Supplementary Information

Synthesis and Evaluation of A Radiolabeled Phosphoramide Mustard with Selectivity for Hypoxic Cancer Cells

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Supplementary contents

Materials

Reagent: All solvents used for reactions and silica gel purification were ACS grade and purchased from Fisher Scientific, unless otherwise stated. Acetonitrile utilized for HPLC analysis and purification was HPLC grade and purchased from Fisher Scientific. Water was deionized by Millipore® Milli Q Biocell Ultrapure Water System before use. TLC silica gel 60 plate was purchased from EMD Millipore. $CDCl_3$, sodium hydride was obtained from ACROS Organics™. Dimethyl sulfoxide was obtained from Cambridge Isotope Laboratories. 4-(Dimethylamino) pyridine (DMAP), Cu (II) chloride, CuI, phosphorus (V) oxychloride, sodium sulfate and tetrabromomethane were obtained from Sigma-Aldrich. Boc anhydride was obtained from Chem-Impex International. Deuterium oxide and triphenylphosphine were purchased from Alfa Aesar®. 2-Bromoethylamine hydrobromide, alamarBlue®, ammonium chloride, ethanolamine, imidazole, L-glutamine, propargyl bromide, sodium chloride, tetrabutylammonium fluoride (TBAF) and triethylamine (Et_3N) were obtained from Thermo Fisher. (1-Methyl-1H-imidazol-5-yl) was purchased from AstaTech. Tert-Butyldimethylchlorosilane (TBSCl) was purchased from Oakwood Chemical. (1-methyl-2-nitro-1H-imidazol-5-yl) methanol was purchased from ChiralStar.), Dulbecco's Modified Eagle Medium (DMEM), bovine serum albumin (BSA), hydrochloric acid and sulfuric acid, Kaighn's Modification of Ham's F-12 Medium (F-12K Medium), 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), Penicillin-Streptomycin, phosphate buffered saline (PBS) and silica gel (230-400 mesh) were obtained from Fisher Scientific. [^{125}I] NaI was purchased from American Radiolabeled Chemicals, Inc. The human prostate cancer PC-3 (CRL-1435™) and DU145 (HTB-81™) was purchased from American Type Culture Collection.

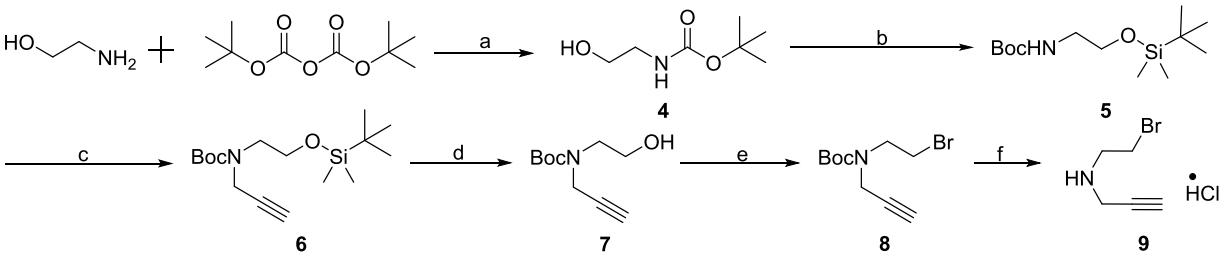
Instrumentation: A Waters e2695 system equipped with a Waters 2489 absorption detector and a Waters Qtof Micro electrospray ionization mass spectrometer was used to perform high performance liquid chromatography/mass spectrometry analyses. ^{1}H, ^{13}C and ^{31}P NMR spectrums were recorded on a Bruker Avance-III HD 500 MHz instrument using as the solvent. Triphenylphospjate was used as ^{31}P standard. A Phenomenex Jupiter 5u C18 300A 250× 10 mm semiprep column was used for the purification of 2-nitroimidazole phosphoramidate mustard and its controls. A Phenomenex Jupiter C12 Proteo 250×10 mm semiprep column was used for the purification of model compounds of 5-[^{Nat}I]iodo-1,2,3-triazoles. Waters 1515 binary pump equipped with a Waters 2489 absorption detector and a Bioscan Flow Count radiometric detector system using a Phenomenex Jupiter C12 Proteo 250×4.6 mm column. Falcon™ Polystyrene Microplates (96-well plate and 6-well plate) were used for in vitro cytotoxicity assay and efflux study separately. SpectraMax® M5 Multi-Mode Microplate Readers was used to quantify fluorescence intensities. EVOS FL Cell Imaging System was used for time-dependent cytotoxicity assay. Hypoxic PC-3 and DU145 cells were incubated in hypoxic glove box with temperature, CO_2 and humidity controller (Coy Laboratory Products INC, Grass Lake, MI). Gamma decay detection of ^{125}I for the efflux studies was accomplished using a LTI (U.S.) Multi-Wiper nuclear medicine gamma counter.

Methods

Chemistry. Compounds **1** through **9** were synthesized on the basis of reported methods.[1-6] The key intermediate compound **9** was successfully synthesized in 80% yield by a series of reactions as shown in **Scheme 1A**. Similar synthetic procedures were applied to the synthesis of both the negative control, imidazole phosphoramidate mustard **1** and the positive control, 2-nitroimidazole phosphoramidate mustard, **2** and are depicted in **Scheme 1B**. Briefly, $POCl_3$ was reacted with 2 equiv of 2-bromoethylamine hydrobromide and the residue was purified several times by filtration through fiberglass. The filtrate was added to the suspension of (1-Methyl-1H-imidazol-5-yl)methanol or its 2-nitroimidazole derivatives in the presence of TEA for overnight. The products were purified by HPLC to produce **1** (13.8% yield) and **2** (11.7% yield). Compound **3** was synthesized by adding 0.5 eq of PCl_3 to compound **9** following by conjugation of with (1-Methyl-2-nitro-1H-imidazol-5-yl)methanol and oxidation. Details of synthesis can be found in the following content.

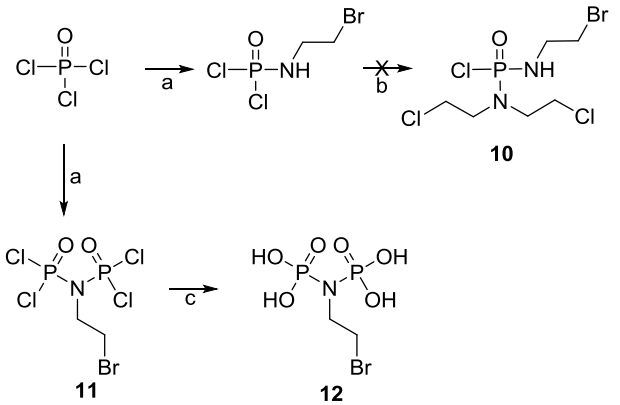
Schemes

Scheme S1. Synthesis of N-(2-bromoethyl)prop-2-yn-1-amine hydrochloride 9[a]



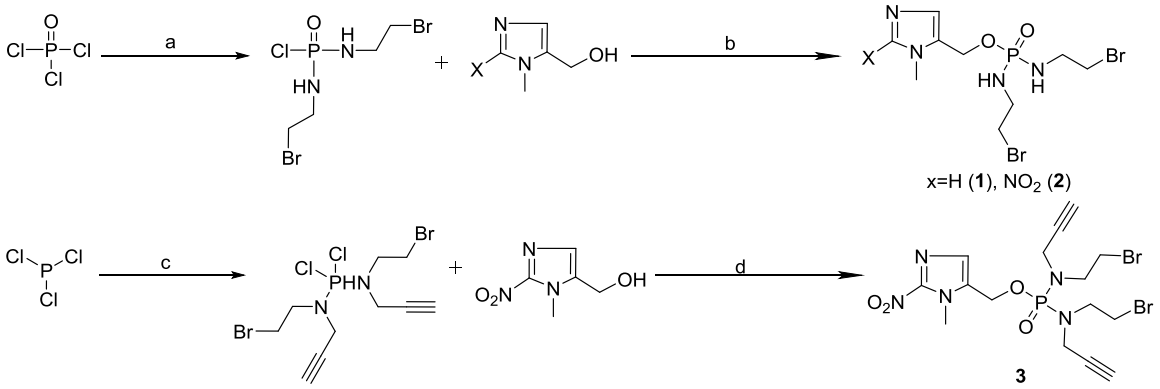
[a]Reagents, conditions, and yields: (a)Et_3N, CH_2Cl_2, 96.0%; (b) Imidazole, 4-DAMP, TBDMSCl, CH_2Cl_2, 98.3%; (c)propargyl bromide, NaH, THF, 58.0%; (d)TBAF, THF,85.0%; (e)CBr_4, PPh_3, ACN, 77.8%; (f)HCl (g), Et_2O, 79.6%

Scheme S2 Attempt to synthesize asymetric phosphoramidate mustard[a]



[a]Reagents, conditions, and yields: (a)propargyl bromide, Et_2O, Et_3N, -5°C; (b)Bis(2-chloroethyl)amine hydrochloride, ACN, Et_3N, pyridine, 40°C; (c) H_2O.

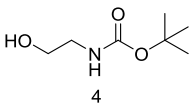
SchemeS3. Synthesis of imidazole and its nitro-derivative conjugated phosphoramidate mustard (1-3)[a]



[a]Reagents, conditions, and yields: (a)propargyl bromide, Et_3N, CH_2Cl_2; (b) Et_3N, CH_2Cl_2, 13.8% (**1**), 11.7% (**2**); (c)compound **9**, Et_3N, CH_2Cl_2; (d)tert-butyl hydroperoxide, Et_3N, CH_2Cl_2, 7.4% (**3**)

Synthesis

^{1}H spectra are reported as chemical shifts (δ) in ppm relative to TMS (δ = 0) and ^{31}P NMR are referenced to H_3PO_4 (δ = 0).



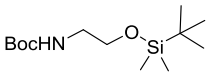
4

***tert*-butyl(2-hydroxyethyl)carbamate (4)**. To a stirred solution of ethanolamine (6.16 g, 100.8 mmol) in 200 mL dry CH_2Cl_2 was added Et_3N (19.2 mL, 137.46 mmol) at rt for 30 min and then the solution was cooled to 0 °C. To this, a solution of Boc_2O (20.0 g, 91.64 mmol) in 60 mL of dry CH_2Cl_2 was added dropwise and stirred at rt overnight. The mixture was quenched with saturated aqueous NH_4Cl, and extracted with CH_2Cl_2 three times. The combined organic extracts were dried over anhydrous Na_2SO_4, filtered and concentrated by rotary evaporation to yield **4** as a colorless oil (15.6 g, 96.8 mmol, 96.0%).

R*f*=0.31(12.5% Et_2O/petroleum ether)

^{1}H NMR (500 MHz, $CDCl_3$): δ 5.10 (s, 1H), 3.66-3.65 (m, 2H), 3.25-3.25 (m, 2H), 3.02 (s, 1H), 1.42 (s, 9H).

MS (ESI+) *m/z* calcd for $C_7H_{15}NO_3$ $[M+H]^+$ 162.11, found 162.09.



5

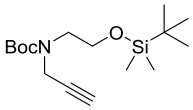
***tert*-butyl(2-((tert-butyldimethylsilyl)oxy)ethyl)carbamate (5)**. To a stirred solution of **4** (10 g, 62.03 mmol) and imidazole (12.67 g, 186.1 mmol) in 200 mL dry CH_2Cl_2 with DMAP (0.4 g, 3.27 mmol), TBSCl (11.22 g, 74.44 mmol) was slowly added at 0 °C. The solution was warmed to rt and stirred overnight, then quenched with saturated aqueous NH_4Cl solution. The mixture was extracted three times with CH_2Cl_2 and the combined organic layers were dried over anhydrous Na_2SO_4, filtered and the solvent removed by rotary evaporation. The product was purified via flash column chromatography on silica gel using 20% Et_2O in petroleum ether to elute **5** (16.8 g, 98.3%) as a clear colorless oil.

