

Photoreactive Polymer Brushes for High Density Surface Patterned Derivatization Using Diels-Alder Photoclick Reaction

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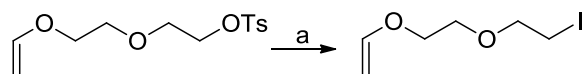
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General Information. All organic solvents were dried and freshly distilled before use. Flash chromatography was performed using 40-63 μm silica gel. All NMR spectra were recorded on 400 MHz instruments in CDCl_3 and referenced to TMS unless otherwise noted. Solutions were prepared using HPLC grade water and acetonitrile.

Materials: Silicon wafers (orientation 100, native oxide) were purchased from University Wafer. Quartz microscope slides were purchased from Technical Glass. Fluorescein NHS ester and Rhodamine B were purchased from Invitrogen. All other chemicals were purchased from Sigma Aldrich and were used as received. 2-(2-(Vinylloxy)ethoxy)ethyl tosylate,¹ 2-(2-(Vinylloxy)ethoxy)ethyl acetate,² (2-Azidoethoxy)ethyl vinyl ether,¹ 9-(Amino-TEG)-2,2-dimethyl-4H-naphtho[2,3-d][1,3]dioxine (NQMP-TEG-amine)¹, ADIBO-Rhodamine³ and azido-Fluorescein⁴ were prepared following the procedure reported previously.

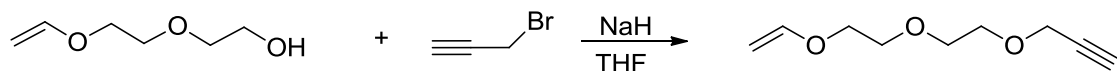
2-(2-(vinylloxy)ethoxy)ethyl iodide



Reagents and conditions: (a) NaI, Acetone, Reflux, 85%

NaI (1.5g, 10 mmol) was added to a solution of 2-(2-(vinylloxy)ethoxy)ethyl tosylate (1.43 g, 5 mmol) in acetone (20 mL) and reaction mixture was refluxed for 8 h. Solvent was removed under reduced pressure, the residue was dissolved in dichloromethane, washed with brine, dried over anhydrous magnesium sulfate, and the solvent was removed in vacuum. The product was purified by chromatography (60% EtOAc in hexanes) to yield 0.99 g (82%) of 2-(2-(vinylloxy)ethoxy)ethyl iodide as yellowish oil. ¹H NMR: 6.40 (dd, $J = 14.3, 6.8$ Hz, 1H), 4.10 (dd, $J = 14.3, 2.0$ Hz, 1H), 3.94 (dd, $J = 6.8, 2.0$ Hz, 1H), 3.81 – 3.73 (m, 2H), 3.73 – 3.63 (m, 4H), 3.18 (t, $J = 7.0$ Hz, 2H). ¹³C NMR: 151.95, 87.18, 72.37, 69.49, 67.54, 2.91. FW calc. ($\text{C}_6\text{H}_{11}\text{IO}_2$): 241.9804, EI-HRMS: 241.9810.

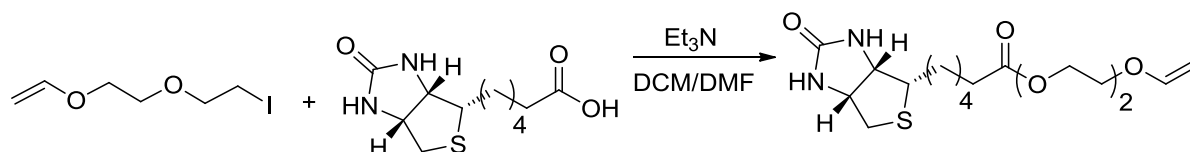
3-(2-(2-(vinylloxy)ethoxy)ethoxy)prop-1-yne.



