Molecular Nanomachines Disrupt Bacterial Cell Wall Increasing Sensitivity of Extensively Drug Resistant *Klebsiella pneumoniae* to Meropenem

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Supplementary Information

Synthesis of Molecular Machines

The molecular motors 1 and 2 were freshly made, followed previously reported literature.[1,2] The molecular motor 3 is newly-designed, synthesized by the following procedures.

General Methods. All glassware was oven-dried overnight prior to use. Reagent grade dichloromethane (DCM, CH_2Cl_2) was distilled from calcium hydride (CaH_2) under N_2 atmosphere. All reactions were carried out under N_2 atmosphere unless otherwise noted. All other chemicals were purchased from commercial suppliers and used without further purification. Flash column chromatography was performed using 230-400 mesh silica gel from EM Science. Thin layer chromatography (TLC) was performed using glass plates pre-coated with silica gel 40 F_{254} 0.25 mm layer thickness purchased from EM Science. ^1H NMR and ^{13}C NMR spectra were recorded at 400/500 and 100/125 MHz, respectively. Chemical shifts (δ) are reported in ppm from tetramethylsilane (TMS).

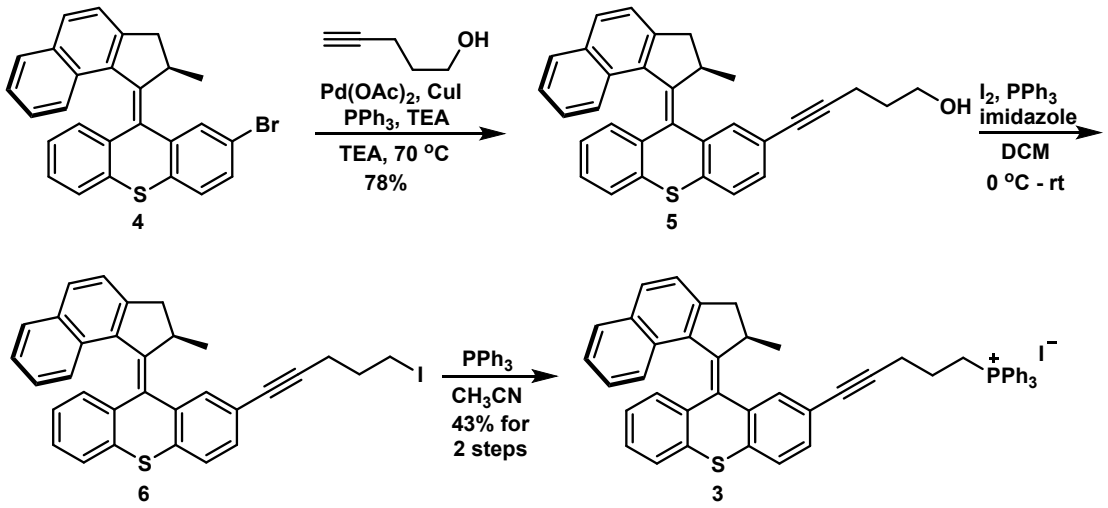
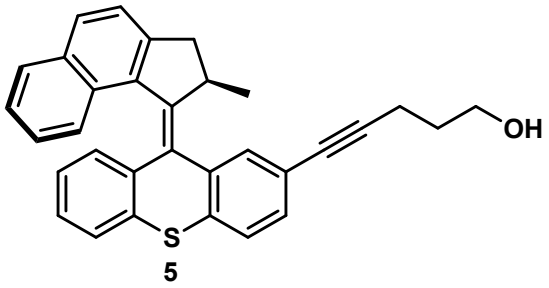


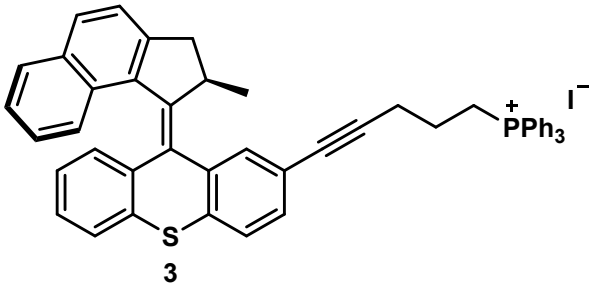
Figure S1. Synthesis of Molecular Nanomolecular Motor 3 (MNM 3).



5

5-(9-(2-methyl-2,3-dihydro-1H-cyclopenta[a]naphthalen-1-ylidene)-9H-thioxanthen-2-yl)pent-4-yn-1-ol (5). An oven dried round-bottom flask equipped with a stir bar was charged with motor **4** (50 mg, 0.11 mmol),[2] palladium(II) acetate (2.5 mg, 0.011 mmol), CuI (2.0 mg, 0.011 mmol), PPh_3 (5.8 mg, 0.022 mmol) and but-4-yn-1-ol (0.03 mL, 0.44 mmol). NEt_3 (3 mL) was added and the mixture was stirred at 70 °C overnight. The resulting mixture was partitioned between DCM (10 mL) and saturated NH_4Cl (aq) (10 mL). The organic layer was dried over anhydrous $MgSO_4$, filtered and the filtrate concentrated *in vacuo*. The resulting concentrate was purified by column chromatography (silica gel; 30% DCM in hexanes) to afford **5** as a pale yellow solid (39.5 mg, 78%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.84-7.67 (m, 3 major isomer protons and 3 minor isomer protons), 7.62-7.55 (m, 1 major isomer proton and 1 minor isomer proton), 7.53-7.40 (m, 2 major isomer protons and 2 minor isomer protons), 7.33 (m, 1 minor isomer proton), 7.24-7.13 (m, 2 major isomer protons and 2 minor isomer protons), 7.04-6.97 (m, 1 major isomer proton and 1 minor isomer proton), 6.86-6.60 (m, 4 major isomer protons and 3 minor isomer protons), 4.33-4.21 (m, 1 major isomer proton and 1 minor isomer proton), 3.85 (t, J = 6.1 Hz, 2 major isomer protons), 3.73-3.60 (m, 1 major isomer proton and 1 minor isomer proton), 3.55 (t, J = 6.1 Hz, 2 minor