R*f*=0.47(20% Et_2O/ petroleum ether).

^{1}H NMR (500 MHz, $CDCl_3$): δ 4.84 (br, 1H), 3.65 (m, 2H), 3.22 (m, 2H), 1.44 (s, 9H), 0.89 (s, 9H), 0.054 (s, 6H).

^{13}C NMR (125 MHz, $CDCl_3$) δ 155.96, 79.12, 62.26, 42.88, 28.41, 25.89, 18.30, -2.96, -5.37.

MS (ESI+) *m/z* calcd for $C_{13}H_{29}NO_3Si$ $[M+H]^+$ 276.19, found 276.05.



6

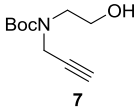
***tert*-butyl(2-((tert-butyldimethylsilyl)oxy)ethyl)(prop-2-yn-1-yl)carbamate (6)**. To a suspension of NaH (2.9 g, 72.61 mmol) in 100mL dry THF was added a solution of **5** (10 g, 36.3 mmol) in 30 mL dry THF at 0 °C. The mixture was stirred at rt for 1.5 h and subsequently cooled down to 0 °C. To this reaction mixture propargyl bromide (13.7 mL of an 80% wt solution in toluene, 90.76 mmol) was added dropwise. Allowing to warm to rt, the reaction mixture was stirred overnight and quenched with 50mL MeOH followed by 100 mL water. The aqueous layer was extracted three times with CH_2Cl_2 and the combined organic layers were dried over anhydrous Na_2SO_4, filtered and the solvent removed by rotary evaporation. The product was purified via flash column chromatography on silica gel using (2% Et_2O in petroleum ether) to provide **6** (6.6 g, 58.0%) as clear colorless oil.

R*f*=0.38(20% Et_2O/ acetone)

^{1}H NMR (500 MHz, $CDCl_3$): δ 4.16 (d, 2H), 3.74 (s, 2H), 3.42 (s, 2H), 2.18 (s, 1H), 1.47 (s, 9H), 0.89 (s, 9H), 0.049 (s, 6H).

^{13}C NMR (125 MHz, $CDCl_3$): δ 155.93, 80.09, 79.05, 62.24, 48.47, ,43.08, 38.08, 32.25, 28.39, 25.87, 18.28, -5.39.

HRMS (ESI+) *m/z* calcd for $C_{16}H_{31}NO_3Si$ $[M+H]^+$ 314.2151, found 314.2155.



7

***tert*-butyl(2-hydroxyethyl)(prop-2-yn-1-yl)carbamate (7)**. To a solution of **6** (5 g, 15.95 mmol) in 100 mL CH_2Cl_2 was added TBAF (1M in THF) (22.33 mL, 22.33 mmol) at 0 °C. The reaction mixture was stirred overnight at rt and quenched with saturated aqueous NH_4Cl solution, extracted three times with CH_2Cl_2 . The organic layers were dried over anhydrous Na_2SO_4, filtered and the solvent was removed by rotary evaporation. The product was purified via flash column chromatography on silica gel using 20% acetone in

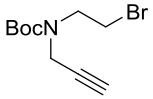
petroleum ether as the eluent to provide **7** (2.7 g, 85.0%) as a clear light yellow oil.

R*f*=0.54(50% Et_2O/ acetone)

^{1}H NMR (500 MHz, $CDCl_3$): δ 4.09 (br, 2H), 3.82 (m, 2H), 3.52 (m, 2H), 2.75 (br, 1H), 2.27 (s, 1H), 1.50 (s, 9H).

^{13}C NMR (125 MHz, $CDCl_3$): δ 207.11, 156.82, 79.54, 71.60, 62.45, 43.13, 30.88, 28.35.

MS (ESI+) *m/z* calcd for $C_{10}H_{17}NO_3$ $[M+H]^+$ 200.12, found 200.14.

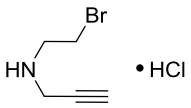


8

***tert*-butyl(2-bromoethyl)(prop-2-yn-1-yl)carbamate (8)**. To a solution of **7** (2 g, 10.04 mmol) and of triphenylphosphine (3.42 g, 13.05 mmol) in 50 mL dry THF a solution of cabon tetrabromide (4.33 g, 13.05 mmol) in 30 mL acetonitrile was added dropwise while the reaction temperature was slightly above ambient (be specific). The reaction was stirred for 2h, and the solvent was removed by rotor evaporation. The residue was purified via flash column chromatography on silica gel using 20% acetone in petroleum ether as the eluent to provide **8** (2.04 g, 77.8%) as clear light yellow oil. The product **8** was immediately used in next step synthesis.

^{1}H NMR (500 MHz, $CDCl_3$): δ 4.15 (dd, 2H), 3.69 (m, 2H), 3.51 (br, 2H), 2.24 (s, 1H), 1.48 (s, 9H).

MS (ESI+) *m/z* calcd for $C_{10}H_{16}BrNO_2$ $[M+H]^+$ 262.04, found 261.91.



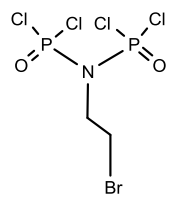
9

N-(2-bromoethyl)prop-2-yn-1-amine hydrochloride (9). To a solution of **8** (0.5 g, 1.92 mmol) in 50 mL Et_2O was added anhydrous hydrogen chloride gas at 0 °C continuously for 3h. **9** (0.3 g, 79.6%) gradually precipitate out of solution as a white solid. The product was filtrated to remove solvent and used for the next reaction without further purification.

^{1}H NMR (500 MHz, DMSO-d_6):9.3245 (s, 2H), 3.99-3.98 (d, 2H), 3.90-3.88 (t, 2H), 3.77 (s, 1H).

^{13}C NMR (125 MHz, DMSO-d_6): δ 80.229, 75.331, 47.622, 36.193, 27.045.

HRMS (ESI+) *m/z* calcd for free base C_5H_8NBr $[M+H]^+$161.9918, found 161.9921.



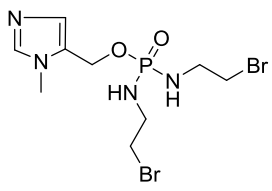
11

(2-bromoethyl)(dichlorophosphoryl)phosphoramidic dichloride (11). To a suspension of 2-Bromoethylamine hydrobromide (3 g, 14.6 mmol) in 50mL Et_2O was added $POCl_3$ (1.4 mL, 14.64 mmol) slowly at -5°C with stirring. Et_3N (4.08 mL, 97.61 mmol) was added and the reaction mixture was allowed to stir overnight. The solution was filtered and the filtrate was concentrateddown. The residue was purified by distillation to provide **8** (2.7 g, 85%) as white crystal.

^{1}H NMR (500 MHz, $CDCl_3$): δ 4.13-4.03 (m, 2H), 3.73-3.70 (t, 2H).

^{13}C NMR (125 MHz, $CDCl_3$): δ 50.41, 26.25.

^{31}P NMR (202 MHz, $CDCl_3$): δ 9.44.



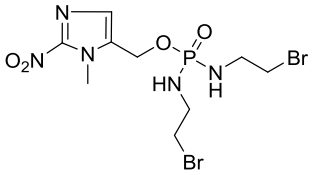
1

1-Methyl-1*H*-imidazol-5-yl)methyl *N,N*-bis(2-bromoethyl)phosphordiami-date (1). To a suspension of 2-Bromoethylamine hydrobromide (10 g, 48.8 mmol) in 100 mL CH_2Cl_2 was added $POCl_3$ (2.28 mL, 24.4 mmol) slowly at -15°C with vigorous stirring. To this reaction mixture a solution of Et_3N (10.96 mL, 97.6 mmol) in 50 mL CH_2Cl_2 was added dropwise, followed by stirring at -10 °C for 2h. The solid residue was filtered and washed with small amount (how much?) of cold CH_2Cl_2, and the filtrate was concentrated by rotor evaporation to about 20 mL. The residue was filtered and washed with cold CH_2Cl_2 again. The resulting filtrate was concentrated down by rotor evaporation. To a suspension of (1-Methyl-1*H*-imidazol-5-yl)methanol (27.5 mg, 0.25 mmol) in 5 mL CH_2Cl_2 was added solution of the residue (20 mg in 5 mL CH_2Cl_2) from the precious step at 0 °C. To the reaction mixture Et_3N (16.9 mg, 0.167 mmol) in 2 mL CH_2Cl_2 was added t, and stirred overnight at 0 °C?. The solvent was removed by rotor evaporation. The residue was purified by a semi-preparative Jupiter 5u C18 HPLC column with a flow rate of 5.0 mL/min. The final yields of **1** was 13.8% (3.4 mg) as colorless oil.

^{1}H NMR (500 MHz, DMSO-d_6): δ 7.63 (s, 1H), 6.938 (s, 1H), 4.91-4.84 (m, 4H), 3.63 (m, 3H), 3.40 (m, 4H), 3.09 (m, 4H).

^{31}P NMR (202 MHz, DMSO-d_6): δ 14.82.

MS (ESI+) *m/z* calcd for $C_9H_{17}Br_2N_4O_2P$ $[M+H]^+$ 402.95, found 402.95.



2

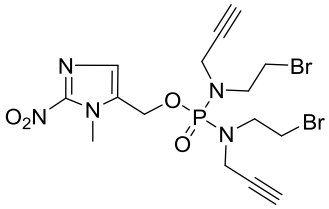
1-Methyl-2-nitro-1*H*-imidazol-5-yl)methyl *N,N*-bis(2-bromo-ethyl)phosphordiamidate (2).

Compound **2** was synthesized by the same synthetic procedure as **1** and obtained as a yellow oil in 11.7% yield.

^{1}H NMR (500 MHz, DMSO-d_6): δ 7.24 (s, 1H), 4.98-4.96 (m, 4H), 3.93 (m, 3H), 3.41 (m, 4H), 2.88 (m, 4H).

^{31}P NMR (202 MHz, DMSO-d_6): δ 15.30.

HRMS (ESI+) *m/z* calcd for $C_9H_{16}Br_2N_5O_4P$ $[M+H]^+$ 447.9385, found 447.9382.



3

1-Methyl-2-nitro-1*H*-imidazol-5-yl)methyl *N,N*-bis((2-bromoethyl)prop-2-yn)phosphordiami-date (3). To a suspension of Compound **9** (94.6 mg, 0.484 mmol) in 0 mL CH_2Cl_2 was added phosphorus trichloride (30 mg, 0.22 mmol) at room temperature, followed by the addition of TEA (397.9 mg, 3.93 mmol) in 10 mL CH_2Cl_2dropwise. The reaction mixture was stirred at room temperature for 1h and then (1-Methyl-2-nitro-1*H*-imidazol-5-yl)methanol (41.2 mg, 0.26 mmol) in 5 mL CH_2Cl_2 was added. After stirring for another 2h, the reaction mixture was cooled to -20°C and the tert-butyl hydroperoxide (48 µL, 0.26 mmol, 5M in decane) was added. The solution was warmed to room temperature and stirred for 1h. The reaction mixture was quenched with saturated aqueous NH_4Cl solution, extracted three times with CH_2Cl_2 . The organic layers were dried over anhydrous Na_2SO_4, filtered and the solvent was removed by rotary evaporation. The residue was purified by a semi-preparative Jupiter 5u C18 HPLC column with a flow rate of 5.0 mL/min. The final yields of **3**

No table of contents entries found. was 7.4% (8.4 mg) as yellow oil.