NaH (65 mg, 2.8 mmol) was added to a solution of diethylene glycol monovinyl ether (300 mg, 2.3 mmol) in 10 ml of THF and the resulting suspension was stirred at 0°C for 30 min, followed by the dropwise addition of propargyl bromide (325 mg, 2.8 mmol). Reaction mixture was

allowed to warm up to room temperature and stirred for 4 h at r.t., was quenched with saturated ammonium chloride, and the product was extracted with ether. Combined organic layers were washed with brine solution, dried over anhydrous sodium sulfate, and the solvent was removed under reduced pressure. The resulting residue was purified by column chromatography using 10% ethyl acetate in hexane as eluent to yield 300 mg (80%) of propargyl-DEG-vinyl ether as colorless oil. ^1H NMR: 6.41 (dd, $J = 14.3, 6.8$ Hz, 1H), 4.15 – 4.05 (m, 3H), 3.92 (dd, $J = 6.8, 2.1$ Hz, 1H), 3.80 – 3.73 (m, 2H), 3.70 – 3.59 (m, 6H), 2.38 (t, $J = 2.3$ Hz, 1H). ^{13}C NMR: 151.88, 86.82, 79.79, 74.81, 70.68, 69.79, 69.26, 67.36, 58.58. FW calc. ($[\text{C}_9\text{H}_{14}\text{O}_3]\text{Na}^+$): 193.0835, EI-HRMS: 193.0834.

2-(2-(vinylloxy)ethoxy)ethylbiotin



Et_3N (69 μL , 0.49 mmol) was added to a solution of biotin (100 mg, 0.41 mmol) in 5 mL $\text{DMF}/\text{Methylene chloride}$ (1:10) at 0°C , the mixture was stirred for 30 min, and 2-(2-(vinylloxy)ethoxy)ethyl iodide (120 mg, 0.49 mmol) was added dropwise. The reaction mixture was warmed up to r.t., stirred overnight, quenched with saturated NH_4Cl solution, and the product was extracted with ether. Combined organic layers were washed with brine, dried over anhydrous sodium sulfate, and the solvent was removed under reduced pressure. The resulting residue was purified by column chromatography using 1% methanol in DCM to yield 120 mg of Biotin-DEG-Vinyl ether (82%) as pale yellow oil. ^1H NMR: 6.48 (dd, $J = 14.3, 6.8$ Hz, 1H), 6.10 (s, 1H), 5.49 (s, 1H), 4.48 (t, $J = 6.3$ Hz, 1H), 4.32 – 4.25 (m, 1H), 4.25 – 4.13 (m, 3H), 4.00 (d, $J = 6.8$ Hz, 1H), 3.87 – 3.79 (m, 2H), 3.71 (dd, $J = 10.9, 5.7$ Hz, 4H), 3.13 (d, $J = 4.6$ Hz, 1H), 2.93 – 2.83 (m, 1H), 2.72 (d, $J = 12.8$ Hz, 1H), 2.36 (t, $J = 7.5$ Hz, 2H), 1.75 – 1.63 (m, 4H), 1.44 (q, $J = 7.8$ Hz, 2H). ^{13}C NMR: 173.82, 163.84, 151.86, 87.06, 77.55, 77.24, 76.92, 69.72, 69.47, 67.49, 63.53, 62.15, 60.33, 55.70, 40.73, 33.98, 28.51, 28.44, 24.95. FW calc. ($[\text{C}_{16}\text{H}_{26}\text{N}_2\text{O}_5\text{S}]\text{H}^+$): 358.1635, ESI-HRMS: 359.1636

Fluorescein vinyl ether conjugate (EV-Fluorescein). DIEA (0.069 g, 0.6 mmol) was added to a solution of Fluorescein NHS ester (0.095 g, 0.2 mmol) followed by 2-(2-(vinylloxy)ethoxy)ethylamine (0.052 g, 0.4 mmol) in DMF (2 mL) at r.t. The reaction mixture was stirred overnight at room temperature. The reaction mixture was concentrated, and the fluorescein vinyl ether was purified by column chromatography (CHCl_3 : MeOH 80:20) to provide

84 mg of EV-Fluorescein as yellow oil (85% yield). The purity of the product was checked by HPLC and was characterized by HRMS. FW calc. ($[C_{27}H_{23}NO_8]H^+$): 490.1496, ESI-HRMS: 490.1502.