isomer protons), 2.68-2.61 (m, 1 major isomer proton and 1 minor isomer proton), 2.59 (t, J = 7.0 Hz, 2 major isomer protons), 2.24 (t, J = 6.8 Hz, 2 minor isomer protons), 1.95-1.85 (m, 2 major isomer protons), 1.66-1.57 (m, 2 minor isomer protons), 0.793 (d, J = 6.8 Hz, 3 major isomer protons), 0.787 (d, J = 6.8 Hz, 3 minor isomer protons). ^{13}C NMR (125 MHz, $CDCl_3$) δ 146.60, 146.48, 146.04, 145.88, 140.08, 140.02, 137.98, 137.67, 135.68, 135.52, 135.23, 135.15, 135.03, 134.90, 133.08, 132.94, 131.67, 130.64, 130.17, 130.13, 129.07, 129.06, 128.86, 128.80, 128.66, 127.87, 127.86, 127.75, 127.74, 127.73, 127.72, 127.70, 127.44, 127.30, 126.48, 126.39, 126.38, 126.12, 126.12, 126.09, 124.84, 124.75, 124.33, 124.15, 123.79, 123.72, 121.82, 121.75, 89.83, 88.88, 81.00, 80.49, 61.88, 61.54, 39.82, 39.68, 37.88, 37.79, 31.39, 30.99, 19.53, 19.52, 16.12, 15.73..



3

(5-(9-(2-methyl-2,3-dihydro-1H-cyclopenta[a]naphthalen-1-ylidene)-9H-thioxanthen-2-yl)pent-4-yn-1-yl)triphenylphosphonium iodide (3). An oven dried round-bottom flask equipped with a stir bar was charged with motor **5** (25 mg, 0.055 mmol), iodine (28 mg, 0.11 mmol) and imidazole (7.5 mg, 0.11 mmol) at 0°C. PPh_3 (28.8 mg, 0.11 mmol) was added slowly at 0°C and the mixture was stirred at room temperature for 30min. Saturated solutions of sodium thiosulfate (10 mL) and sodium

bicarbonate (10 mL) were added to the reaction mixture. The organic phase was separated and the aqueous phase was extracted with methylene chloride. The combined organic layers were washed with brine, dried over MgSO4, and then concentrated in vacuo. The resulting concentrate was purified by flash column chromatography and the resulting iodide product **6** was used for the next step due to its instability.

To a stirred solution of compound **6** in dry acetonitrile (4 mL) at room temperature, PPh_3 (56 mg, 0.22 mmol) was added, and then the mixture was allowed to stir under reflux for 48 h. After TLC analysis indicated the consumption of the starting material, the solvent was subsequently removed under reduced pressure, and the residue was purified by flash chromatography to afford compound **3** as a white solid (19.5 mg, 43% for two steps).

^{1}H, ^{13}C, DEPT-135 ^{13}C, ^{1}H-^{13}C HSQC, and ^{31}P spectra were acquired in order to make the assignments below for **3**.

In the HSQC spectrum, the separation in Hz in the ^{13}C dimension for the contours with the same ^{1}H chemical shift is the $^{n}J_{CP}$ coupling and enables a specific pair of ^{13}C methylene signals in the 1D ^{13}C spectra to be confidently assigned as a $^{n}J_{CP}$ doublet.[3-5] This frequency separation in the ^{13}C dimension is large enough for the $^{1}J_{CP}$ and $^{3}J_{CP}$ couplings for two sets of contours to be readily evident (**Figure S2**) for each of the isomers of **3**. In contrast, the much smaller $^{2}J_{CP}$ couplings result in two contours that severely overlap for each isomer (**Figure S2**) because the FID digital resolution in the ^{13}C dimension, although very high (0.0215 ppm = 2.71 Hz), is not high enough to

resolve the two contours. Thus, for the indicated C≡C-CH_2*CH_2*CH_2-P methylene group, one isomer gives a contour centered at δ_H2.01 / δ_C22.27, and the other isomer gives a contour centered at δ_H1.73 / δ_C21.91.

