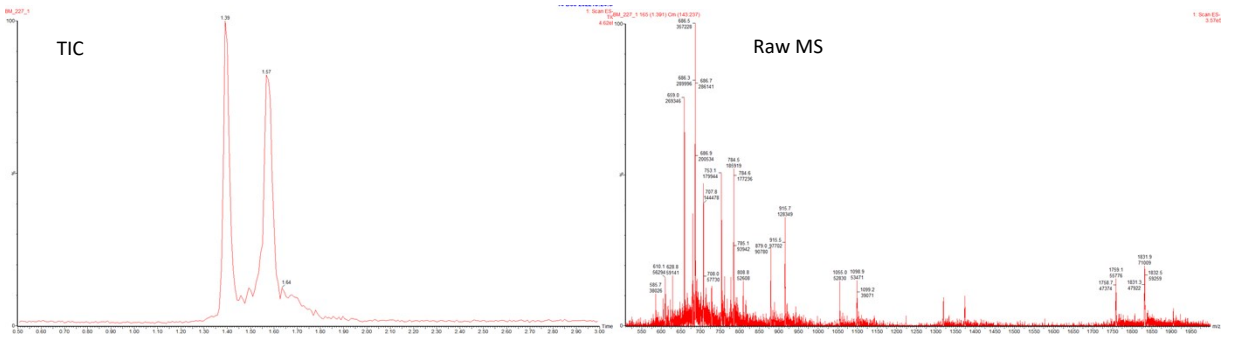


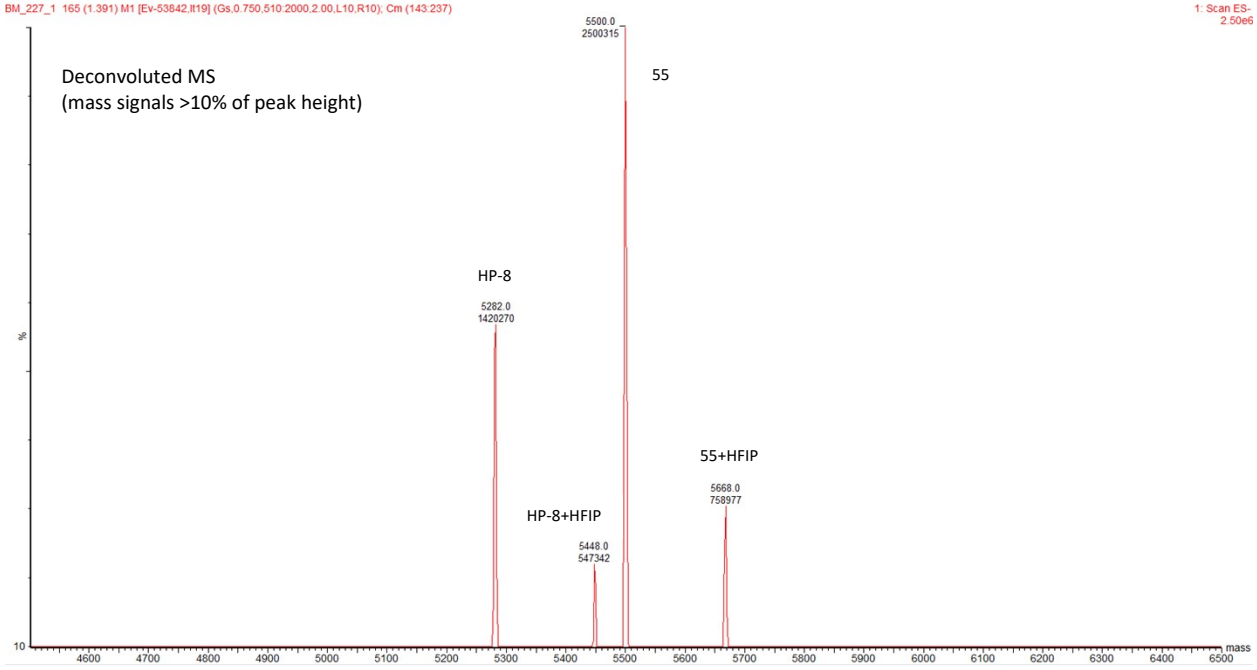
HP-8 (10 nmol, 1.0 equiv)
Molecular Weight: 5278.63

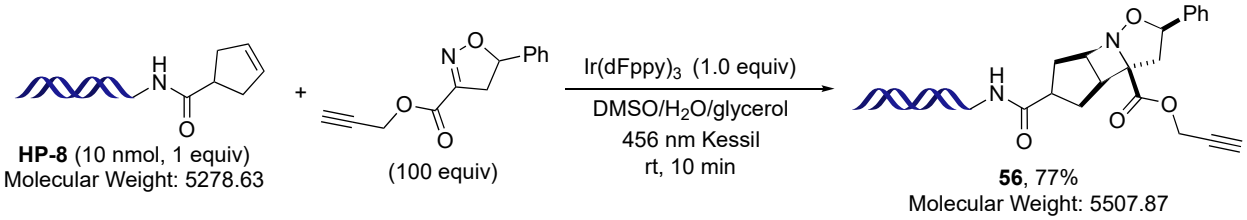
(100 equiv)

55, 62%
Molecular Weight: 5497.87

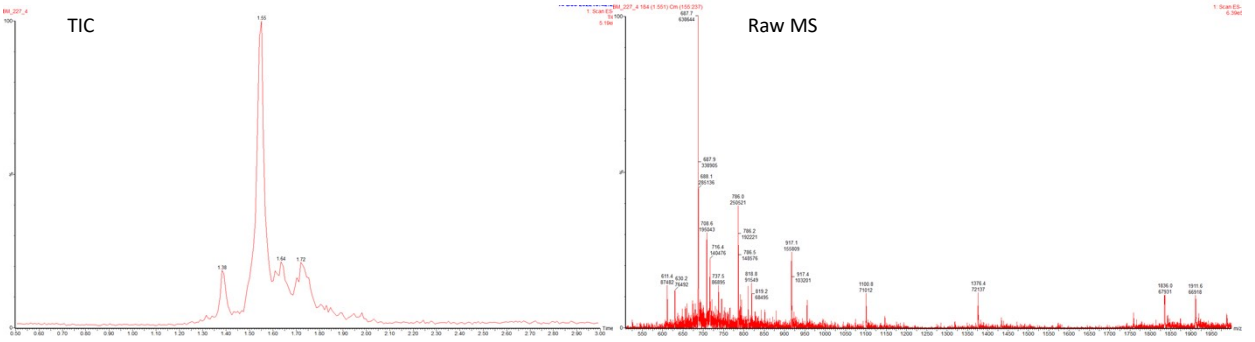
The title compound **55** was synthesized according to **GP-IV** from ethyl 5-phenyl-4,5-dihydroisoxazole-3-carboxylate and the DNA-tagged alkene **HP-8**. The product was formed with 62% conversion (38% recovered **HP-8**).

