Materials. All chemicals used in the synthesis of target compounds were reagent grade unless stated otherwise. Hexa(ethylene glycol), potassium hydroxide, 2,2,2-trifluoroacetamide, sodium hydride, p-toluenesulfonyl chloride, 20% palladium hydroxide on carbon (wet), thionyl chloride, 1.5 M HCl in EtOH, carbon tetrabromide, triphenylphosphine, triethyl phosphite, oxalyl chloride, triethylamine, p-nitrophenol, pyridine, succinic anhydride, O' O'- bis(carboxyethyl)dodecaethylene glycol, N-methylmorpholine, 6-bromohexanoyl chloride, benzyl alcohol, aluminum chloride, anisole, nitromethane, diisopropylcarbodiimide, N-hydroxysuccinimide, sodium chloride, sodium azide, Triton X-100, tetracycline, carbenicillin, kanamycin, isopropyl thiogalactoside (IPTG), dimethyl sulfoxide, and Cell Lytic B, were purchased from Sigma-Aldrich (www.sigmaaldrich.com). Hexa(ethylene glycol) monobenzyl ether was purchased from TCI America (www.tciamerica.com). AlexaFluor 647 cadaverine sodium salt, Mega X DH10b T1^{R} electrocompetent cells, and 10x PBS buffer were purchased from Invitrogen (www.invitrogen.com) All primers used for cloning protein inserts were purchased from Integrated DNA Technologies (www.idtdna.com) with standard desalting as means of purification unless HPLC or SDS-PAGE was recommended by the manufacturer for primers that exceeded 60 bp in length. Novagen's pET21-d expression vector, Origami B (DE3) chemically competent cells, and KOD HotStart PCR Master Mix were purchased from EMD Biosciences (www.emdchemicals.com). The restriction enzymes NcoI, BamHI, and XhoI; and T4 DNA ligase were purchased from New England Biolabs (www.neb.com). Tryptone, yeast extract, LB agar, agarose, and 1M Tris-HCl buffer (pH 8.0) were purchased from Thermo Fisher Scientific (www.fishersci.com). Complete-Mini EDTA-free protease inhibitor tablets were purchased from Roche Applied Science (www.roche-applied-science.com). Talon cobalt IMAC resin was purchased from Clontech (www.clontech.com). 4-12% PAGEr Gold precast polyacrylamide gels were purchased from Lonza (www.lonza.com). HaloTag AlexaFluor 488 ligand, HaloTag O4 Amine ligand and the HaloTag pHT2 vector were purchased from Promega (www.promega.com). MicroBioSpin 30 columns were purchased from Pierce (www.piercenet.com) All organic solvents were purchased from VWR (www.vwr.com).

Cloning of HaloTag-Cutinase. The following PCR protocol was used to amplify an N-terminal HaloTag (HT) from template pHT2 plasmid. Forward primers (N-terminal) containing an NcoI cut site and reverse primers (C-terminal) containing a BamHI cut site (1 uL each, 100 uM final) were added to a reaction mixture containing template DNA (2 uL, ~ 50 ng), 2 uL DMSO (2 µL), KOD HotStart Master Mix (25 µL) and deionized ultra-filtered water (DIUF) (19 µL) to give a final volume of 50 µL. This reaction mixture was denatured at 95°C for 2 min then subjected to 25 repetitions of the following thermal cycles: 95°C for 20 s (denaturation), 65°C for 10 s (primer annealing), and 70°C for 15 s (primer extension). The reactions were then purified via agarose gel electrophoresis. Gel bands corresponding to PCR products of the correct size were excised from the gel using a razor blade and the DNA extracted from the gel slice using a Gel Extraction Kit (Qiagen). Purified PCR products (50 µL, ~5 ug) and uncut circular pET21-d (~1 ug) were then digested with NcoI (20 U) and BamHI (40 U) for two hours at 37°C. These reactions were again purified via agarose gel electrophoresis. Digested product bands corresponding to the correct size were excised from the gel using a razor blade and the DNA isolated via Gel Extraction Kit. The digested plasmid (200 ng) and PCR insert at a 5:1 insert : vector molar ratio were ligated at room temperature for 16 hr with T4 DNA ligase (100 U). The ligation reactions were dialyzed over DIUF for 20 min using floating dialysis membrance circles (Millipore) and the dialyzed ligation reactions (5 µL) were transformed into Mega X DH10b electrocompetent cells (50 µL) using an Eppendorf Electroporator 2510. Time constants of > 4 ms were deemed successful transformations. The transformants were then incubated in SOC media (500 µL) (Invitrogen) for 45 min at 37°C, plated on LB agar containing carbenicillin (100 µg / mL), and incubated for ~16 hr at 37°C. Colonies were picked and grown in overnight cultures containing carbenicillin (100 µg / mL), and their plasmid DNA isolated by Miniprep Spin Kit (Qiagen). Positive transformants were determined by DNA sequencing at the Cancer Research Center DNA sequencing facility at the University of Chicago. The transformants' plasmid DNA containing the N-terminal HaloTag reading frame was then used as the vector for the next cloning step. PCR products for the C-terminal cutinase of the fusion protein was generated from a preexisiting plasmid encoding cutinase from *Fusarium s. pisi* in a similar manner as above, though with the forward primer containing a region

coding for an $(EAAAK)_4$ helical linker after the BamHI cut site and prior to the annealing sequence for the target gene. Following ligation, transformation and sequencing of plasmids containing the full-length HaloTag-Cutinase (HC) reading frame, these plasmids were transformed into chemically competent Origami B (DE3) cells following the manufacturers instructions. The transformants were then plated on agar containing tetracycline (12.5 µg / mL), kanamycin (50 µg / mL), and carbenicillin (100 µg / mL); and incubated overnight at 37°C. Resulting colonies were grown overnight in 8 mL cultures containing the aforementioned antibiotics. A portion of these cultures (600 µL) were reserved and diluted by half using 20% glycerol and frozen at -80°C. The remaining volume of culture was used to verify the presence of the expression construct in the cultures by isolation of plasmid DNA via miniprep kit and sequencing.

HaloTag7-Cutinase Sequence.

M A E I G T G F P F D P H Y V E V L G E R M H Y V D V G P R D G T P V L F L H G N P T S S Y V W R N I I P H V A P T H R C I A P D L I G M G K S D K P D L G Y F F D D H V R F M D A F I E A L G L E E V V L V I H D W G S A L G F H W A K R N P E R V K G I A F M E F I R P I P T W D E W P E F A R E T F Q A F R T T D V G R K L I I D Q N V F I E G T L P M G V V R P L T E V E M D H Y R E P F L N P V D R E P L W R F P N E L P I A G E P A N I V A L V E E Y M D W L H Q S P V P K L L F W G T P G V L I P P A E A A R L A K S L P N C K A V D I G P G L N L L Q E D N P D L I G S E I A R W L S T L E I S G G S E A A A K E A A A K E A A A K E A A A K A A A G L P T S N P A Q E L E A R Q L G R T T R D D L I N G N S A S C A D V I F I Y A R G S T E T G N L G T L G P S I A S N L E S A F G K D G V W I Q G V G G A Y R A T L G D N A L P R G T S S A A I R E M L G L F Q Q A N T K C P D A T L I A G G Y S Q G A A L A A A S I E D L D S A I R D K I A G T V L F G Y T K N L Q N R G R I P N Y P A D R T K V F C N T G D L V C T G S L I V A A P H L A Y G P D A R G P A P E F L I E K V R A V R G S A L E H H H H H H

Cyan – HaloTag, Purple – restriction site encoded amino acids, Green – helical linker, Red – cutinase, **Black – His-Tag**