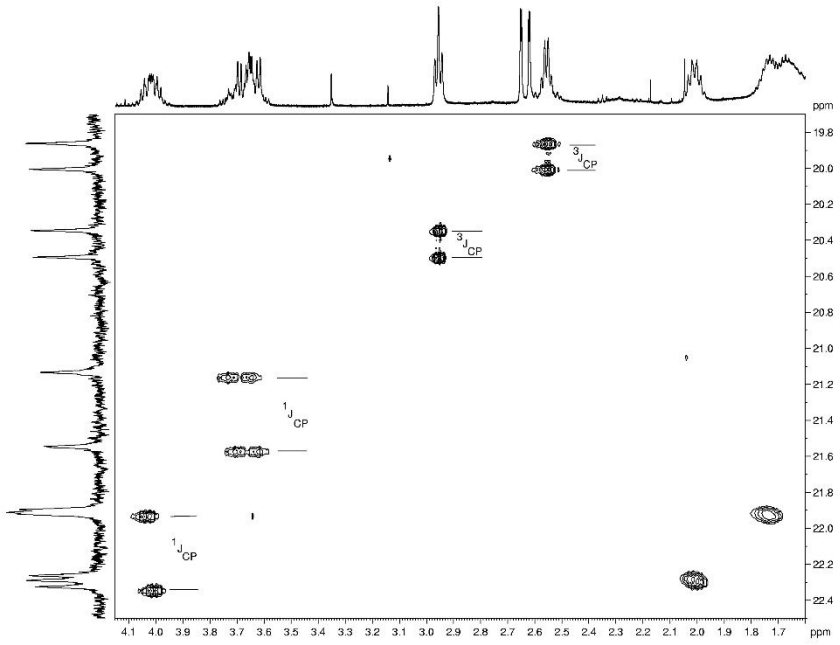


Figure S2. Highly expanded plot of part of the ^{1}H-^{13}C HSQC spectrum of the two isomers of **3** showing the correlations for the C≡C-$CH_2CH_2CH_2$-P moiety. The corresponding regions of the 1D ^{1}H and ^{13}C spectra are shown on top and to the left. The relatively large $^1J_{CP}$ and $^3J_{CP}$ couplings result in clearly visible contours at different ^{13}C frequencies for the ^{1}H-^{13}C correlation of CH_2-P in each isomer and for the ^{1}H-^{13}C correlation of *CH_2*CH_2CH_2-P in each isomer.

Figure S2 clearly indicates that at 11.7 T (500.1 MHz ^{1}H and 125.8 MHz ^{13}C), the downfield component of the $^1J_{CP}$ doublet centered at δ22.118 is just slightly downfield of the $^2J_{CP}$ doublet centered at δ22.27, and the upfield component of the $^1J_{CP}$ doublet centered at 22.118 is within the $^2J_{CP}$ doublet centered at δ21.91. A significantly lower

or significantly higher field strength would be required to completely separate the two components of the $^{1}J_{CP}$ doublet from the two components of each $^{2}J_{CP}$ doublet.

Additional support that the correct pairs of signals have been identified (below) for the various doublets resulting from ^{13}C-^{31}P coupling comes from the ^{13}C satellites in the ^{31}P spectrum (**Figure S3**), which exhibit the same $^{n}J_{CP}$ values (within experimental error) as in the ^{13}C spectrum and negative, one-bond ^{13}C vs. ^{12}C isotope effects on the ^{31}P chemical shift that are clearly larger (in an absolute sense) than the longer range ^{13}C vs. ^{12}C isotope effects on the ^{31}P chemical shift. The ^{13}C satellites are most easily detected for the *ortho* and *meta* carbons of the phenyl groups (6-fold equivalence with 3 equivalent phenyl groups). The ^{13}C satellites for the substituted carbons of the phenyl groups are reduced in intensity by about a factor of two (3-fold symmetry with 3 equivalent phenyl groups). The ^{13}C satellites for the P-$CH_2CH_2CH_2$ groups are the weakest: no symmetry and the signals are also broader.

The ^{13}C chemical shifts and $^{n}J_{CP}$ values for the phenyl groups in the two isomers of **3** are very similar to those reported previously for *n*-propyl(triphenyl)phosphonium and *n*-butyl(triphenyl)phosphonium salts.[6] The $^{1}J_{CP}$ value for the P-CH_2 group in these salts (49.6 and 50.3 Hz)[6] is also very similar to the corresponding $^{1}J_{CP}$ value for the two isomers of **3** (below). Indeed, these salts and both isomers of **3** exhibit very similar values for the $^{1}J_{CP}$, $^{2}J_{CP}$, and $^{3}J_{CP}$ coupling constants for the CH_2 groups. In all cases, $^{1}J_{CP} >> {}^{3}J_{CP} >> {}^{2}J_{CP}$ for the CH_2 groups.