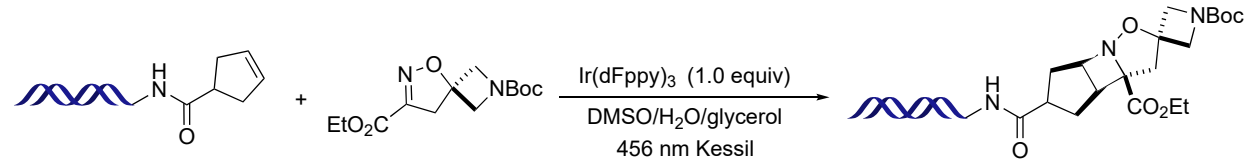






The title compound **56** was synthesized according to **GP-IV** from prop-2-yn-1-yl 5-phenyl-4,5-dihydroisoxazole-3-carboxylate and the DNA-tagged alkene **HP-8**. The product was formed with 77% conversion (23% double addition product).



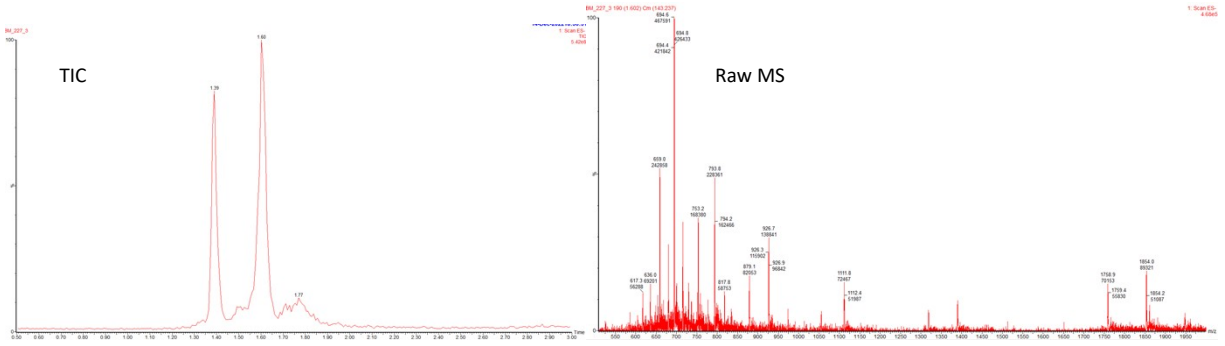


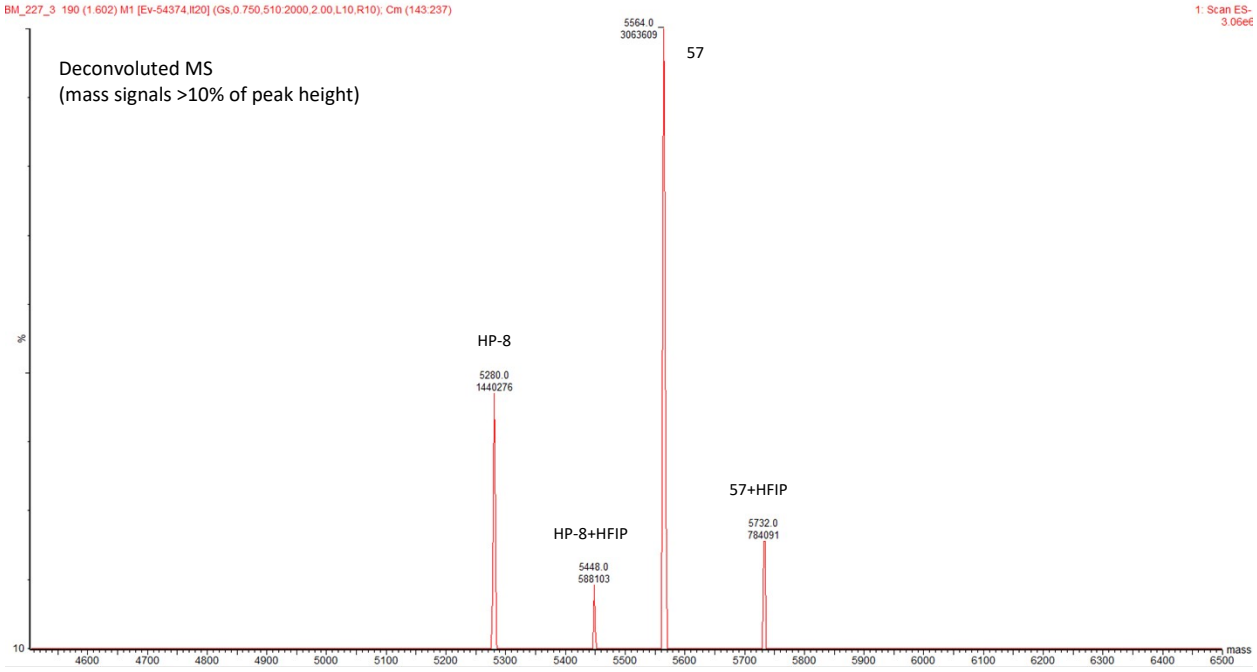
HP-8 (10 nmol, 1.0 equiv)
Molecular Weight: 5278.63

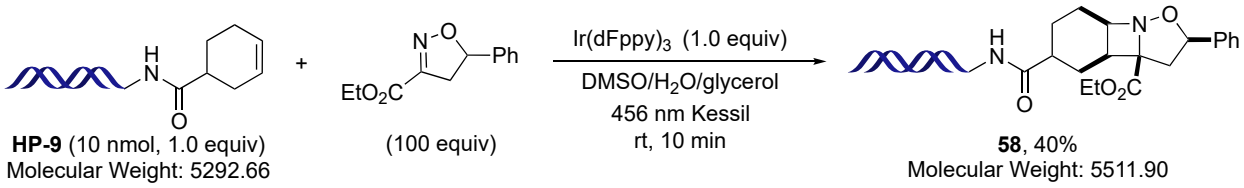
(100 equiv)

57, 65%
Molecular Weight: 5562.95

The title compound **57** was synthesized according to **GP-IV** from 2-(*tert*-butyl) 7-ethyl 5-oxa-2,6-diazaspiro[3.4]oct-6-ene-2,7-dicarboxylate and the DNA-tagged alkene **HP-8**. The product was formed with 65% conversion (35% recovered **HP-8)**.