Expression and Purification of HaloTag-Cutinase (HC). Media (2xYT, 15 mL) supplanted with tetracycline (12.5 µg / mL), kanamycin (50 µg / mL), and carbenicillin (100 µg / mL) were inoculated with a stab of HT-Cut Origami B (DE3) and allowed to grow overnight at 37°C with shaking. The culture was then was added to 2xYT (1 L) media in a Thompson flask containing the same concentration of antibiotics and shaken at 37°C until the OD_{600} reached ~ 1.0 (about 4hr). The cultures were cooled to ~18°C in an ice / water bath and induced with IPTG (1 mL, 1 M) and shaken at 18°C for 16 hr in an Innova 44R (New Brunswick Scientific) incubator. The bacteria were harvested by centrifugation and the pellet subsequently lysed with Cell Lytic B (40 mL) containing Complete-Mini Protease inhibitor tablets according to the manufacturer's instructions. The resulting bacterial lysate was centrifuged (17000 rpm, 20 min) to remove cellular debris, and the cleared lysate applied to a 50 mL Kontes Flex column (Kimbal Kontes Glassware) containing Talon IMAC resin (4 mL bed volume) preequillibrated with 1x PBS buffer. This column was then placed on a nutating shaker at 4°C for 1 hr. The supernatant was drained from the column using positive pressure from a 60 mL syringe and the column washed with four times with of 1x PBS buffer (50 mL). The bound protein was then eluted from the resin using elution buffer (25 mM Tris-HCl, 150 mM NaCl, 150 mM imidazole, 0.05% NaN_3, pH 7.8 @ 25°C) (2 x 20 mL). The elution fractions were pooled and concentrated to 1 mL in a 25 mL Amicon 10 kDa cutoff Centrifugal Filter Unit (Millipore). Buffer exchange was then carried out by adding low salt buffer (25 mM Tris-HCl, 0.05% (w/v) NaN_3, pH 7.8 @ 25°C) (20 mL) to the concentrated eluate and centrifugation back to 1mL, 4x, in the same ultrafiltration unit. The low salt eluate was then diluted to 10 mL and applied to a Resource Q (GE Healthcare) anion exchange column at 4°C via an Akta FPLC (GE Healthcare). The protein was bound to the column in low salt buffer (25 mM Tris-HCl, 0.05% NaN_3, pH 8.2 @ 4°C) and eluted with a linear gradient of high salt buffer (25 mM Tris-HCl, 500 mM NaCl, 0.05% NaN_3, pH 8.2 @ 4°C). Fractions containing

reasonably pure (as determined by SDS-PAGE) protein were pooled and concentrated to 2 mL in a 5 mL Microcon 10kDa Centrifugal Filter Unit (Millipore) and subsequently purified by size exclusion chromatography via an Akta FPLC, on a Hi-Load 16/60 Superdex 200 (GE Healthcare) column using 25 mM Tris-HCl, 150 mM NaCl and 0.05% NaN_3, pH 8.0 @ 4°C as the running buffer. Pure fractions were determined by SDS-PAGE, pooled together, and concentrated to 500 uL using a 10 kDa cutoff Microcon 5 mL centrifugal filter unit.

Determination of Kinetic Parameters. The HaloTag-Cutinase (HC) protein used in the protein-ligand rate experiments was first blocked at one domain to permit measurement of the rate constant for reaction at the other domain. For example, HaloTag-blocked HC (^{b}HC) was prepared by treating HC (2 nmol) with HaloTag O4 Amine ligand (4 nmol) for 1 hr. The excess HaloTag ligand was subsequently removed by size exclusion chromatography using a MicroBioSpin 30 column according to the manufacturers instructions. Similarly, cutinase-blocked HC (HC^{b}) was prepared by treating HC (2 nmol) with compound **20** (4 nmol) for 1-2 hr. The excess ligand was subsequently removed by size exclusion chromatography using a MicroBioSpin 30 column.

Rate data were obtained for the protein-ligand reactions by treating the blocked protein (25 pmol) with compound **14** (500 pmol) in reaction buffer (25 μL, 1 μM protein, 20 μM ligand, 25 mM Tris HCl, 150 mM NaCl, 0.05% NaN_3, pH 7.8 @ 25°C) for an allotted time period. Reactions of ^{b}HC with **14** were quenched after this period with compound **20** (1 nmol)**.** Reactions of HC^{b} with compound **14** were quenched by the addition of HaloTag AlexaFluor 488 (HTAF 488) (1 nmol) ligand. Reported rate constants for the HaloTag – HTAF 488 reaction are on the order of 10^5 $M^{-1}s^{-1}$ and the determined rate constant for the cutinase – compound **20** reaction is 181 $M^{-1}s^{-1}$ (Figure S1 (A)). At the aforementioned concentrations we expect the quenching reaction rate to exceed the ligand reaction rate by at least ten fold so that these data points yield good approximations of the reaction extent at a given time point. Ten time points were taken for each of these two reactions and repeated in duplicate. Each time series was run on a denaturing 4-12% Tris HCl polyacrylamide gel. These gels were scanned on a Pharos FX gel scanner (BioRad) and the corresponding fluorescent bands quantified using the Quantity One (BioRad) software package. Fluorescence intensity for each data point was normalized to the maximum fluorescence intensity for that particular experiment. The normalized intensity data were plotted against time and then fit using Origin (Origin) software to a pseudo-first order kinetic decay model according to equation (1):

$$I_t = I_0 e^{-kt} \quad (1)$$

where I_t is the normalized fluorescence intensity of the band corresponding to a particular time point. Pseudo-first order rate constants obtained by fits to these kinetic data were then divided by the concentration of the excess species to obtain 2nd order rate constants for the reactions.

A similar procedure was followed to obtain rate constants for the reaction of two fusion proteins and started by first blocking HC (~50μM, 2 nmol) at one enzyme functionality using 1.5 eq either HTAF 488 or compound **20** to generate an AlexaFluor 488 labeled HC at the HaloTag (*HC) or AlexaFluor 647 labeled HC at cutinase (HC*) respectively. This labeled substrate was subsequently treated with two equivalents of compound **14**. For linker functionalization through HaloTag to generate phosphonate-HC*, the ligand reaction was allowed to proceed for 1 hr. For linker functionalization through cutinase to generate *HC-alkylchloride, the reaction was allowed to proceed for 5 hr. The labeled, linker- functionalized proteins were passed through a Microbiospin 30 column to remove excess linker and fluorophore. Kinetic experiments were performed by treating either phosphonate-HC* or *HC-alkylchloride (25 pmol) with HC (500 pmol, 25 μL final reaction volume) for specified periods of time. Nine time points were taken for each protein-protein dimerization reaction and each kinetic run was performed in duplicate. Reactions were quenched at the given time point by the addition of 5x Laemmli sample buffer (5 μL). Each time series was run on a denaturing 4-12% Tris HCl polyacrylamide gel. These gels were scanned on a Pharos FX gel scanner and the corresponding fluorescent bands quantified using the Quantity One software package. Fluorescence intensity for each data point was normalized to the maximum fluorescence

intensity for that particular experiment. Normalized intensity data vs. time was then fit using Origin (Origin) software to a pseudo-first order kinetic growth model according to equation (2):

$$I_t = I_0(1 - e^{-kt}) \quad (2)$$

where I_t is the normalized fluorescence intensity of the band corresponding to a particular time point. Pseudo-first order rate constants obtained by fits to these kinetic data were then divided by the concentration of the excess species to obtain 2nd order rate constants for the reactions.