^{1}H NMR (500 MHz, $CDCl_3$): δ 5.15-5.14(d, 2H), 4.10 (s, 3H), 3.93-3.82 (m, 8H), 3.52 (m, 4H), 2.35 (m, 2H).

^{31}P NMR (202 MHz, $CDCl_3$): δ 15.62.

^{13}C NMR (125 MHz, $CDCl_3$): δ 129.44, 78.95, 73.39, 56.58, 47.81, 36.28, 34.52, 29.41.

HRMS (ESI+) *m/z* calcd for $C_{15}H_{20}Br_2N_5O_4P$ $[M+Na]^+$ 546.9698, found 546.9689.

X-Ray Crystallographic Analysis. The single crystal generated from synthesis was stored in -80°C before characterization. Crystal data for compound **9:** $C_6H_9NBr_6$, MW = 574.60 g/mol, orthorhombic, space group $P2_12_12_1$, a = 7.30499(16) Å, b = 11.3505(2) Å, c = 16.9283(3) Å, $\alpha = \beta = \gamma = 90°$, V = 1403.62(5) Å, Z = 4, D_{calc} = 2.719 g/cm^3, μ = 20.381 mm^{-1}, T = 100 (2) K, Data was collected on a Bruker SMART 1K CCD. Refinement of data with $I > 2\sigma(I)$ (2909 independent reflections, R_{int} = 0.0758) gave a $R_1(F)$ = 0.0346 and a $wR_2(F^2)$ = 0.0925 for all data with a GOF = 1.061. Crystallographic data for the structural analysis has been deposited with the Cambridge Crystallographic Data Center (CCDC 1566695). Crystal data for compound 11**:** $C_2H_4Br_1C_{l4}N_1O_2P_2$, MW = 354.71 g/mol, orthorhombic, space group P21/c, a = 11.2875(5) Å, b = 7.8601(4)Å, c = 12.5373(6)Å, $\alpha = \gamma = 90°$, β = 102.528(5), V = 1085.84(9)Å, Z = 4, T = 100 (2) K, D_{calc} = 2.191 g/cm^3, μ = 16.776 mm^{-1}, Data was collected on a Bruker SMART 1K CCD. Refinement of data with $I > 2\sigma(I)$ (2212 unique reflections, R_{int} = 0.048) gave a $R_1(F)$ = 0.0464 and a $wR_2(F^2)$ = 0.1524 for all data with a GOF = 1.176. Crystallographic data for the structural analysis has been deposited with the Cambridge Crystallographic Data Center (CCDC 1566696). Copies of this information may be obtained free of charge from the CCDC (www: http://www.ccdc.cam.ac.uk) or from e-mail jcgarrison@unmc.edu.

Table S1 Crystal data and structure refinement for compound 9.		
Identification code	wenting-1	
Empirical formula	$C_5H_8NBr_6$	
Formula weight	574.6	
Temperature/K	100	
Crystal system	orthorhombic	
Space group	$P2_12_12_1$	
Unit cell dimensions	a/Å= 7.30499(16)	α/°= 90.00
	b/Å= 11.3505(2)	β/°= 90.00
	c/Å= 16.9283(3)	γ/°= 90.00
Volume/Å^3	1403.62(5)	
Z	4	
Density (calculated) g/cm^3	2.719	
Absorption coefficient /mm^{-1}	20.381	
F (000)	1048.0	
Crystal size/mm^3	0.4 × 0.05 × 0.05	
Index ranges	$-9 \le h \le 8$, $-14 \le k \le 13$, $-21 \le l \le 21$	
Reflections collected	21627	
Independent reflections	2909 [R_{int} = 0.0758, R_{sigma} = 0.0346]	
Radiation	CuKα (λ = 1.54184)	
2Θ range for data collection/°	9.38 to 152.66	
Data / restraints / parameters	2909/0/118	
Goodness-of-fit on F^2	1.061	
Final R indices [I>=2σ (I)]	R_1 = 0.0346, wR_2 = 0.0907	
R indices (all data)	R_1 = 0.0369, wR_2 = 0.0925	
Largest diff. peak and hole/ e.Å^{-3}	0.94 and -0.58	

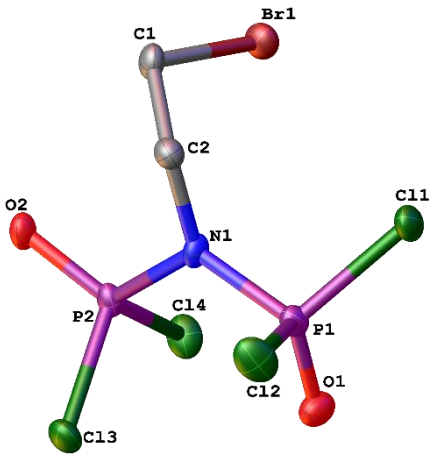


Figure S1 Crystal structure of side product **11**

Table S2 Crystal data and structure refinement for compound 11.		
Identification code	JG	
Empirical formula	$C_2H_4NO_2P_2Cl_4Br$	
Formula weight	357.71	
Temperature/K	100	
Crystal system	monoclinic	
Space group	$P2_{1/c}$	
Unit cell dimensions	a/Å= 11.2852(7)	α/°= 90.00
	b/Å= 7.8547(5)	β/°=102.540(7)
	c/Å= 12.5336(9)	γ/°= 90.00
Volume/$Å^3$	1084.49(12)	
Z	4	
Density (calculated) g/cm^3	2.191	
Absorption coefficient /mm^{-1}	16.776	
F (000)	688.0	
Crystal size/mm^3	0.2 × 0.06 × 0.03	
Index ranges	$-9 \le h \le 14$, $-9 \le k \le 9$, $-15 \le l \le 13$	
Reflections collected	7033	
Independent reflections	2212 [R_{int} = 0.0480, R_{sigma} = 0.0378]	
Radiation	CuKα (λ = 1.54184)	
2Θ range for data collection/°	8.026 to 152.386	
Data / restraints / parameters	2212/0/109	
Goodness-of-fit on F^2	1.176	
Final R indices [I>=2σ (I)]	R_1 = 0.0464, wR_2 = 0.1409	
R indices (all data)	R_1 = 0.0502, wR_2 = 0.1524	
Largest diff. peak and hole/ e.$Å^{-3}$	0.81 and -1.01	

***In vitro* cytotoxicity study.** PC-3 and DU145 cells were separately cultured in F-12K and DMEM medium supplemented with 10% fetal bovine serum, 2.5 mM L-glutamine, 15 mM HEPES and 1% penicillin/streptomycin. Cells were seeded 24h before the experiment in 96-well plates at a density of 15,000/mL with 200µL medium of PC-3 cells and 12,500/mL with 200µL medium. On the day of the experiment, serial dilutions of the test compound with medium were administrated to cells. The plates were incubated for 2h, or longer if indicated, under either normoxic (95% air, 5% CO_2) or hypoxic (94.9% N_2, 0.1% O_2, 5% CO_2) conditions. After washing with fresh medium, cells were cultured for 72h in 200µL complete medium under normoxic conditions. Cell viability was determined by AlamarBlue. The 50% inhibitory concentration (IC_{50}) of the test compound relative to the untreated control were calculated by Graphpad PRISM 5.

Radiolabeling of 2-NIPAM. The general procedure for the preparation of $^{Nat/125}$I-labeled Azide-Alkyne Cycloaddition conjugates were adapted from previous reported synthetic strategy.[7] To 1.2 mL anhydrous acetonitrile, copper(II) chloride (4.02 mg, 30 µmol) was added to make $CuCl_2$/TEA solution. To this mixture, anhydrous triethylamine (4.53 mg, 45 µmol) was added and sonicated until a clear burgundy solution was formed. The solution was gently mixed before adding slowly over 5 min a solution of 2-NIPAM (0.1 µmol) in 20 µL acetonitrile. To this mixture, $Na^{125}I$ (7.4 MBq) in 6.5 µL H_2O was added followed by the addition of another 0.5 µL benzyle azide in 20 µL of anhydrous acetonitrile. The resultant solution was heated at 60 °C for 90 min. The purification of the radiolabeled azide-alkyne cycloaddition conjugates was performed by using RP-HPLC (≥10%).

Distribution Coefficient. The distribution coefficient was determined (n = 5) for each ^{125}I-labeled radioconjugate. In

a 1.5 mL centrifuge tube, 0.5 mL of 1-octanol was added to 0.5 mL of phosphate-buffered saline (pH 7.4) containing the radiolabeled peptide (400 000 cpm). The solution was vigorously stirred for 2 min at room temperature and subsequently centrifuged (8000g, 5 min) to yield two immiscible layers. Aliquots of 100 μL were taken from each layer, and the radioactivity of each was quantified by an LTI (Elburn, IL) Multi-Wiper nuclear medicine gamma counter.

In vitro efflux study. PC-3 and DU145 cells were incubated in 6-well plates (0.5×10^6/well) under normoxic (95% air, 5% CO_2) or hypoxic (94.9 % N_2, 0.1 % O_2, 5 % CO_2) conditions overnight in RPMI 1640 media (pH 7.4, 4.8 mg/mL HEPES, and 2 mg/mL BSA). On the day of the experiment, the medium was replaced with fresh medium and incubated for 2 h under normoxic or hypoxic conditions, respectively. The cells were pre-incubated for 2h at 37 °C in the presence of 100,000 cpm of ^{125}I-radioconjugates. Upon completion of the incubation, cells were washed thrice with medium to discard the unbound peptide. At time points 1, 2, 4, 6 and 8 h, the medium was harvested for quantitative analysis as the effluxed ligand. Surface bound radioactivity was collected by washing the cells twice with an acid wash (50 mM glycine-HCl/0.1 M NaCl buffer, pH 2.8). The cells were then lysed at 37 °C using a 10% aqueous SDS solution and the lyses were collected as internalized ligand. The radioactivity of the effluxed, surface bounded and internalized fractions for each radioconjugate was determined using a Multi Viper gamma counter. Statistical analyses were performed by two-way analysis of variance (ANOVA) using Graphpad PRISM 5 (U.S.).

Statistical Analysis. IC_{50} values were determined by nonlinear regression using the 1-binding-site model of GraphPad PRISM 5 (GraphPad Software, Inc). Comparisons of the 2 groups for efflux studies were analyzed by the 2-tailed Student t test, and P values of less than 0.05 were considered statistically significant.

(1) Duan, J.-X.; Jiao, H.; Kaizerman, J.; Stanton, T.; Evans, J. W.; Lan, L.; Lorente, G.; Banica, M.; Jung, D.; Wang, J. Potent and highly selective hypoxia-activated achiral phosphoramidate mustards as anticancer drugs. *Journal of Medicinal Chemistry* **2008**, *51*, 2412-2420.

(2) Cambeiro, F.; Lopez, S.; Varela, J. A.; Saa, C. Vinyl dihydropyrans and dihydrooxazines: cyclizations of catalytic ruthenium carbenes derived from alkynals and alkynones. *Angew Chem Int Ed Engl* **2014**, *53*, 5959-5963.

(3) Molander, G. A.; Cormier, E. P. Ketyl-allene cyclizations promoted by samarium(II) iodide. *J Org Chem* **2005**, *70*, 2622-2626.

(4) Rengasamy, R.; Curtis-Long, M. J.; Seo, W. D.; Jeong, S. H.; Jeong, I. Y.; Park, K. H. New building block for polyhydroxylated piperidine: total synthesis of 1,6-dideoxynojirimycin. *J Org Chem* **2008**, *73*, 2898-2901.

