Supplementary Material (ESI) for Organic & Biomolecular Chemistry
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Supporting Information

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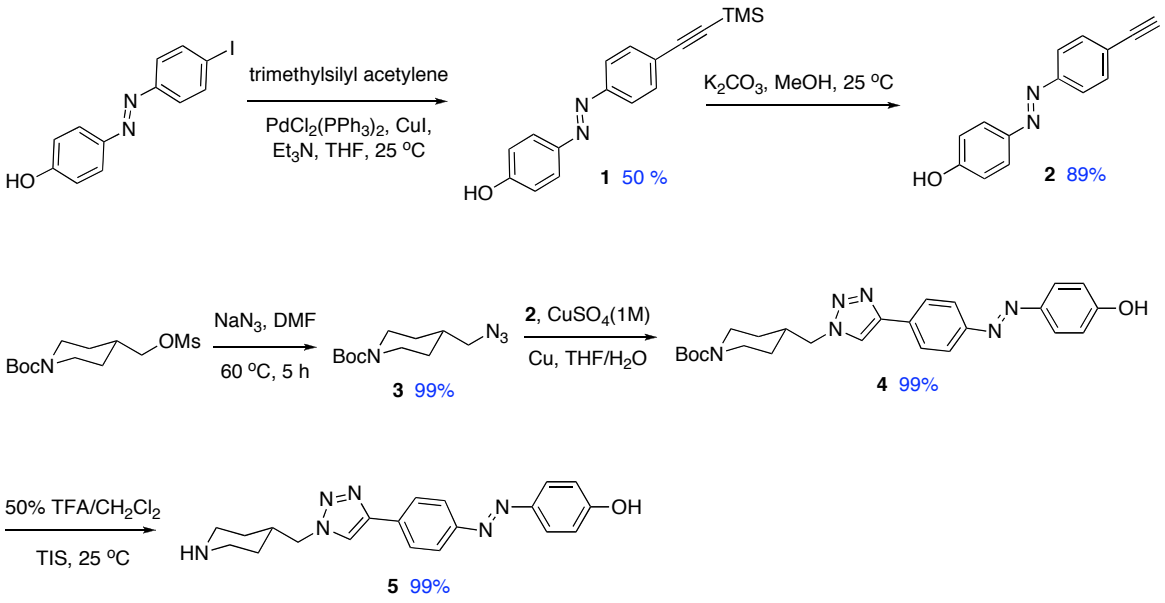
1. General Procedures

Anion Exchange Resin, IONAC A-554, Cl^- Form, type II, beads (16-50 mesh) were bought from Sigma-Aldrich. Dry DMF, (< 50 ppm water) was purchased from Acros. THF was dried with molecular sieves and Et_3N was distilled from CaH_2. Other solvents and reagents were used as received. All reactions were carried out under an atmosphere of dry nitrogen. Unless otherwise indicated, common reagents or materials were obtained from commercial source and used without further purification.

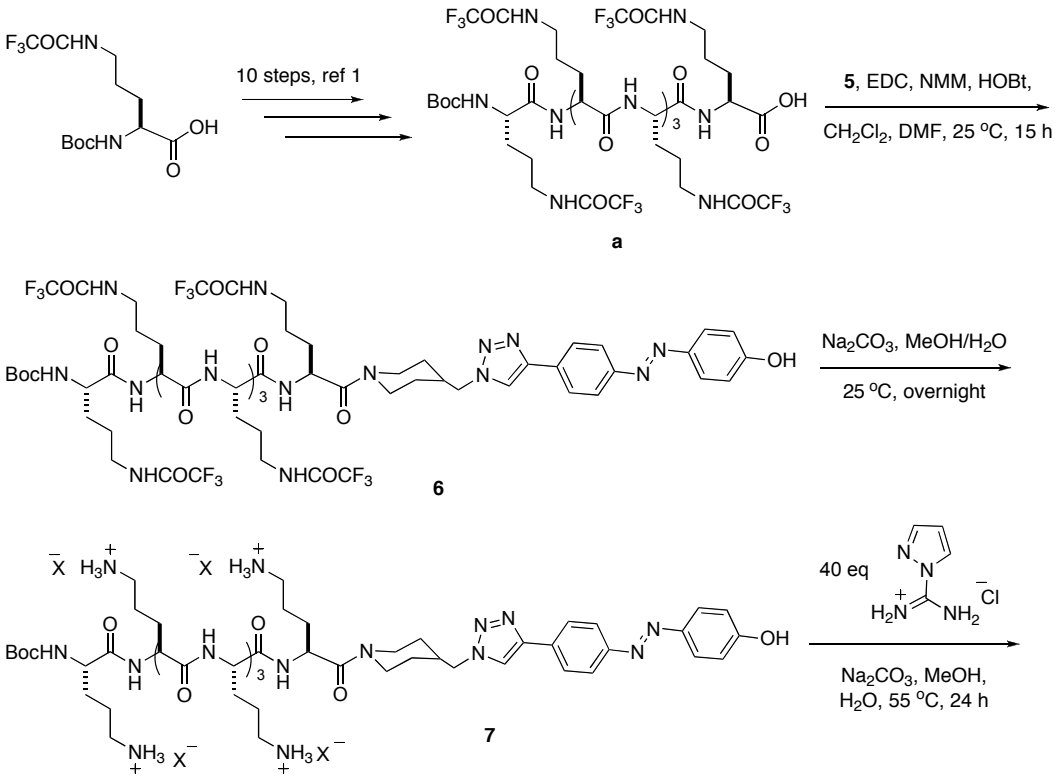
NMR spectra were recorded on a VXP-300 MHz and Inova-500 MHz spectrometers (^1H at 300 MHz or 500 MHz, and ^{13}C at 75 or 125 MHz) at room temperature unless other mentioned. Chemical shifts of ^1H NMR spectra were recorded and chemical shifts are reported in ppm from the solvent resonance ($CDCl_3$ 7.26 ppm, CD_3OD 3.30 ppm, CD_3SOCD_3 2.49 ppm). Data are reported as follows: chemical shift, multiplicity (s = singlet, bs = broad singlet d = doublet, t = triplet, q = quartet, m = multiplet), coupling constants, and number of protons. Proton decoupled ^{13}C NMR spectra were also recorded in ppm from tetramethylsilane resonance ($CDCl_3$ 77.0, CD_3OD 49.1, DMSO-d_6 39.5 ppm). Analytical thin layer chromatography (TLC) was performed on EM Reagents 0.25 mm silica-gel 60-F plates, and visualized with UV light or stained with $KMnO_4$. Flash chromatography was performed using silica gel 60 (230–400 mesh). HPLC (Beckman Coulter) analysis of samples was subjected to reverse phase analytical HPLC column [protein & peptide C18, VYDAC] eluting with solvents CH_3CN (0.1 % TFA) and H_2O (0.1 % TFA). Some compounds were purified using prep HPLC ((Beckman Coulter, X Terra Prep-MS-C18 Column, 5mm, 19 x 160 mm) eluting with solvents A (H_2O, 0.1 % TFA) and B (CH_3CN, 0.1 % TFA). MS were measured under ESI or MALDI conditions.