In the HSQC spectrum, the separation in Hz in the ^{1}H dimension between the centers of the contours separated by $^{n}J_{CP}$ in the ^{13}C dimension is $^{n+1}J_{HP}$.[3-5]

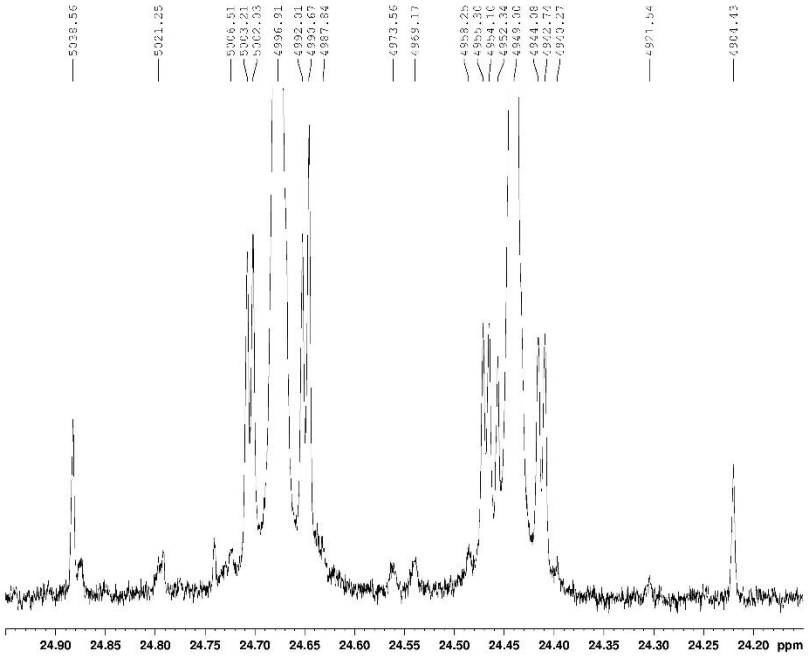


Figure S3. The ^{31}P signals from the two isomers of **3**. The vertical scale has been expanded so as to more clearly reveal the various ^{31}P-^{13}C doublets; their frequencies (in Hz) are displayed. As a result, the intense signals for the all-^{12}C phenyl isotopomers (δ24.6765 and δ24.4399) are cut off. At 11.7 T (202.4 MHz ^{31}P), two satellites clearly overlap (at 4990.67 Hz).

^{1}H NMR (500.1 MHz, $CDCl_3$). δ 7.87-7.63 (complex patterns from numerous overlapping signals, perhaps 34H); 7.592, d (J = 7.8 Hz) of d (J ≈ 1.2 Hz) of d (J ≈ 0.3 Hz), 1H; 7.559, d (J = 7.8 Hz) of d (J = 1.2 Hz) of d (J = 0.5 Hz), 1H; 7.517, d (J = 8.0 Hz) of d (J = 0.5 Hz), 1H; 7.514, d (J = 8.0 Hz) of d (J ≈ 0.4 Hz), 1H; 7.487, d (J = 8.2 Hz), 1H; 7.439, d (J = 8.2 Hz), 1H; 7.37-7.33, highly overlapping signals from 2H; 7.264, d (J ≈ 8.2 Hz) plus many much smaller couplings, apparently 1H, overlapped by signal from residual $CHCl_3$; 7.231, d (J = 7.7 Hz) of d (J = 7.4 Hz) of d (J = 1.4 Hz),

1H; 7.182, d (J = 8.0 Hz) of d (J = 1.7 Hz), 1H; 7.157, d (J = 8.1 Hz) of d (J = 6.6 Hz) of d (J = 1.2 Hz), 1H; 7.03-6.99, highly overlapping signals from 2H; 6.928, d (J = 8.1 Hz) of d (J = 6.2 Hz) of d (J = 1.8 Hz), 1H; 6.83, d ($J \approx 8.5$ Hz) plus many much smaller couplings, 1H; 6.81-6.75, complex pattern from highly overlapping signals, perhaps 3H; 6.73-6.71, highly overlapping signals from 2H; 6.633, d (J = 7.7 Hz) of d (J = 7.4 Hz) of d (J = 1.2 Hz), 1H; 4.270 and 4.242, highly overlapping multiplets from rotor methine in the two isomers, 2H; 4.017, complex multiplet from P-CH_2 in one isomer ($^2J_{HP} \approx 13.0$ Hz and multiple ^{1}H-^{1}H J couplings), 2H; 3.683, complex multiplet from P-CH_2 in other isomer ($^2J_{HP} \approx 13.8$ Hz and multiple ^{1}H-^{1}H J couplings), 2H; 3.676, one rotor CH_2 proton in one isomer, d (J = 15.6 Hz) of d (J = 6.4 Hz), 1H; 3.636, one rotor CH_2 proton in other isomer, d (J = 15.6 Hz) of d (J = 6.0 Hz), 1H; 2.956, -C≡C-CH_2- in one isomer, t ($^3J_{HH}$ = 6.5 Hz) of d ($^4J_{HP} \approx 1$ Hz), 2H; 2.637, other rotor CH_2 proton in one isomer, d (J = 15.6 Hz), 1H; 2.633, other rotor CH_2 proton in other isomer, d (J = 15.6 Hz), 1H; 2.556, complex multiplet from -C≡C-CH_2- in other isomer (^{1}H-^{1}H J couplings and $^4J_{HP} \approx 1$ Hz), 2H; 2.010, complex multiplet from -C≡C-$CH_2C\mathit{H}_2CH_2$-P in one isomer (^{1}H-^{1}H J couplings and $^3J_{HP}$), 2H; 1.731, complex multiplet from -C≡C-$CH_2C\mathit{H}_2CH_2$-P in other isomer (^{1}H-^{1}H J couplings and $^3J_{HP}$), 2H; 0.778, CH_3 protons in one isomer, d (J = 6.8 Hz), 3H; 0.768, CH_3 protons in other isomer, d (J = 6.8 Hz), 3H.