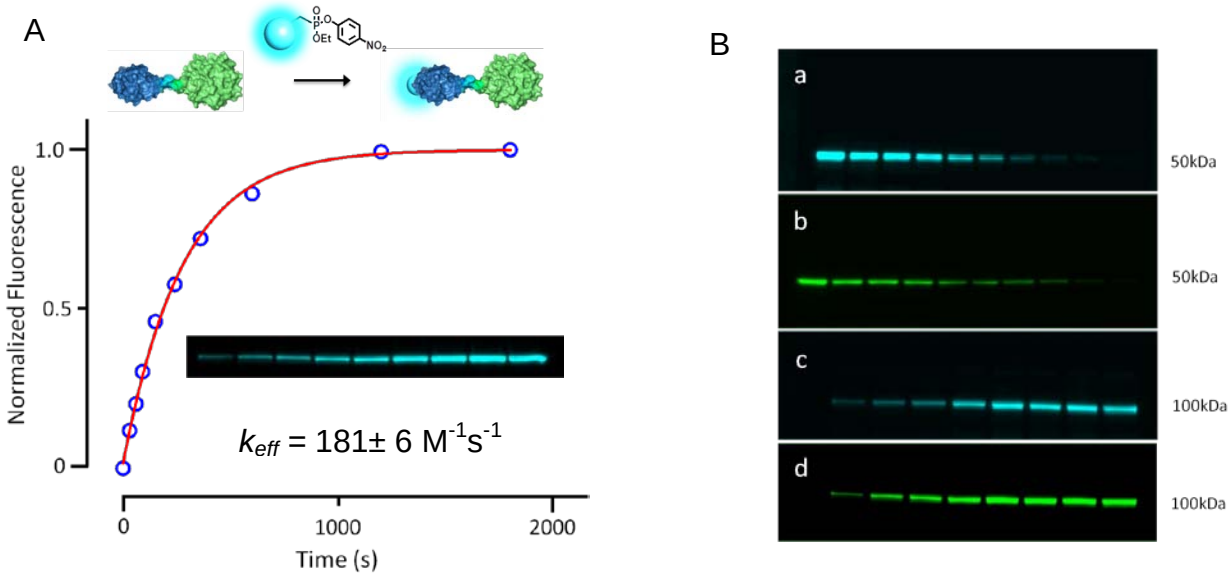


Figure S1. Kinetic analysis of the reaction of HC with compound **20** (p-nitrophenylphosphonate-AlexaFluor 647 conjugate) (A). Part (B) shows expanded gel scans of the kinetic experiments outlined in the text and supporting information. Each band represents a different time point in the course of the reaction; (a): 1 µM ^{b}HC + 20 µM **14**; (b): 1 µM HC^{b} + 20 µM **14**; (c) 1 µM phosphonate-HC* + 20 µM HC; (d) 1 µM *HC-alkylchloride + 20 µM HC. The absence of oligomeric products at higher molecular weight illustrates the regioselectivity of the protein modification reactions.

Determination of Hydrodynamic Radii from Size Exclusion Chromatography. The High Molecular Weight Gel Filtration Calibration Kit (GE Healthcare) was used to generate a hydrodynamic radius calibration curve for known protein calibrants according to the manufacturers instructions listed in the product manual. All experiments were performed at 4°C on an Akta FPLC (GE Healthcare) at a flow rate of 1 mL / min using TBS-Azide (25 mM Tris-HCl, 150 mM NaCl, 0.05% (w/v) NaN_3, pH 7.8 @ 25°C) as the mobile phase. Hydrodynamic radii for the HC oligomers were determined by substituting experimental elution volumes into the fit of the calibration curve. The scaling coefficient for the oligomers was determined by plotting the log of the protein hydrodynamic radii vs. log MW for each species. A linear fit to this data was performed using Origin 8.5 graphing software.

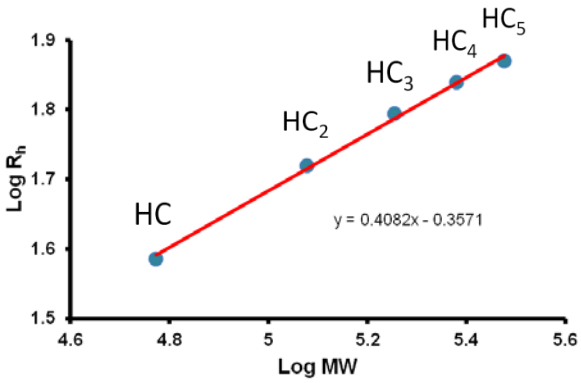
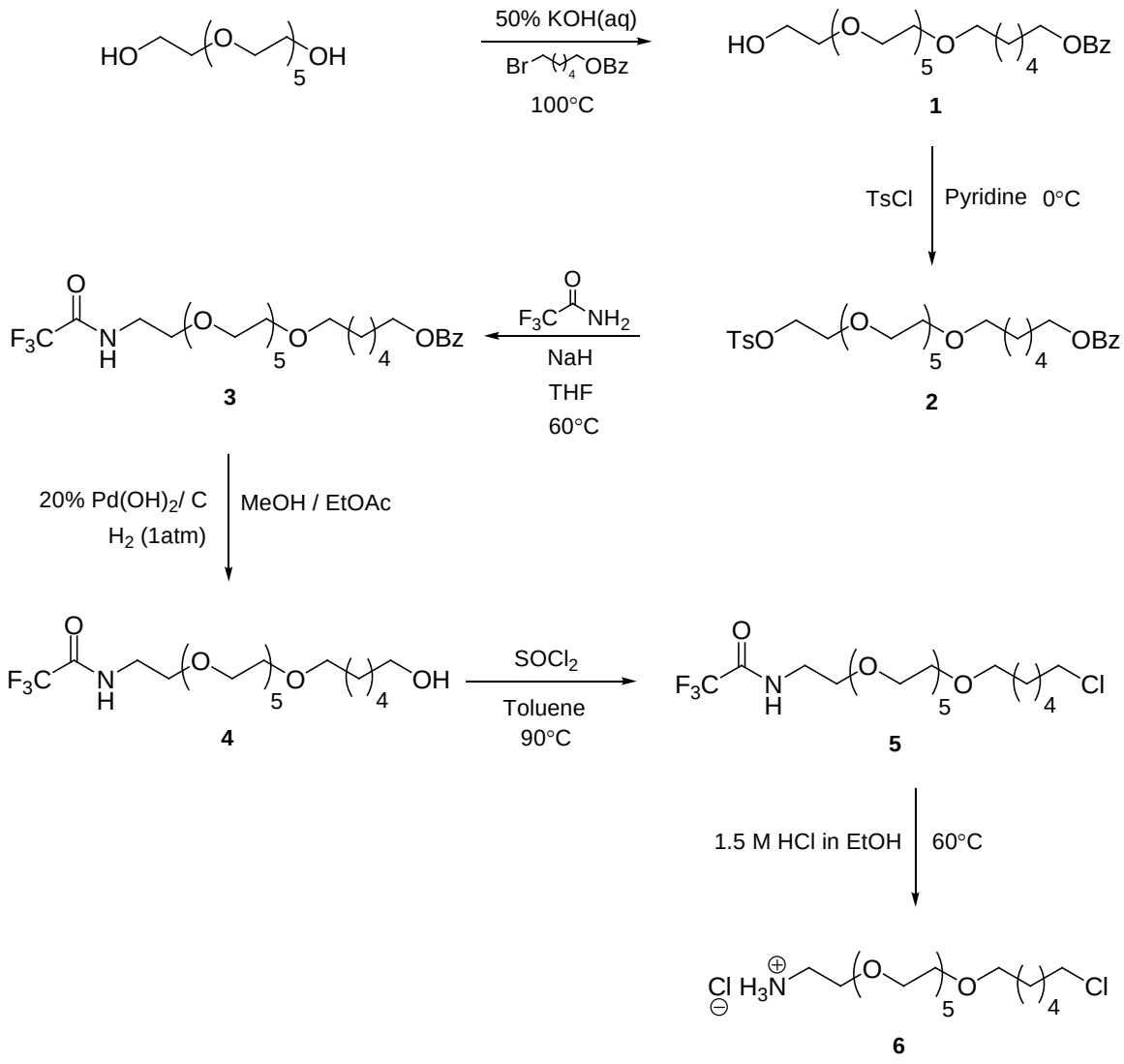


Figure S2. A plot of log R_h versus log MW for the HC oligomers. A fit to this data yields a straight line with a scaling coefficient of v = 0.41.

Matrix-assisted Desorption Ionization Mass Spectrometry (MALDI-MS): 50 uL of an approximately 5 µM solution of protein was desalted using a Microbiospin 30 gel filtration column preequilibrated with deionized ultra-filtered water. 1 uL of this solution was placed on a 100 spot MALDI target. To this spot was added 1 uL of sinapic acid matrix (20 mg / mL sinapic acid in 50:50 (v/v) acetonitrile : water + 0.01% trifluoroacetic acid). The spots were then allowed to dry under ambient conditions. These spots were then analyzed using a Voyager DE Pro (Applied Biosystems) MALDI-TOF spectrometer. Spectra were acquired in linear mode using an acceleration voltage of 20 kV. Typical spectra consisted of ~1000 acquisitions. M/z values were obtained by fitting the centroid to each peak.

Syntheses.