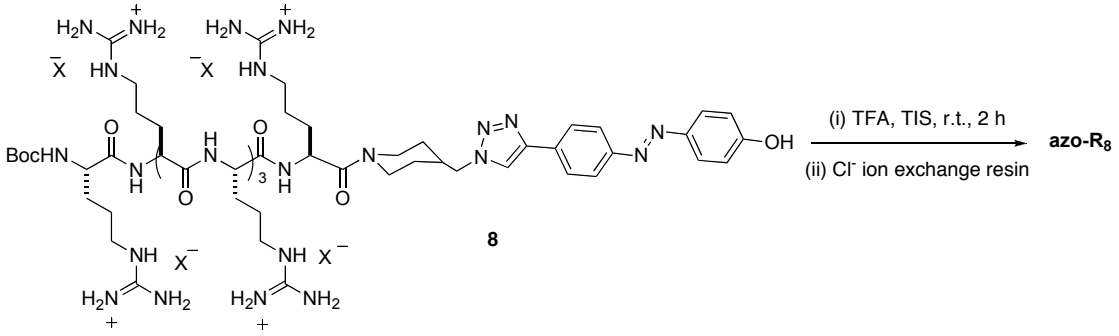
2. Synthetic scheme for azo-R_8 and R_8

i. Synthesis of azo-triazolyl piperidin 5

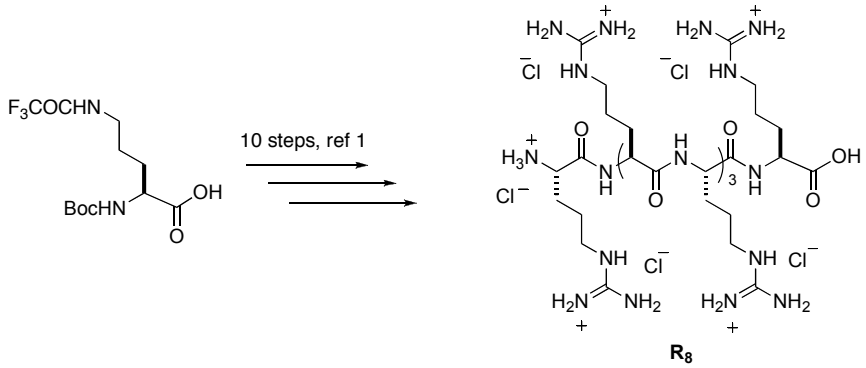


ii Synthesis of azo-R_8



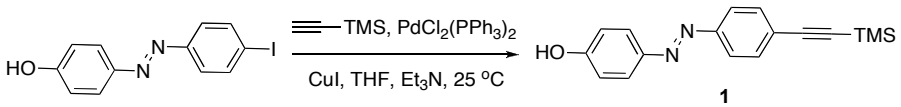


iii. Synthesis of R_8



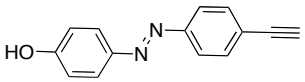
Synthesis of R_8 was according the literature procedure.[1]

3. Preparation and Characterization of Compounds 1 - 8 and azo-R_8



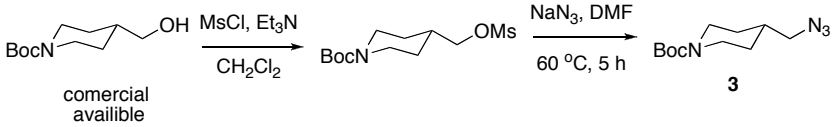
1

(*E*)-4-((4-((Trimethylsilyl)ethynyl)phenyl)diazenyl)phenol (1). (*E*)-4-((4-iodophenyl)diazenyl)phenol (257 mg, 0.793 mmol), which was prepared by the coupling reaction of phenol and 4-iodophenyl diazonium salt, trimethylsilyl acetylene (1.12ml, 7.93 mmol), $PdCl_2(PPh_3)_2$ (28 mg, 0.04 mmol), CuI (15 mg, 0.08 mmol), Et_3N (1.1 ml, 7.93 mmol) and 8 ml THF were added into a 50 mL round bottom flask. After degassed three times via the freeze-thaw method, the mixture was stirred at r.t. for 12 h. The reaction solvent was removed under reduced pressure and the crude product was purified by flash column eluting with 30% hexane : ethyl acetate to give the desired product as a red solid 115 mg (49%). ^{1}H NMR (300 MHz, $CDCl_3$), δ 7.89 (d, *J* = 9.0 Hz, 2H), 7.82 (d, *J* = 8.1 Hz, 2H), 7.59 (d, *J* = 8.1 Hz 2H), 6.96 (d, *J* = 6.3 Hz, 2H), 0.27 (s, 9H).