^{13}C NMR (125.8 MHz, $CDCl_3$). 46 of 48 non-phenyl sp^2 signals are clearly visible: δ 146.7899 (quaternary), 146.7441 (quaternary), 146.2124 (quaternary), 146.0556 (quaternary), 140.2517 (quaternary), 139.9897 (quaternary), 138.0868 (quaternary),

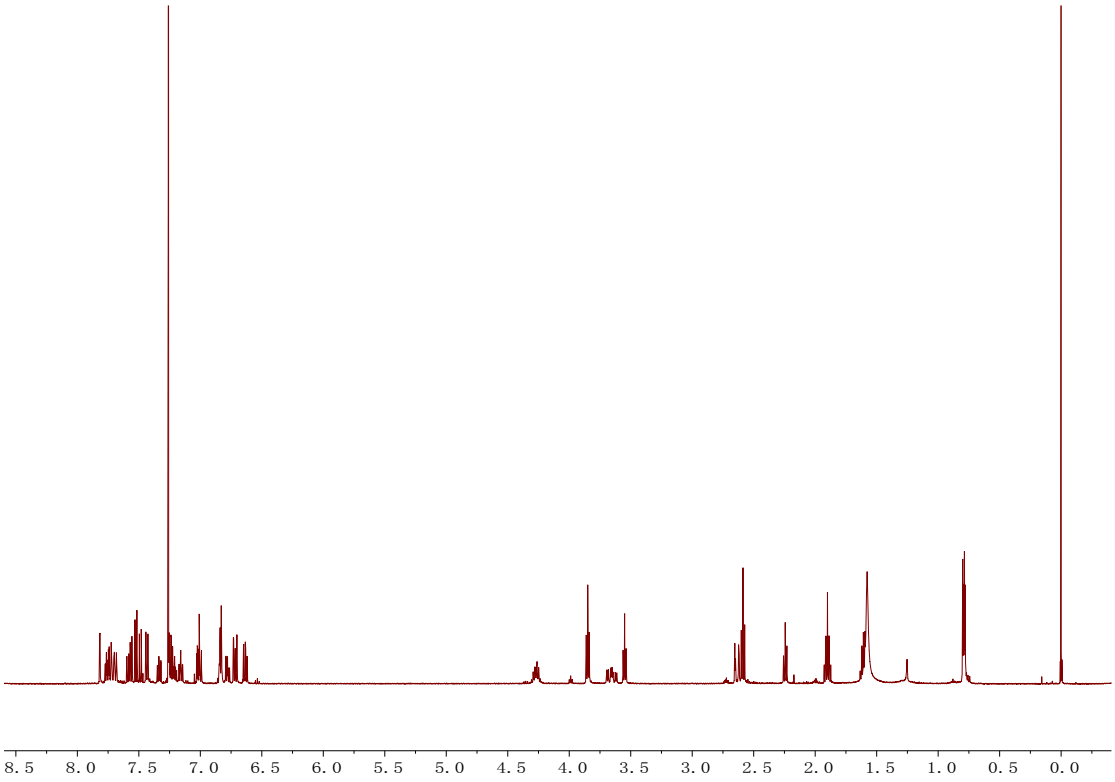
137.5957 (quaternary), 136.0726 (quaternary), 135.7561 (quaternary), 135.4097 (quaternary), 135.136 (CH, $^{4}J_{CP} \approx 2.8$ Hz) in one isomer, 135.113 (CH, $^{4}J_{CP} \approx 2.8$ Hz) in other isomer, 134.9000 (quaternary), 134.6666 (quaternary), 133.7563 (CH, $^{2}J_{CP} = 10.0$ Hz) in one isomer, 133.7273 (CH, $^{2}J_{CP} = 10.0$ Hz) in other isomer, 132.9394 (quaternary), 132.9002 (quaternary), 131.3830 (CH), 130.8797 (CH), 130.5495 (CH, $^{3}J_{CP} = 12.6$ Hz) in one isomer, 130.5071 (CH, $^{3}J_{CP} = 12.6$ Hz) in other isomer, 130.3669 (CH), 130.2294 (CH), 129.3388 (CH), 128.8748 (CH), 128.8192 (quaternary), 128.6863 (CH), 128.6682 (quaternary), 127.8157 (CH), 127.7964 (CH), 127.7453 (CH), 127.6965 (CH), 127.6576 (CH), 127.5630 (quaternary), 127.4801 (CH), 127.4491 (CH), 126.5724 (CH), 126.4403 (CH), 126.4123 (CH), 126.1809 (CH), 126.048 (CH), 126.039 (CH), 124.772 (CH), 124.757 (CH), 124.3036 (CH), 124.1440 (CH), 123.8462 (CH), 123.7571 (CH), 121.3505 (quaternary), 121.2021 (quaternary), 118.0423 (quaternary, $^{1}J_{CP} = 86.2$ Hz) in one isomer, 117.9461 (quaternary, $^{1}J_{CP} = 86.1$ Hz) in other isomer; 88.9455 (alkyne), 87.7646 (alkyne), 82.1226 (alkyne), 81.6068 (alkyne), 39.8679 (rotor CH_2 in one isomer), 39.6997 (rotor CH_2 in other isomer), 37.8823 (rotor CH in one isomer), 37.8308 (rotor CH in other isomer), 22.2744 ($^{2}J_{CP} \approx 3.5$ Hz) -*C*$H_2$$CH_2$-P in one isomer, 22.1177 ($^{1}J_{CP} = 51.7$ Hz) -CH_2-P in one isomer, 21.9081 ($^{2}J_{CP} \approx 3.1$ Hz) -*C*$H_2$$CH_2$-P in other isomer, 21.3402 ($^{1}J_{CP} = 52.0$ Hz) -CH_2-P in other isomer, 20.4197 ($^{3}J_{CP} = 18.8$ Hz) -*C*$H_2$$CH_2$$CH_2$-P in one isomer, 19.9323 ($^{3}J_{CP} = 18.0$ Hz) -*C*$H_2$$CH_2$$CH_2$-P in other isomer, 19.5120 (CH_3) in one isomer, 19.4723 (CH_3) in other isomer.

