Supporting Information

for

Regioselective Covalent Immobilization of Catalytically Active Glutathione S-Transferase on Glass Slides

Rajesh Viswanathan,[‡] Guillermo R. Labadie,[¶] and C. Dale Poulter[†]*

[†]315 South, 1400 East RM 2020; Department of Chemistry; University of Utah, Salt Lake City, UT, 84112.

[‡]Current address: Department of Chemistry; Case Western Reserve University; 10900 Euclid Ave.; Cleveland, OH 44106.

[¶]Current address: Instituto de Química Rosario (IQUIR-CONICET); Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario, Suipacha 531, 2000; Rosario, Santa Fe; Argentina.

*Corresponding author: e-mail: poulter@chemistry.utah.edu.

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General Experimental Section

Adhesive-coated silicone mats were purchased from Grace Biolabs. Tris(2-carboxyethyl)phosphine hydrochloride (TCEP•HCl) was purchased from Aldrich Chemical Co. and used as received. Glutathione (GT) and 1-chloro 2,4-dinitro benzene (CDNB) were purchased from Sigma. BlockIT solution was purchased from ArrayIT and was diluted according to manufacturers' recommendations. Anti-GST-AlexaFluor conjugate was purchased from Molecular Probes.

Protein concentrations were measured by Bradford and BCA methods.[1] In addition to these estimations measurement of UV absorbance at 280 nm was performed to verify protein concentration. UV measurements were performed using an Agilent UV-Vis 845X equipped with chemstation software. Expression and purification of yeast protein farnesyltransferase (PFTase) was performed according to previously reported procedures.[2] All protein purification buffers were based on Qiagen's recommended protocols for recombinant protein purification.

Mono BOC-protected hexyl diamine, HOBt, Hunig's base, EDAC, trifluoro acetic acid (TFA), tetraethyleneglycol, triphenylphosphine, diisopropylazodicarboxylate (DIAD), hydrazine hydrate and disuccinimidyl dicarbonate (DSC) were purchased from Aldrich Chemical Co. and were used without further purification. All organic reactions involving air/moisture sensitive materials were conducted under nitrogen. Commercial reagents and solvents were analytical grade or purified by standard procedures prior to use.[3] NaH was dried *in vacuo* after several washes with pentane under inert atmosphere to yield a grey powder. This was sufficiently reactive for alkylation reactions. All organic

solvents were dried and purified prior to use as described in literature.[4] TLC analyses for non-diphosphate compounds were performed on glass-backed plates coated with 0.1 mm Merck silica gel 60 GF254. TLC plates were visualized by UV, iodine, 10% ammonium molybdate solution in ethanol or *p*-anisaldehyde with heating. TLC analyses for diphosphates were carried out on cellulose F 0.1 mm plastic sheets available from EM separations. Cellulose TLC plates were visualized by sulfosalicylic acid-ferric chloride combination using a manual spray. Flash chromatography was carried out on silica gel grade 60, 230-400 mesh (Merck) with elution by EtOAc/hexanes unless otherwise specified. Cellulose column chromatography was performed on Whatman CF-11 fibrous cellulose. NMR spectra were recorded on a Varian Unity Inova FT NMR controlled by a Sun Sparcstation at 300 MHz (^{1}H NMR), 75 MHz (^{13}C NMR) or 121 MHz (^{31}P NMR). Chemical shifts are reported in parts per million (ppm) and coupling constants (*J*) in hertz (Hz). ^{1}H NMR spectra are referenced to residual C*H*Cl$_3$ (δ 7.27 ppm) in CDCl$_3$ or the *H*OD resonance (δ 4.80 ppm) in D$_2$O, ^{13}C NMR are referenced to *C*DCl$_3$ (δ 77.16 ppm) or external *C*H$_3$OH (δ 49.5 ppm) in D$_2$O.[5] ^{31}P NMR is referenced to external phosphoric acid (δ 0.00 ppm) in D$_2$O.

Cloning and Expression of His_6-GSTase-CVIA

The open reading frame of *Schistosoma japonicum* glutathione S-transferase (GSTase) was cloned into pQE-30Xa.[6] The resulting construct pQE-His_6-GSTase-CVIA encodes for GSTase with an N-terminal His_6 tag and a C-terminal CVIA recognition motifs. The amino acid sequence of His_6-GSTase-CVIA is shown below. Hexahistidine motif and CaaX recognition motifs are shown in italics and underscore respectively.

MRGS*HHHHHH*GSGSGSGIEG RPYNGTGSMSPILGYWKIKGLVQPTRLLLE
YLEEKYEEHLYERDEGDKWRNKKFELGLEFPNLPYYIDGDVKLTQSMAII
RYIADKHNML GGCPKERAEI SMLEGAVLDI RYGVSRIAYS KDFETLKVDF
LSKLPEMLKMFEDRLCHKTYLNGDHVTHPDFMLYDALDVVLYMDPMCLDA
FPKLVCFKKRIEAIPQIDKYLKSSKYIAWPLQGWQATFGGGDHPPKSDRTRCVIA.

Plasmid pQE-His_6-GSTase-CVIA encoding for the above recombinant protein was transformed into *E. coli* M15[pREP4]. The resulting cellular composite is referred to as M15-pQE(His_6-GSTase-CVIA). Then, expression and purification of recombinant GSTase was carried out.[6] The quality of purified GSTase-CVIA was checked by SDS-PAGE analysis in addition to ESI-MS analysis (see Figure S1). Protein immobilization was carried out first with purified His_6-GSTase-CVIA, followed by corresponding immobilizations on cell-free homogenates of M15-pQE(His_6-GSTase-CVIA).

SDS-PAGE Analysis of Purified His_6-GSTase-CVIA

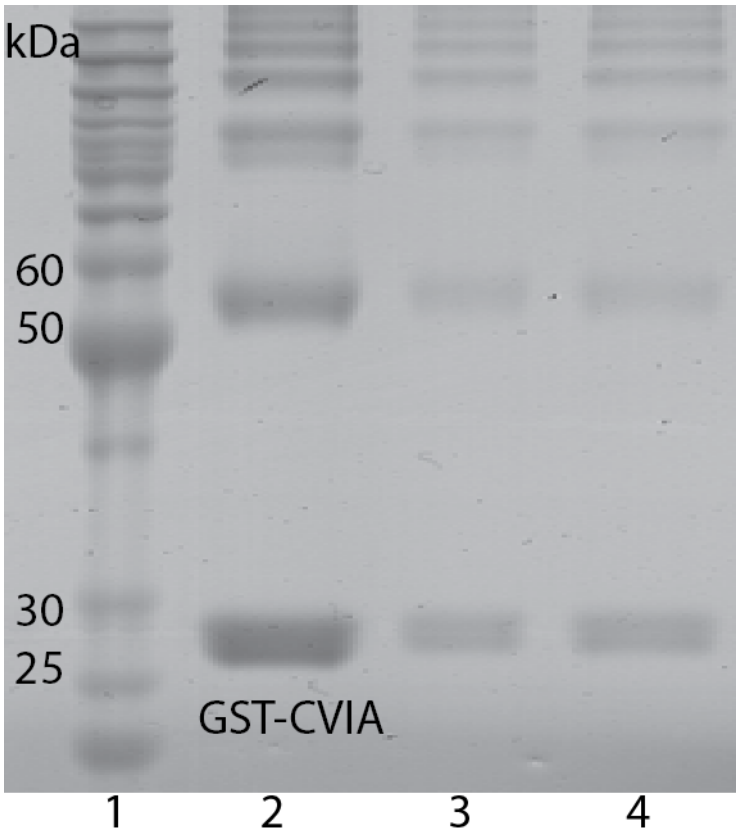


Figure S1. SDS-PAGE analysis of His_6-GSTase-CVIA expressed after transformation with pQE-His_6-GSTase-CVIA followed by IPTG induction in *E. coli* M15[pREP4] cells. Benchmark MW ladder (1); Lanes 2, 3 and 4 – collected elute from Ni-NTA purification. Note: *higher molecular weight peaks are observed even after protein purification due to the presence of dimeric and higher order multimeric complexes of* His_6-GSTase-CVIA. Also, a truncated form of His_6-GSTase-CVIA is observed as a co-eluting band. ESI-MS analysis suggests the presence of this truncated protein in all farnesylation experiments performed in this study.

General Procedure for PFTase-Catalyzed Prenylation of His_6-GSTase-CVIA

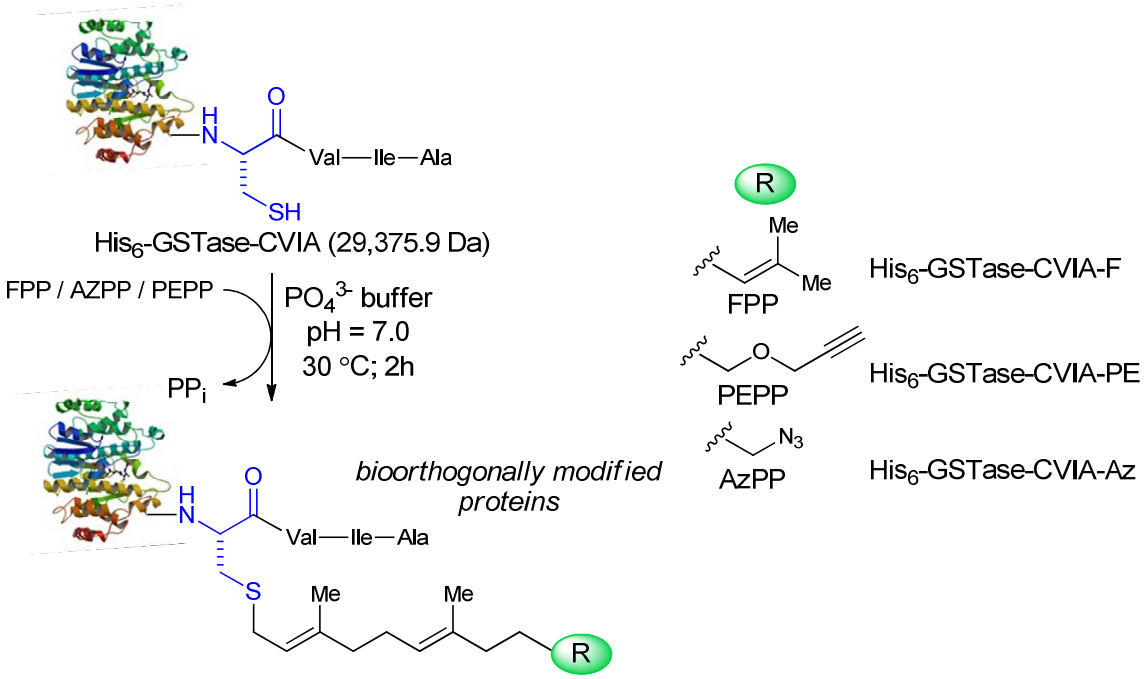
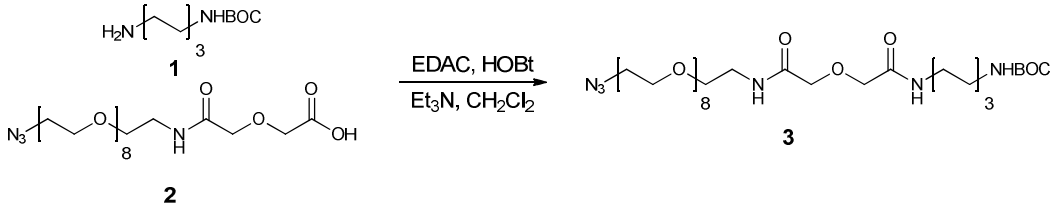


Figure S2. PFTase-catalyzed prenylation of His_6-GSTase-CVIA with FPP and its analogues.