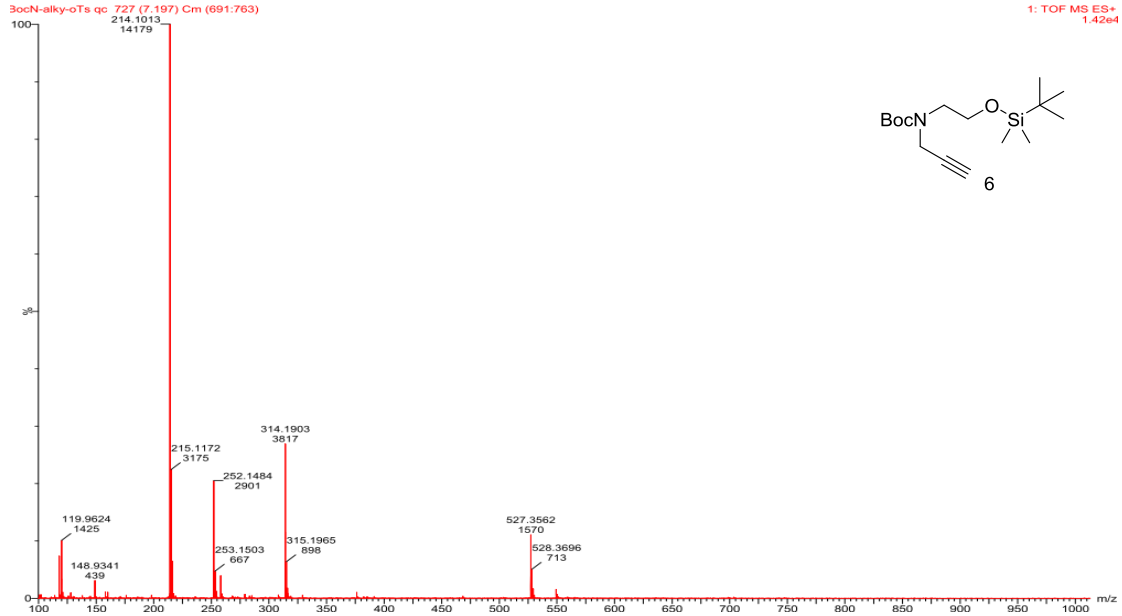
(5) Fedotenko, I. A., Zaffalon, P. L., Favarger, F., & Zumbuehl, A. The synthesis of 1, 3-diamidophospholipids. *Tetrahedron Letters* **2010**, *51*, 5382-5384.

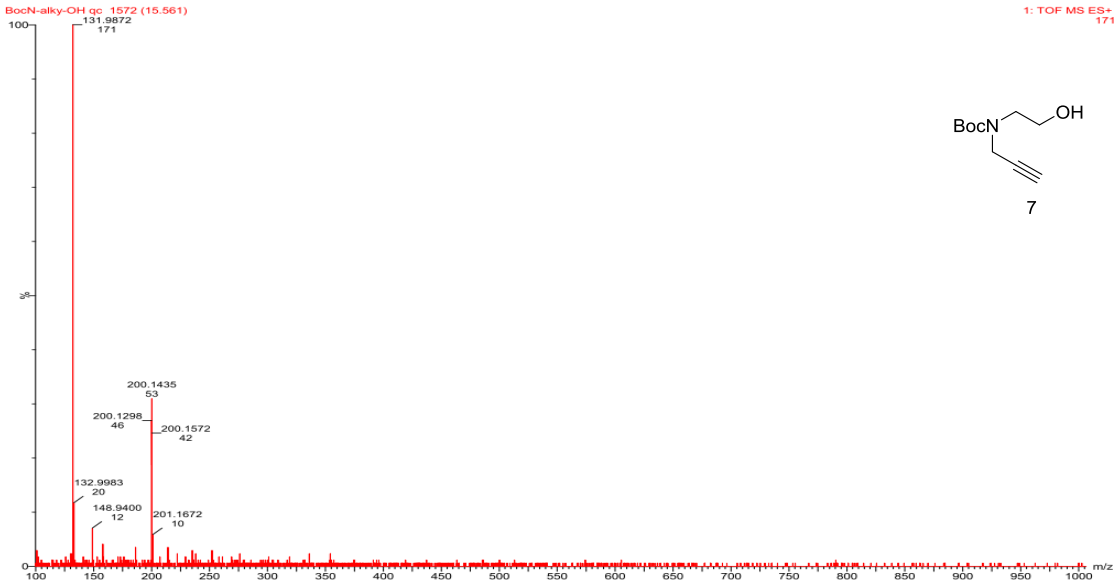
(6) Vedejs, E., & Stults, J. S. Synthesis of azocine derivatives from thio aldehyde Diels-Alder adducts. *The Journal of Organic Chemistry* **1988**, *53*, 2226-2232.

(7) Yan, R.; Sander, K.; Galante, E.; Rajkumar, V.; Badar, A.; Robson, M.; El-Emir, E.; Lythgoe, M. F.; Pedley, R. B.; Arstad, E. A one-pot three-component radiochemical reaction for rapid assembly of 125I-labeled molecular probes. *Journal of the American Chemical Society* **2013**, *135*, 703-709.

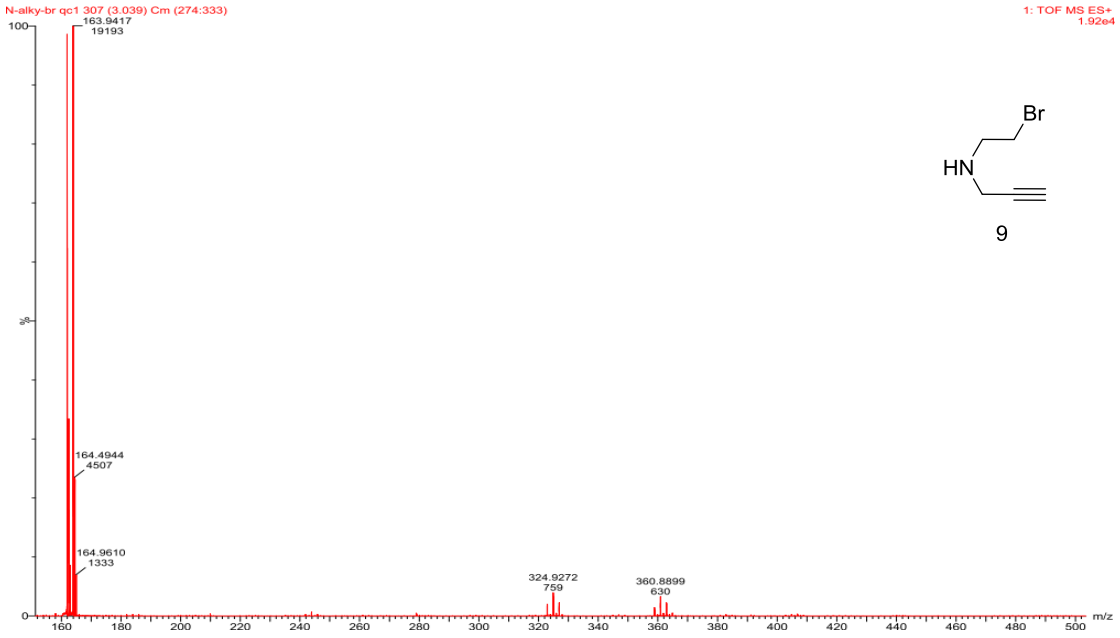

BocN-OH qc1 316 (3.127) Cm (272:316)
1: TOF MS ES+
1.14e3
162.0851
1136
218.1297
226
240.1196
260
184.0607
133
279.0537
75
301.0916
48
%
m/z
HO
N
H
O
O
4

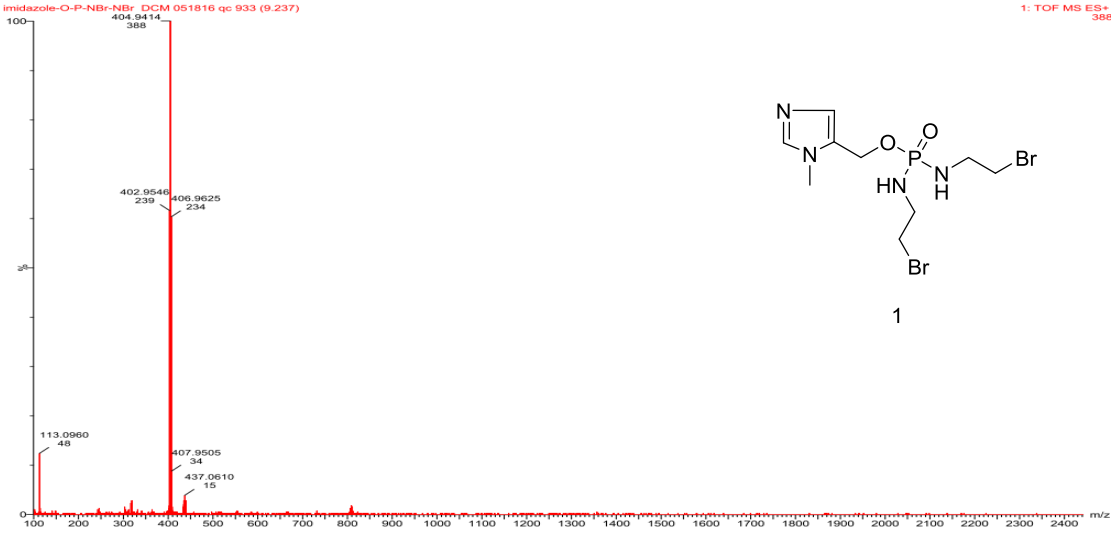

BocN-oTs qc 509 (5.273) Cm (507:666)
1: TOF MS ES+
6.14e4
176.0539
61376
176.6273
31930
220.0819
28519
177.0789
18323
160.0345
16429
221.0975
5979
222.0937
1911
%
m/z
BocHN
O
Si
5


BocN-alky-oTs qc 727 (7.197) Cm (691:763)
1: TOF MS ES+
1.42e4
214.1013
14179
314.1903
3817
215.1172
3175
252.1484
2901
119.9624
1425
527.3562
1570
315.1965
898
253.1503
667
528.3696
713
148.9341
439
%
m/z
BocN
O
Si
6

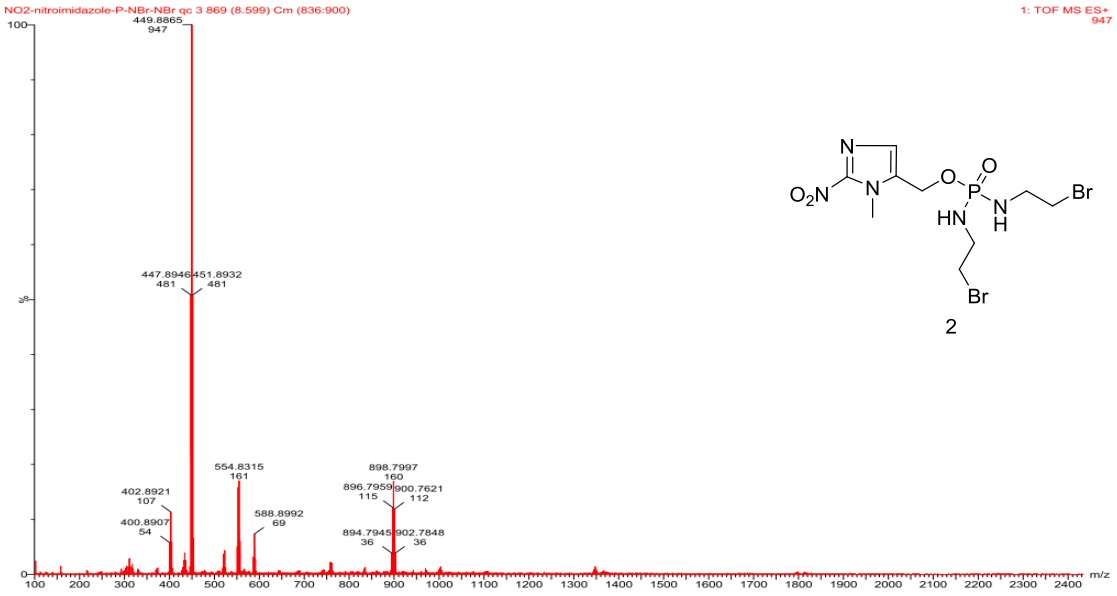

BocN-alky-OH qc 1572 (15.561)
1: TOF MS ES+
171
131.9872
171
200.1435
53
200.1298
46
200.1572
42
132.9983
20
148.9400
12
201.1672
10
BocN
OH
7
m/z