Rhodamine B vinyl ether – conjugate (EV-Rhodamine B). 2-(2-(vinylloxy)ethoxy)ethylamine (0.052 g, 0.4 mmol) was added to a solution of Lissamine Rhodamine B sulfonyl chloride (0.115 g, 0.2 mmol) in DMF (3 mL) followed by *N,N*-diisopropylethylamine (0.069 g, 0.6 mmol) and the reaction mixture was stirred overnight at r.t.. The reaction mixture was concentrated and the product was purified by chromatography ($CHCl_3$: MeOH 15:1) to provide 108 mg of EV-Rhodamine B as dark red viscous oil (80% yield). The structure of rhodamine B vinyl ether is shown below. The structure of fluorescein is shown below. The purity of the product was checked by HPLC and was characterized by HRMS. FW calc. ($[C_{33}H_{42}N_3O_8S_2]H^+$): 672.2408, ESI-HRMS: 672.2403.

Methods

Functionalization of poly-(NHS4VB) with (NQMP-TEG-amine). Poly-(NHS4VB) polymer brushes on silicon wafers were prepared following the procedure reported previously.⁴ The poly(NHS4VB) was submerged in 1 mL of dry DMF under nitrogen atmosphere. NQMP-TEG-amine (40 mg, 0.11 mmol) was added, followed by Et_3N (15 μ L, 0.11 mmol), and stirred overnight at 40°C. Substrates were removed, rinsed well with DMF and dried under a stream of nitrogen. The acetal was then deprotected by incubating the functionalized slide in 0.1M HCl in DMF. Substrates were removed, rinsed well with DMF and dried under a stream of nitrogen.

Photochemical derivatization of Poly-(NQMP-4VB) brushes. Flood irradiation of the substrates immersed in 0.1 mM aqueous solution of vinyl ether-derivatized label was conducted using mini-Rayonet photochemical reactor equipped with 8 fluorescent UV lamps (4W, 300 or 350 nm). For photopatterning, the TEM grid (250 or 12.5 micron pitch) was placed in intimate contact with the polymer brush surface by placing the silicon wafer polymer brush on a slab of poly(dimethylsiloxane) (PDMS). A TEM grid was placed on top of the brush and the whole surface was covered with a quartz slide. By clamping the quartz slide to the PDMS, no space exists between the grid and the brush surface, giving clean pattern lines. Handheld UV lamp set up fitted with a 300 nm Rayonet UV fluorescent lamp was used as light source.

Sequential Click using Copper-catalyzed Alkyne-Azide Cycloaddition (CuAAC). Propargyl-functionalized poly-(NQMP-4VB) coated wafer was immersed in a solution containing 5 mM of

the azide, 5 mol % of CuBr, 5 mM of PMDETA, and 10 mM of sodium ascorbate for 4 h at r.t. The substrate was removed from the solution, rinsed well and dried.

Sequential Click using Catalyst-free Alkyne-Azide Cycloaddition (SPAAC). Azide-functionalized poly-(NQMP-4VB)-coated wafer was immersed in a solution containing 1 mM of the ADIBO-Rhodamine B for 4 h. The substrate was removed from the solution, rinsed well, and dried.

Patterned immobilization of avidin on Poly-(NQMP-4VB) brushes: EV-biotin was photo-patterned on poly-(NQMP-4VB)-coated wafers. The resulting slide was immersed in a solution of FITC-avidin (5µg/ mL in PBS buffer) for 15 min at 2⁰C. The non-specifically adsorbed avidin was removed following the washing procedure reported previously.¹

Characterization of Functionalized Polymer Brush Films.

Characterization. Thickness measurements were taken by spectroscopic ellipsometry, which was performed on a J. A. Woollam M-2000V spectroscopic ellipsometer with a white light source at 65°, 70°, and 75° angles of incidence to the silicon wafer normal. Delta (Δ) and psi (Ψ) were measured as a function of wavelength between 400 and 1000 nm. Polymer film thicknesses were determined using a three layer model, where the polymer's refractive index was modeled using the Cauchy equation (Equation 1),

$$n = a + \frac{b}{\lambda^2} \quad (1)$$

Static contact angle measurements were taken on a Krüss DSA 100 using a 1 µL drop. The experimental error range for contact angle measurements was ± 3°. GATR-FTIR measurements were taken with a Nicolet Model 6700 with a grazing angle attenuated total reflection accessory (GATR) at 128 scans with 4 cm⁻¹ resolution.

The contact angle and thickness changes upon brush functionalization are summarized in Table 1 below.

Scheme S1. Chemical functionalities of brush polymer films.