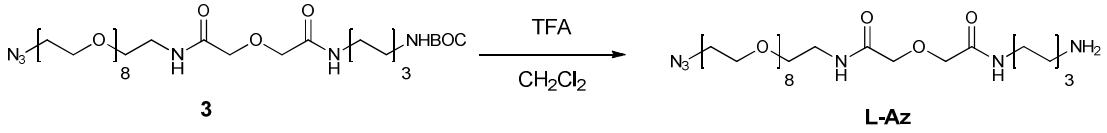
Prenylation of purified His_6-GSTase-CVIA. Figure S2 provides a schematic summary of PFTase-catalyzed prenylations. Recombinantly constructed pQE-His_6-GSTase-CVIA was expressed under IPTG induction in *E. coli* M15-pQE(His_6-GSTase-CVIA) cells and, following cell lysis in the presence of protease inhibitor cocktail, GSTase was purified to homogeneity using a Ni-NTA matrix. Farnesyl diphosphate or its bioorthogonal analogue (35 μM) and purified His_6-GSTase-CVIA (35 μM) were incubated with 12 nM yeast protein farnesyltransferase at 30 °C for 3 h in 5.0 mL of farnesylation buffer (50 mM sodium phosphate, pH 7.0, containing 10 mM $MgCl_2$, 10 μM $ZnCl_2$ and 5 mM β-

mercapto ethanol (BME)). Following this incubation, the mixture was spun at 8000 rpm at 4 °C in an Amicon Ultra®-4 mL microconcentrator (10 kDa cutoff). The protein concentration in the sample was measured by UV absorbance at 280 nm and by Bradford analysis.[1] Protein samples were then analyzed by high resolution ESI-MS. Modified His_6-GSTase-CVIAs were flash frozen with liquid N_2 and stored in 50 μL aliquots at -80 °C until further use.

Sequential synthesis of L-Az

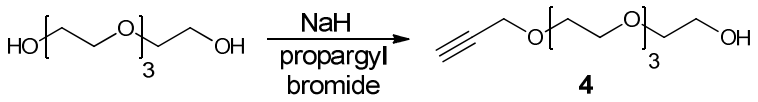


To mono BOC-protected hexyl diamine **1** (163 mg, .645 mmol) in CH_2Cl_2 was added 103 mg (0.763 mmol) of HOBt and 2.93 g of Hunig's base. This mixture was cooled to 0 °C before addition of 325 mg (0.587 mmol) of azido-PEG-acid **2**. After stirring for 15 min, 146 mg (0.763 mmol) of EDAC was added and the mixture was stirred overnight. Then CH_2Cl_2 was added and the organic layer was washed twice with brine, dried and concentrated in vacuo to yield a yellow oil. Purification by flash chromatography over Silica (0 to 20% MeOH/EtOAC) gave 411 mg (94 %) of a colorless oil.

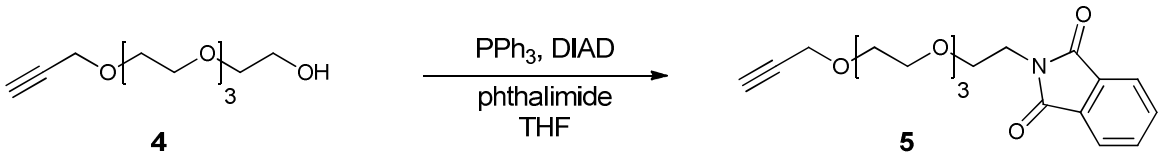


(**L-Az**) The azido Boc-protected amine **3** (74 mg, 98 μmol) obtained from the previous step was dissolved in CH_2Cl_2 and treated with 73 μL (980 μmol) of trifluoroacetic acid (TFA). The mixture was stirred for 16 h at room temperature. Next, solvent was removed in vacuo and 5 mL of 1M HCl was added. The aqueous mixture was extracted twice with EtOAc and lyophilized to yield **L-Az** in quantitative yield as a white powder.

Sequential Synthesis of L-PE



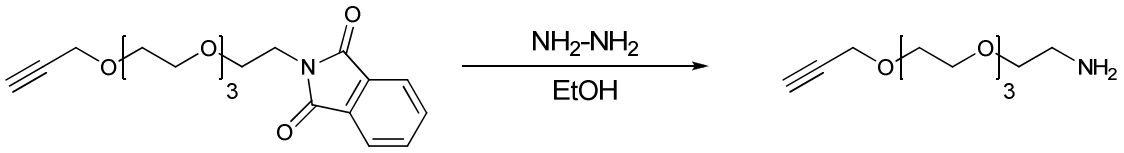
(**monopropargyl-tetratehyleneglycol**) Tetraethyleneglycol (5.00 g, 25.8 mmol) was dissolved in 50 mL of THF. The solution was cooled to 0 °C and 639 mg (25.7 mmol) of NaH was added slowly with vigorous stirring. After 15 min, 1.83 mL (20.53 mmol) of propargylbromide were added over 4 h using a syringe pump. The reaction was allowed to warm to room temperature and stirring was continued overnight. THF was removed in vacuo and 100 mL water was added to the residue. This mixture was washed with EtOAc. The organic layers were combined and dried. Solvent was removed in vacuo and the residue was chromatographed on silica gel (30% EtOAc/Hexanes to 100% EtOAc) to give 2.84 g (60 %) of a colorless oil.



(**N-phthalimido-monopropargyl-tetraethyleneglycol**) Monopropargyl-tetraethyleneglycol obtained from the previous step (2.63 g, 11.35 mmol) was dissolved in 75 mL of THF. Triphenylphosphine (2.68 g, 10.2 mmol) and phthalimide (1.5 g, 10.2 mmol) were added to this solution. The solution was cooled to 0 °C before (2.02 mL, 10.2 mmol) of DIAD was added dropwise. The mixture was allowed to be stirred

overnight before solvent was removed in vacuo. Following dilution with CH_2Cl_2, the organic layer was washed twice with brine, dried and concentrated in vacuo. The residue was chromatographed on silica gel (25% EtOAc/Hexanes to 100% EtOAc then to 20% MeOH/Hexanes) to give 2.12 g (53 %) of a colorless oil. The remaining 20% of starting pegylated alkyne was recovered.

Analytical data: ^{1}H NMR ($CDCl_3$, δ): 7.74-7.69 (m, 2H, ArCH); 7.63-7.59 (m, 2H, ArCH); 4.06 (d, *J* = 6.0 Hz, 2H, OC*H*$_2$CCH); 3.78 (t, *J* = 6.0 Hz, 2H, OC*H*$_2$CH$_2$); 3.63 (t, *J* = 6.0 Hz, 2H, C*H*$_2$Nphth); 3.58-3.45 (m, 14H, OC*H*$_2$C*H*$_2$O); 2.38-2.37 (m, 1H, CC*H*); ^{13}C NMR ($CDCl_3$, δ): 168.1, 133.9, 132.0, 123.1, 79.6, 74.6, 70.5 (3C), 70.3, 69.9 (2C), 67.8, 58.3, 37.2; IR (cm^{-1}, neat): 2867, 2113, 1772, 1716, 1616. HRMS (MALDI, $[M+H]^+$) Calcd for $C_{19}H_{24}O_6N$: 362.1604; found: 362.1589.



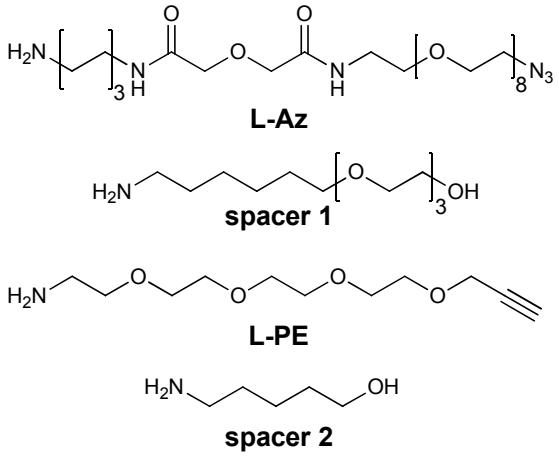
[**L-PE**] Phthalimido-monopropargyl-tetraethyleneglycol **5** obtained from the previous step (528 mg, 1.46 mmol) was dissolved in 20 mL of EtOH. Hydrazine hydrate (330 mL) was added and the solution was heated at reflux for 8 h, during which time a white gelatinous precipitate formed. One mL of 3N HCl was added to dissolve the precipitate. The mixture was lyophilized and the residue was purified by chromatography on silica gel (20 % MeOH/CH_2Cl_2) to give 280 mg (83 %) of a colorless oil.