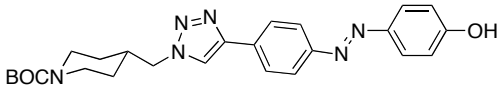


2

(*E*)-4-((4-Ethynylphenyl)diazenyl)phenol (2). Compound **1** (78 mg, 0.265 mmol) was dissolved in methanol (20 mL) in a 50 mL flask. Then potassium carbonate (110 mg, 0,796 mmol) was added to the solution and the mixture was stirred at r.t. until the reaction went to completion. The solvent was removed under reduced pressure. And the product was dissolved in CH_2Cl_2 (50 mL), which was then washed with water (25 mL x 2). The organic phase was dried with $MgSO_4$ and the product was achieved as a pure orange solid after removing the organic solvent under reduced pressure. ^{1}H NMR (300 MHz, CD_3OD), δ 7.81 (d, *J* = 9.0 Hz, 2H), 7.78 (d, *J* = 8.5 Hz, 2H), 7.57 (d, *J* = 8.5 Hz, 2H), 6.91 (d, *J* = 9.0 Hz, 2H); ^{13}C NMR (125 MHz, CD_3OD), δ 162.6, 153.8, 147.6, 134.0, 126.3, 125.5, 123.6, 116.9, 84.2, 80.8. MS (ESI) calcd for $C_{14}H_{11}N_2O$ $(M+H)^+$ 223.25, found 223.24.

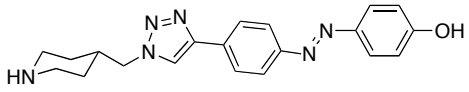


***tert*-Butyl 4-(azidomethyl)piperidine-1-carboxylate (3).** A mixture of *tert*-butyl 4-((methylsulfonyloxy)methyl)piperidine-1-carboxylate (2.90 mg, 0.1 mol, synthesized as shown in the scheme using the commercial available s.m.), NaN_3 (1.95g, .0.3 mol) and dry DMF (100 mL) in a 250 mL flask were stirred at 60 ^oC until the reaction went to completion. Water (200 mL) was added to the reaction mixture, and the product was extracted from water with ethyl acetate (50 mL x 3). The combined organics were washed with water (50 ml x 3), and then dried over MgSO4. The crude product after removing the organic solvent under reduced pressure was purified by flash chromatography eluting with hexane and ethyl acetate (4:1) to give desired product (2.37 g, 99%) as colorless oil. ^1H NMR (300 MHz, $CDCl_3$), δ 4.13 (bs, 2H), 3.19 (d, J = 6.3 Hz, 2H), 2.69 (bs, 2H), 1.73-1.66 (m, 3H), 1.45 (s, 9H), 1.24-1.10 (m, 2H). ^{13}C NMR (125 MHz, $CDCl_3$), δ 154.6, 79.3, 56.9, 43.7, 42.9, 36.4, 29.5, 28.3. MS (ESI) calcd for $C_{11}H_{21}N_4O_2^+$ $(M+H)^+$, 241.17, found, 241.16. TLC (1:1 EtOAc:hexane) R_f = 0.78.



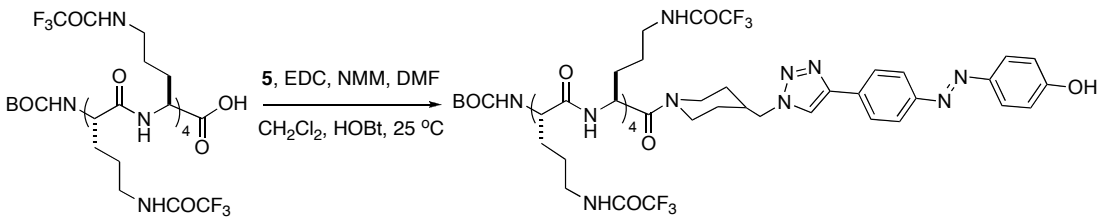
4

(*E*)-*tert*-Butyl 4-((4-(4-((4-hydroxyphenyl)diazenyl)phenyl)-1*H*-1,2,3-triazol-1-yl)methyl)piperidine-1-carboxylate (4). A mixture of terminal alkyne **2** (100 mg, 0.455 mmol), organic azide **3** (110mg, 0.455 mmol), Cu (29mg, 0.455 mmol) and $CuSO_4$ (0.046 mL, 1M) in THF/H_2O (1:1, 10 mL) was stirred in a flask for overnight. The product was isolated pure as an orange solid by filter out of Cu and other impurity through celite. ^{1}H NMR (500 MHz, CD_3OD), δ 8.19 (s, 1H), 7.92 (d, *J* = 9.0 Hz, 2H), 7.88 (d, *J* = 9.0 Hz, 2H), 7.81 (d, *J* = 9.0 Hz, 2H), 6.90 (d, *J* = 9.0 Hz, 2H), 4.32 (d, *J* = 7.5 Hz, 2H), 4.10 (bs, 1H), 4.07 (bs, 1H), 2.71 (bs, 2H), 2.18-2.09 (m, 1H), 1.63 (bs, 1H), 1.60 (bs, 1H), 1.42 (s, 9H), 1.27-1.08 (m, 2H), ^{13}C NMR (125 MHz, CD_3OD), δ 161.4, 155.8, 153.1, 147.7, 146.9, 132.6, 126.9, 125.6, 123.7, 122.5, 116.4, 80.8, 60.6, 56.1, 37.8, 30.0, 28.6. MS (ESI) calcd for $C_{25}H_{31}N_6O_3^+$ $(M+H)^+$, 463.25, found, 463.15. TLC (1:1 EtOAc-Hexane) R_f = 0.30.