1-phenyl-2,9,12,15,18,21,24-heptaoxahexacosan-26-ol (1). 50% KOH $_{(aq)}$ (1.75 mL, 2.48 g, 22.2 mmol) prepared by dissolving 2 g solid KOH in deionized water (2 mL), was added to hexaethylene glycol (31.2 g, 110.9 mmol) and stirred vigorously until heat dissipated and a yellow color developed, approximately 1 hr. To this solution was added benzyl-6-bromohexyl ether (6.0 g, 22.2 mmol) and the mixture heated to 100°C and held at this temperature for 3 hr. The resulting mixture was allowed to cool to room temperature and poured into 1 N HCl (200 mL) in a seperatory funnel. The water was extracted with EtOAc (4 x 50 mL), the organic fractions pooled, dried over $MgSO_4$, vacuum filtered and concentrated on a rotary evaporator. The resulting crude yellow oil was then purified via flash chromatography on silica using EtOAc first to remove non-polar impurities and then by elution with 10:1 EtOAc : MeOH to afford the product as a light yellow oil (7.6 g, 73%). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 1.37 (m, 4H), 1.59 (m, 4H), 3.45 (dt, 4H), 3.57 (t, 2H), 3.65 (m, 20H), 3.73 (t, 2H), 4.50 (s, 2H), 7.28 (t, 1H), 7.34 (m, 4H). MALDI-MS: *m/z* 495.5995 ($[M + Na]^+$, calcd for $C_{25}H_{44}O_8Na$ 495.6018).

1-phenyl-2,9,12,15,18,21,24-heptaoxahexacosan-26-yl 4-methylbenzenesulfonate (2). Compound **1** (5.4 g, 11.5 mmol) was dissolved in anhydrous pyridine (30 mL) and cooled to 0°C in an ice water bath. Tosyl chloride (4.38 g, 23.0 mmol) was added to the solution and the resulting mixture was stirred at 0°C for 1 hr. After this period, the reaction was placed in a 4°C refrigerator overnight (~16 hr). The reaction was then poured into 1 N HCl (150 mL), the water extracted with EtOAc (4 x 30 mL), the organic fractions pooled, dried over $MgSO_4$, and concentrated on a rotary evaporator to yield a yellow oil. This oil was purified via flash chromatography on silica gel by using 1:1 hexane :

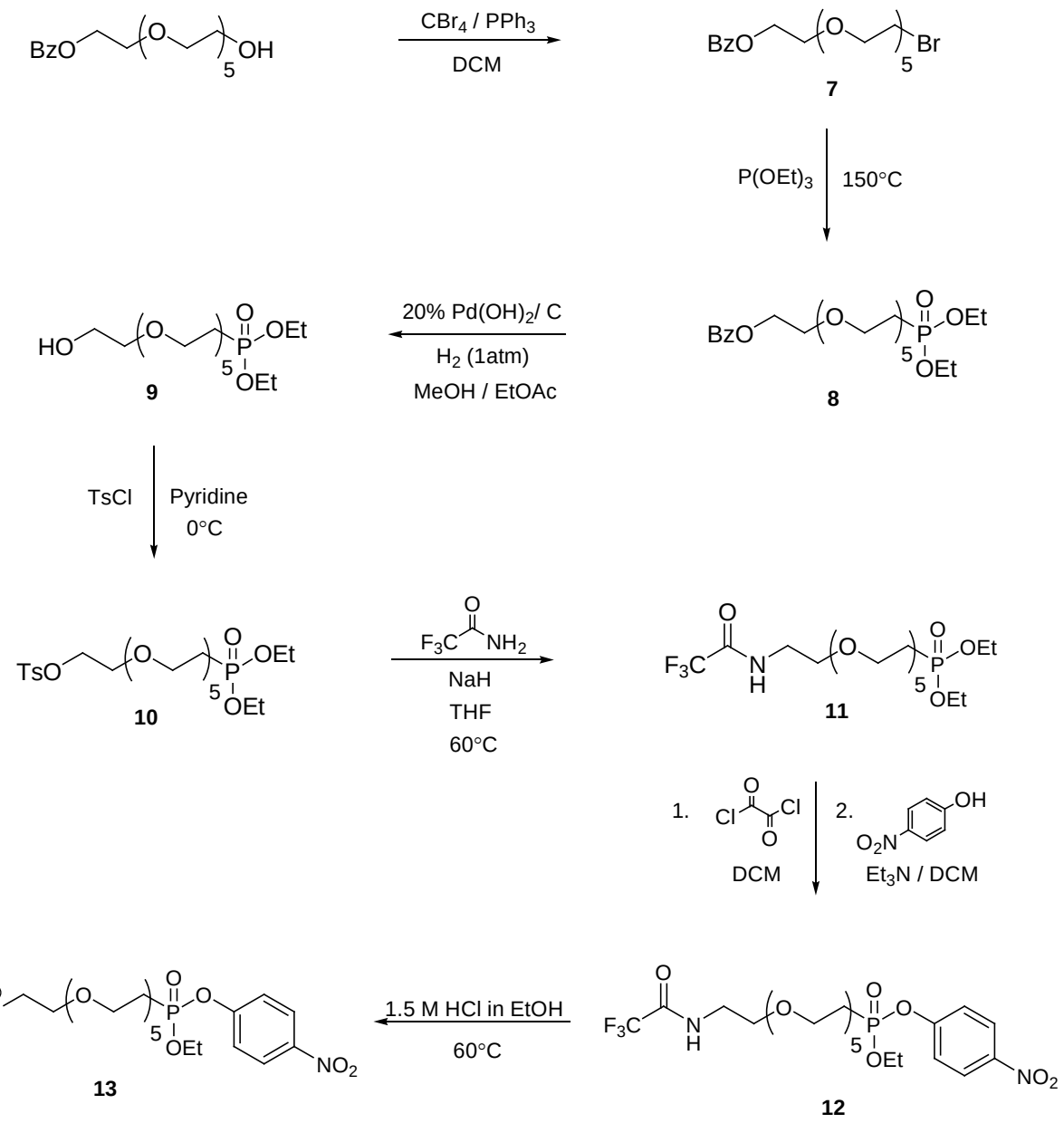
EtOAc first to remove non-polar impurities and then by elution with EtOAc to afford a nearly colorless yellow oil (4.81 g, 67%): ^{1}H NMR ($CDCl_3$, 500 MHz): δ 1.36 (m, 4H), 1.59 (m, 4H), 2.45 (s, 3H), 3.45 (dt, 4H), 3.58 (m, 6H), 3.64 (m, 14H), 3.68 (d, 2H), 4.16 (t, 2H), 4.5 (s, 2H), 7.28 (t, 1H), 7.34 (m, 6H), 7.8 (d, 2H). MALDI-MS: *m/z* 649.7060 ($[M + Na]^+$ calcd for $C_{32}H_{50}O_{10}SNa$ 649.7882).

2,2,2-trifluoro-N-(1-phenyl-2,9,12,15,18,21,24-heptaoxahexacosan-26-yl)acetamide (3). 2,2,2-Trifluoroacetamide (912 mg, 8.07 mmol) was dissolved in anhydrous THF (40 mL) and cooled to 0°C in an ice water bath. To this solution at 0°C was added hexane-washed and vacuum dried NaH (258 mg, 10.76 mmol from 60% dispersion in mineral oil) in three portions resulting in vigourous evolution of hydrogen gas. The mixture was stirred another 5 min until the evolution of gas ceased and to this mixture added compound **2** (3.37 g, 5.38mmol) dissolved in anhydrous THF (10 mL). The reaction was then fitted with a reflux condenser and heated to 60°C in an oil bath and held at this temperature for 14 hr. After this period, the slightly green reaction mixture was allowed to cool to room temperature and the excess hydride quenched by the addition of saturated $NH_4Cl_{(aq)}$ (2 mL). This was then poured into water (50 mL), extracted with EtOAc (3 x 15 mL), the resulting organic fractions pooled, dried over $MgSO_4$ and concentrated via rotary evaporator to yield a colorless oil. This oil was then purified via flash chromatography on silica by using 1:1 hexane : EtOAc first to remove any non-polar impurities and then by elution with EtOAc to afford a colorless oil (1.9 g 62%): ^{1}H NMR ($CDCl_3$, 500 MHz): δ 1.37 (m, 4H), 1.60 (m, 4H), 3.45 (dt, 4H), 3.57 (m, 4H), 3.65 (m, 20H), 4.50 (s, 2H), 7.28 (t, 1H), 7.34 (m, 4H), 7.77 (bs, 1H). MALDI-MS: *m/z* 590.5535 ($[M + Na]^+$, calcd for $C_{27}H_{44}F_3NO_8Na$ 590.6251).