Analytical data: ^{1}H NMR ($CDCl_3$, δ): 4.16 (d, *J* = 2.4 Hz, 2H, OC*H*$_2$CCH); 3.65-3.58 (m, 14H, OC*H*$_2$C*H*$_2$O); 3.46 (t, *J* = 5.1 Hz, 2H, OC*H*$_2$CH$_2$); 2.81 (t, *J* = 5.4 Hz, 2H, C*H*$_2$NH$_2$); 2.40 (s, 1H, CC*H*) 1.58 (br, s, 2H, N*H*$_2$); ^{13}C NMR ($CDCl_3$, δ): 79.7, 74.7, 73.6, 70.7,

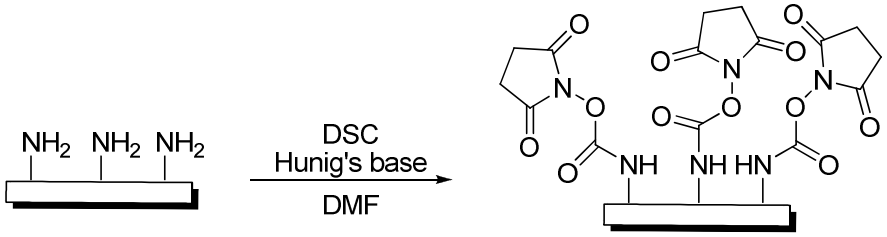
70.69, 70.5, 70.4, 69.2, 58.5, 58.48, 41.9; IR (cm^{-1}, neat): 3247, 2868, 2112. HRMS (MALDI, $[M+H]^+$) Calcd for $C_{19}H_{24}O_6N$: 362.1604; found: 362.1589.

Glass Surface Derivatization

For ease of understanding, the structures of the four linkers used for glass surface derivatization are provided here:



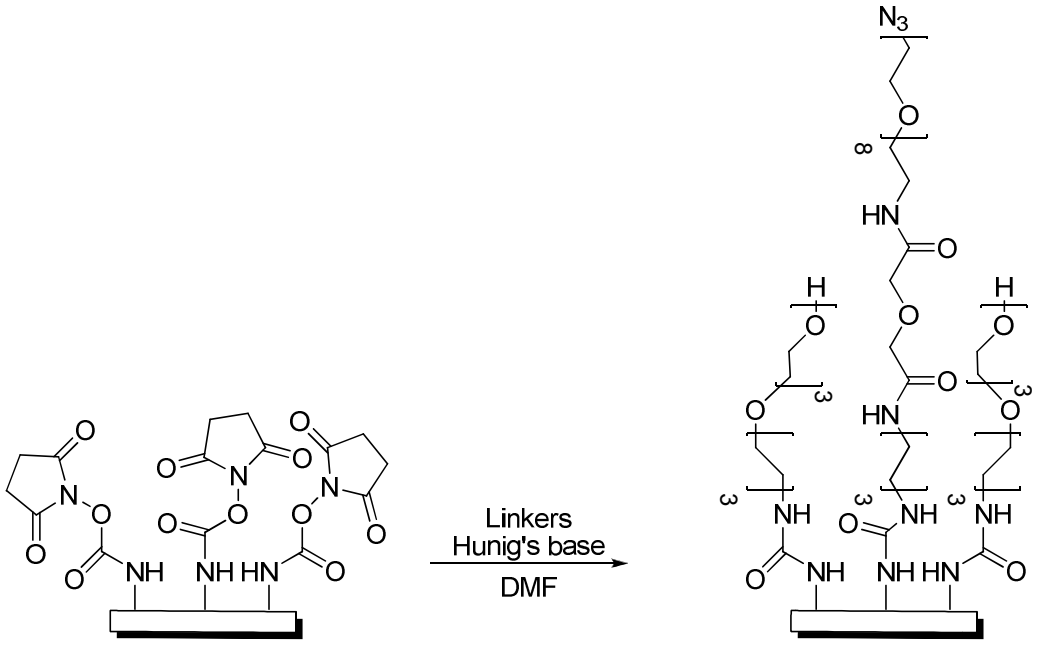
Activation of amine-coated glass surface.



Amine derivatized glass slides were purchased from ARRAYIT (www.arrayit.com). According to the manufacturer, the amino group density of the slide is 5 x10^{12} units per mm^2. Disuccinimidyl dicarbonate (DSC) (17.2 mg, 0.067 mmol) was dissolved in 1.8 mL of DMF and Hunig's base (0.202 mL, 1.16 mmol) was added. This solution was mixed until all DSC dissolved completely and the solution was then added to a chamber containing a Fisher microscope glass slide with the amine-coated surface facing up under

N_2 atmosphere. The chamber was sealed and shaken at 75 rpm overnight. Following this incubation, the slide surface was rinsed with five 3 mL portions of DMF over 1 h in periodic 15 -20 minute intervals.

Preparation of azide-coated glass surface.



To 1.05 mL of DMF was added 4.65 µL of a 10.9 mM stock solution of linker **L-Az** (50.6 nmole) and 48.6 µL of a 21 mM stock solution of **spacer 1** (1.02 µmol), followed by 9.3 µL of Hunig's base. The resulting mixture was added to the chamber containing the activated slide. The slide chamber was sealed under N_2 and shaken overnight at room temperature. The slide was rinsed several times with DMF (~3.0 mL per rinse) and treated with a pre-mixed solution of 5 µL of ethanolamine in 1.5 mL of DMF. This mixture was kept under shaking for 4 h at ambient temperature. Finally, all the slides were rinsed several times with DMF (~3.0 mL per rinse) followed by isopropyl alcohol

(~5 mL per rinse). Derivatized slides were immersed in 2 mL of 3:1 H_2O:glycerol and stored at 4 °C for further use. Prior to use in protein immobilization experiments, the slides were dried under a stream of N_2 and a multi-welled silicone mat, which divided the surface of the slide into 24 separate wells, was attached directly over the surface of the glass slide.

Preparation of alkyne-coated glass surface.

Similar to the procedure described for **L-Az**, the surface-activated slides were treated with a 1:20 molar ratio of alkyne containing active linker **L-PE** and 6-amino hexanol (**spacer 2**). The slides were stored under the same conditions used for the azide-derivatized surfaces. Contact angle measurements were performed using a standard calibrated microscope with water droplet providing the angle measurements. In all cases the contact angle increases from 0 ° for the free amino derivatized slides to ~ 50 ° upon the formation of the monolayer.

CDNB Assay for His_6-GSTase-CVIA

For immobilized as well as non-immobilized enzymes the chloro-2,4-dinitro benzene (CDNB) assays for GSTase activity measurement was adapted from methods reported by Pabst *et al.*[7] The background rate of conjugation was measured by incubating CDNB (20-160 μM) and reduced glutathione (GT) (1 mM) in 100 mM NaH_2PO_4 buffer, pH 6.5, without the enzyme and the reaction was allowed to proceed for 24 h. Product formation was measured from the increase in absorbance at 340 nm. Average ε for CDNB-GT adduct was calculated using Beer's Law ($\varepsilon = 9.60\ mM^{-1}\ cm^{-1}$).

In order to estimate the difference between rates of enzyme-catalyzed and the background non-enzymatic conjugation, the chromogenic substrate CDNB (160 μM) was incubated with reduced glutathione (GT) (1 mM) with His_6-GSTase-CVIA (735 nM) and without the enzyme in parallel experiments in 100 mM NaH_2PO_4; pH = 6.5 buffer. Product formation was measured by the increase in absorbance at 340 nm. The enzymatic reaction was complete within 30 min while the non-enzymatic reaction was incomplete even after 480 minutes. This indicated minimal interference due to background non-enzymatic reaction rates. The assays performed with immobilized GSTase were designed to fit in the Michaelis-Menten kinetic regime. The substrate concentrations were kept saturated at least 3 times the K_m of respective substrates. For example, the K_m for CDNB is 60 μM for GSTase, and the enzyme assays were performed at 200-250 μM CDNB.

Protein immobilization

TBTA necessary for Click chemistry was prepared according to literature[8]. TBTA, as white crystalline solid, was dissolved in 1:1 ^{t}BuOH:H_2O prior to the click reaction. This stock solution required warming to 60 °C in order to dissolve the ligand (TBTA) completely. A solution of His_6-GSTase-CVIA-PE or His_6-GSTase-CVIA-F (3 µL, 10 µM) was spotted into individual wells. Click reagent cocktail (7 µL of a solution prepared by mixing 40 µL of 100 mM $CuSO_4$, 8.0 µL of 500 mM TCEP•HCl and 400 µL of 10 mM TBTA in 3.6 mL of 3:1 H_2O:glycerol) was added to each well. The characteristic color change for the effective formation of this Click reagent is from colorless to green. This green solution was freshly made before each conjugation attempt. The slide was kept humid (in a cold room) at 4 °C with gentle swirling for 6 h. Each well was washed with 10 µL of phosphate-buffered saline containing Tween20® (PBST: 50 mM KH_2PO_4; 150 mM sodium chloride; 0.1% Tween 20; pH = 7.2) followed by the addition of 4 µL of Block IT solution and gentle shaking for 4 h at 4 °C. Each well was shaken for 15 min three times with 10 µL of PBST before 4 µL of anti-GSTase Alexa Fluor conjugate [prepared as a 40 µg/mL in Tris-buffered saline (TBS: 20 mM Tris•HCl; 150 mM sodium chloride; pH = 7.5.] was added. The slide was incubated overnight at 4 °C. The silicone mat was carefully removed; the slide was washed with several portions of PBST over 2h, and the slide was scanned with a Typhoon phosphorimager. After scanning, the slide was immersed in stripping solution (125 mM glycine, 500 mM sodium chloride, 2.5% Tween20® pH=2.0) for 6 h at 80 °C. The slide was scanned again after rinsing with PBST. The slide was blocked, washed, incubated with anti-GST (40

μg/mL) overnight at 4 °C and imaged. Spots were quantified using ImageQuant® software.

General Procedure for Assay of immobilized GSTase

Picture 1. Slide assembly using MMH4x24 ArayIT kit.

ArrayIT glass slides derivatized with **L-Az** or **L-PE** were immersed in 2 mL of 3:1 H_2O:glycerol for 1 h at 4 °C with constant swirling under N_2. The slides were matted in a 96-well microarray format using a commercially available hardware sandwich and gasket system (ArrayIT, MMH4x24, see Picture 1). A stock solution of His_6-GSTase-CVIA-PE or His_6-GSTase-CVIA-Az (986 μM) was serially diluted with a buffer consisting of 100 mM sodium phosphate and 1 mM dithiothreitol (DTT), at pH = 6.5 to give solutions containing 33, 67, 133, and 267 μM protein. Thirty μL portions of these samples were spotted into wells on the slide. A 70 μL portion of click reagent cocktail was added to each well and the slide was incubated at 4 °C for 4 h with gentle swirling. The wells were rinsed once with PBS (200 μL per well) and with PBST (200 μL per well) overnight. The wells were emptied and replenished with 100 μL of a solution containing 250 μM 1-chloro-2,4-dinitrobenzene (CDNB), 1 mM glutathione in 100 mM sodium phosphate buffer, pH 6.5. The plates were gently shaken at 21 °C for 30 min. A 75 μL portion was

removed from each well and the absorbance at 340 nm was measured in order to calculate the activity of the immobilized enzyme.