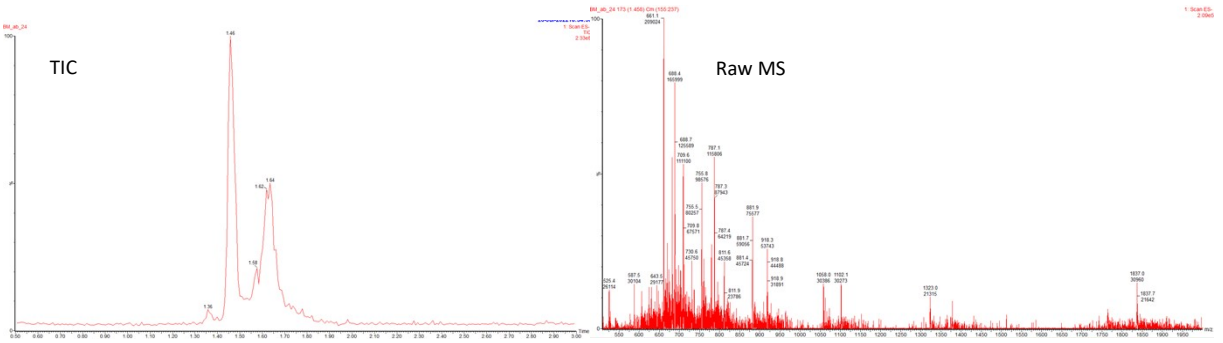


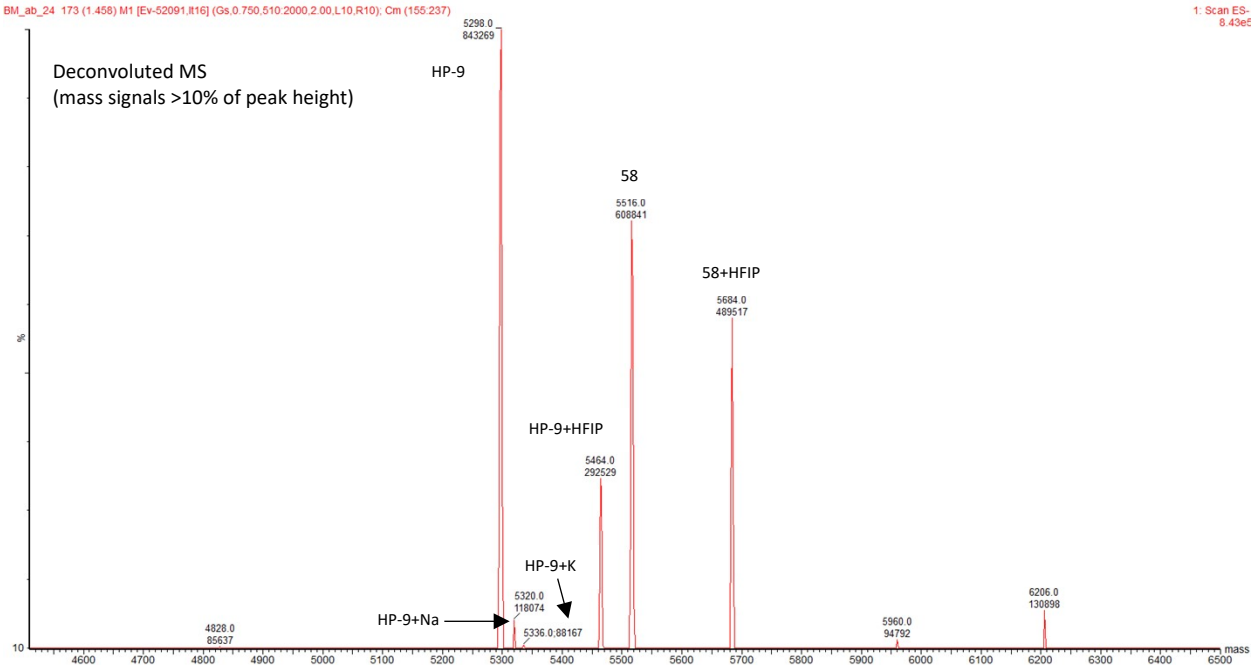
HP-9 (10 nmol, 1.0 equiv)
Molecular Weight: 5292.66

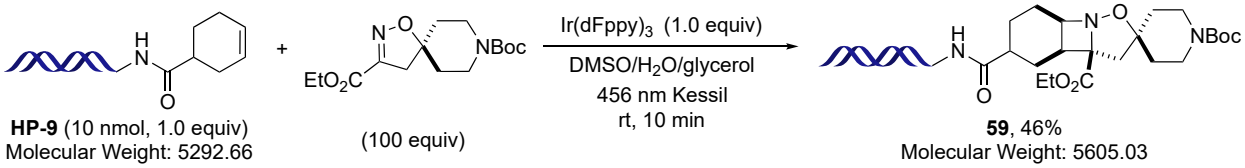
(100 equiv)

58, 40%
Molecular Weight: 5511.90

The title compound **58** was synthesized according to **GP-IV** from ethyl 5-phenyl-4,5-dihydroisoxazole-3-carboxylate and the DNA-tagged alkene **HP-9**. The product was formed with 40% conversion (49% recovered **HP-9**).





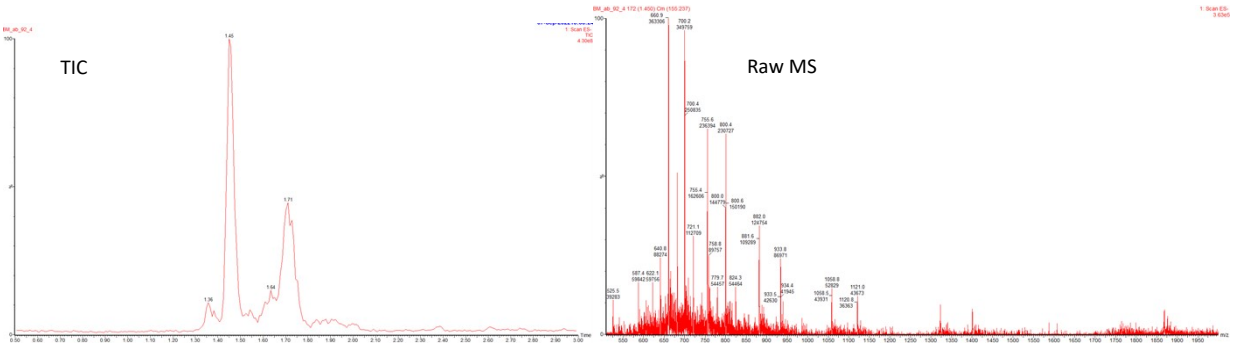


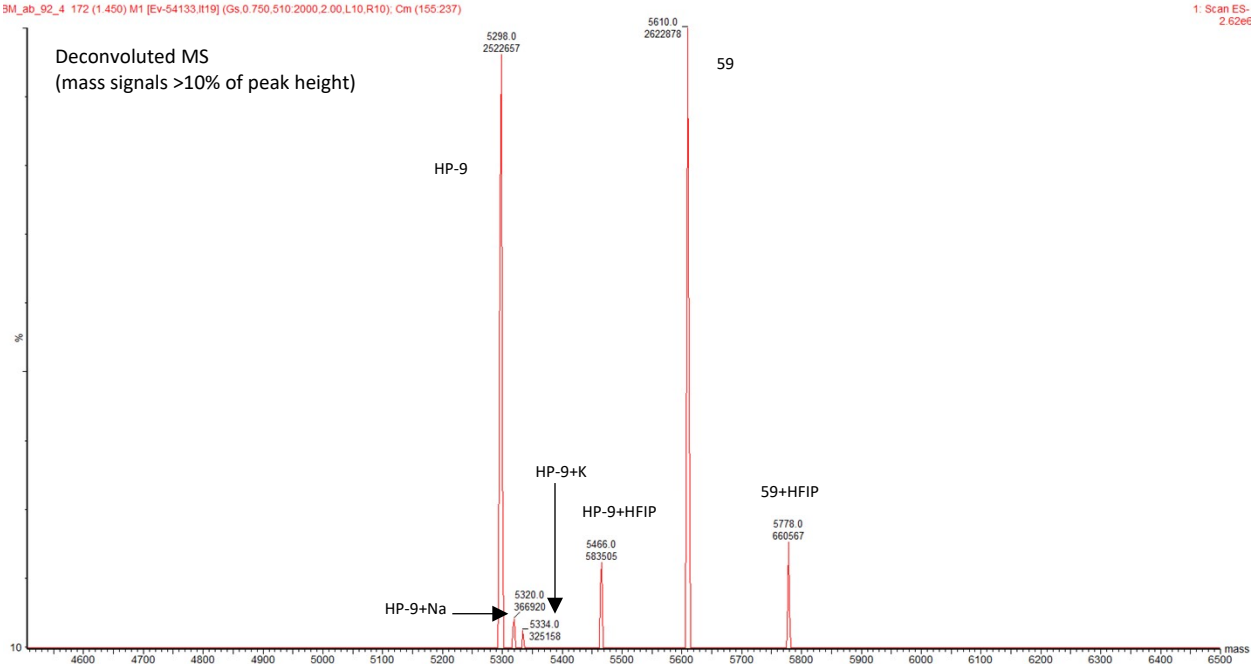
HP-9 (10 nmol, 1.0 equiv)
Molecular Weight: 5292.66