BocN-alky-br qc 1 254 (2.515) Cm (239:275)
1: TOF MS ES+
3.71e3
161.9181
3711
163.9183
3569
-Boc
164.9260
253
402.1683
216
487.2238
229
502.3820;113
BocN
Br
8
m/z

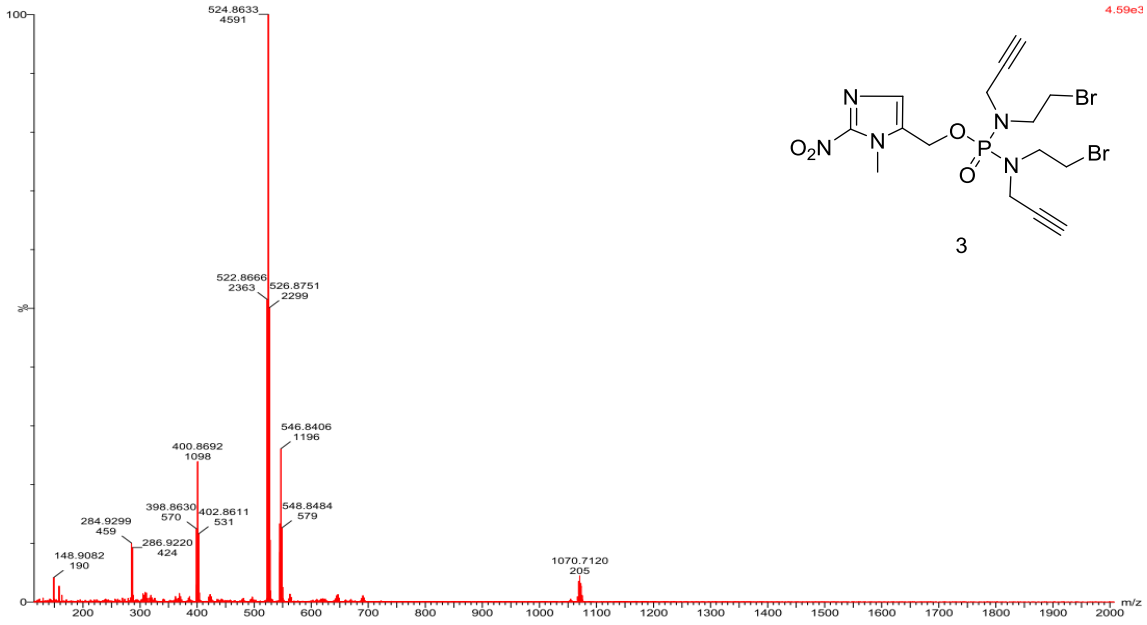

N-alky-br qc1 307 (3.039) Cm (274:333)
1: TOF MS ES+
1.92e4
163.9417
19193
164.4944
4507
164.9610
1333
324.9272
759
360.8899
630
Br
HN
9
m/z


imidazole-O-P-NBr-NBr DCM 051816 qc 933 (9.237)
1: TOF MS ES+
388
404.9414
388
402.9546
239
406.9625
234
113.0960
48
407.9505
34
437.0610
15
%
m/z

1


NO2-nitroimidazole-P-NBr-NBr qc 3 869 (8.599) Cm (836:900)
1: TOF MS ES+
947
449.8865
947
447.8946
481
451.8932
481
554.8315
161
898.7997
160
896.7959
115
900.7621
112
402.8921
107
400.8907
54
588.8992
69
894.7945
36
902.7848
36
%
m/z

2


4.59e3
524.8633
4591
522.8666
2363
526.8751
2299
546.8406
1196
400.8692
1098
398.8630
570
402.8611
531
548.8484
579
284.9299
459
286.9220
424
148.9082
190
1070.7120
205
%
m/z

3

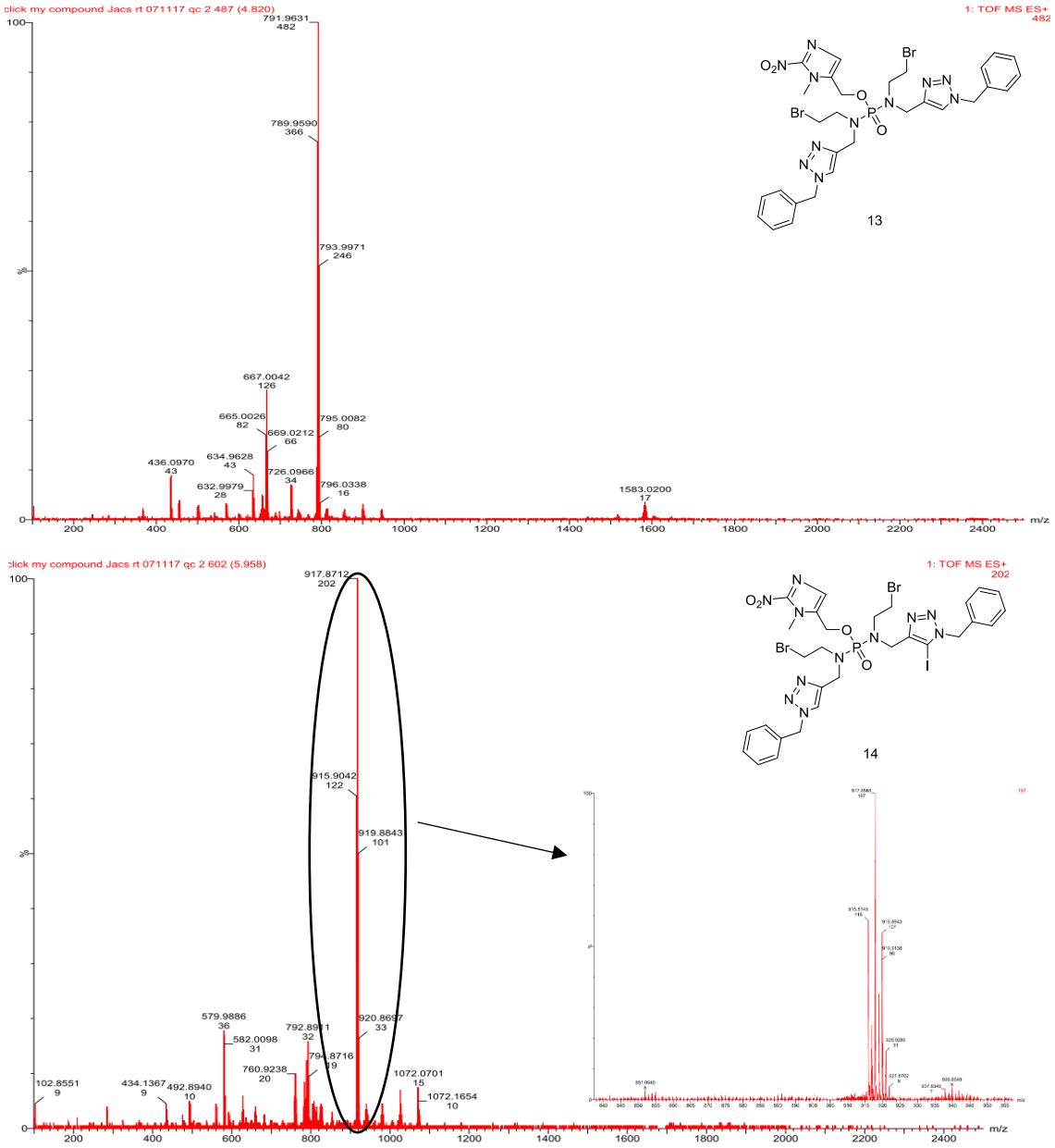
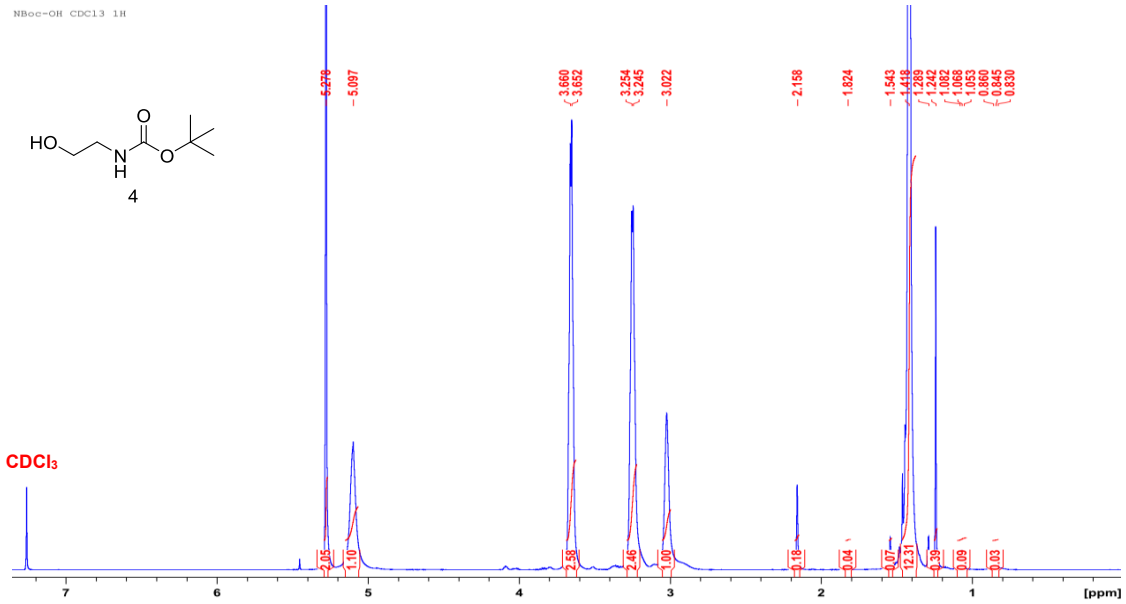
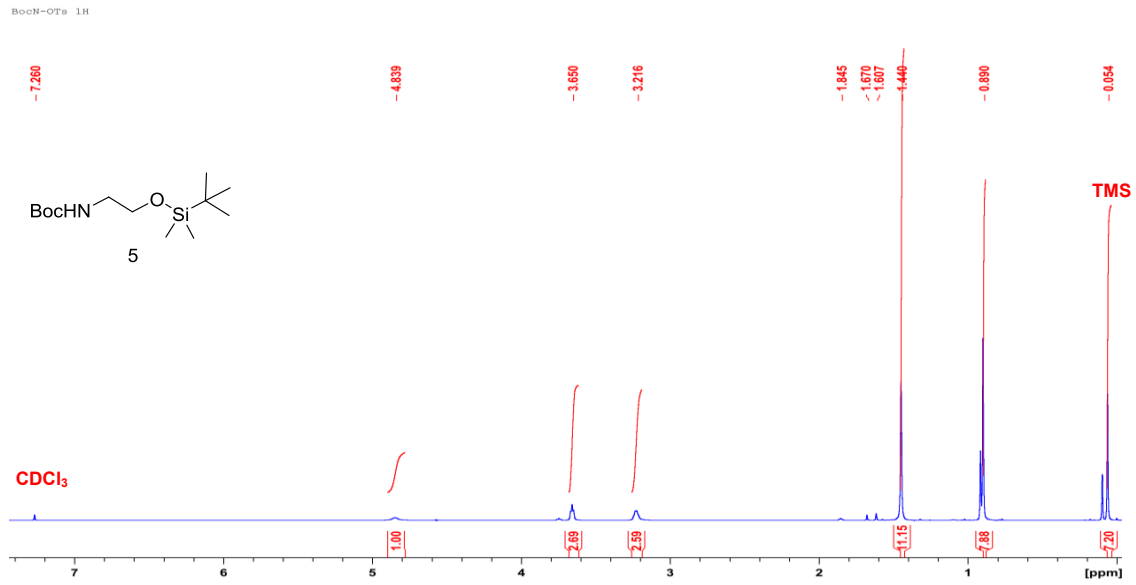
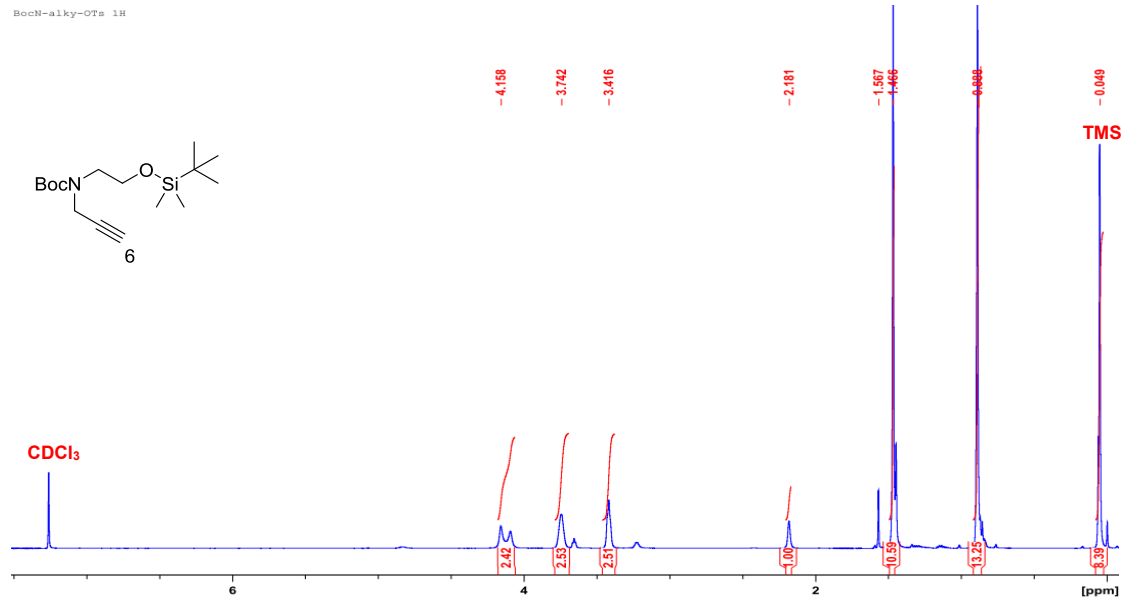
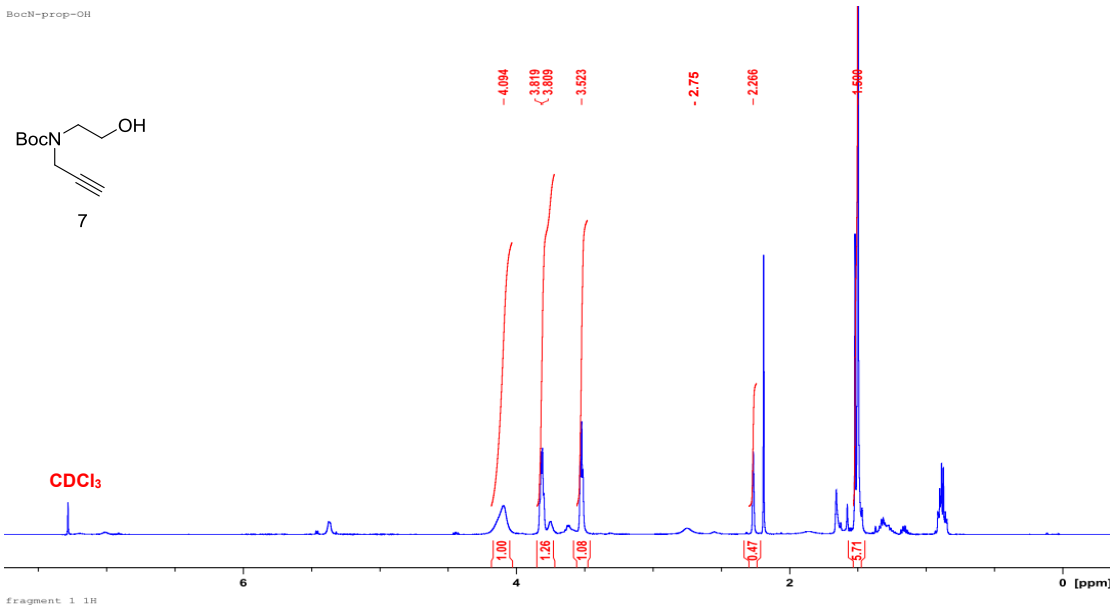