Specific activity of prenylated His_6-GSTase-CVIA in solution

Figure S3 shows results from the enzymatic reaction upon incubation of GT (1 mM) with CDNB (200 μM) and 735 nM of GSTase enzyme in solution prior to immobilization. Samples run in triplicate and average absorbance at 340 nm were converted to the concentration of GT-CDNB adduct using ε value for GT-CDNB adduct calculated from a standard curve for the product. Based on data shown in Figure S3, for His_6-GSTase-CVIA-PE, the specific activity measured in solution is 2.1 μmole product min^{-1} μmol^{-1} enzyme.

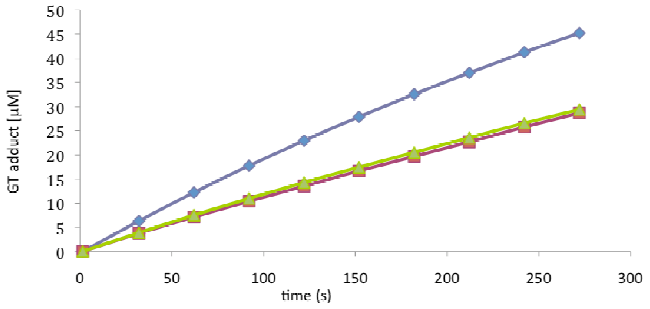


Figure S3. Plot showing GSTase catalyzed GT adduct formation with CDNB for His_6GSTase-CVIA, His_6GSTase-CVIA-PE and His_6GSTase-CVIA-F as a function time. Activity determined by measurement of absorbance at 340 nm in 100 mM NaH_2PO_4 buffer, pH = 6.5, containing 250 μM CDNB and 1 mM GT after incubation for 30 min at 21 °C. 735 nM of enzyme was used in each run. His_6GSTase-CVIA-PE - ■; His_6GSTase-CVIA-F - ■; His_6GSTase-CVIA - ■. Samples run in triplicate and average absorbance at 340 nm are used to calculate each value in the plot.

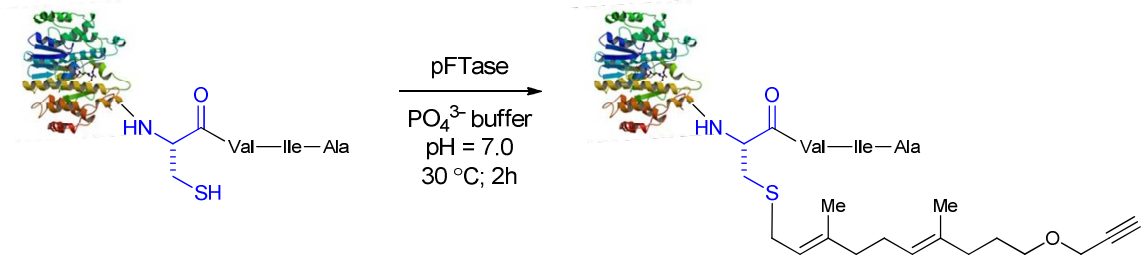
Specific activity of immobilized His_6-GSTase-CVIA

For data shown in Figure 2 of the manuscript, we calculate the specific activity of immobilized His_6-GSTase-CVIA-PE based on the surface area of each well over which the enzyme is spotted and the number of reactive amino groups present in this surface area. (Table S1). The average specific activity of immobilized His_6-GSTase-CVIA-PE is 1.4 μmole product min^{-1} μmol^{-1} enzyme. This correlates with the specific activity of His_6-GSTase-CVIA-PE measured in solution (2.1 μmole product min^{-1} μmol^{-1} enzyme) as shown in Figure S2.

Table S1. Specific activity of immobilized His_6-GST-CVIA-PE with 250 μM CDNB and 1 mM GT as substrates at pH 6.5

His_6-GSTase-CVIA-PE [μM]	μg/ml of His_6-GSTase-CVIA-PE	Amount of enzyme immobilized/area of glass surface ($\mu g/mm^2$)	Specific activity μmoles product min^{-1} μmol $GSTase^{-1}$
10	8.8	0.148	1.29
20	17.6	0.296	1.97
40	35.2	0.593	1.46
80	70.4	1.187	0.95

SDS-PAGE of Prenylated GSTase from cell-free homogenates



Farnesylation of cell-free homogenates containing His_6-GSTase-CVIA.

E. Coli M15-pQE(His_6-GSTase-CVIA) were incubated at 37 °C and protein synthesis was induced with IPTG (1 mM) over 4 h. Cells were lysed under sonication in lysis buffer (45 s pulse, 4 cycles at 4 °C). A 200 μL sample of the cell-free homogenate was added to 5 mL of farnesylation buffer containing the appropriate farnesyl diphosphate analogue (35 μM), followed by yeast protein farnesyltransferase (35 nM). The mixture was incubated at 30 °C for 3 h. The solution was concentrated by ultrafiltration and the concentration of protein was determined using a combination of UV and Bradford assays.[1] Protein samples were further analyzed by ESI-MS and the results are summarized in Table S1. Modified His_6-GSTase-CVIAs were flash frozen with liquid N_2 and stored in 50 μL aliquots at -80 °C until further use. The His-tagged GSTase in the *E. coli* cell-free homogenate after treatment with PFTase and PEPP was loaded onto a Ni-NTA column, purified, and analyzed by SDS-PAGE. (See Figure S4).

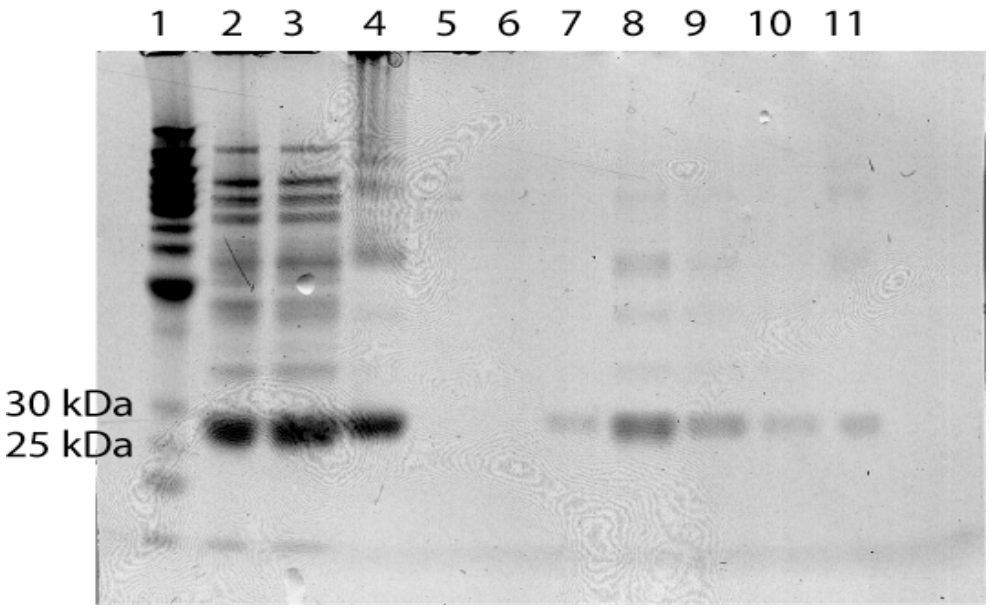


Figure S4. SDS-PAGE analysis of His_6-GSTase-CVIA-PE in *E. coli* cell-free homogenate. Benchmark MW ladder (1); *E. coli* cell-free homogenate incubated with PEPP and PFTase in buffer (2-3); His6-GSTase-CVIA-PE (pure protein sample) control (4); wash fractions (5-6); collected elute fractions (7-11). Note: *higher molecular weight peaks are observed even after protein purification due to the presence of dimeric and higher order multimeric complexes of* His_6-GSTase-CVIA-PE (lanes 4 and 8).

Summary of ESI-MS Analysis of Protein Prenylation

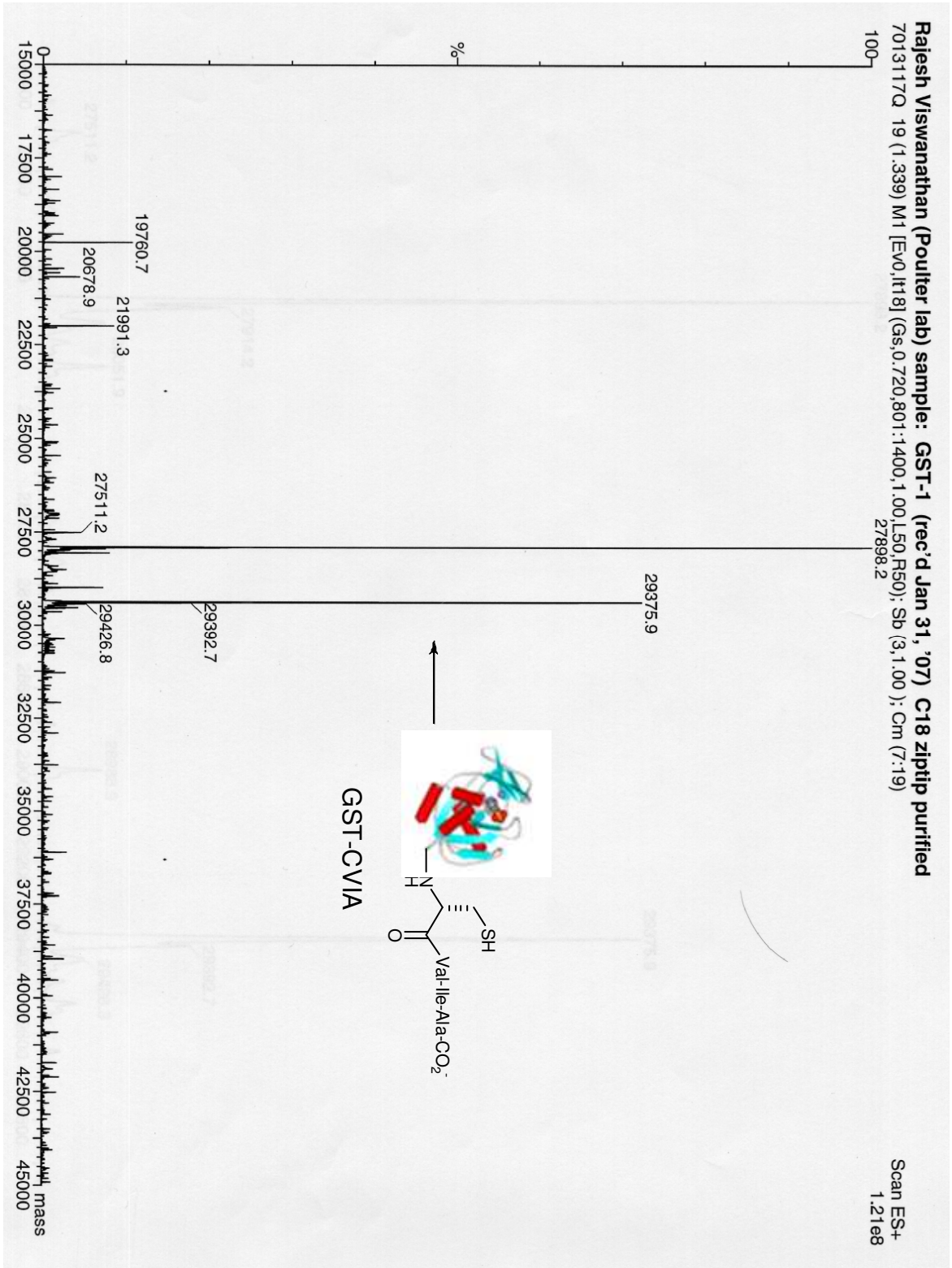
Table S2. ESI-MS analyses of His_6-GST-CVIA and prenylated products.