2,2,2-trifluoro-N-(24-hydroxy-3,6,9,12,15,18-hexaoxatetracosyl)acetamide (4). Compound **3** (1.7 g, 2.99 mmol) was dissolved in nitrogen-sparged 1:1 EtOAc : MeOH (20 mL). To this solution was added 20% $Pd(OH)_2$ on carbon (wet) (100 mg) and the reaction was stirred under an atmosphere of H_2 (1 atm) for 14 hr. After this period, the reaction was filtered through a pad of celite, and concentrated in vacuo to yield a colorless oil that was used without further purification (1.4 g, 98%): ^{1}H NMR ($CDCl_3$, 500 MHz): δ 1.38 (m, 4H), 1.59 (m, 4H), 2.84 (m, 3H), 3.47 (t, 2H), 3.59 (m, 4H), 3.65 (m, 20H), 8.02 (br s, 1H). MALDI-MS: *m/z* 500.5385 ($[M + Na]^+$, calcd for $C_{20}H_{38}F_3NO_8Na$ 500.5026).

N-(24-chloro-3,6,9,12,15,18-hexaoxatetracosyl)-2,2,2-trifluoroacetamide (5). Thionyl chloride (597 mg, 364 μL, 5.02 mmol) was added to a solution of compound **4** (1.2 g, 2.51 mmol) in dry toluene (25 mL). The mixture was heated to 90°C in a sand bath for 4 hr with stirring. After this period, the reaction was allowed to cool to room temperature and the solvent removed in vacuo on a rotary evaporator. The resulting yellow oil was purified via flash chromatography on silica by using 2:1 hexane : EtOAc first to remove non-polar impurities and then by elution with EtOAc to afford the product as a golden oil (1.04 g, 83%): ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.35 (quintet, 2H), 1.43 (quintet, 2H), 1.59 (quintet, 2H), 1.77 (quintet, 2H), 3.46 (t, 2H), 3.55 (m, 6H), 3.65 (m, 20H), 7.76 (br s, 1H). MALDI-MS: *m/z* 518.4673 ($[M + Na]^+$, calcd for $C_{20}H_{37}ClF_3NO_8Na$ 518.2108).

24-chloro-3,6,9,12,15,18-hexaoxatetracosan-1-aminium chloride (6). Compound **5** (528 mg, 1.06 mmol) was dissolved in 1.25 M HCl in EtOH (30 mL) and heated to 60°C in a sand bath with stirring for 3 hr. After this period, the reaction was concentrated on a rotary evaporator to yield a yellow oil. This oil was taken up in EtOH (30 mL) and concentrated again on a rotary evaporator. This process was repeated until pH paper yielded only a faint trace of HCl vapor in the sample. The resulting oil was purified via flash chromatography on a small plug of silica by using 10:1 EtOAc : MeOH first to remove impurities then by elution with MeOH to afford the product as a yellow oil (460 mg, 99%): ^{1}H NMR (CD_3OD, 500MHz) δ 1.39 (quintet, 2H), 1.46 (quintet, 2H), 1.61 (quintet, 2H), 1.76 (quintet, 2H), 3.15 (t, 2H), 3.49 (t, 2H), 3.55 (t, 2H), 3.59 (m, 2H), 3.61 (m, 16H), 3.71 (m, 2H), 3.76 (m, 2H). No signals observed for the aminium group. MALDI-MS: *m/z* 400.4512 ($[M + H]^+$, calcd for $C_{18}H_{39}ClNO_6$ 400.9578).



19-bromo-1-phenyl-2,5,8,11,14,17-hexaoxanonadecane (7). Monobenzyl hexaethylene glycol (2.0 g, 5.37 mmol), and CBr_4 (3.56 g, 10.74 mmol) were dissolved in CH_2Cl_2 (30 mL) and cooled to 0°C in an ice water bath. To this solution was added triphenylphosphine (2.82 g, 10.74 mmol), the reaction allowed to warm to room temperature, and stirred for 14 hr. After this period, the reaction was concentrated on a rotary evaporator to yield a crude white oil. This oil was resuspended in Et_2O to precipitate triphenylphosphine oxide, filtered, concentrated, and then purified via flash chromatography first using a gradient of 10:1 → 3:1 hexane : ethyl acetate to remove non-polar impurities then by elution with 1:1 hexane : EtOAc to yield a colorless oil (1.98 g, 85%): ^{1}H NMR ($CDCl_3$, 500MHz): δ 3.47 (t, 2H), 3.67 (m, 20H), 3.81 (t, 2H), 4.57 (s, 2H), 7.28 (m, 1H), 7.35 (m, 4H). MALDI-MS: *m/z* 458.4077 ([M + Na]$^+$, calcd for $C_{19}H_{31}BrO_6Na$ 458.3396).

Diethyl 1-phenyl-2,5,8,11,14,17-hexaoxanonadecan-19-yl-phosphonate (8). Compound **7** (2.61 g, 6.00 mmol) was dissolved in triethyl phosphite (20 mL, excess), the reaction flask fitted with a reflux condenser and the mixture heated to 150°C for 4 hr. After this period, the mixture was allowed first to cool to room temperature and then the excess triethyl phosphite removed by short path distillation under reduced pressure at 70°C. The resulting clear and colorless oil was then purified via flash chromatography on silica by using EtOAc first to remove non-polar impurities then by elution with 10:1 EtOAc : MeOH to afford the product as a light yellow oil (2.57 g, 87%): ^{1}H NMR ($CDCl_3$,

500MHz): δ 1.32 (t, 6H), 2.13 (dt, 2H), 3.66 (m, 20H), 3.72 (m, 2H), 4.10 (m, 4H), 4.57 (s, 2H), 7.28 (m, 1H), 7.33 (m, 4H). MALDI-MS: *m/z* 515.5947 ([M + Na]$^+$, calcd for $C_{23}H_{41}O_9PNa$ 515.5298).

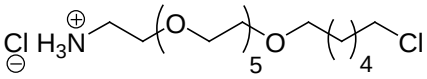
Diethyl 17-hydroxy-3,6,9,12,15-pentaoxaheptadecylphosphonate (9). Compound **7** (1.98 g, 4.02 mmol) was dissolved in 20mL of nitrogen-sparged 1:1 EtOAc : MeOH. To this solution was added 20% $Pd(OH)_2$ on carbon (wet) (100 mg) and the reaction was stirred under an atmosphere of H_2 (1 atm) for 14 hr. After this period, the reaction was filtered through a pad of celite, and concentrated in vacuo to yield a colorless oil that was used without further purification (1.49 g, 92%): ^{1}H NMR ($CDCl_3$, 500MHz): δ 1.31 (t, 6H), 2.13 (dt, 2H), 3.65 (m, 19H), 3.72 (m, 4H), 4.09 (m, 4H). MALDI-MS: *m/z* 425.5341 ([M + Na]$^+$, calcd for $C_{16}H_{35}O_9PNa$ 425.4072).

17-(diethoxyphosphoryl)-3,6,9,12,15-pentaoxaheptadecyl 4-methylbenzenesulfonate (10). Compound **9** (1.25 g, 3.11 mmol) was dissolved in anhydrous pyridine (15 mL) and cooled to 0°C in an ice water bath. Tosyl chloride (1.19 g, 6.22 mmol) was added to the solution and the resulting mixture was stirred at 0°C for 1 hr. After this period, the reaction was placed in a 4°C refrigerator overnight (~14 hr). The reaction was then poured into 1 N HCl (50 mL), the water extracted with EtOAc (3 x 10 mL), the organic fractions pooled, dried over $MgSO_4$, and concentrated on a rotary evaporator to yield a yellow oil. This oil was purified via flash chromatography on silica gel by using EtOAc first to remove non-polar impurities then by elution with 20:1 EtOAc: MeOH to afford a light yellow oil (1.20 g, 69%): ^{1}H NMR ($CDCl_3$, 500 MHz): δ 1.32 (t, 6H), 2.13 (dt, 2H), 2.45 (s, 3H), 3.64 (m, 20H), 4.11 (m, 4H), 4.16 (m, 2H), 7.34 (d, 2H), 7.80 (d, 2H): MALDI-MS: *m/z* 579.4820 ([M + Na]$^+$, calcd for $C_{23}H_{41}O_{11}PSNa$ 579.5936).