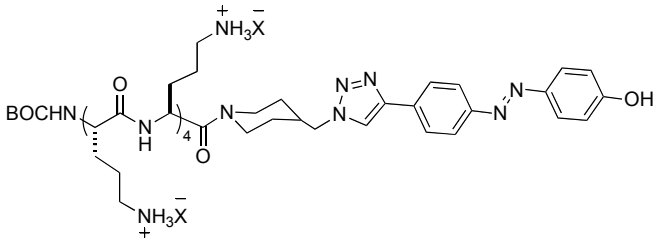
5

(*E*)-4-((4-(1-(Piperidin-4-ylmethyl)-1*H*-1,2,3-triazol-4-yl)phenyl)diazenyl)-phenol (5). BOC protected compound **4** (157 mg, 0.339 mmol), and one drop of triisopropylsilane (TIS) was dissolved in CH_2Cl_2/TFA (1:1, 20 mL) and the mixture was stirred for 5 h at room temperature. The solvent was removed under reduced pressure. Then the residue was washed with ether (10 ml x 1) to provide the desired product as a yellow solid (117 mg, 95%). ^{1}H NMR (500 MHz, CD_3OD), δ 8.44 (s, 1H), 7.97 (d, *J* = 8.5 Hz, 2H), 7.91 (d, *J* = 8.5 Hz, 2H), 7.83 (d, *J* = 8.5 Hz, 2H), 6.92 (d, *J* = 9.0 Hz, 2H), 4.45 (d, *J* = 7.0 Hz, 2H)), 3.42 (d, *J* = 13 Hz, 2H), 3.00 (td, *J* = 13 Hz, 2.5Hz, 2H), 2.37-2.29 (m, 1H), 1.90 (d, *J* = 14Hz, 2H), 1.59-1.50 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$), δ 162.4, 153.8, 147.5, 133.3, 127.3, 126.1, 124.1, 123.5, 123.5, 116.8, 55.6, 44.7, 36.0, 27.4. MS (ESI) calcd for $C_{20}H_{23}N_6O^+$ $(M+H)^+$, 363.19, found, 363.19.



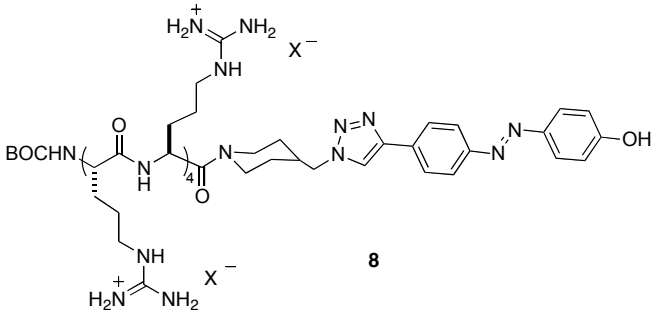
a **6**

BocNH-(OrnNHCOCF$_3$)$_8$-azo (6). To a 25 mL flask was added the known compound BocNH-(OrnNHCOCF$_3$)$_8$-CO$_2$H **a [1]** (324 mg, 0.18 mmol), AZO compound **5** (86 mg, 0.18 mmol), 1-ethyl-3-(3'-dimethylaminopropyl)carbodiimide (EDC, 52 mg, 0.27 mmol),1-hydroxybenzotriazol (HOBt, 26 mg, 0.189 mmol), and CH_2Cl_2/DMF (5:1, 6 mL). The mixture was stirred at 0 ^oC for 10 min, then N-methyl morpholine (NMM, 0.038 ml, 0.54 mmol) was added to the reaction mixture dropwise, which was then slowly warmed to r.t., and kept stirring for overnight. The reaction mixture was treated with 100 mL CH_2Cl_2. The organic solution was washed with $NaHCO_3$ (1M, 50 mL x 3), and then washed with $NaHSO_4$ (1M, 50 mL x 3). At last, the dichloromethane phase was washed with brine (25 ml x 1) and then dried over $MgSO_4$. The product was afforded as a yellow solid (331 mg, 85%, > 90% purity) after removing the organic solvent under reduced pressure. ^1H NMR (500 MHz, DMSO), δ 8.39 (s, 1H), 7.96 (d, *J* = 8.5 Hz, 2H), 7.90 (d, *J* = 8.5 Hz, 2H), 7.83 (d, *J* = 9.0 Hz, 2H), 6.92 (d, *J* = 9.0 Hz, 2H), 4.61-3.97 (m, 11H), 3.70-3.69 (m, 1H), 3.32-3.30 (m, 16H), 2.68-2.64 (bm, 2H), 2.32-2.24 (m, 1H), 1.87-1.63 (m, 34H), 1.42 (s, 9H), 1.32-1.26 (M, 2H). MS (MALDI) calcd for $C_{81}H_{102}F_{24}N_{22}O_{19}Na^+$ $(M+Na)^+$, 2165.74, found, 2165.94.



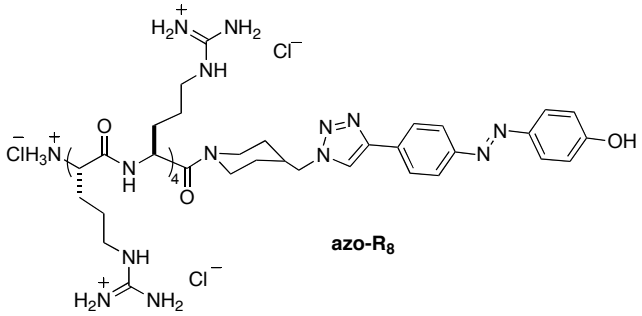
7

BocNH-(Orn)$_8$-azo (•8TFA salt) (7). To a solution of **6** (17 mg, 0.008 mmol) in MeOH/H_2O (2:1, 3 mL) was added Na_2CO_3 (34 mg, 0.317 mol). The solution was stirred at 20 ^oC for 24 h. A yellow solid was achieved after removing the reaction solvent under reduced pressure. The product was purified by RP-prep HPLC using Wender and his coworker's method (isocratic: 5% solvent B, 5 min; gradient: 5% solvent B to 50% solvent B, 19 min). Removing the solvent through lyophilization process gave the desired product as a red solid (retention time 9.9 min, 10 mg, 90%). ^1H NMR (300 MHz, CD_3OD), δ 8.78 (s, 1H), 7.93 (d, *J*= 6.6 Hz, 2H), 7.86 (d, *J* = 7.5 Hz, 2H), 7.75 (d, *J* = 6.6 Hz, 2H), 6.84 (d, *J* =7.5 Hz, 2H), 4.48-4.31 (bm, 8H), 4.08-4.0 (bm, 2H), 3.07-2.79 (m, 18H), 2.79 (bs, 2H) 2.60 (bs, 2H), 2.35-2.23 (bm, 1H), 2.1-1.61 (bm, 32H), 1.49 (s, *J* = 4.5 Hz, 9H), 1.21-1.15 (m, 2H). MS (MALDI) calcd for $C_{59}H_{100}N_{23}O_{11}$ (M-BOC+H) 1275.90, found 1275.86.