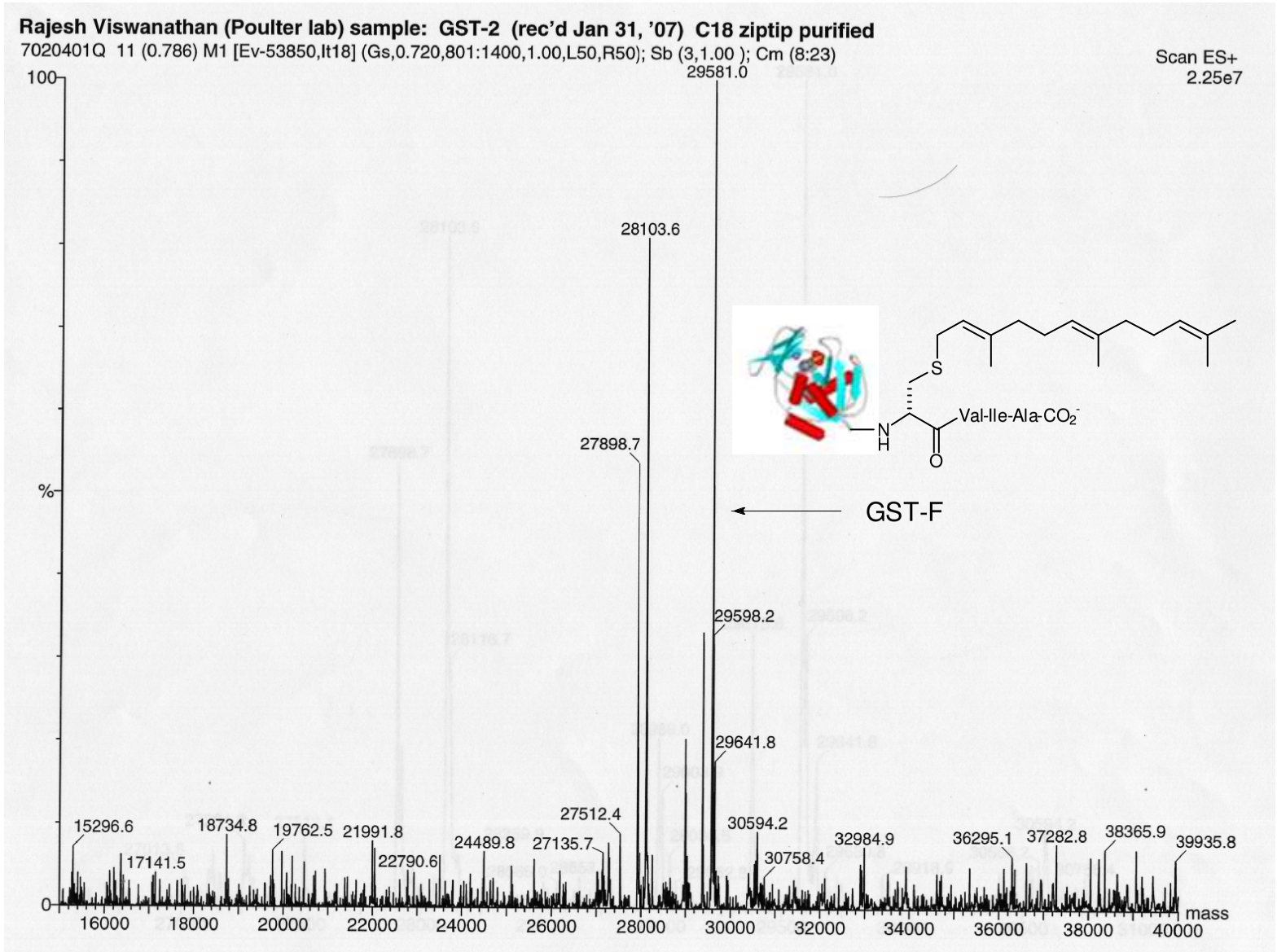
Sample	Expected M^+	Found M^+ (mass difference in Da)	
	Da	purified protein	in cell free homogenate
His_6-GST-CVIA	29376.8	29375.9 (0.9)	-
His_6-GST-CVIA-F	29581.8	29581.0 (0.8)	-
His_6-GST-CVIA-PE	29594.8	29594.8 (0.0)	29594.3 (0.5)
His_6-GST-CVIA-AZ	29581.8	29582.1 (0.3)	29584.0 (2.2)

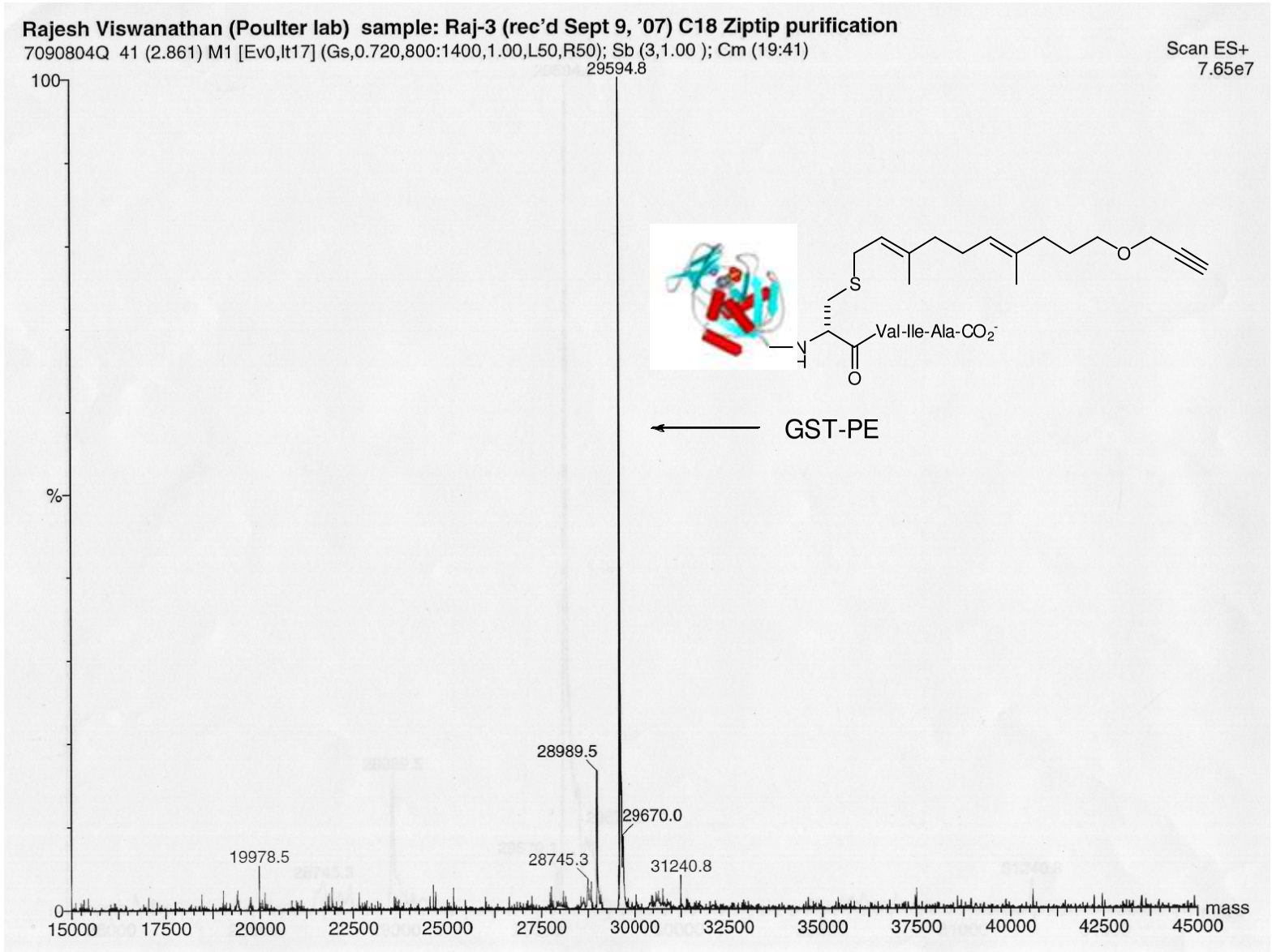
The peaks at M^+ in Table S2 were accompanied by lower mass peaks (see ESI-MS traces) as follows: purified His_6-GST-CVIA, M^+ at 29375.9 and (M^+ - 1477.7) at 27898.2; purified His_6-GST-CVIA-F, M^+ at 29581.0 and (M^+ - 1477.4) at 28103.6; unpurified His_6-GST-CVIA-PE, M^+ at 29594.3 and (M^+ - 1477.2) at 28117.1; His_6-GST-CVIA-AZ, M^+ at 29582.1 and (M^+ - 1478.4) at 28103.7; and His_6-GST-CVIA-AZ, M^+ at 29584.0 and (M^+ - 1479.7) at 28104.3. The average difference between M^+ and the lower mass fragments is 1478.0 ± 0.8, which corresponds to a deletion of amino acids ^{37}KIKGLVQPTRLL^{49}L (1477.6 Da) from His_6-GST-CVIA and its modified derivatives. The deletion occurs in a helix near the N-terminus. The deletion mutant was produced along with the full-length sequence, was modified by FPP, PEPP, and AZPP, and is probably catalytically inactive. Since the deletion mutant co-occurs with the full-length protein, the comparisons reported for His_6-GST-CVIA, the modified proteins in solution, and the immobilized enzymes should reflect the relative activities of each form of the enzyme.