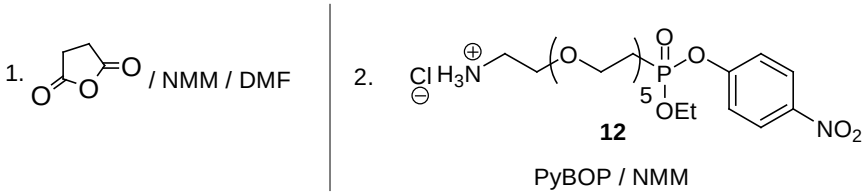
Diethyl 1,1,1-trifluoro-2-oxo-6,9,12,15,18-pentaoxa-3-azaicosan-20-yl-phosphonate (11). 2,2,2-Trifluoroacetamide (305 mg, 2.70 mmol) was dissolved in anhydrous THF (40 mL) and cooled to 0°C in an ice water bath. To this solution at 0°C was added hexane-washed and vacuum dried NaH (86 mg, 3.58 mmol from 60% dispersion in mineral oil) in three portions resulting in vigourous evolution of hydrogen gas. The mixture was stirred another 5 min until the evolution of gas ceased and to this mixture added compound **10** (1.00 g, 1.80 mmol) dissolved in anhydrous THF (5 mL). The reaction was then fitted with a reflux condenser and heated to 60°C in an oil bath and held at this temperature for 14 hr. After this period, the slightly green reaction mixture was allowed to cool to room temperature and the excess hydride quenched by the addition of saturated $NH_4Cl_{(aq)}$ (2 mL). This was then poured into water (50 mL), extracted with EtOAc (3 x 15 mL), the resulting organic fractions pooled, dried over $MgSO_4$ and concentrated via rotary evaporator to yield a colorless oil. This oil was then purified via flash chromatography on silica by using 1:1 hexane : EtOAc first to remove any non-polar impurities then by elution with EtOAc to afford a colorless oil (1.9 g 62%): ^{1}H NMR ($CDCl_3$, 500 MHz): δ 1.32 (t, 6H), 2.13 (m, 2H), 3.54 (m, 2H), 3.65 (m, 20H), 4.10 (m, 4H), 7.84 (broad s, 1H): MALDI-MS: *m/z* 520.3158 ([M + Na]$^+$, calcd for $C_{18}H_{35}F_3NO_9PNa$ 520.4305).

Ethyl 4-nitrophenyl 1,1,1-trifluoro-2-oxo-6,9,12,15,18-pentaoxa-3-azaicosan-20-ylphosphonate (12). Compound **11** (536 mg, 1.08 mmol) was dissolved in CH_2Cl_2 (20 mL) and to the solution was added oxalyl chloride (282 μL, 410 mg, 3.23 mmol) resulting in an immediate and rapid evolution of gas. The reaction was vented and stirred for 5 hr. After this period, the reaction was concentrated on a rotary evaporator and excess volatiles removed with stirring under high vacuum overnight (~14 hr). The resulting orange oil was redissolved in CH_2Cl_2 (20 mL) and cooled to 0°C in an ice water bath. To this solution was then added dropwise over 2 min a solution of 4-nitrophenol (150 mg, 1.08 mmol) and triethylamine (188 μL, 140 mg, 1.08 mmol) in CH_2Cl_2 (5 mL). The reaction was allowed to come to room temperature with stirring and continued until TLC of the reaction showed no more progress, approximately 1 hr. The reaction was then concentrated on a rotary evaporator to yield a deep orange / brown oil. This oil was then purified via flash chromatography on a short column of silica using CH_2Cl_2 first to remove unreacted 4-nitrophenol and other yellow impurities and then by elution with 10:1 CH_2Cl_2 : MeOH to yield the product as an orange oil (507 mg, 79%): ^{1}H NMR ($CDCl_3$, 400MHz) δ 1.33 (t, 3H), 2.32 (dt, 2H), 3.55 (m, 2H), 3.62 (m, 18H), 3.82 (dt, 2H), 4.25 (m, 2H), 7.41 (d, 2H), 7.61 (broad s, 1H), 8.24 (d, 2H): MALDI-MS *m/z* 613.3841 ([M + Na]$^+$, calcd for $C_{22}H_{34}F_3N_2O_{11}PNa$ 613.4709).

17-(ethoxy(4-nitrophenoxy)phosphoryl)-3,6,9,12,15-pentaoxaheptadecan-1-aminium chloride (13). Compound **12** (346 mg, 0.59 mmol) was dissolved in 1.25 M HCl in EtOH (20 mL) and heated to 55°C with stirring for 5 hr. After this period, the reaction was concentrated on a rotary evaporator to yield a dark orange oil. This oil was taken up in EtOH (30 mL) and concentrated again on a rotary evaporator. This process was repeated until pH paper yielded only a faint trace of HCl vapor in the sample. The resulting oil was purified via flash chromatography on a small plug of silica by using 10:1 EtOAc : MeOH first to remove impurities then by elution with MeOH to afford the product as a deep yellow oil (254 mg, 82%): ^{1}H NMR ($CDCl_3$, 500MHz) δ 1.32 (t, 3H), 2.43 (m, 2H), 3.15 (broad m, 2H), 3.65(m, 16H), 3.75 (m, 2H), 3.88 (m, 2H), 4.22 (m, 2H), 7.41 (d, 2H), 7.88 (broad s, 3H), 8.25 (d, 2H): MALDI-MS *m/z* 495.8415 ($[M + H]^+$, calcd for $C_{20}H_{36}N_2O_{10}P$ 495.4805).