8

BocNH-((Arg)$_8$-azo (8•TFA) (8). A mixture of **7** (24 mg, 0.018 mmol), pyrazole-1-carboxamidine hydrochloride (107 mg, 0.728 mmol), and Na_2CO_3 (77 mg, 0.728 mmol) were dissolved in MeOH/H_2O (8 mL, 1:1). Then the mixture was heated up to 55 ^oC for 24 h. The reaction solvent was removed under reduced pressure and the crude product is purified by RP-prep HPLC (isocratic: 5% solvent B, 5 min; gradient: 5% solvent B to 50% solvent B, 19 min) to give the desired product as a yellow solid (25 mg, 78%, retention time 11 min in analytical HPLC spectra, > 99% pure). MS (MALDI) calcd for $C_{73}H_{127}N_{38}O_{11}$ $(M+H)^+$, 1712.05 found 1712.24.

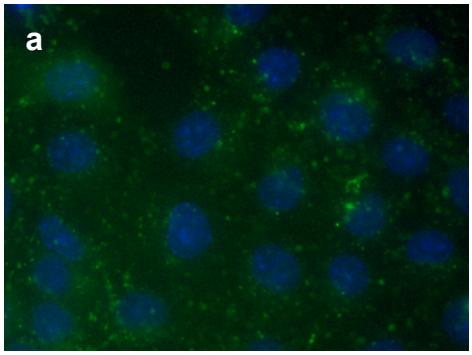


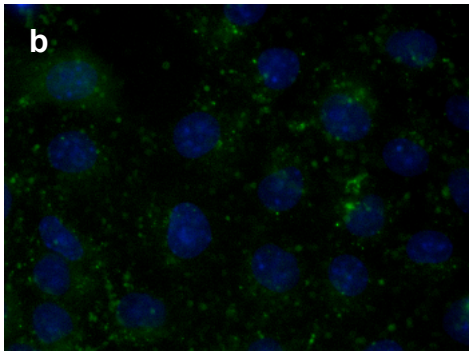
azo-R_8

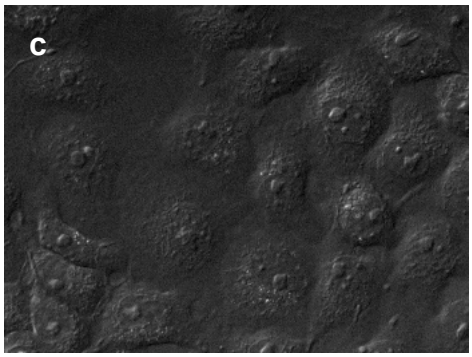
NH_3-($(Arg)_8$-AZO (9•Cl) (azo-R_8). A solution of **8** (10 mg, 0.0058 mmol) in 1 mL trifluoroacetic acid with one drop of triisopropylsilane (TIS) was stirred at room temperature for 2 h. The solvent was removed under the reduced pressure and the crude product was purified by RP-prep HPLC (isocratic: 5% solvent B, 5 min; gradient: 5% solvent B to 50% solvent B, 19 min) to provide a yellow solid 9 mg (retention time is 11 min, > 99% purity, 90%), which was dissolved in DI water (1 mL). Then 500 mg Cl^- exchange resin was added and the resulting mixture was stirred for 10 h to make sure that the Cl^- and $CF_3CO_2^-$ exchange was complete. The desired product with Cl^- counter anions was achieved after removing the resin by filtration. MS (MALDI) calcd for $C_{69}H_{119}N_{38}O_9$ $(M+H)^+$ 1611.99, found 1612.04.

4. Delivery of BSA-F*, β-gal-F* and rec. Streptavidin-F* at 4 ºC

A.







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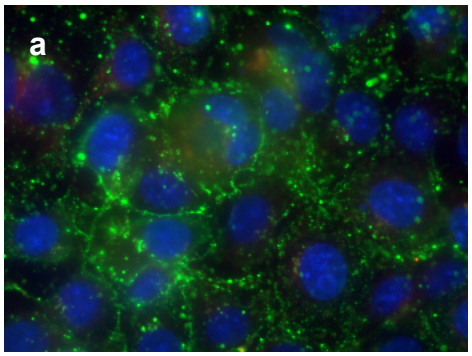

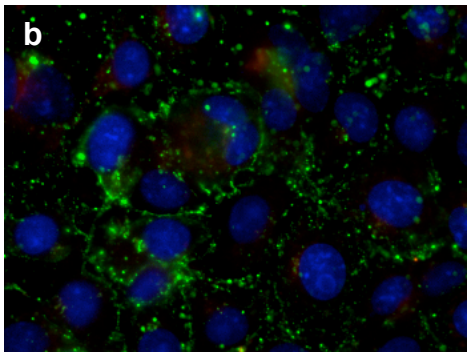

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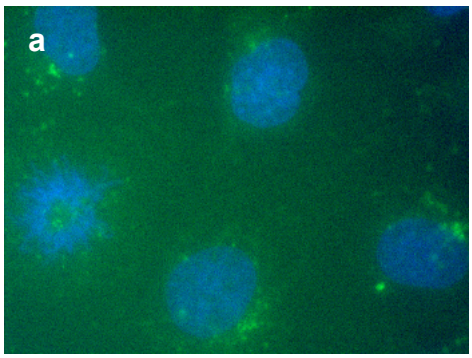
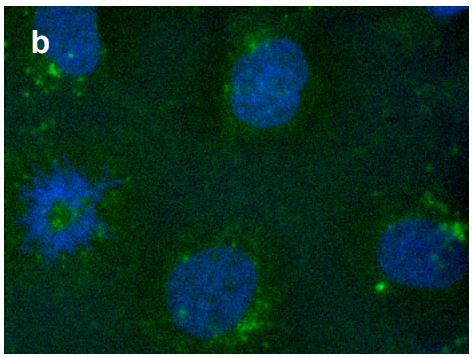
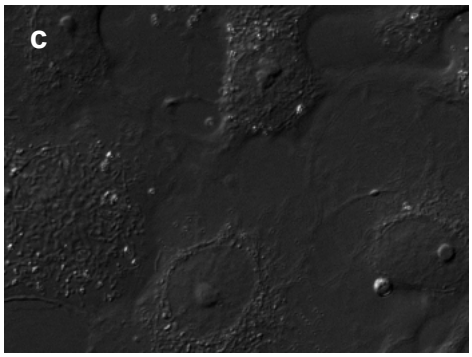
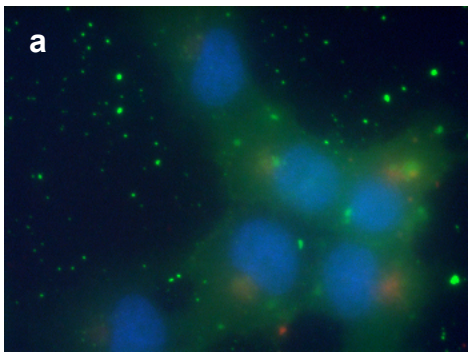
C.