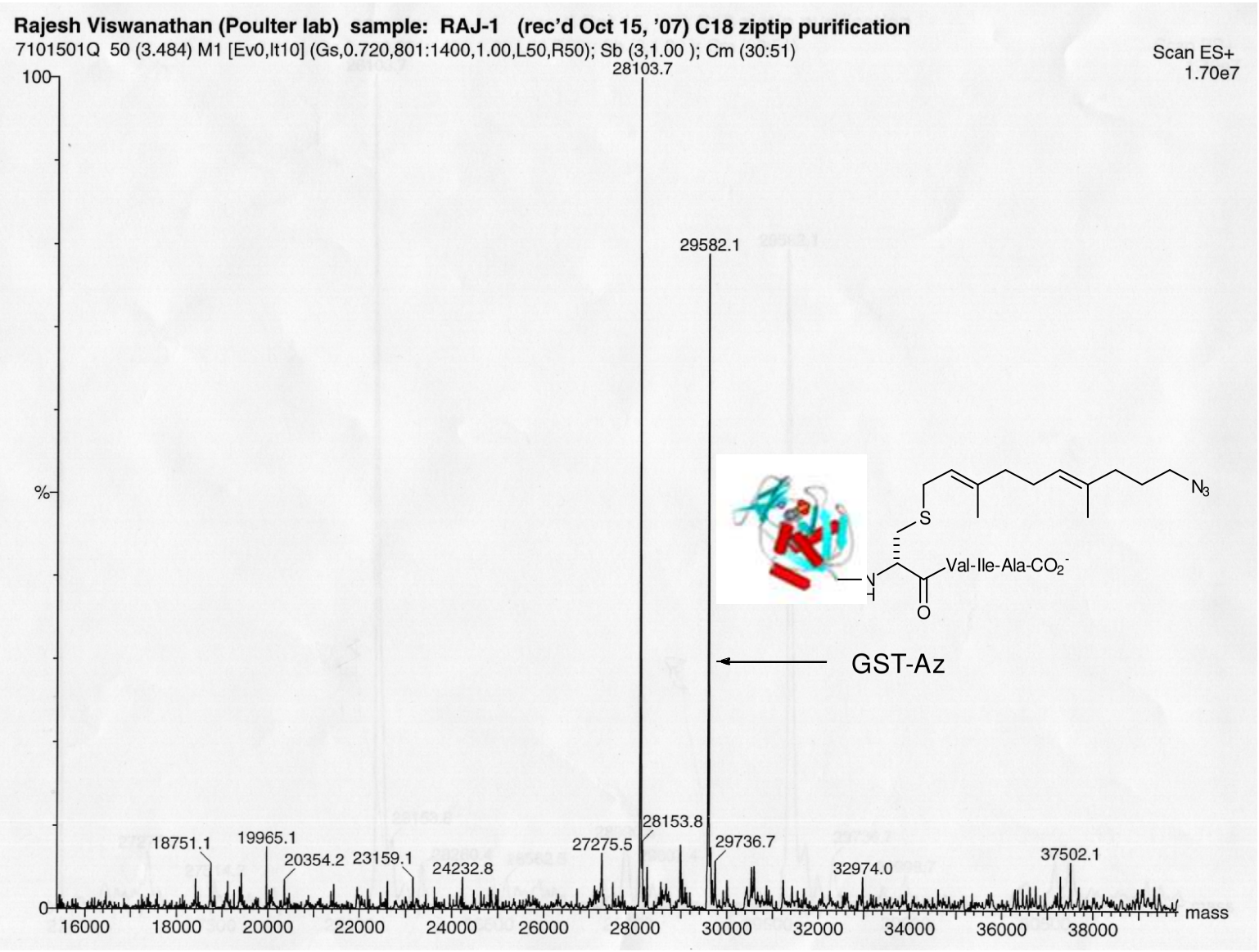
Figure S4. Ribbon diagram of *S. japonicum* GSTase with region ^{37}KIKGLVQPTRLL^{49}L highlighted in red.