^{1}H-^{13}C HSQC correlations (aliphatic region only): δ_H4.270 / δ_C37.8308 (rotor CH in one isomer), δ_H4.242 / δ_C37.8823 (rotor CH in other isomer); δ_H4.017 / δ_C22.1177 (-CH_2-P in one isomer), δ_H3.683 / δ_C21.3402 (-CH_2-P in other isomer); δ_H3.676 and δ_H2.637 / δ_C39.8679 (rotor CH_2 in one isomer), δ_H3.636 and δ_H2.633 / δ_C39.6997 (rotor CH_2 in other isomer); δ_H2.956 / δ_C20.4197 (-C≡C-CH_2- in one isomer), δ_H2.556 / δ_C19.9323 (-C≡C-CH_2- in other isomer); δ_H2.010 / δ_C22.2744 (-C≡C-CH_2*CH_2*CH_2-P in one isomer), δ_H1.731 / δ_C21.9081 (-C≡C-CH_2*CH_2*CH_2-P in other isomer); δ_H0.778 / δ_C19.5120 (CH_3 in one isomer), δ_H0.768 / δ_C19.4723 (CH_3 in other isomer).

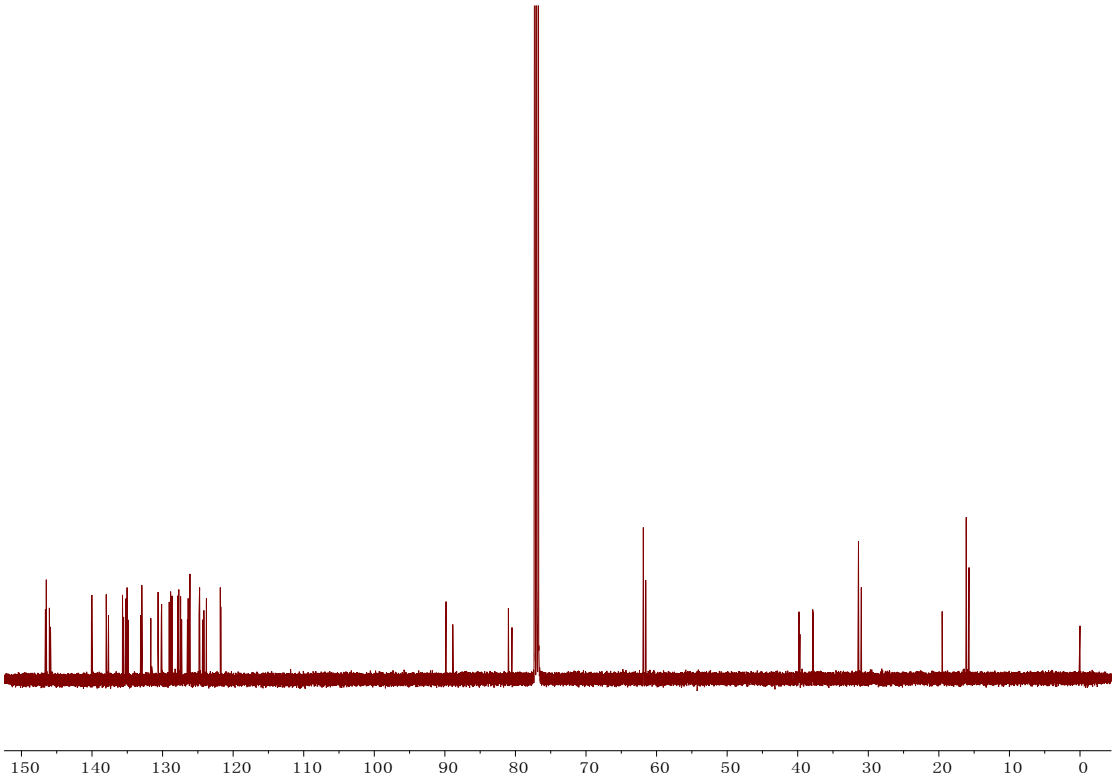
^{31}P (202.4 MHz, $CDCl_3$). δ24.6765 (one isomer) with associated coupling constants and isotope effects for the phenyl group: $^{1}J_{CP}$ = 86.2 Hz, $^{1}\Delta P(^{13/12}C)$ = -0.0072 ppm; $^{2}J_{CP}$ = 10.0 Hz, $^{2}\Delta P(^{13/12}C)$ < 0.001 ppm; $^{3}J_{CP}$ ≈ 12.5 Hz (two satellites overlap), $^{3}\Delta P(^{13/12}C)$ not measurable because two satellites overlap; $^{4}J_{CP}$ obscured by other signals; associated coupling constants and isotope effects for the P-$CH_2CH_2CH_2$ group: for P-CH_2: $^{1}J_{CP}$ = 52.1 Hz, $^{1}\Delta P(^{13/12}C)$ = -0.0084 ppm; for P-CH_2CH_2: $^{2}J_{CP}$ obscured by other signals; for P-$CH_2CH_2CH_2$: $^{3}J_{CP}$ = 18.7 Hz, $^{3}\Delta P(^{13/12}C)$ ≈ 0.001 ppm. δ24.4399 (other isomer) with associated coupling constants and isotope effects for the phenyl group: $^{1}J_{CP}$ ≈ 86.2 Hz (two satellites overlap), $^{1}\Delta P(^{13/12}C)$ ≈ -0.007 ppm; $^{2}J_{CP}$ = 10.0 Hz, $^{2}\Delta P(^{13/12}C)$ < 0.001 ppm; $^{3}J_{CP}$ = 12.6 Hz, $^{3}\Delta P(^{13/12}C)$ < 0.001 ppm; $^{4}J_{CP}$ obscured by other signals; associated coupling constants and isotope effects for the P-$CH_2CH_2CH_2$ group: for P-CH_2: $^{1}J_{CP}$ = 52.0 Hz, $^{1}\Delta P(^{13/12}C)$ = -0.0072 ppm; for P-CH_2CH_2: $^{2}J_{CP}$ obscured by other signals; for P-$CH_2CH_2CH_2$: $^{3}J_{CP}$ = 18.0 Hz, $^{3}\Delta P(^{13/12}C)$ ≈ 0.001 ppm.