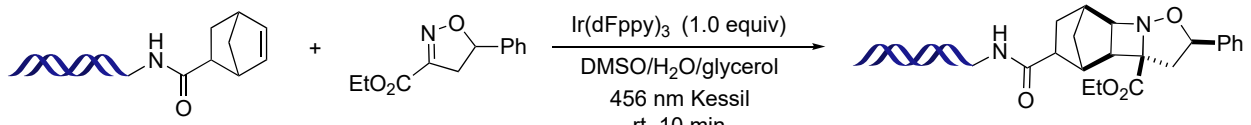
(100 equiv)

59, 46%
Molecular Weight: 5605.03

The title compound **59** was synthesized according to **GP-IV** from 8-(*tert*-butyl) 3-ethyl 1-oxa-2,8-diazaspiro[4.5]dec-2-ene-3,8-dicarboxylate and the DNA-tagged alkene **HP-9**. The product was formed with 46% conversion (54% recovered **HP-9**).





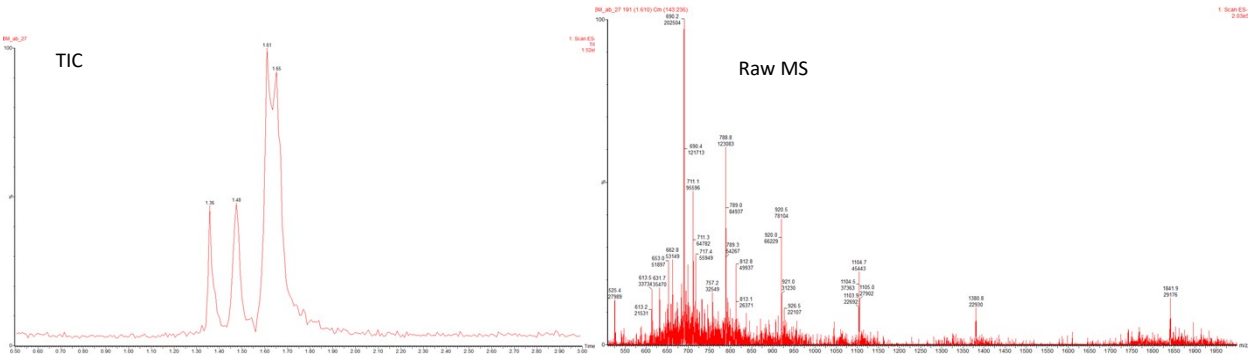


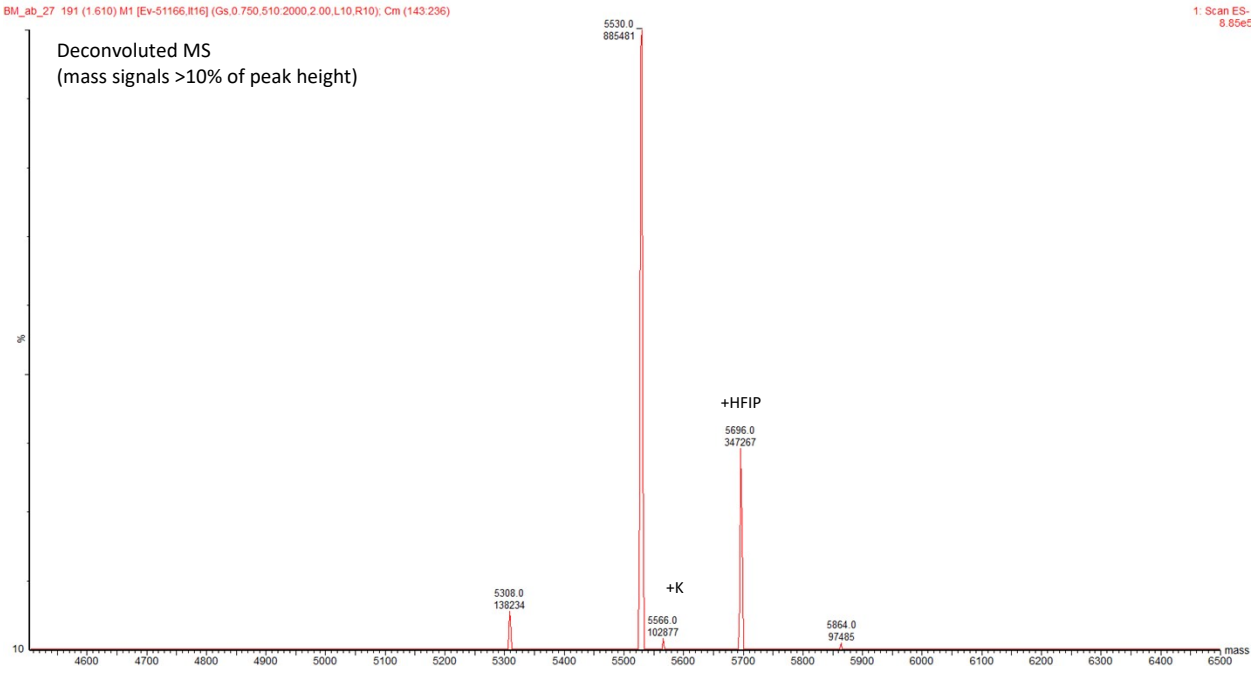
HP-10 (10 nmol, 1.0 equiv)
Molecular Weight: 5304.67

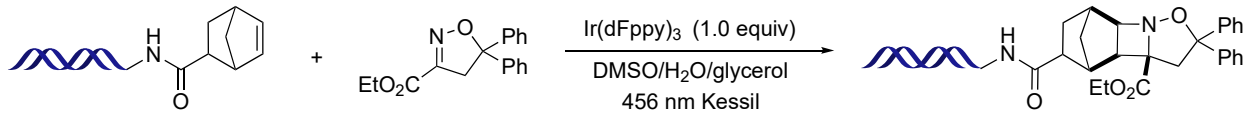
(100 equiv)

60, 85%
Molecular Weight: 5523.91

The title compound **60** was synthesized according to **GP-IV** from ethyl 5-phenyl-4,5-dihydroisoxazole-3-carboxylate and the DNA-tagged alkene **HP-10**. The product was formed with 85% conversion (9% recovered **HP-10**).