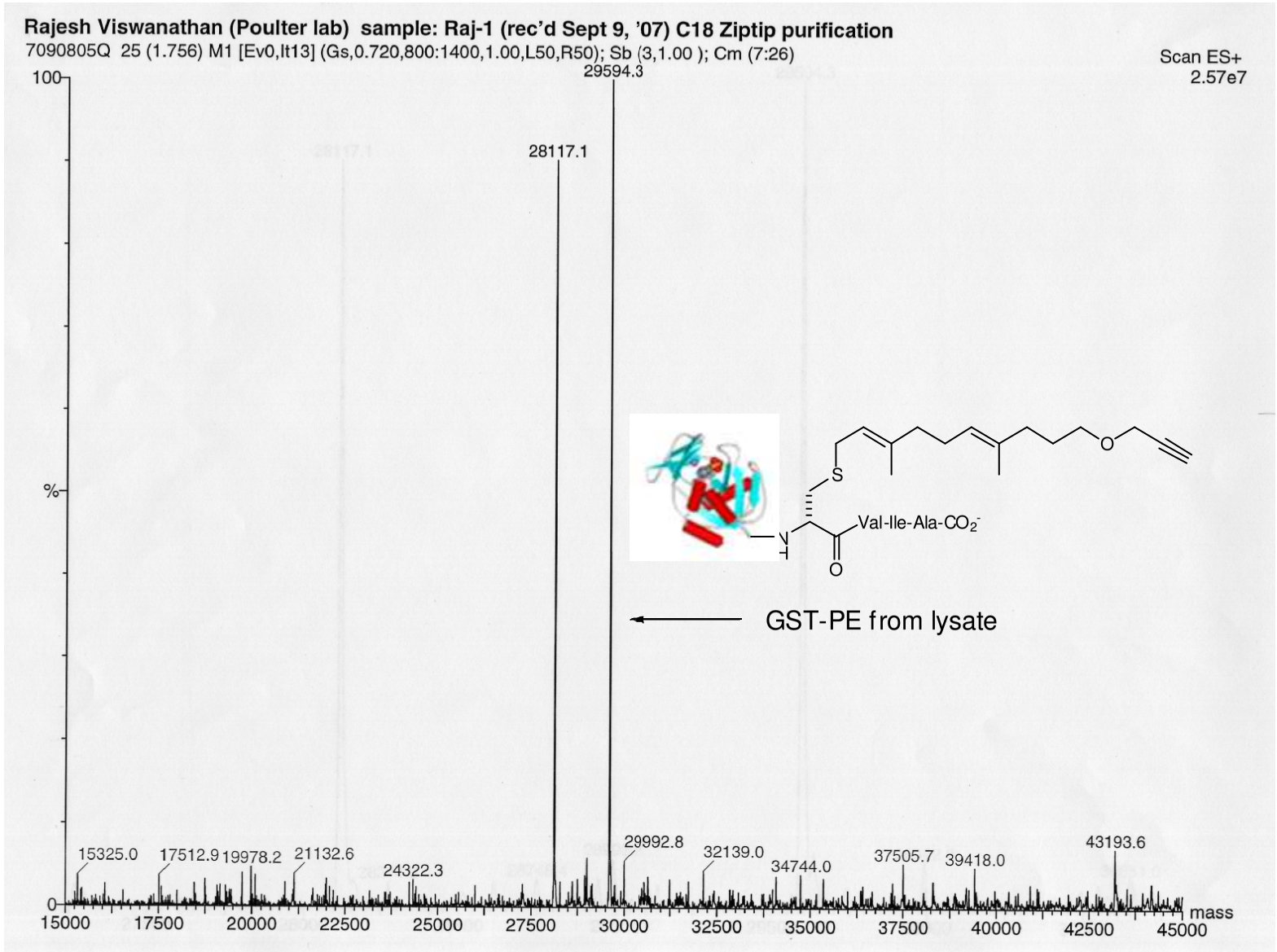
ESI-MS data for proteins

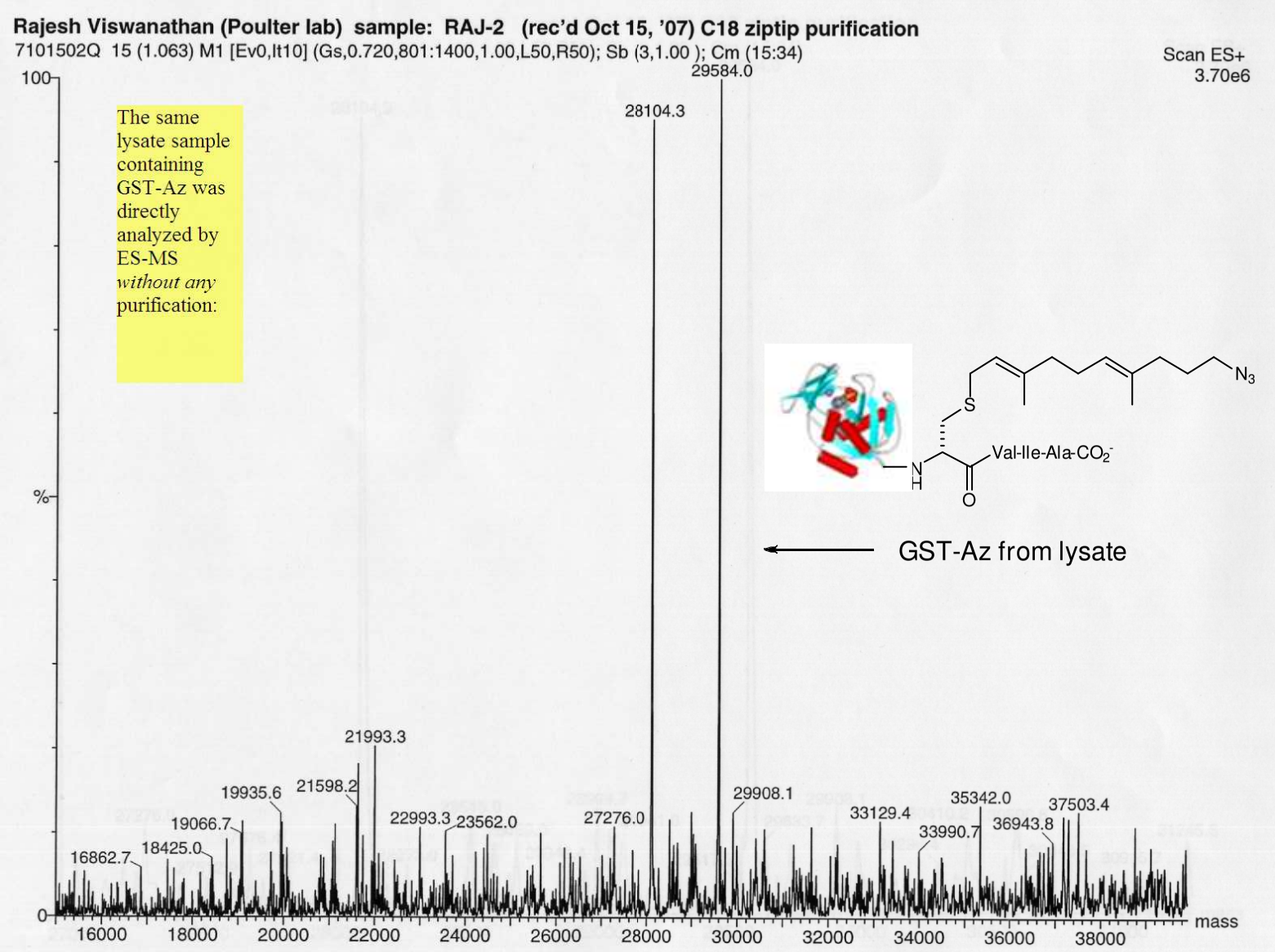



Rajesh Viswanathan (Poulter lab) sample: GST-2 (rec'd Jan 31, '07) C18 ziptip purified
7020401Q 11 (0.786) M1 [Ev-53850,It18] (Gs,0.720,801:1400,1.00,L50,R50); Sb (3,1.00); Cm (8:23)
Scan ES+
2.25e7
100
%
0
29581.0
28103.6
27898.7
29598.2
29641.8
27512.4
30594.2
15296.6
18734.8
19762.5
21991.8
17141.5
22790.6
24489.8
27135.7
30758.4
32984.9
36295.1
37282.8
38365.9
39935.8
16000
18000
20000
22000
24000
26000
28000
30000
32000
34000
36000
38000
40000
mass
S
N
H
O
Val-Ile-Ala-CO2-
GST-F

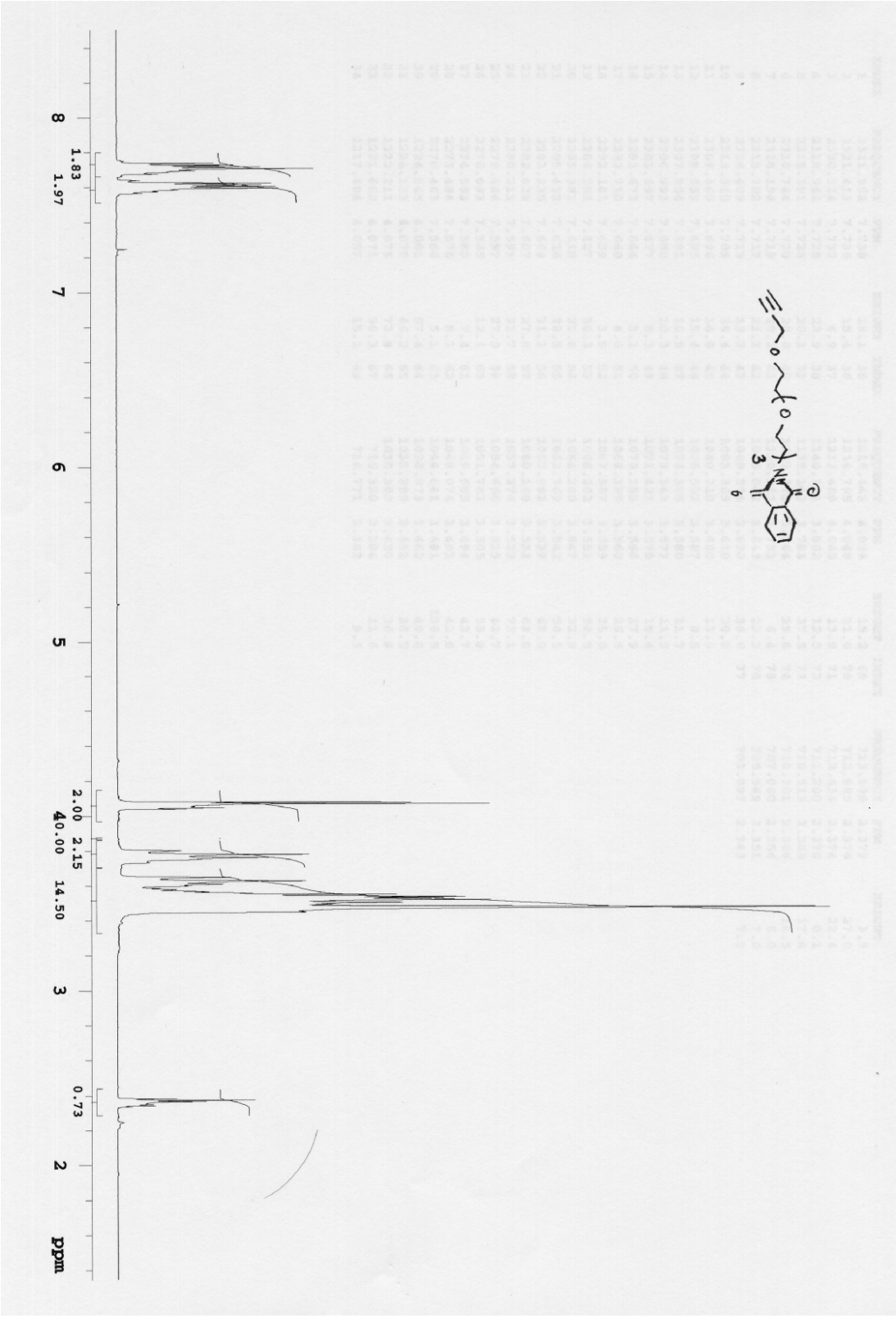

Rajesh Viswanathan (Poulter lab) sample: Raj-3 (rec'd Sept 9, '07) C18 Ziptip purification
7090804Q 41 (2.861) M1 [Ev0,It17] (Gs,0.720,800:1400,1.00,L50,R50); Sb (3,1.00); Cm (19:41)
Scan ES+
7.65e7
29594.8
100
%
0
28989.5
29670.0
19978.5
28745.3
31240.8
mass
15000
17500
20000
22500
25000
27500
30000
32500
35000
37500
40000
42500
45000
S
O
Val-Ile-Ala-CO2-
O
GST-PE

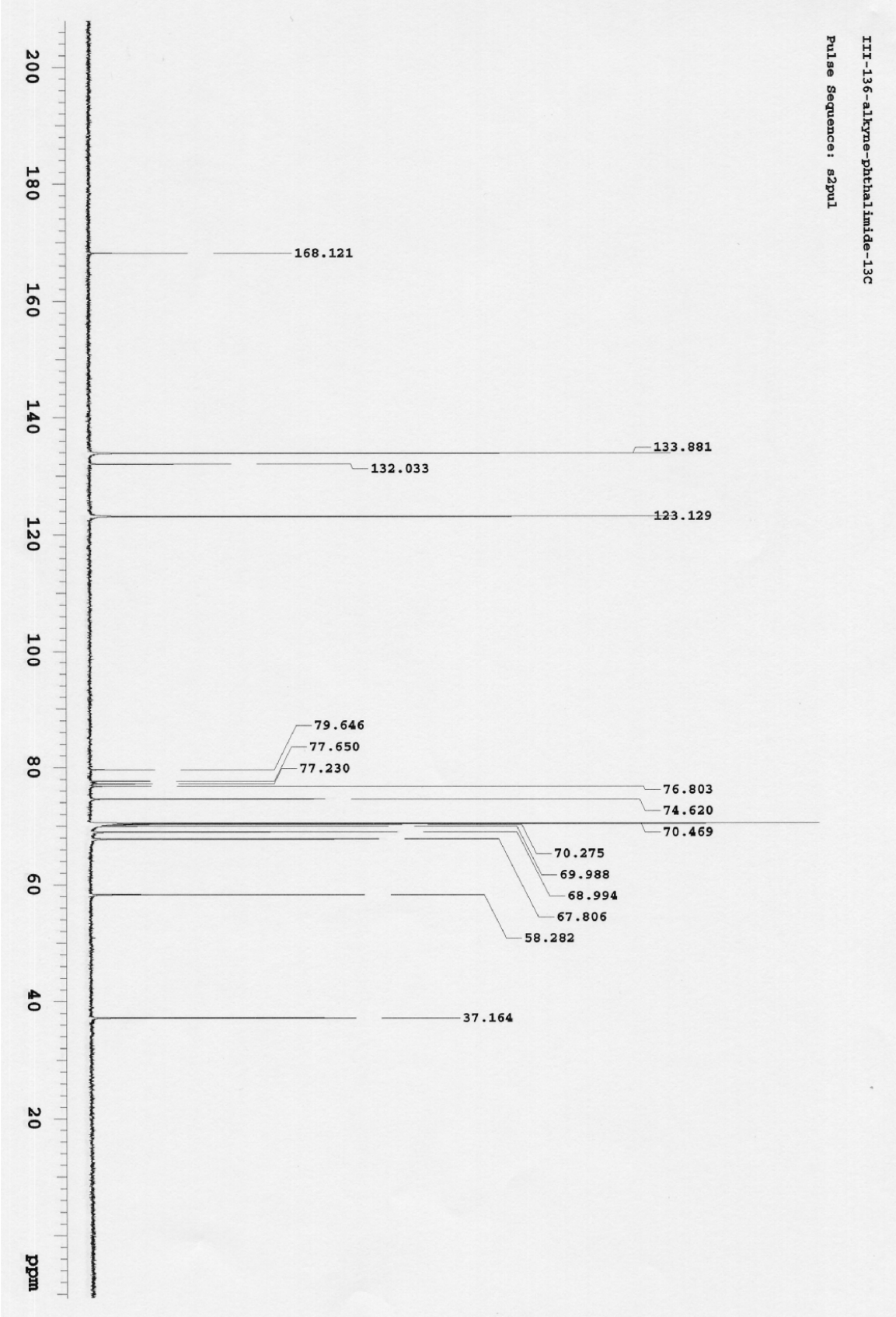

Rajesh Viswanathan (Poulter lab) sample: RAJ-1 (rec'd Oct 15, '07) C18 ziptip purification
7101501Q 50 (3.484) M1 [Ev0,It10] (Gs,0.720,801:1400,1.00,L50,R50); Sb (3,1.00); Cm (30:51)
Scan ES+
1.70e7
100
%
0
28103.7
29582.1
18751.1
19965.1
20354.2
23159.1
24232.8
27275.5
28153.8
29736.7
32974.0
37502.1
16000
18000
20000
22000
24000
26000
28000
30000
32000
34000
36000
38000
mass
GST-Az
S
N3
Val-Ile-Ala-CO2-
O