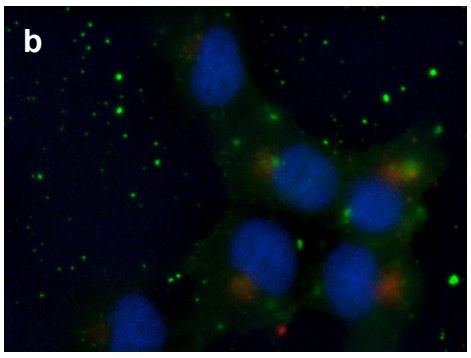






D.





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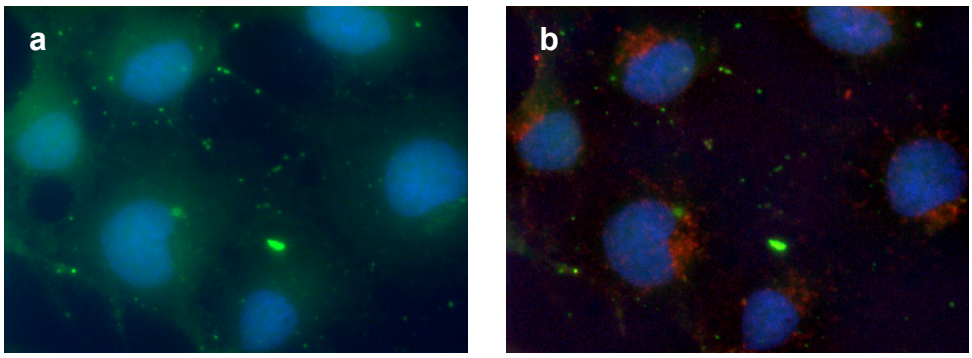
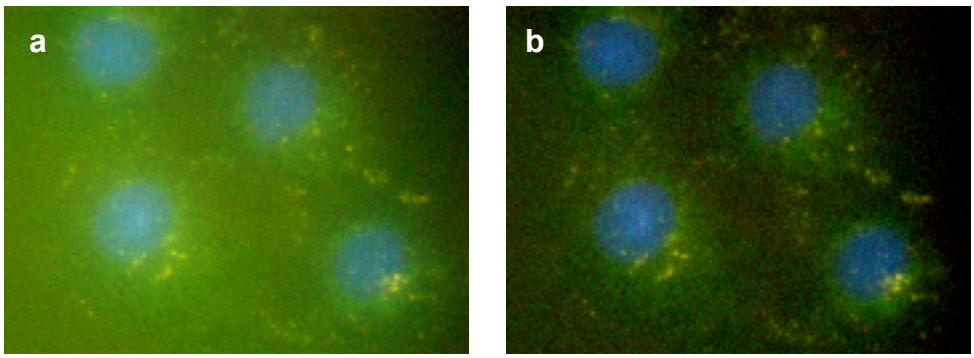
E.



F.



G.

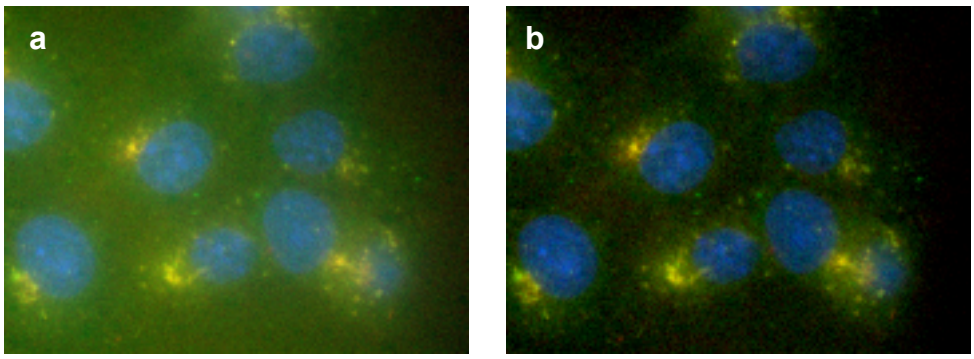


Figure S1. Cellular uptake of **A.** BSA-F*-R_8, **B** BSA-F*-pep-1, **C.** β-gal-F*-R_8, **D.** β-gal-F*-azoR_8, **E.** β-gal-F*-pep-1, **F.** rec. Streptavidin-F*-R_8, **G.** rec. Streptavidin-F*-pep-1 at 4°C in living COS-7 cells. A 1.0:10 protein:carrier ratio was used for the import of BSA-F* and rec. Streptavidin-F*. A 1.0:20 mol:mol protein:carrier ratio was used for the import of β-gal-F*. Images labeled "a" are shown before deconvolution; images "b" are after deconvolution, and images "c" are Differential Interference Contrast microscopy (DIC). In some of the experiments, the cells were also incubated with the marker for endosomes FM-464 for 1h, at 4 ºC.

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5. Delivery of avidin-F* at 37°C mediated by (A) R_8, (B) azo-R_8 and, (C) pep-1.