Assigning ^{1}H, ^{13}C, and ^{31}P chemical shifts to specific sites in a specific isomer will require additional 2D NMR experiments, which are planned.

^{1}H NMR of **5**



^{13}C NMR of **5**



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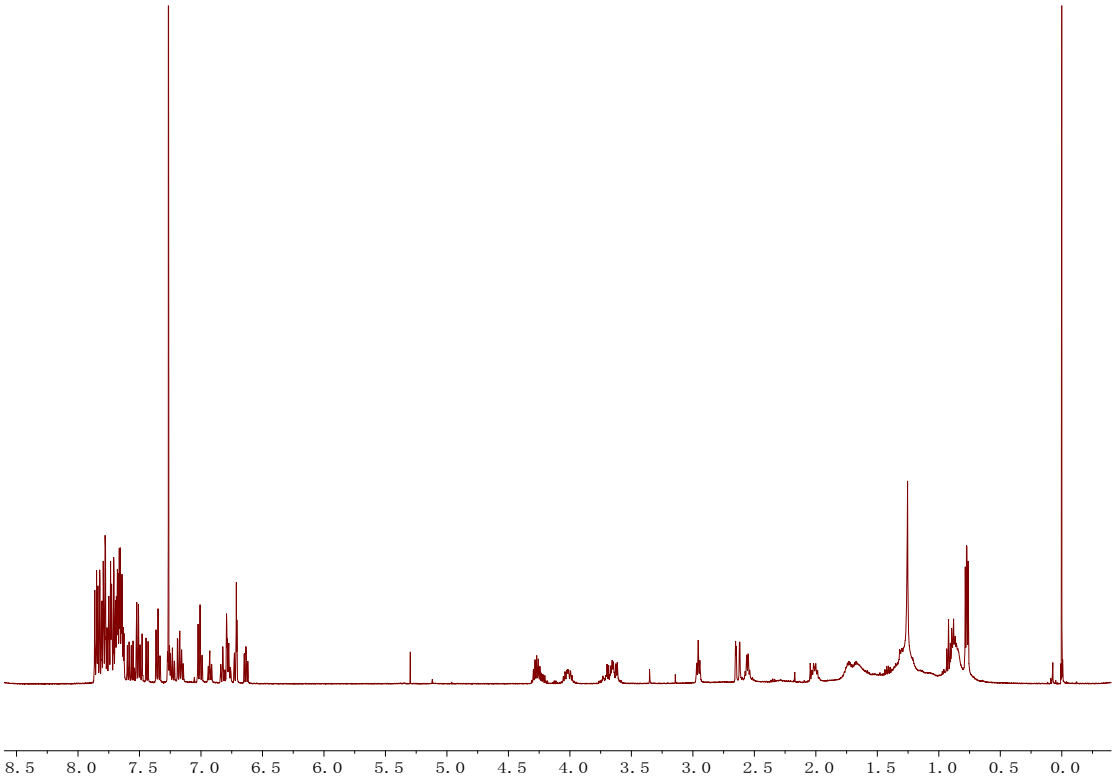