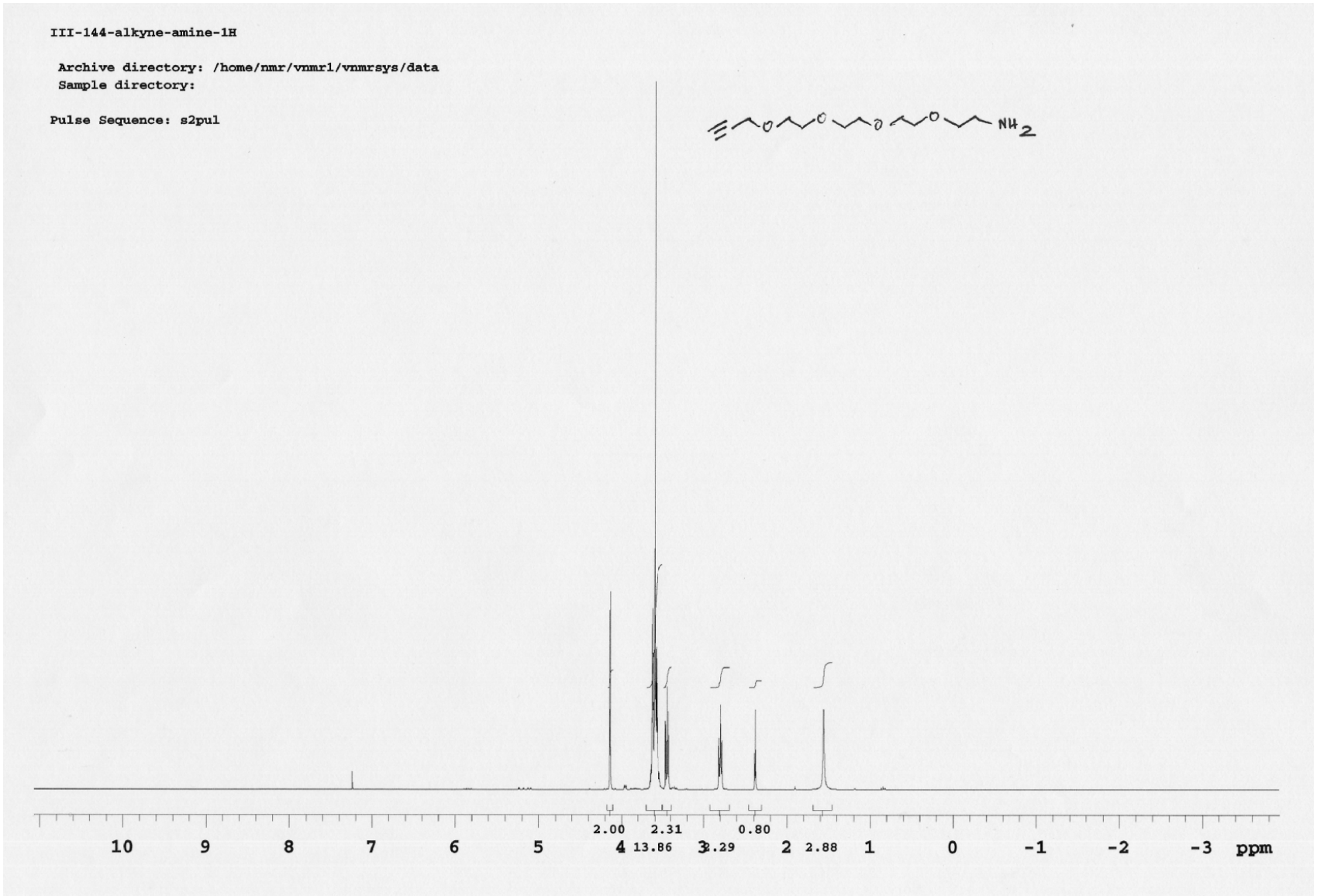

Rajesh Viswanathan (Poulter lab) sample: Raj-1 (rec'd Sept 9, '07) C18 Ziptip purification
7090805Q 25 (1.756) M1 [Ev0,It13] (Gs,0.720,800:1400,1.00,L50,R50); Sb (3,1.00); Cm (7:26)
Scan ES+
2.57e7
100
%
0
29594.3
28117.1
15325.0
17512.9
19978.2
21132.6
24322.3
29992.8
32139.0
34744.0
37505.7
39418.0
43193.6
15000
17500
20000
22500
25000
27500
30000
32500
35000
37500
40000
42500
45000
mass
S
O
Val-Ile-Ala-CO2-
GST-PE from lysate

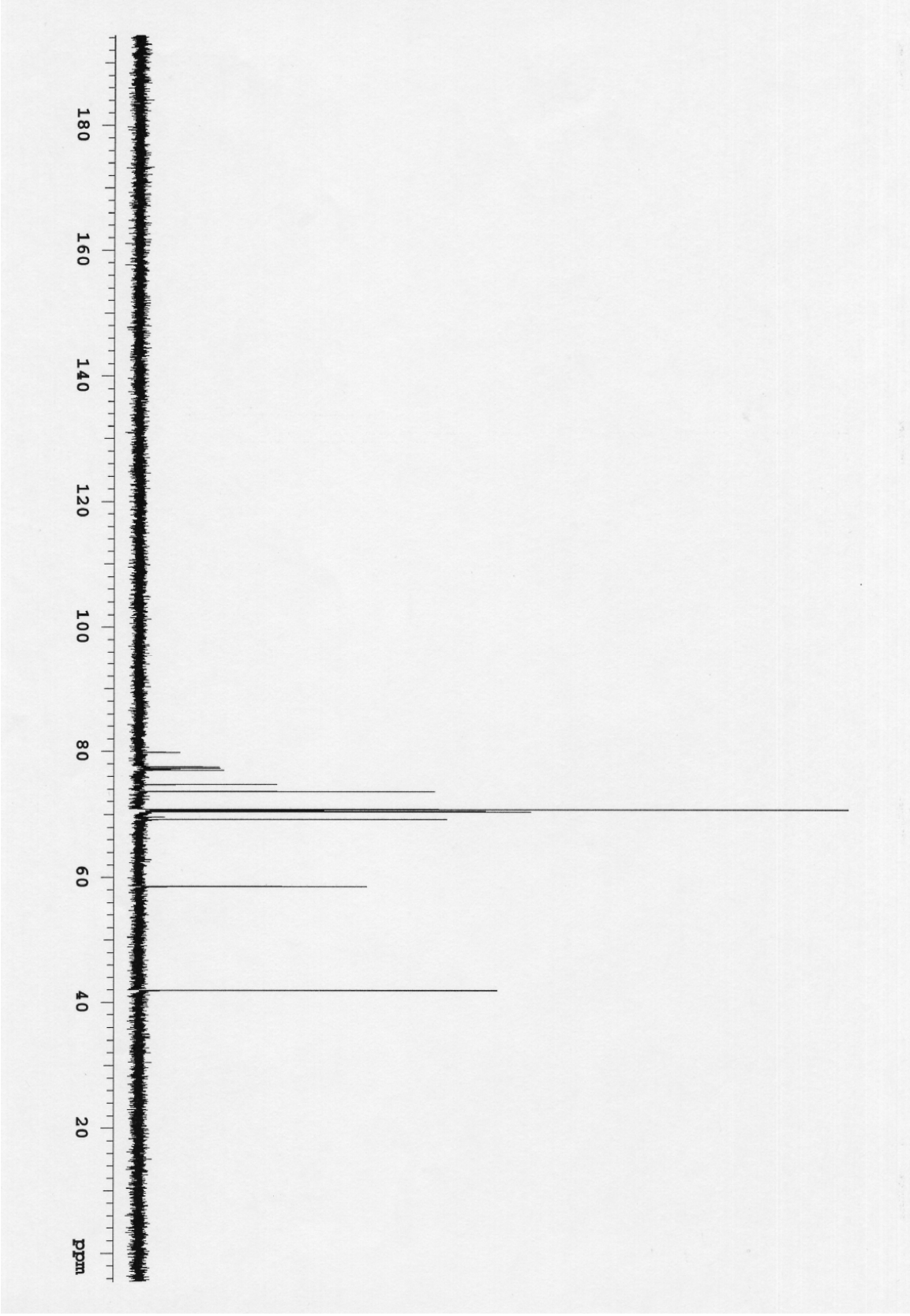

Rajesh Viswanathan (Poulter lab) sample: RAJ-2 (rec'd Oct 15, '07) C18 ziptip purification
7101502Q 15 (1.063) M1 [Ev0,It10] (Gs,0.720,801:1400,1.00,L50,R50); Sb (3,1.00); Cm (15:34)
Scan ES+
3.70e6
The same lysate sample containing GST-Az was directly analyzed by ES-MS *without any* purification:
100
%
0
29584.0
28104.3
21993.3
19935.6
21598.2
19066.7
22993.3
23562.0
27276.0
29908.1
33129.4
35342.0
37503.4
33990.7
36943.8
16862.7
18425.0
16000
18000
20000
22000
24000
26000
28000
30000
32000
34000
36000
38000
mass
S
N3
Val-Ile-Ala-CO2-
N
H
O
GST-Az from lysate

^{1}H and ^{13}C NMR data for small molecule linkers




III-136-alkyne-phthalimide-13C
Pulse Sequence: s2pul
168.121
133.881
132.033
123.129
79.646
77.650
77.230
76.803
74.620
70.469
70.275
69.988
68.994
67.806
58.282
37.164
200
180
160
140
120
100
80
60
40
20
ppm


III-144-alkyne-amine-1H
Archive directory: /home/nmr/vnmr1/vnmrsys/data
Sample directory:
Pulse Sequence: s2pul
NH2
2.00
2.31
13.86
3.29
0.80
2.88
10
9
8
7
6
5
4
3
2
1
0
-1
-2
-3
ppm


180
160
140
120
100
80
60
40
20
ppm

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