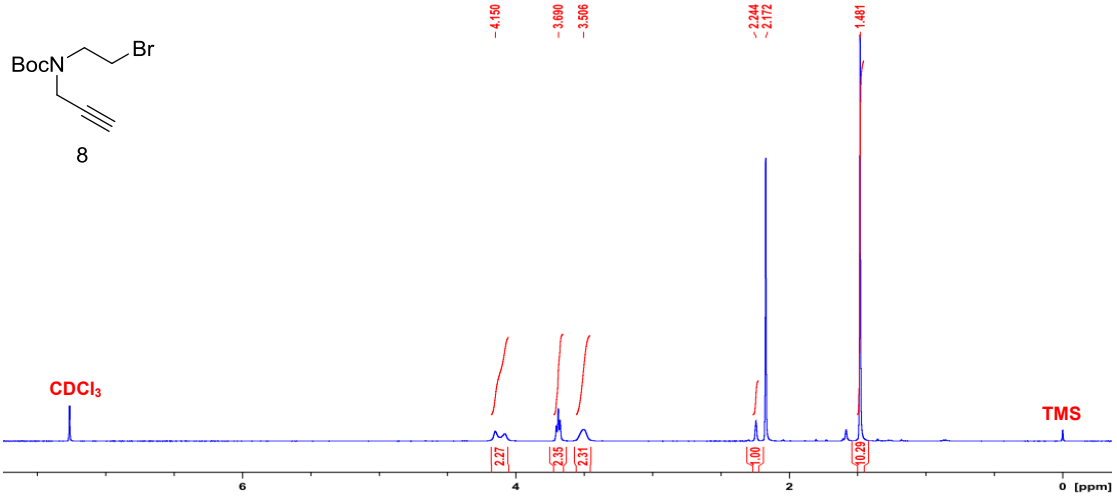
Fig S2. Mass spectrometry of compound **1--9**, **13** and **14**.

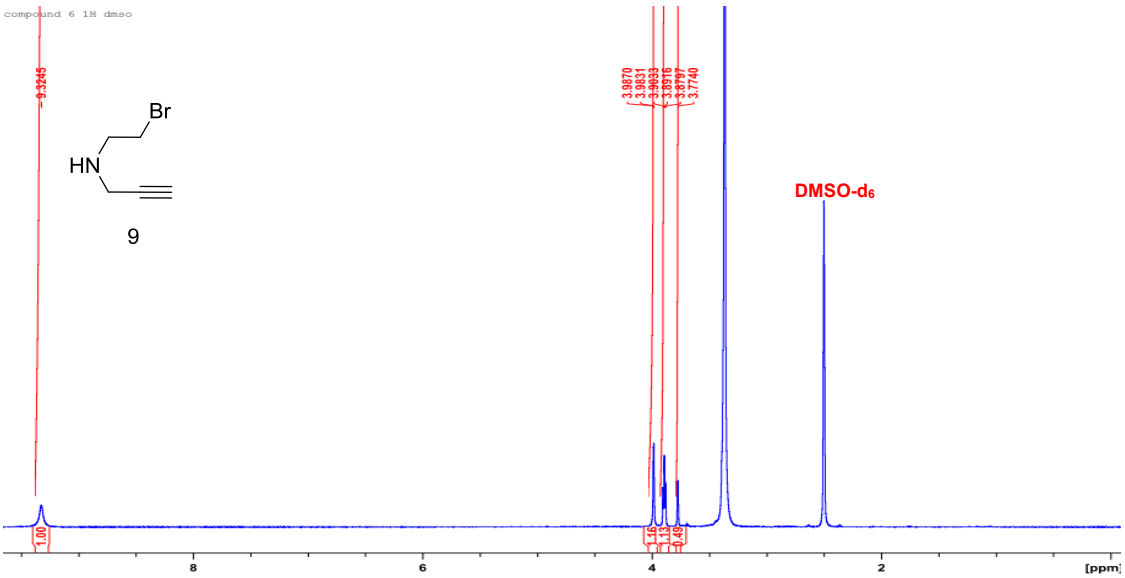

NBoc-OH CDCl3 1H
HO
N
H
O
O
4
CDCl3
5.270
5.097
3.660
3.652
3.254
3.245
3.022
2.158
1.824
1.543
1.418
1.289
1.242
1.082
1.068
1.053
0.866
0.845
0.830
2.05
1.10
2.58
2.46
1.00
0.18
0.04
0.07
12.31
0.39
0.09
0.03
7
6
5
4
3
2
1
[ppm]

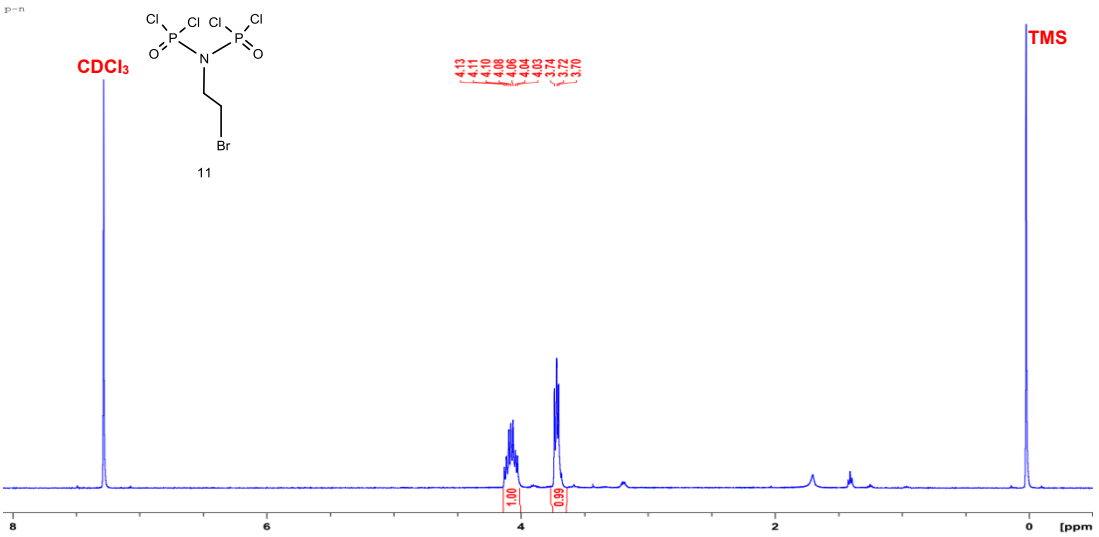

BocN-OTs 1H
7.260
4.839
3.650
3.216
1.845
1.670
1.607
1.440
0.890
0.054
BocHN
O
Si
5
CDCl3
TMS
1.00
2.69
2.59
11.15
7.88
7.20
7
6
5
4
3
2
1
[ppm]