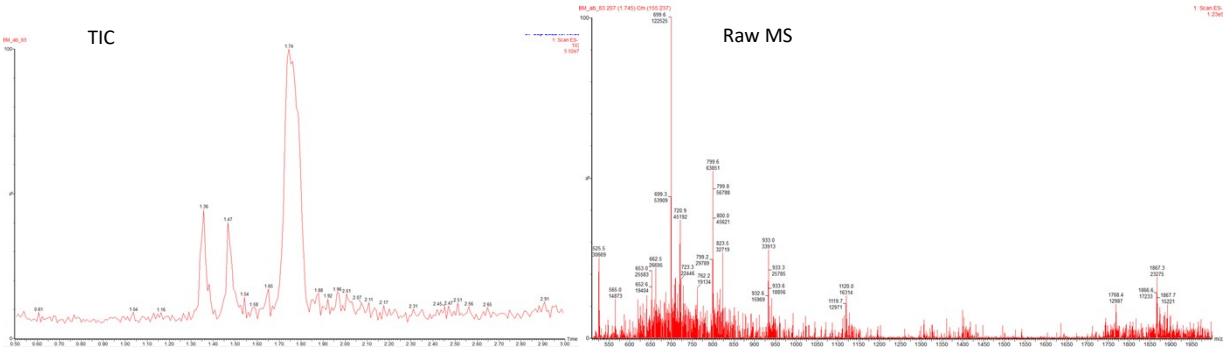


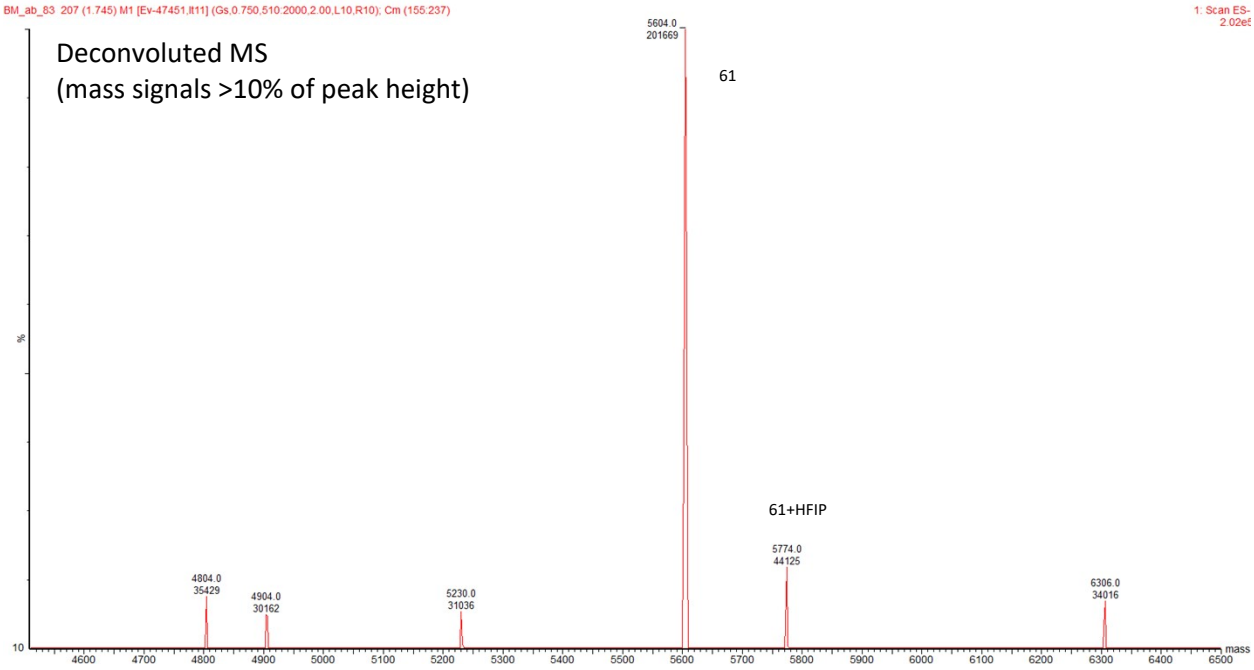
HP-10 (10 nmol, 1.0 equiv)
Molecular Weight: 5304.67

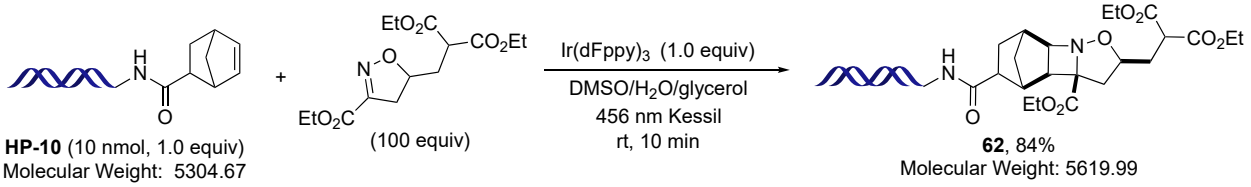
(100 equiv)

61, 65%
Molecular Weight: 5600.01

The title compound **61** was synthesized according to **GP-IV** from ethyl 5,5-diphenyl-4,5-dihydroisoxazole-3-carboxylate and the DNA-tagged alkene **HP-10**. The product was formed with 65% conversion.