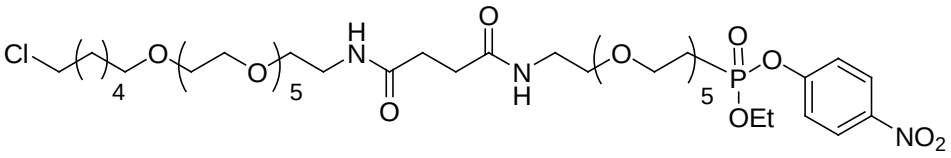


6



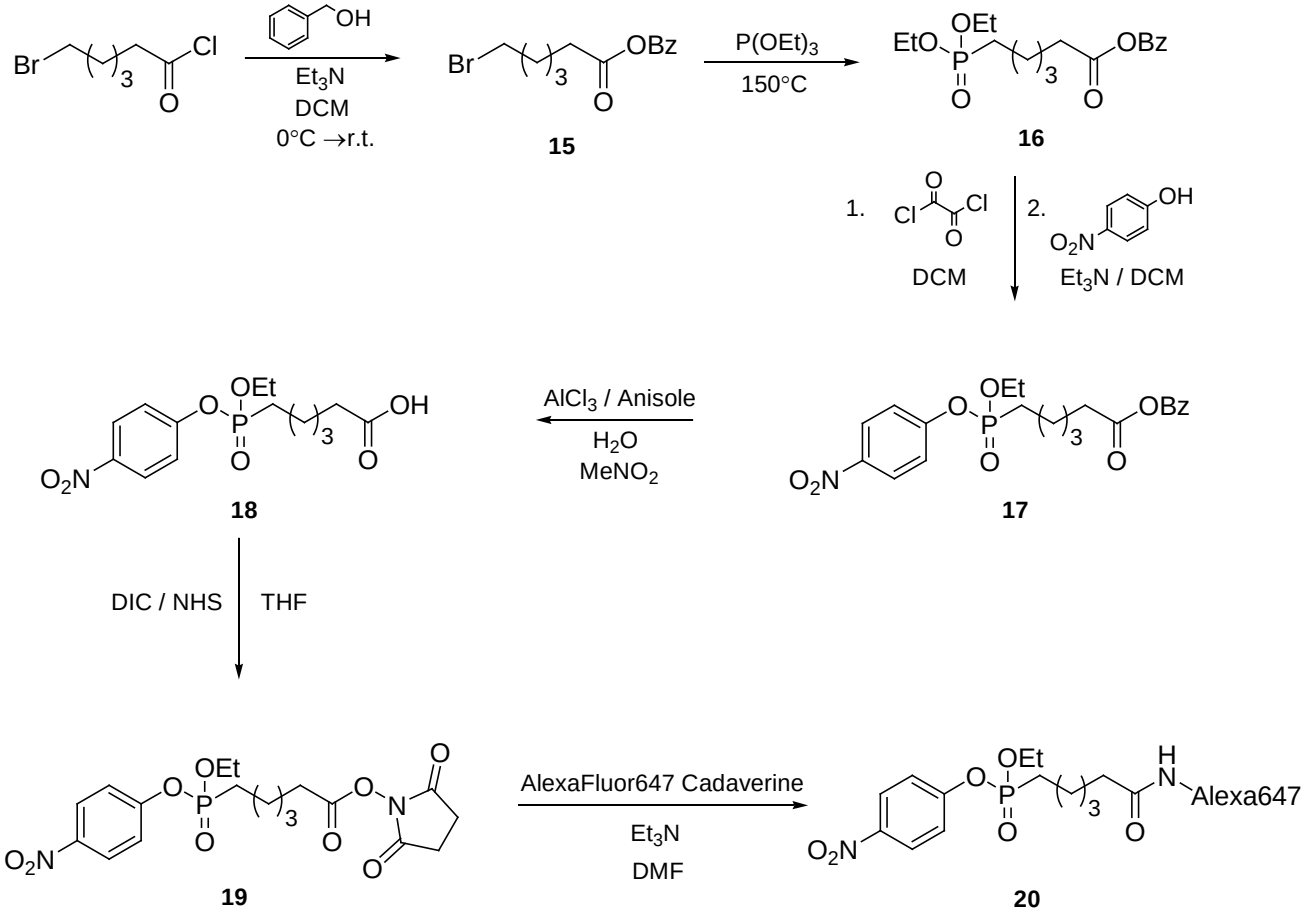
12

PyBOP / NMM



14

ethyl (4-nitrophenyl) (47-chloro-19,22-dioxo-3,6,9,12,15,26,29,32,35,38,41-undecaoxa-18,23-diazaheptatetracontyl)phosphonate (14). Compound **6** (20 mg, 46 μmol) and succinic anhydride (4.6 mg, 46 μmol) were dissolved in DMF (5 mL). To this solution was added 4-methylmorpholine (5.1 μL, 4.7 mg, 46 μmol), and the reaction allowed to stir for 3 0min. After this period, PyBOP (29 mg, 55 μmol) was added followed by 4-methylmorpholine (5.1 μL, 4.7 mg, 46 μmol) and the reaction allowed to stir for another 30 min. To the solution of the activated ester was then added compound **13** (24 mg, 46 μmol) dissolved in DMSO (24 μL) followed by 4-methylmorpholine (5.1 μL, 4.7 mg, 46 μmol). The reaction was then allowed to stir o/n. The majority of solvent was then stripped off the reaction by passing a stream of N_2 over the flask for 2 hr. The resulting light yellow oil was then redissolved in CH_2Cl_2 and purified via flash chromatography on silica by using 20:1 EtOAc : MeOH first to remove any non-polar impurities then by elution with 5:1 EtOAc : MeOH to afford a light yellow oil (11 mg, 24%): ^{1}H NMR ($CDCl_3$, 500 MHz): δ 1.33 (t, 3H), 1.36 (m, 2H), 1.44 (m, 2H), 1.58 (quintet, 2H), 1.76 (quintet, 2H), 2.32 (m, 4H), 3.45 (m, 6H), 3.52-3.65 (m, 44H), 3.82 (m, 2H), 4.24 (m, 2H), 6.75 (broad s, 2H), 7.41 (d, 2H), 8.23 (d, 2H): ^{31}P NMR (DMSO, 500 MHz): δ 29.39 MALDI-MS *m/z* 999.0272 ($[M + Na]^+$, calcd for $C_{42}H_{75}N_3O_{18}PNa$ 999.4707).



Benzyl 6-bromohexanoate (15). A solution of 6-bromohexanoyl chloride (3.00 g, 14.05 mmol) dissolved in CH_2Cl_2 (125 mL) was cooled to 0°C in an ice water bath. To this solution was added benzyl alcohol (1.52 g, 14.05 mmol) followed by triethylamine (1.96 mL, 1.42 g, 14.05 mmol). The reaction was allowed to stir for 3 hr. After this period, the reaction was concentrated on a rotary evaporator, the resulting thick orange oil resuspended in cold Et_2O, filtered and reconcentrated to yield an orange oil. This oil was purified via flash chromatography on silica using 10:1 hexane : EtOAc as eluent to afford a colorless oil (2.9 g 72%): ^{1}H NMR ($CDCl_3$, 500 MHz): δ 1.49 (m, 2H), 1.69 (quintet, 2H), 1.87 (quintet, 2H), 2.39 (t, 2H), 3.40 (t, 2H), 5.13 (s, 2H), 7.37 (m, 5H): HRMS: *m/z* 205.1 ($[M - Br]^+$, calcd for $C_{13}H_{17}O_2$ 205.3).

Diethyl-6-carbobenzyloxyhexyl-1-phosphonate (16). Compound **17** (2.8 g, 9.82 mmol) was dissolved in triethyl phosphite (20 mL, excess), the reaction flask fitted with a reflux condenser and the mixture heated to 150°C for 4 hr. After this period, the mixture was allowed first to cool to room temperature and then the excess triethyl phosphite removed by short path distillation under reduced pressure at 70°C. The resulting clear and colorless oil was then purified via flash chromatography using 1:2 hexane : EtOAc as eluent to afford the product as a light yellow oil (2.97 g, 88%): ^{1}H NMR ($CDCl_3$, 500MHz): δ 1.32 (t, 6H), 1.41 (m, 2H), 1.58-1.78 (m, 6H), 2.37 (t, 2H), 4.08 (m, 4H), 5.12 (s, 2H), 7.36 (m, 5H): HRMS: *m/z* 343.2 ($[M + H]^+$, calcd for $C_{17}H_{28}O_5P$ 343.4).

Ethyl 4-nitrophenyl-(6-carbobenzyloxyhexyl)-1-phosphonate (17). To a solution of compound **18** (2.87 g, 8.38 mmol) dissolved in CH_2Cl_2 (100 mL) was added oxalyl chloride (2.19 mL, 3.19 g, 25.15 mmol) which resulted in an immediate and rapid evolution of gas. The reaction was vented and stirred for 4 hr. After this period, the reaction was concentrated on a rotary evaporator and excess volatiles removed with stirring under high vacuum for 30 min. The resulting orange oil was redissolved in CH_2Cl_2 (100 mL) and cooled to 0°C in an ice water bath. To this solution was then added dropwise over 2 min a solution of 4-nitrophenol (1.22 g, 8.8 mmol) and triethylamine (1.23 mL, 890 mg, 8.8 mmol) in CH_2Cl_2 (20 mL). The reaction was allowed to come to room temperature with stirring and continued until TLC of the reaction showed no more progress, approximately 2 hr. After this period, the reaction was concentrated on a rotary evaporator, the resulting orange oil resuspended in cold Et_2O, filtered and reconcentrated to yield a dark orange oil. This oil was purified via flash chromatography on silica using

1:1 hexane : EtOAc as eluent to afford a yellow oil (3.53 g 97%): ^{1}H NMR ($CDCl_3$, 500 MHz): δ 1.32 (t, 3H), 1.46 (m, 2H), 1.69 (m, 4H), 1.91 (m, 2H), 2.38 (t, 2H), 4.14 (m, 1H), 4.23 (m, 1H), 5.12 (s, 2H), 7.35 (m, 7H), 8.23 (d, 2H): HRMS: *m/z* 436.2 ($[M + H]^+$, calcd for $C_{21}H_{27}NO_7P$ 436.4).

Ethyl 4-nitrophenyl-(6-carboxyhexyl)-1-phosphonate (18). To a solution of compound **19** (3.43 g, 7.88 mmol) and anisole (2.56 g, 23.63 mmol) in nitromethane (60 mL) was added $AlCl_3$ (3.15 g, 23.63 mmol). The resulting deep red reaction was stirred at r.t. for 1.5 hr. After this period H_2O (20 mL) was carefully added and the mixture stirred vigorously for 20 min. The reaction was then poured into water (100 mL) in a separatory funnel and the aqueous phase extracted with Et_2O (3 x 50 mL). The organic fractions were pooled and dried over $MgSO_4$ and filtered to yield an orange solution consisting mostly of the product dissolved in nitromethane. This excess nitromethane was removed by passing a stream of N_2 over the flask for 12 hr. The resulting orange oil was purified via flash chromatography on silica using 1:1 hexane : EtOAc as eluent to afford an off-white solid (2.72 g 47%): ^{1}H NMR ($CDCl_3$, 500 MHz): δ 1.32 (t, 3H), 1.48 (m, 2H), 1.66 (m, 2H), 1.72 (m, 2H), 1.94 (m, 2H), 2.35 (t, 2H), 4.17 (m, 1H), 4.24 (m, 1H), 7.38 (d, 2H), 8.23 (d, 2H), 8.96 (v broad s, 1H): HRMS: *m/z* 346.1 ($[M + H]^+$, calcd for $C_{14}H_{21}NO_7P$ 346.3).