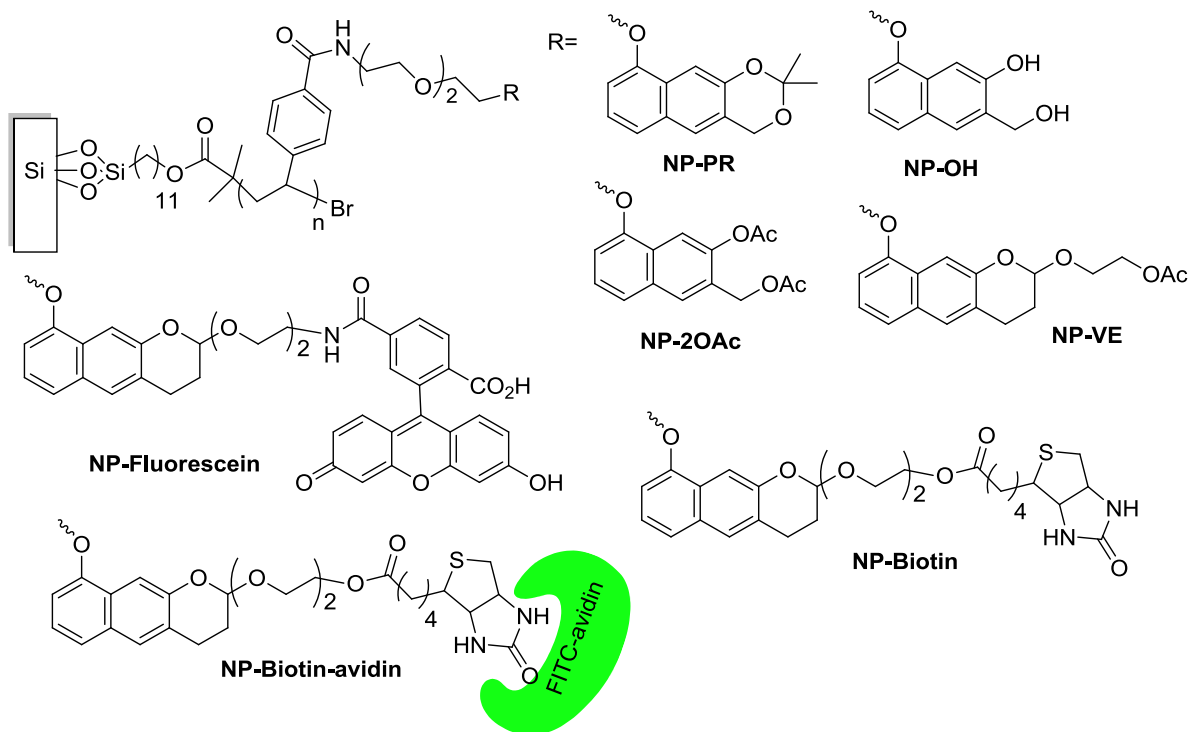


Table S1: Properties of brush polymer films.

Functionality (R)	Thickness / nm	Contact angle / deg
NHS	50	75
NP-PR	89	81
NP-OH	65	55
NP-Fluorescein	153	69
NP-2OAc	87	77
NP-VE	85	75
NP-Biotin	175	78
NP-Biotin-Avidin	185	57

IR Characterization: Chemical composition of the polymer film was analyzed using GATR-FTIR spectroscopy. The quantitative conversion of poly-(NHS4VB) brushes to poly-(NP-PR-4VB) is confirmed by the disappearance of imide and ester C=O stretching vibrations at 1802, 1768, and 1737 cm^{-1} and the formation of amide I and II bands at 1641 and 1538 cm^{-1} . The loss of methyl stretches at 2965 and 2897 cm^{-1} , in addition to the OH stretch observed at 3148 cm^{-1} , confirm deprotection of poly-(NP-PR-4VB) to form poly-(NP-OH-4VB). poly-(NP-OH-4VB) functionalization with a variety of vinyl ether adducts is indicated by absorbances of the attached functional groups: poly-(NP-2OAc-4VB) shows ester bands at 1765 and 1740 cm^{-1} ; poly-(NP-VE-4VB) has ester C=O stretching vibration at 1738 cm^{-1} and C-O-C stretch of cyclic ethers at 1198 cm^{-1} . Functionalization to form poly-(NP-Fluorescein-4VB) is supported by the α -unsaturated ketone at 1713 cm^{-1} and the C=O stretch of the carboxylic acid at 1767 cm^{-1} . Biotin functionalization is confirmed by the increased amide I and II stretching from biotin and the N-C-N stretching at 1347 and 1132 cm^{-1} . Functionalization of poly-(NP-biotin-4VB) with avidin gave very broad amide stretching due to the secondary structure of the protein. Complete assignments of IR spectra are presented in Table S2.