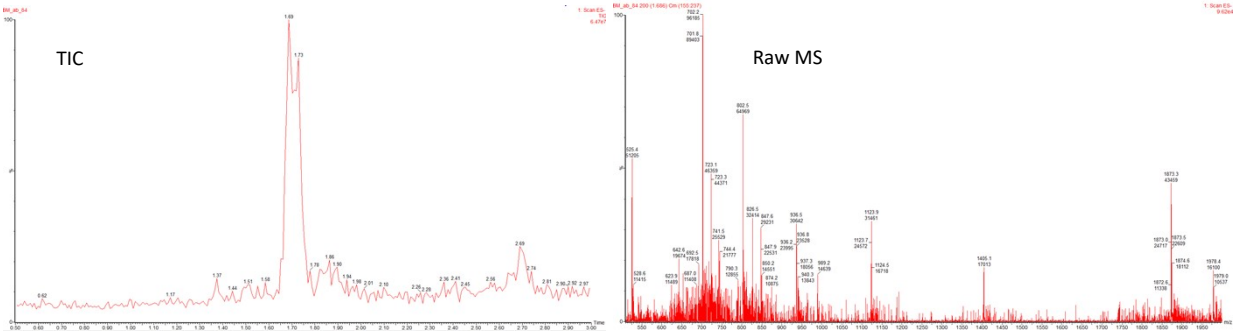


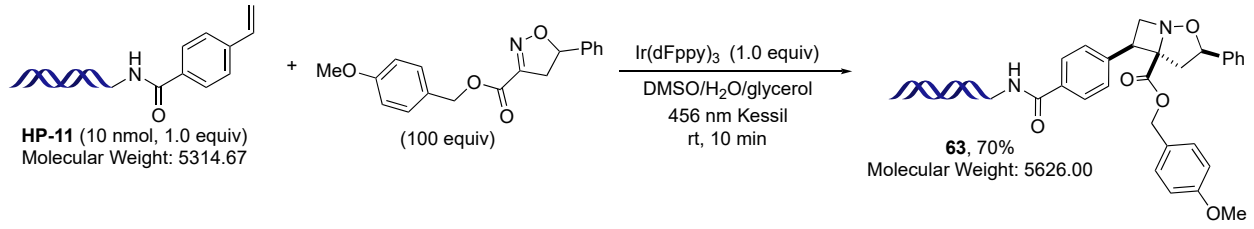


HP-10 (10 nmol, 1.0 equiv)
Molecular Weight: 5304.67

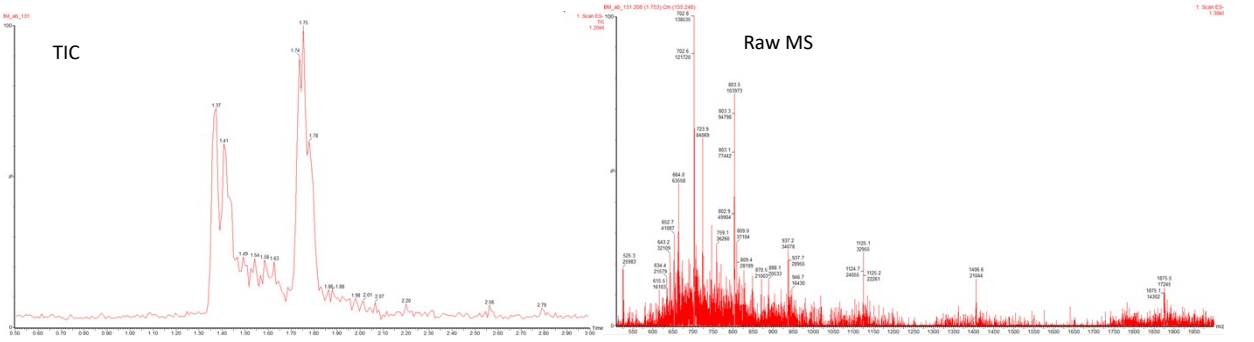
62, 84%
Molecular Weight: 5619.99

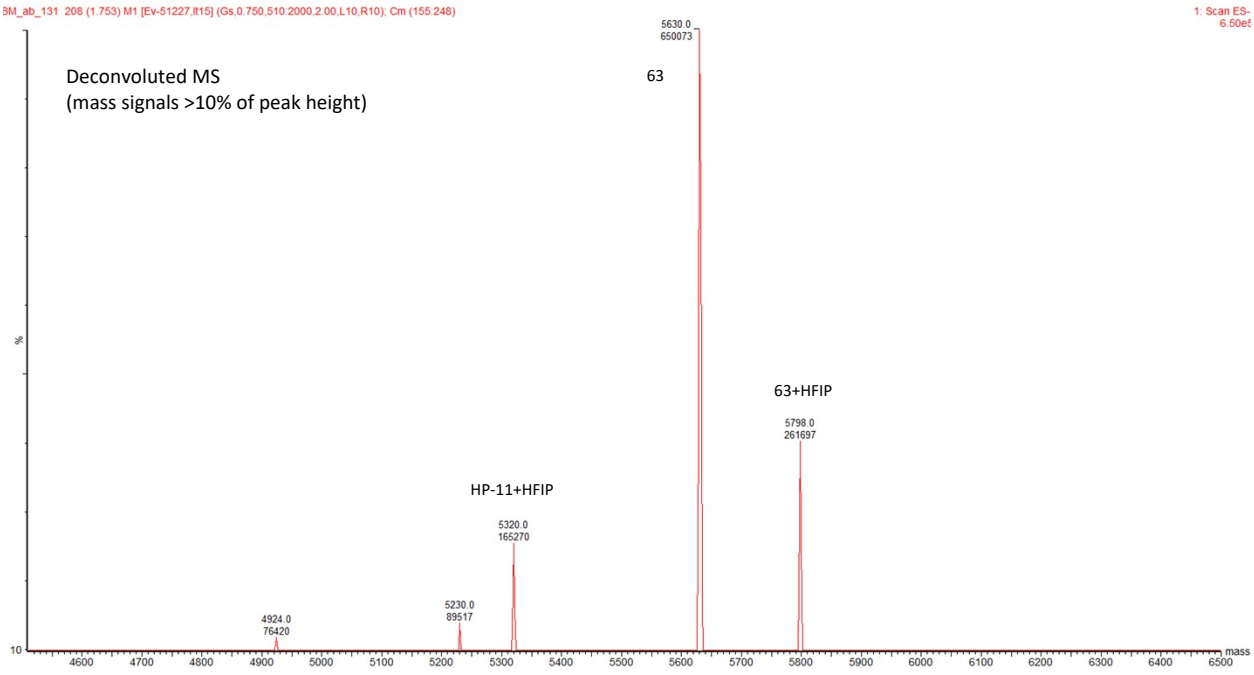
The title compound **62** was synthesized according to **GP-IV** from diethyl 2-((3-(ethoxycarbonyl)-4,5-dihydroisoxazol-5-yl)methyl)malonate and the DNA-tagged alkene **HP-10**. The product was formed with 84% conversion (8.6% double addition product)

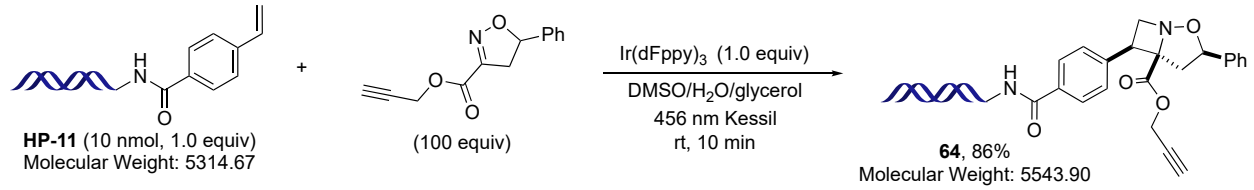




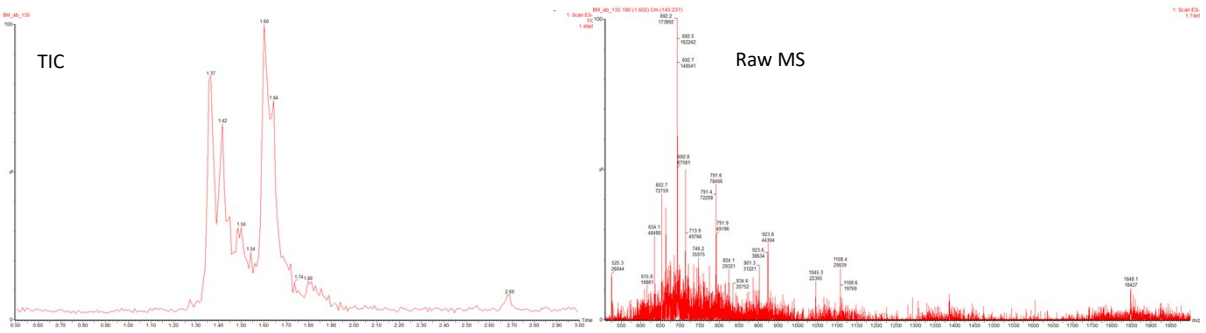
The title compound **63** was synthesized according to **GP-IV** from 4-methoxybenzyl 5-phenyl-4,5-dihydroisoxazole-3-carboxylate and the DNA-tagged alkene **HP-11**. The product was formed with 76% conversion (13% recovered **HP-11**).