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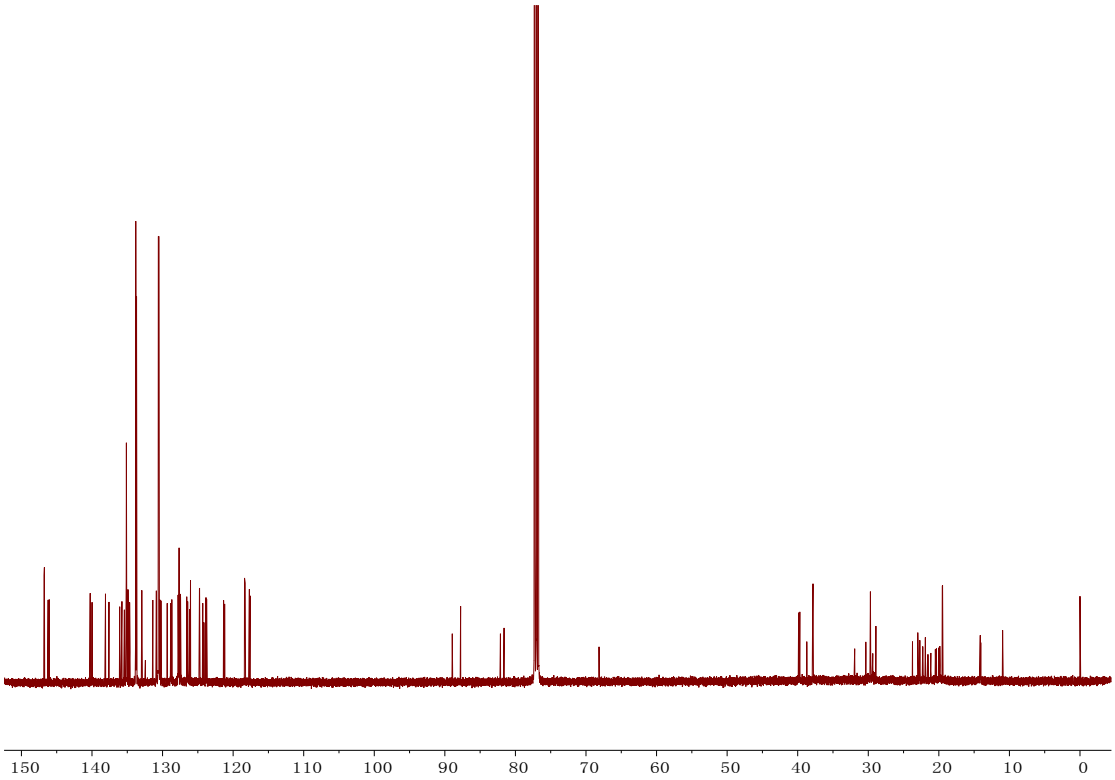
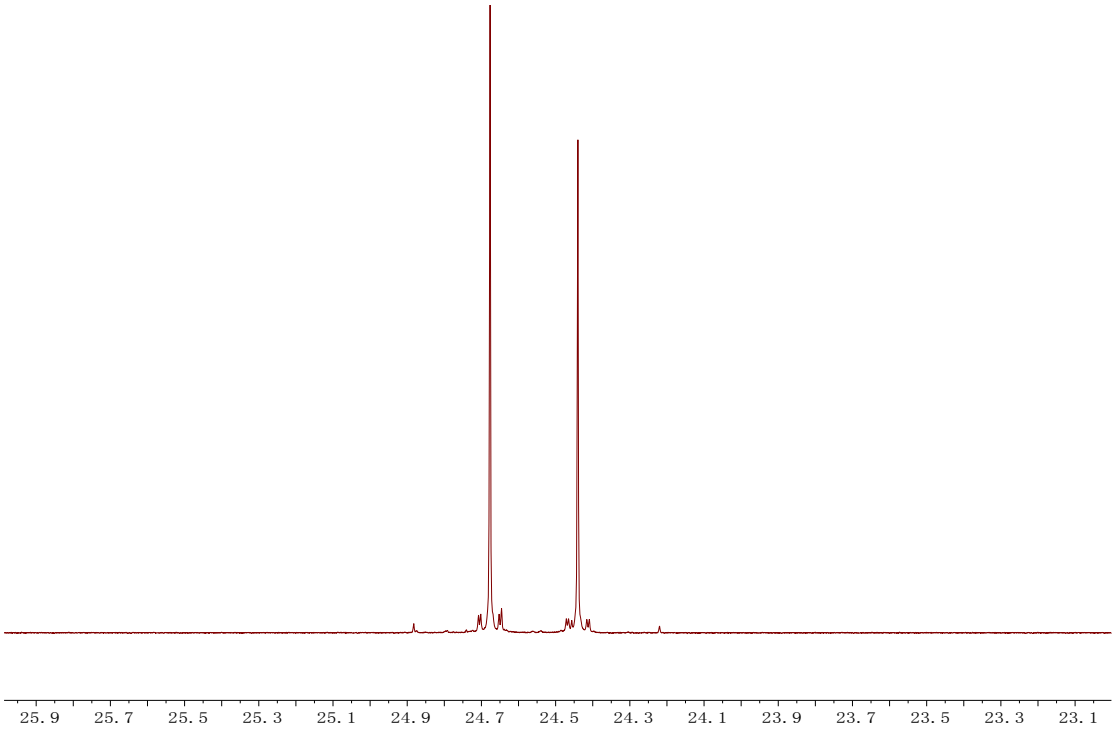


^{31}P of **3**



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