Table S2. List of Important Vibration Modes and Mode Assignments for the GATR-FTIR Spectrum of Surface Attached Poly(NHS4VB) and subsequent derivatives

Surface	Frequency (cm^{-1})	Assignment ^a
poly(NHS4VB)	1802	C=O in phase str of imide
	1768	C=O out of phase str of imide
	1737	C=O str of ester
	1607	p-subst. aromatic
	1258, 1237	Asym. C-O str
	1205	C-N str, Amide III
	1026	Sym. C-O str
	997	Si-O-C str.
NP-PR	2962, 2877	CH_3 str.
	2928, 2858	CH_2 str.
	1641	C=O str, Amide I
	1608, 1574, 1506, 1448	C=C ring str. of arom.

	1541	Amide II, NH bend
	1387,1373	CH ₃ def.
	1280, 1262	C-O-C str alkyl aryl ether
	1200	C-O-C str cyclic ether
	1134	C-O-C str dialkyl ether
	1096, 1053	C-O-C str. alkyl aryl ether
NP-OH	3418	OH str.
	2919, 2852	CH ₂ str.
	1641	Amide I
	1606,1538,1504, 1451	C=C ring str. of arom.
	1538	Amide II
	1383	CH deformation
	1279, 1204	C-O-C str. alkyl aryl ether
	1131	C-O-C str. dialkyl ether
	1056	R-C-O str, alkyl aryl ether
NP-2OAc	2926, 2854	CH ₂ str.
	1766, 1740	C=O str. ester
	1641	C=O, Amide I
	1607,1535,1500, 1446	C=C ring aro str.
	1535	NH bend, amide II
	1370	CH def
	1240	C-O-C str, asymm. Ester
	1201, 1183	C-O-C str. alkyl aryl ether
	1139	C-O-C str. alkyl ether
	1068	R-C-O alkyl aryl ether
NP-VE	2927, 2757	CH ₂ str.
	1738	C=O str. of ester
	1642	C=O str, amide I
	1609,1542, 1502, 1446	C=C ring str. arom.
	1542	NH bend, amide II
	1368	CH deformation

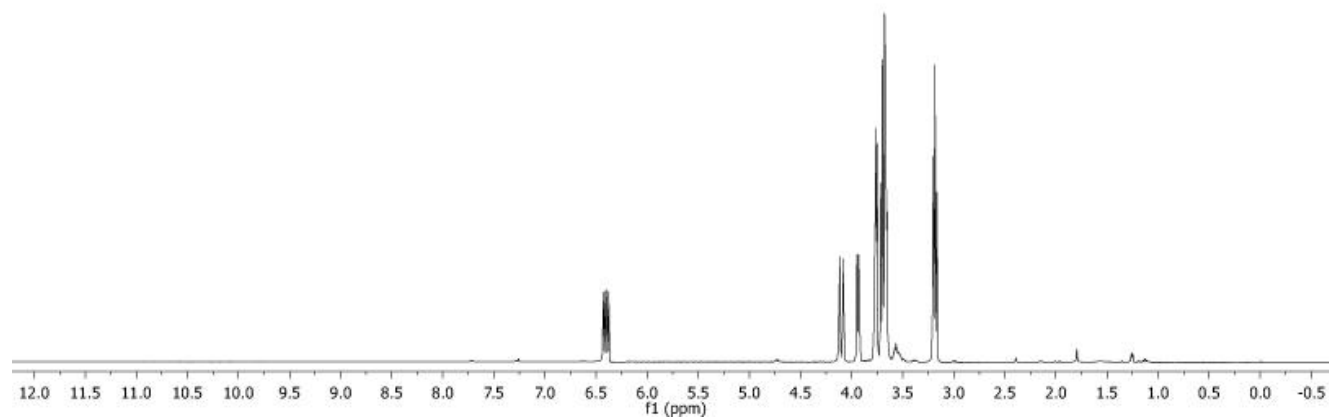
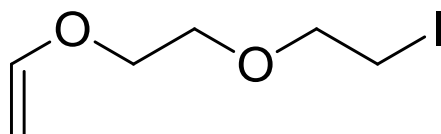
1271	C-O-C alkyl aryl ether
1198	C-O-C str. of cyclic ether
1142, 1107	C-O-C str. dialkyl ethers
1048	R-C-O str. alkyl aryl ethers