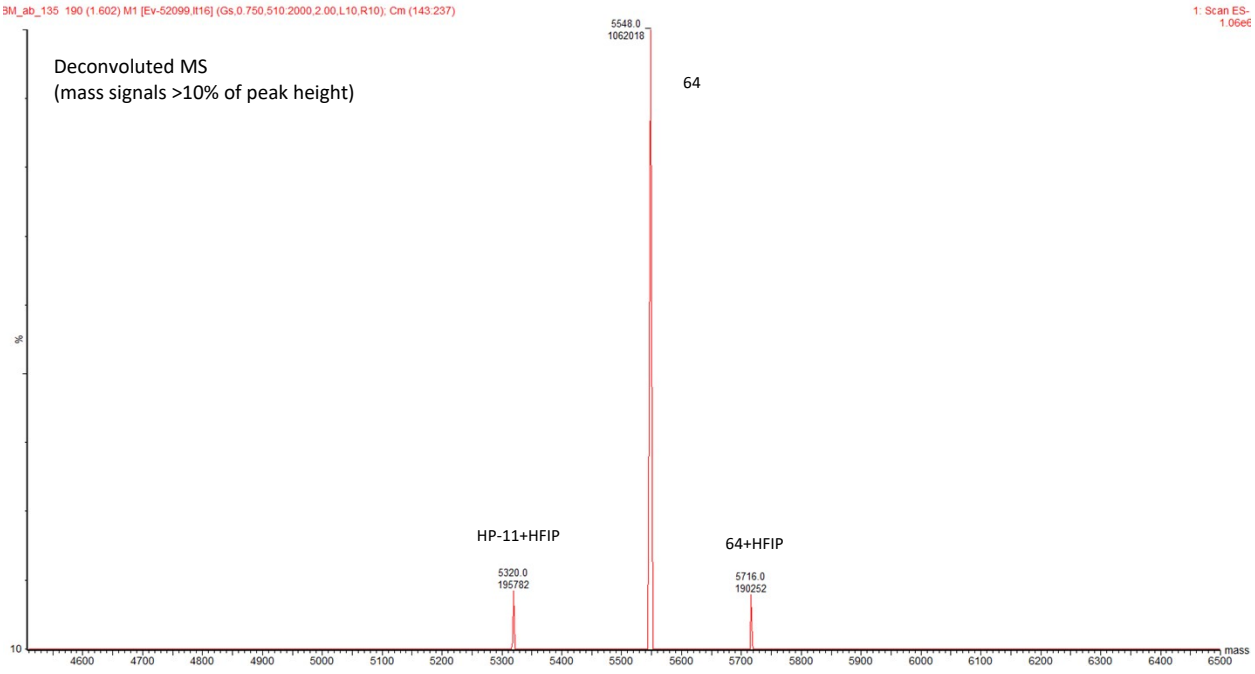


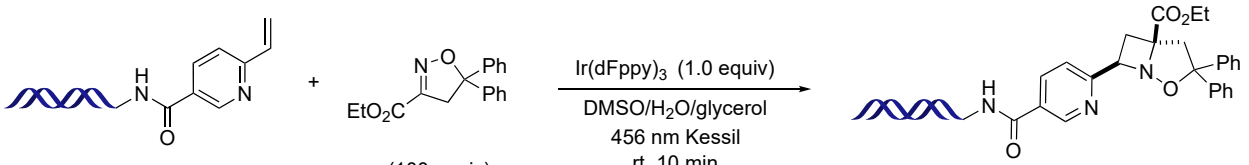




The title compound **64** was synthesized according to **GP-IV** from prop-2-yn-1-yl 5-phenyl-4,5-dihydroisoxazole-3-carboxylate and the DNA-tagged alkene **HP-11**. The product was formed with 86% conversion (13% recovered **HP-11**).