The cells were co-incubated with the complex avidin-F*:carrier and FM 4-64 at 37°C for 1h, then washed several times with PBS before imaging.

The following images are illustrative of the labeling of the mitochondria when the cells were incubated with FM 4-64 for 1h.

Under those conditions, no co-localization of avidin-F* with endosomes can be observed. It can be seen however, that avidin-F* is concentrated around the nucleus of the cell.

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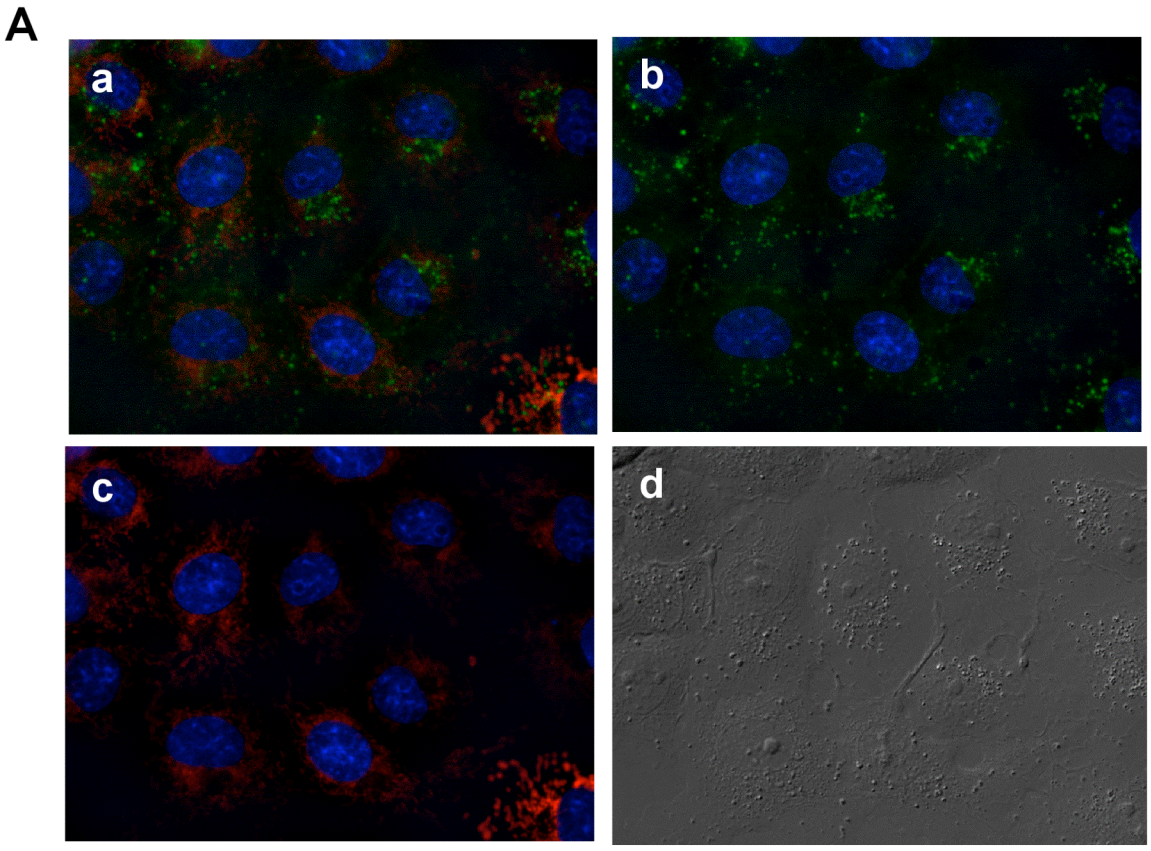