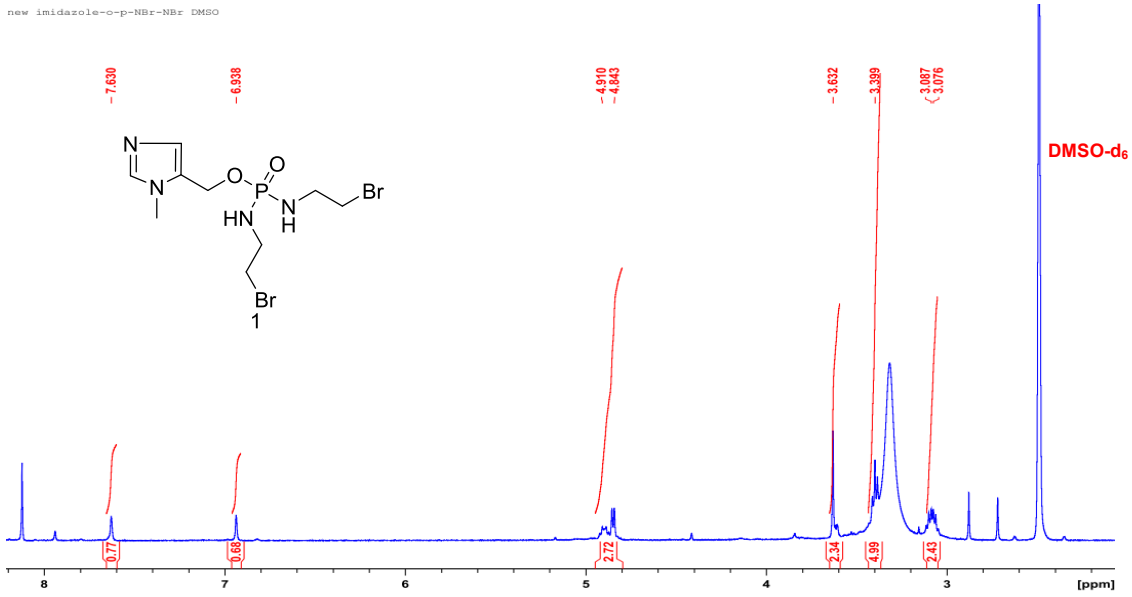

BocN-alky-OTs 1H
4.158
3.742
3.416
2.181
1.567
1.466
0.868
0.049
BocN
O
Si
6
CDCl3
TMS
2.42
2.53
2.51
1.00
10.59
13.25
8.39
6
4
2
[ppm]

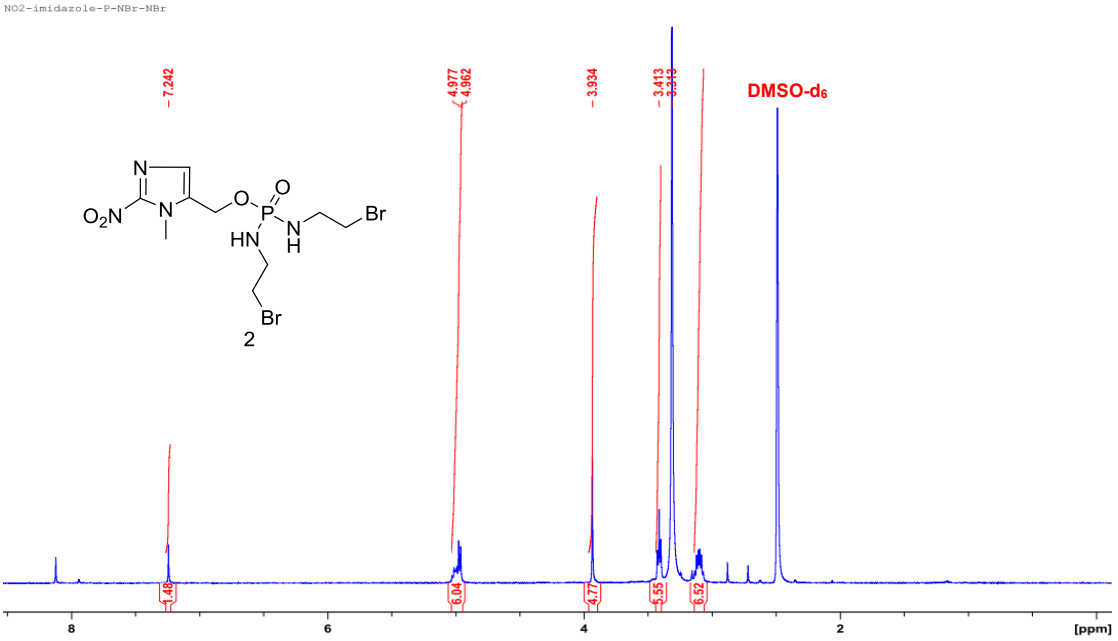

BocN-prop-OH
BocN
OH
7
4.094
3.819
3.809
3.523
2.75
2.266
1.500
CDCl3
1.00
1.26
1.08
0.47
5.71
6
4
2
0 [ppm]


fragment 1 1H
BocN
Br
8
4.150
3.690
3.506
2.244
2.172
1.481
CDCl3
TMS
2.27
2.35
2.31
1.00
10.29
6
4
2
0 [ppm]


compound 6 1H dmso
9.3245
Br
HN
9
3.9870
3.9831
3.9033
3.8916
3.8797
3.7740
DMSO-d6
1.00
1.16
1.13
0.49
8
6
4
2
[ppm]


p-n
CDCl3
TMS
11
4.13
4.11
4.10
4.08
4.06
4.04
4.03
3.74
3.72
3.70
1.00
0.99
8
6
4
2
0
[ppm]


new imidazole-o-p-NBr-NBr DMSO
7.630
6.938
4.910
4.843
3.632
3.399
3.087
3.076
DMSO-d6
1
0.77
0.68
2.72
2.34
4.99
2.43
8
7
6
5
4
3
[ppm]


NO2-imidazole-P-NBr-NBr
7.242
4.977
4.962
3.934
3.413
3.393
DMSO-d6
2
1.46
6.04
4.77
5.55
6.52
8
6
4
2
[ppm]

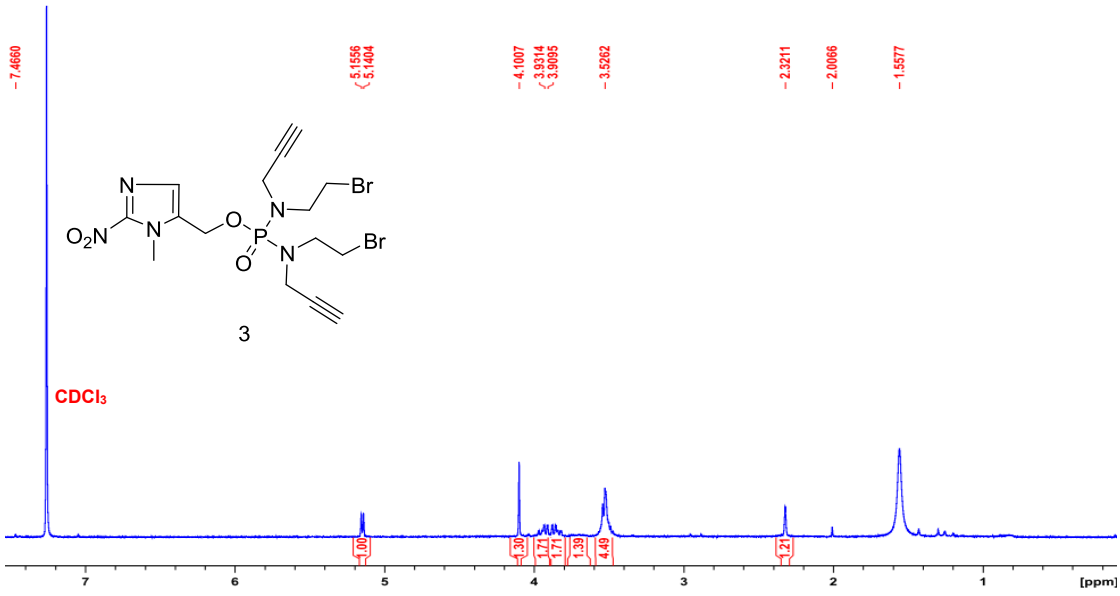
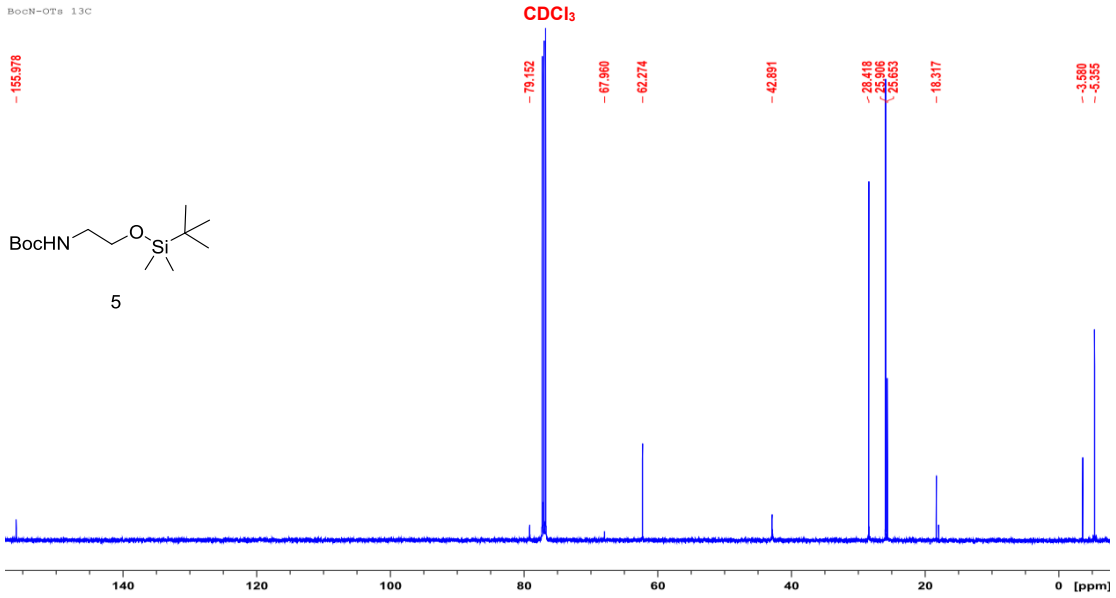
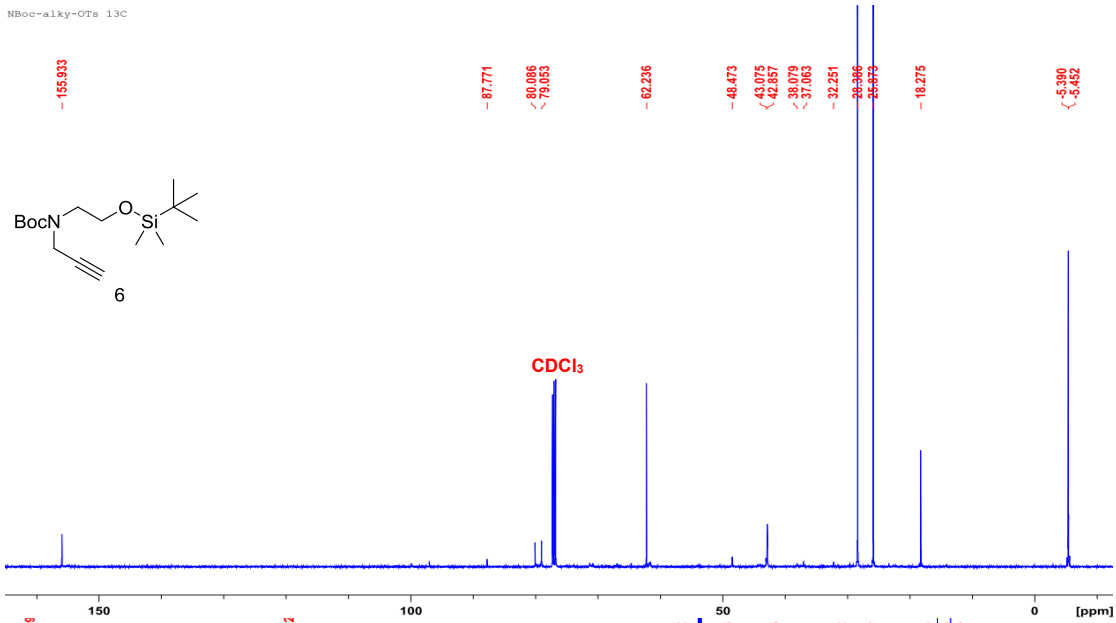