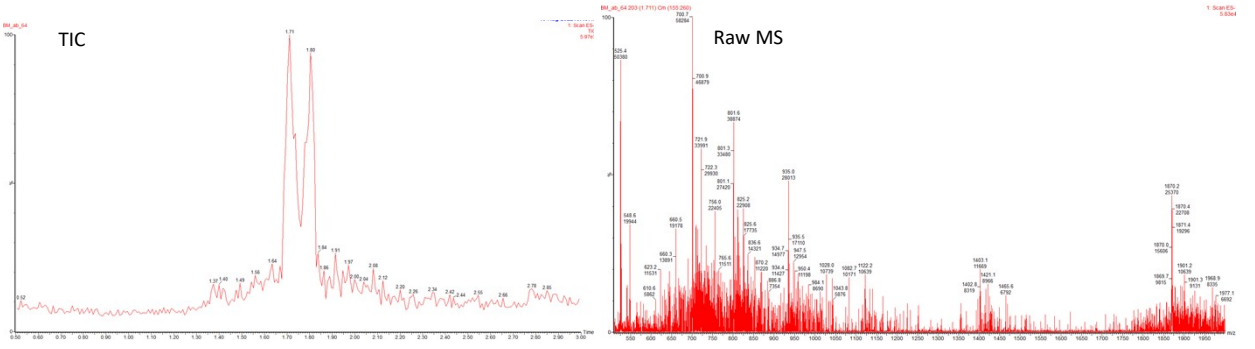


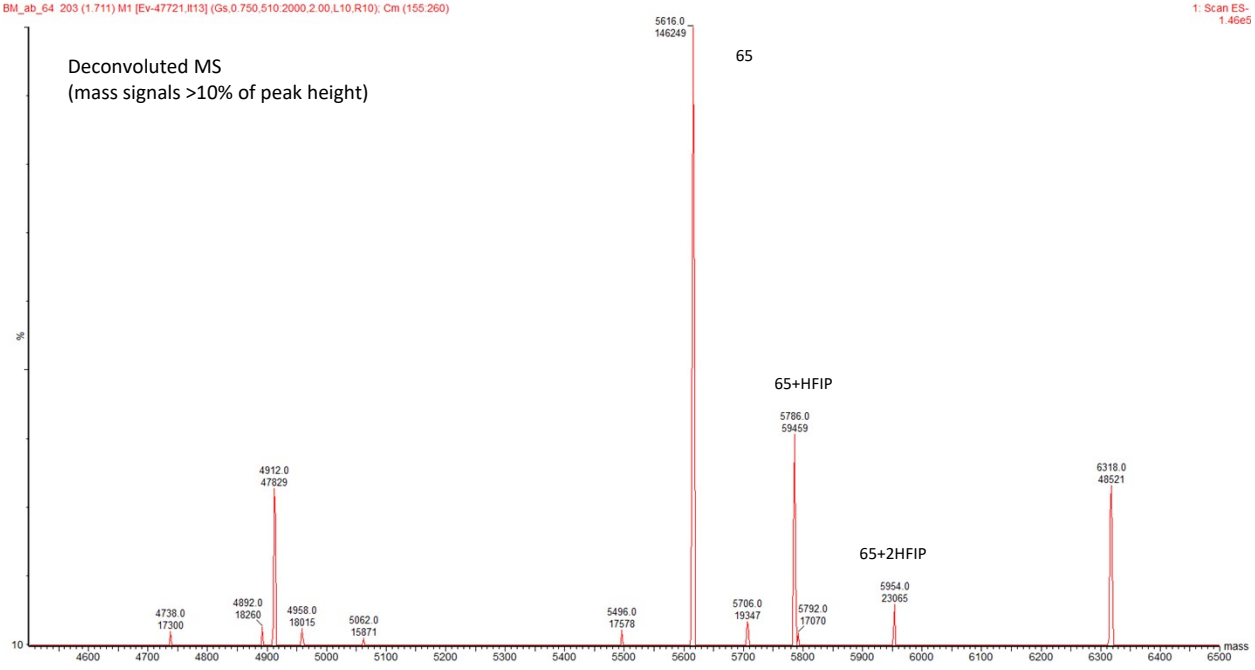
HP-16 (10 nmol, 1.0 equiv)
Molecular Weight: 5315.66

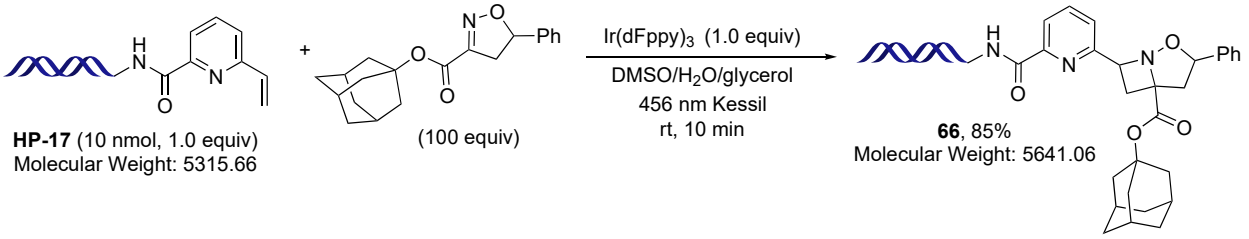
(100 equiv)

65, 51%
Molecular Weight: 5610.99

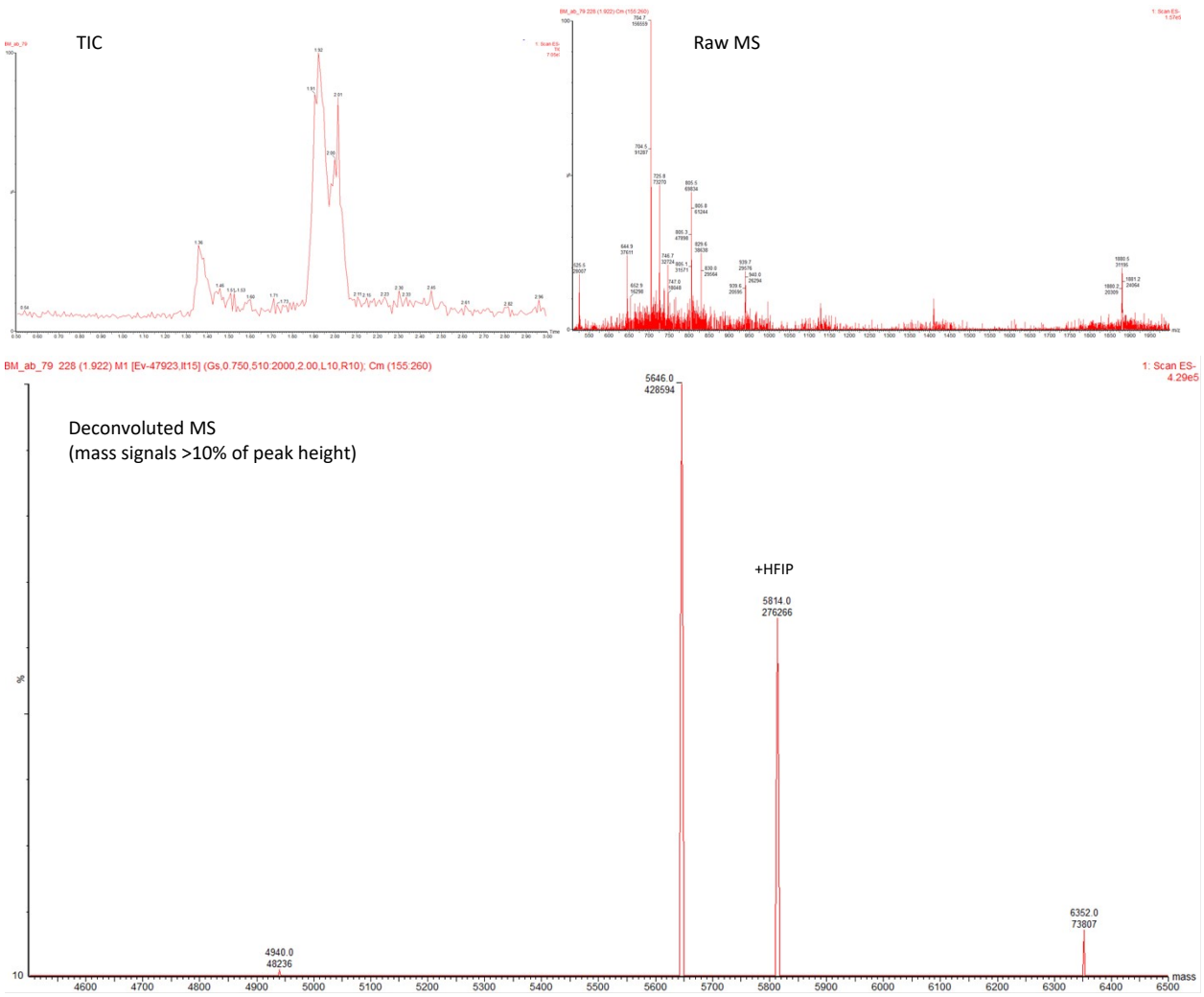
The title compound **65** was synthesized according to **GP-IV** from ethyl 5-phenyl-4,5-dihydroisoxazole-3-carboxylate and the DNA-tagged alkene **HP-16**. The product was formed with 51% conversion.







The title compound **66** was synthesized according to **GP-IV** from (3s,5s,7s)-adamantan-1-yl 5-phenyl-4,5-dihydroisoxazole-3-carboxylate and the DNA-tagged alkene **HP-17**. The product was formed with 85% conversion.



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References for **Section 9**: X-ray Structure Determination (only)

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