2,5-dioxopyrrolidin-1-yl 6-(ethoxy(4-nitrophenoxy)phosphoryl)hexanoate (19). To a solution of compound **20** (200 mg, 0.58 mmol) and N-hydroxysuccinimide in dry distilled THF (10 mL) was added diisopropylcarbodiimide (99 μL, 80 mg, 0.64 mmol). This reaction was stirred overnight at r.t. After this period, the mixture was filtered and concentrated to yield a brown oil. This oil was purified via flash chromatography on silica using EtOAc as eluent to afford a brown / yellow oil (174 mg, 68%): ^{1}H NMR ($CDCl_3$, 500 MHz): δ 1.32 (t, 3H), 1.55 (m, 2H), 1.78 (m, 4H), 1.95 (m, 2H), 2.62 (t, 2H), 2.84 (broad d, 4H), 4.17 (m, 1H), 4.24 (m, 1H), 7.38 (d, 2H), 8.24 (d, 2H): HRMS: *m/z* 443.2 ($[M + H]^+$, calcd for $C_{18}H_{24}N_2O_9P$ 443.4).

AlexaFluor 647 cadaverine-[21] conjugate (20). A solution of compound **21** (10 mg, 23 μmol) in DMSO (230 μL) was added to an aliquot of AlexaFluor 647 cadaverine sodium salt (1 mg, 1.01 μmol). The mixture was shaken and left at r.t. overnight. After this period, the mixture was diluted with DIUF (270 μL) and injected onto a C_{18} semi-prep scale column via a Waters Delta 4000 HPLC and purified using a linear gradient of 75% aqueous ACN + 0.1% TFA over 70 min with DIUF + 0.1% TFA as the mobile phase at a flow rate of 10 mL / min. Fractions were collected at a rate of 1 / min. Fractions around 45 min were then analyzed by MALDI. Those containing clean product were pooled, frozen at -80°C and lyophilized to yield a blue solid (~ 1 mg, 16%): MALDI-MS: *m/z* 1270.4658 ($[M (diacid) + H]^+$, calcd for $C_{55}H_{76}N_5O_{19}PS_4$ 1270.4483).

Protein Oligomer Synthesis.

All proceeding reactions were carried out in the following buffer: 25 mM Tris HCl, 150 mM NaCl, 0.05% NaN_3, pH 7.8 @ 25°C at room temperature. Stock solutions of compounds **20** (10 mM) and **14** (1 mM) were prepared using DMSO as diluent. All size-exclusion purification steps of products were carried out using the aforementioned buffer as the mobile phase at 4°C on an Akta FPLC. Yields listed below correspond to the molar mass of oligomeric protein products isolated as > 95% pure and not crude yields for the reaction.

HaloTag-Cutinase Dimer (HC_2). HaloTag-Cutinase (HC) (120 nmol, 1.2 mL, 100 μM) was treated with compound **20** (12 μL, 120 nmol) at room temperature for 2 hr. This reaction resulted in blocking of the cutinase activity by covalent modification of the active site serine. After this period, compound **14** (120 μL, 120 nmol) was added to the solution and allowed to react 1 hr. Free HC (1.2 mL, 100 μM) was then added and allowed to react overnight. After this period, the reaction was concentrated to 2 mL and applied to a Hi-Load Superdex 200 16/60 gel filtration column using a flow rate of 1 mL / min. Fractions containing pure HC_2, as determined by SDS-PAGE were pooled and concentrated to ~1 mL using a 5 mL Amicon ultrafiltration unit. The concentration of dimer was determined in this solution by dividing the measured absorbance at 280 nm (via NanoDrop UV / Vis spectrometer) by the calculated extinction coefficient for this species, ε = 148740 $M^{-1}cm^{-1}$. (92.4 nmol, 77%) MALDI-MS: *m/z* 119203.02, calcd for $C_{5356}H_{8308}N_{1466}O_{1573}P_2S_{30}$, 119429 g / mol

HaloTag-Cutinase Trimer (HC_3). **HC_2** (90 nmol, 1.8 mL, 50 μM) was treated with compound **14** (135 μL, 135 nmol) for 1 hr. The resulting ligand-functionalized dimer was then purified by size-exclusion chromatography on a Hi-Load Superdex 200 column. Fractions corresponding to the ligand-modified dimer were then concentrated to 1 mL in a 5 mL 10 kDa Amicon ultrafiltration unit into which free HC (90 nmol) was added and allowed to react overnight. After this period, the reaction was concentrated to 2 mL and applied to a Hi-Prep Sephacryl S300 26/60 gel filtration column using a flow rate of 0.5 mL / min. Fractions containing pure HC_3, as determined by SDS-PAGE were pooled and concentrated to ~1 mL using a 5 mL Amicon ultrafiltration unit. The concentration of trimer was determined in this solution by dividing the measured absorbance at 280 nm (via NanoDrop UV / Vis spectrometer) by the calculated extinction coefficient for this species, ε = 223110 M^{-1} cm^{-1}. (45 nmol, 55%) MALDI-MS: *m/z* 180926.75, calcd for $C_{8048}H_{12489}N_{2200}O_{2365}P_3S_{45}$ 179441 g / mol

HaloTag-Cutinase Tetramer (HC_4). **HC_3** (40 nmol, 1 mL, 40μM) was treated with compound **14** (60 nmol, 60 μL) for 1 hr. The resulting ligand-functionalized trimer was then purified by size-exclusion chromatography on a Hi-Load Superdex 200 column. Fractions corresponding to the ligand-modified trimer were then concentrated to 1mL in a 5 mL 10 kDa Amicon ultrafiltration unit into which free HC (40 nmol) was added and allowed to react overnight. After this period, the reaction was applied to a Hi-Load Superdex 200 16/60 column *in series* with a Hi-Prep Sephacryl S300 26/60 gel filtration column during one continuous chromatography run using a flow rate of 0.5 mL / min. Fractions containing pure HC_4, as determined by SDS-PAGE were pooled and concentrated to ~1 mL using a 5 mL Amicon ultrafiltration unit. The concentration of tetramer was determined in this solution by dividing the measured absorbance at 280nm (via NanoDrop UV / Vis spectrometer) by the calculated extinction coefficient for this species, ε = 297480 M^{-1} cm^{-1}. (15 nmol, 30%) MALDI-MS: *m/z* 242773.98, calcd for $C_{10740}H_{16670}N_{2934}O_{3157}P_4S_{60}$ 239453 g/mol

HaloTag-Cutinase Pentamer (HC_5). **HC_4** (10 nmol, 0.5 mL, 20 μM) was treated with compound **14** (25 nmol, 25 μL) for 1 hr. The resulting ligand-functionalized tetramer was then purified by size-exclusion chromatography on a Hi-Load Superdex 200 column. Fractions corresponding to the ligand-modified tetramer were then concentrated to 1 mL in a 5 mL 10 kDa Amicon ultrafiltration unit into which 10 nmol of free HC was added and allowed to react overnight. After this period, the reaction was applied to a Hi-Load Superdex 200 16/60 column *in series* with a Hi-Prep Sephacryl S300 26/60 gel filtration column during one continuous chromatography run using a flow rate of 0.5 mL / min. Fractions containing pure HC_5, as determined by SDS-PAGE were pooled and concentrated to ~1 mL using a 5 mL Amicon ultrafiltration unit. The concentration of pentamer was determined in this solution by dividing the measured absorbance at 280 nm (via NanoDrop UV / Vis spectrometer) by the calculated extinction coefficient for this species, ε = 371850 M^{-1} cm^{-1}. (900 pmol, 9%) MALDI-MS: *m/z* 305455.04, calcd for $C_{13432}H_{20860}N_{3668}O_{3949}P_5S_{75}$ 299465 g/mol