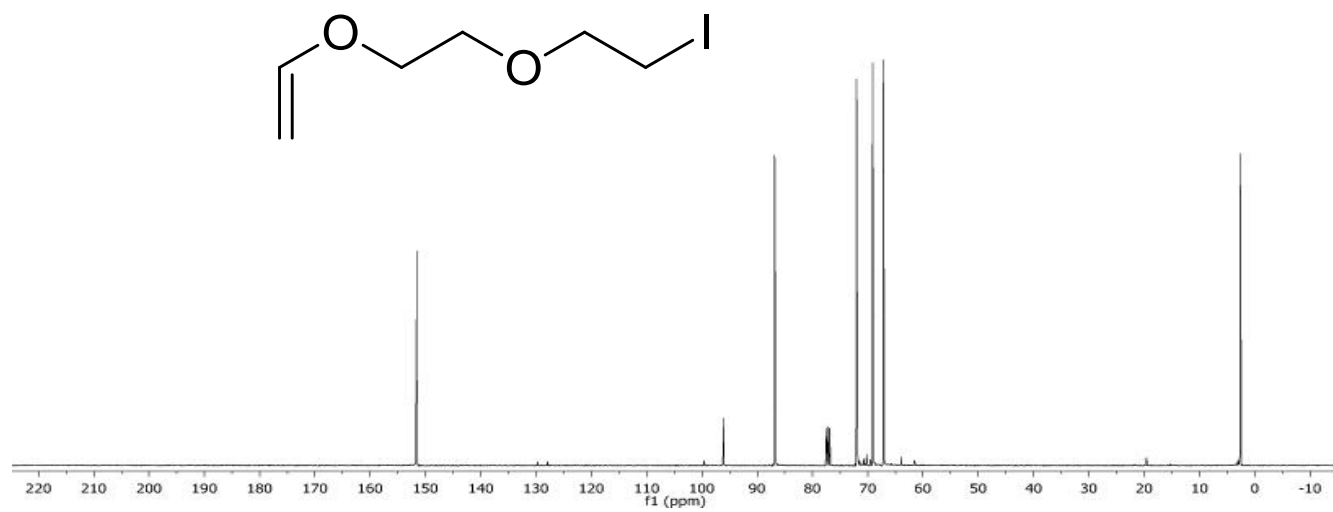
NP-Fluorescein	2924, 2854	CH ₂ str..
	1767	C=O str, COOH
	1713	α unsaturated ketone (fluorescein)
	1657	C=O str, Amide I
	1607, 1565, 1509, 1465	C=C ring str. of arom.
	1565	Amide II, NH bend
	1387.1373	CH ₃ def.
	1270, 1235	C-O-C str alkyl aryl ether
	1142 (br)	C-O-C str cyclic ether, dialkyl ethers
NP-Biotin	2956, 2869	OH str.
	2930, 2852	CH ₂ str.
	1641	C=O str, Amide I and cyclic ureas
	1606, 1549, 1499, 1454	C=C ring str. of arom.
	1549	NH bend, Amide II
	1397	CH ₂ scissor
	1347	N-C-N asym. str. cyclic urea
	1244	C-O-C str. alkyl aryl ether
	1132	N-C-N sym. Str. of cyclic urea.
	1110 (br)	R-C-O str, dialkyl ethers
NP-Biotin-Avidin	3020	Arom. Str.
	2956, 2871	CH ₃ str.
	2925, 2854	CH ₂ str.
	1680-1665 (br)	C=O str. Amide I
	1590-1571 (br)	C=O, NH bend Amide II
	1465	CH ₂ scissor
	1280	NH bend, amide II
	1253	CH def
	1204	C-O-C str
	967	Si-O-C str.

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^1H and ^{13}C NMR Spectra

as-DEg-Ovinyl-I_Proton_20081219_01





ANJ-PROpargyl-vinyl_Proton_20101117_01