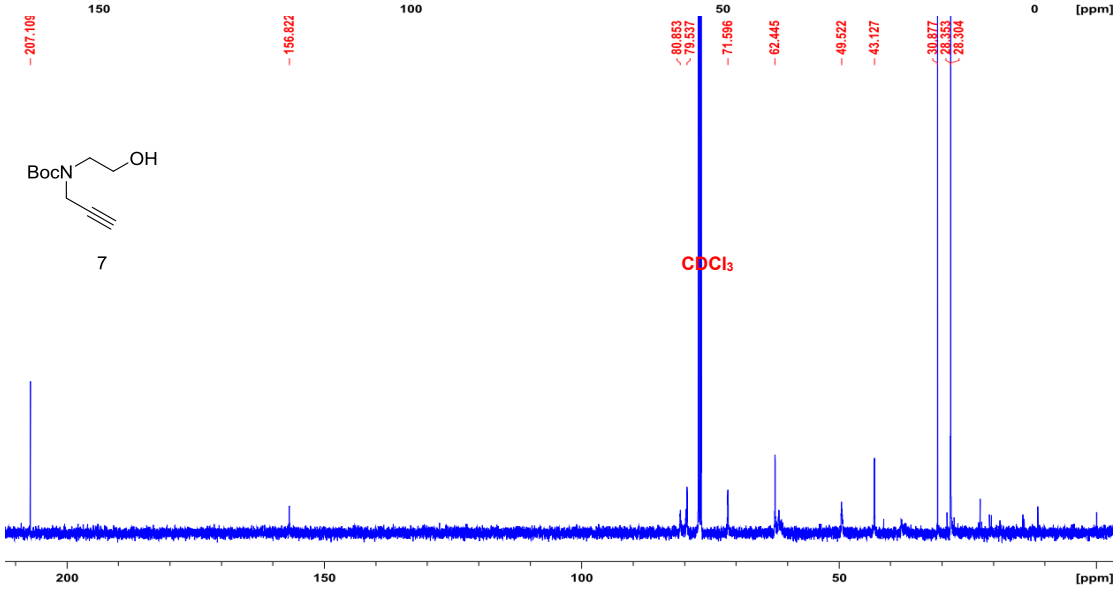
Fig. S3. [1]H-NMR spectra of compound **1-9** and **11**.


BocN-OTs 13C
CDCl3
155.978
79.152
67.960
62.274
42.891
28.418
25.906
25.653
18.317
-3.588
-5.355
BocHN
O
Si
140
120
100
80
60
40
20
0
[ppm]

5


NBoc-alky-OTs 13C
155.933
87.771
80.086
79.053
62.236
48.473
43.075
42.857
38.079
37.063
32.251
28.366
25.873
18.275
-5.390
-5.452
BocN
O
Si
CDCl3
150
100
50
0
[ppm]

6


207.108
156.822
80.853
79.537
71.596
62.445
49.522
43.127
30.877
28.353
28.304
BocN
OH
CDCl3
200
150
100
50
[ppm]

7

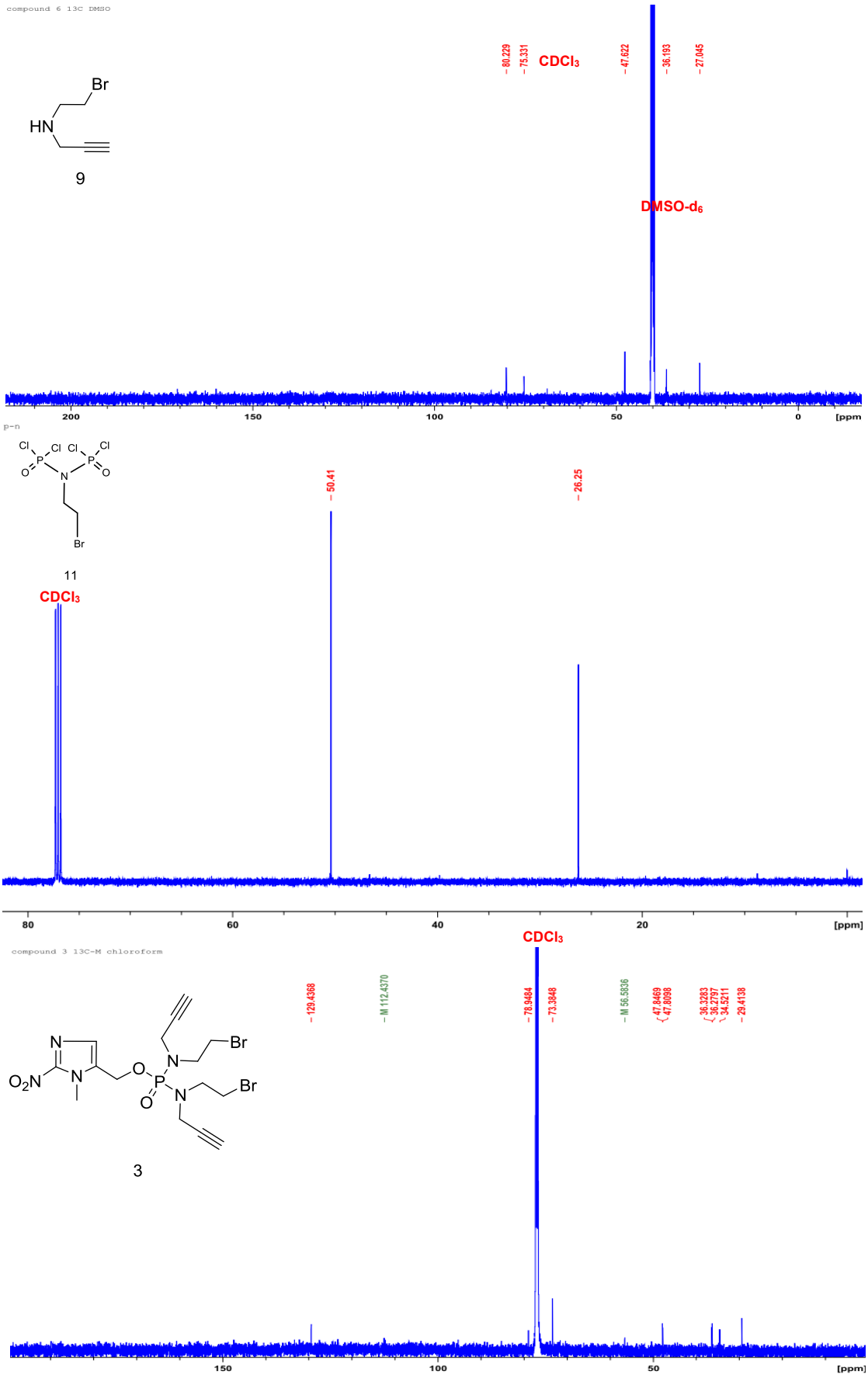
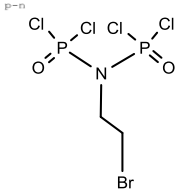


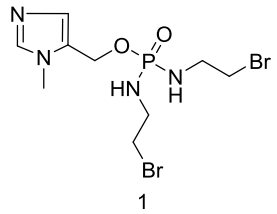
Fig. S4. ^{13}C-NMR spectra of compound **3**, **5-7, 9** and **11.**

p-n



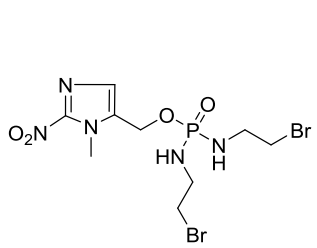
11

new imidazole-o-p-NBr-NBr DMSO



1

NO2-imidazole-P-NBr-NBr



2

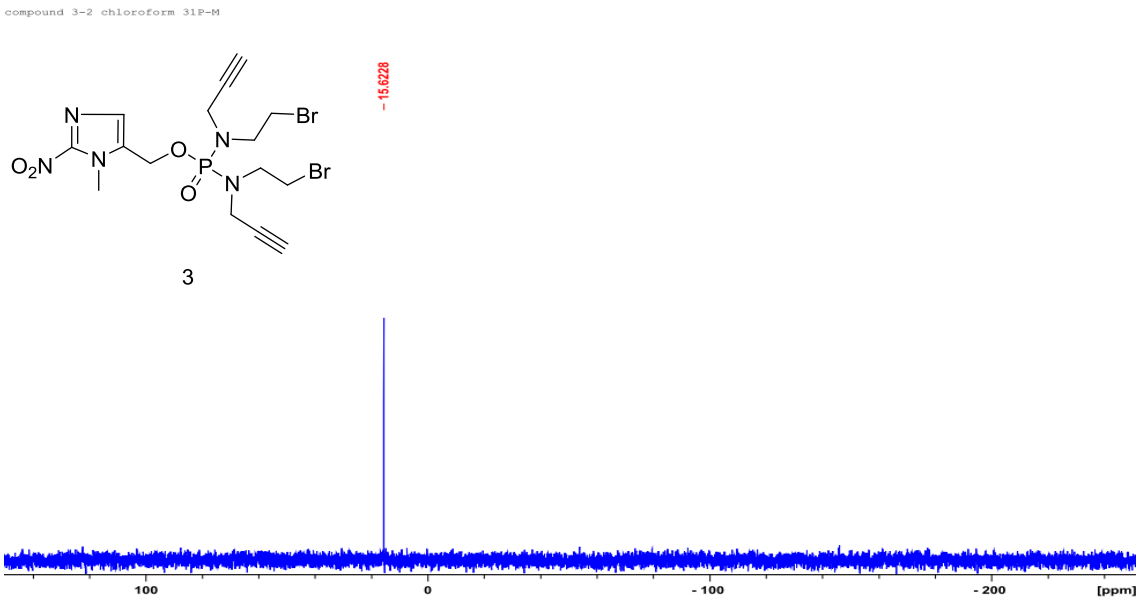


Fig. S5. ^{31}P-NMR spectra of compound **1-3 and 11.**