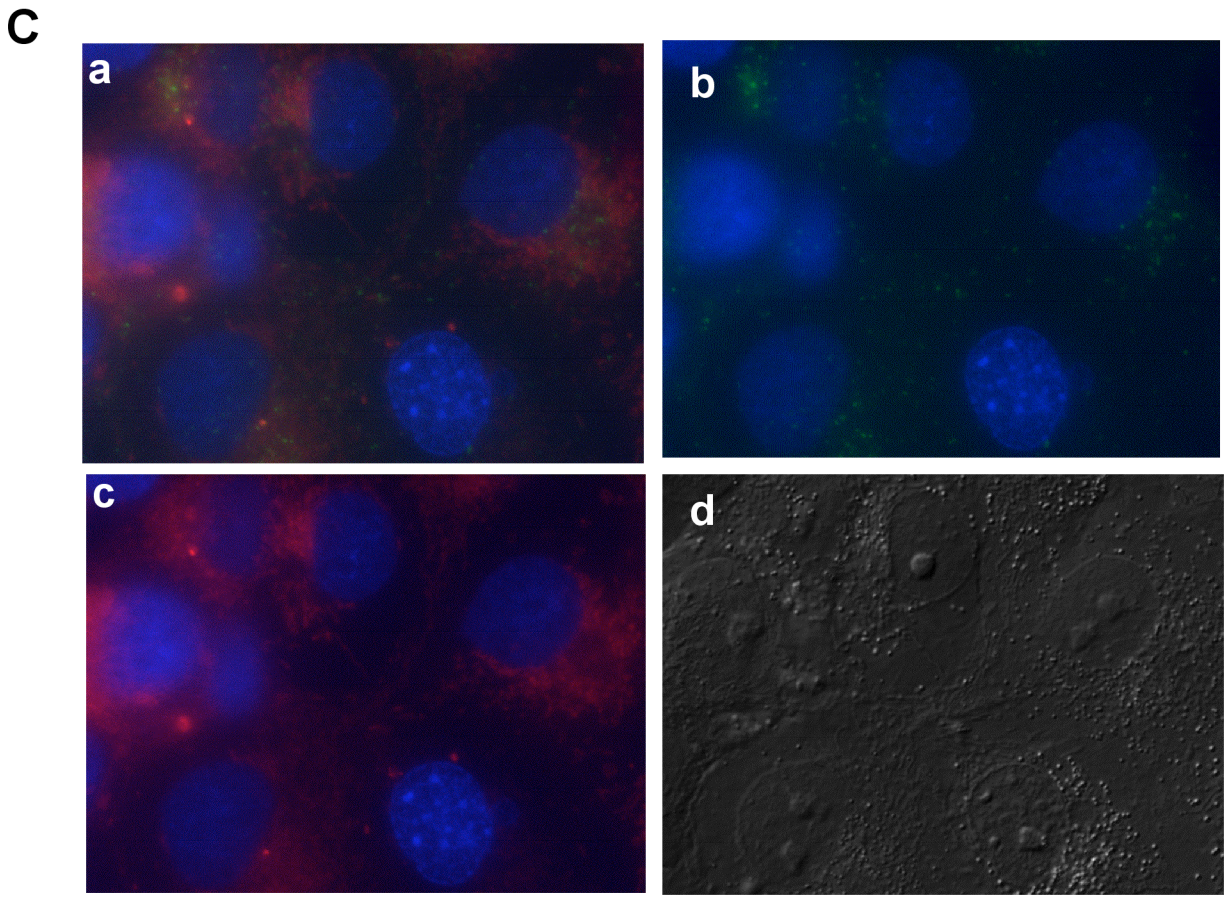
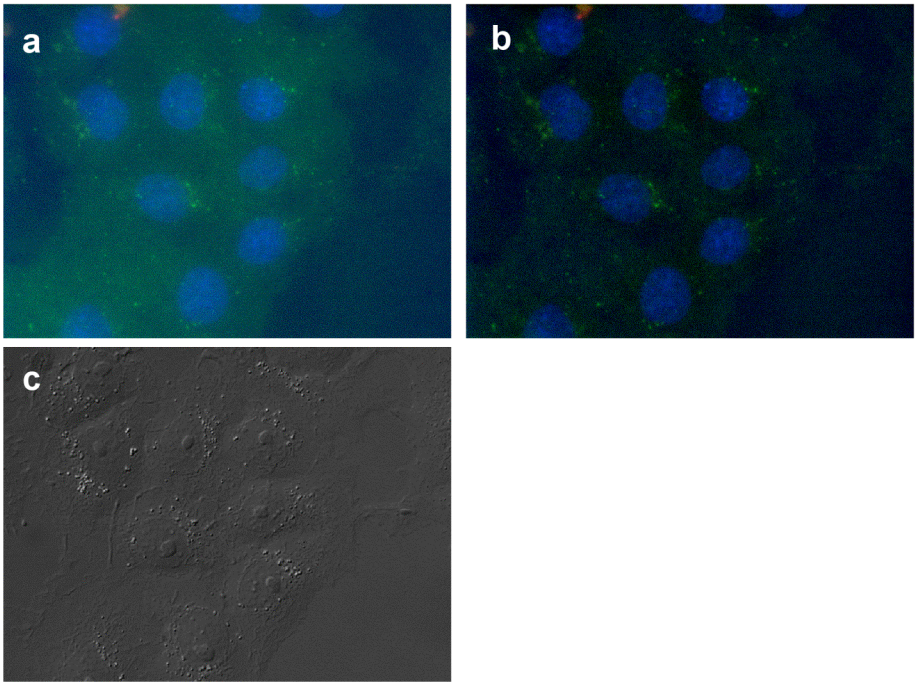


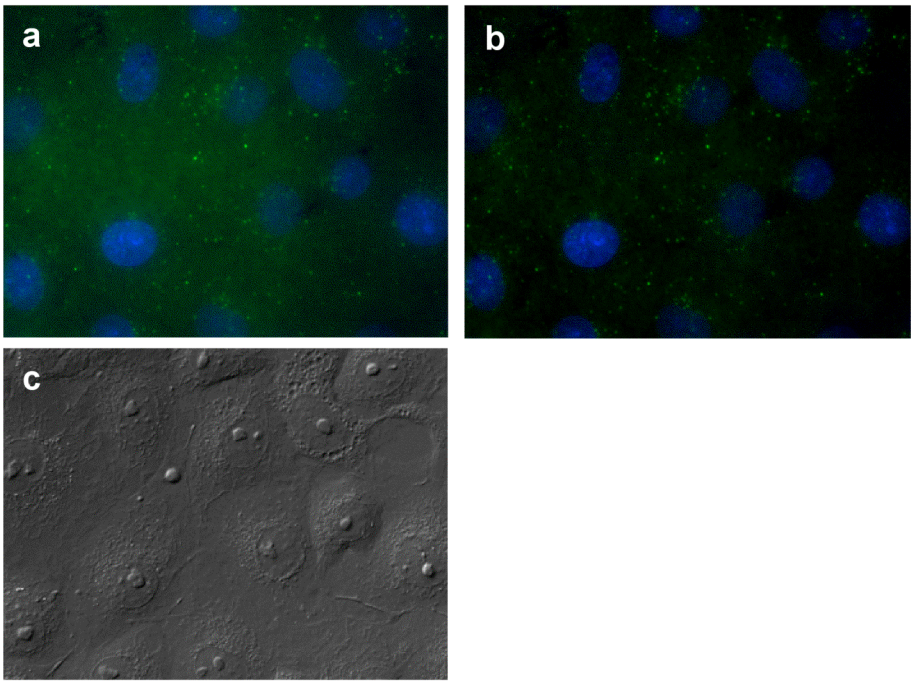
Figure S2. Delivery of avidin-F* in COS-7 cells at 37°C mediated by (**A**) R_8 (**B**) azo-R_8 and (**C**) pep-1. Throughout the carrier (5.0 μM), avidin-F* (0.5 μM) and the COS-7 cells were incubated at 37°C for 1 h with FM 4-64 and Hoechst 33342; the cells were then washed with PBS and analyzed by fluorescence microscopy. **a** Overlaid images of the vesicles (green), the nuclei (blue, Hoescht marker), and the endosomyl marker FM-464 (red). **b** Images of only the vesicles and the nuclei. **c** Images of the mitochondria and the nuclei. **d** Differential interference contrast (DIC) images.

6. R_8 mediated delivery of avidin-F* at 4 ºC.

A



B



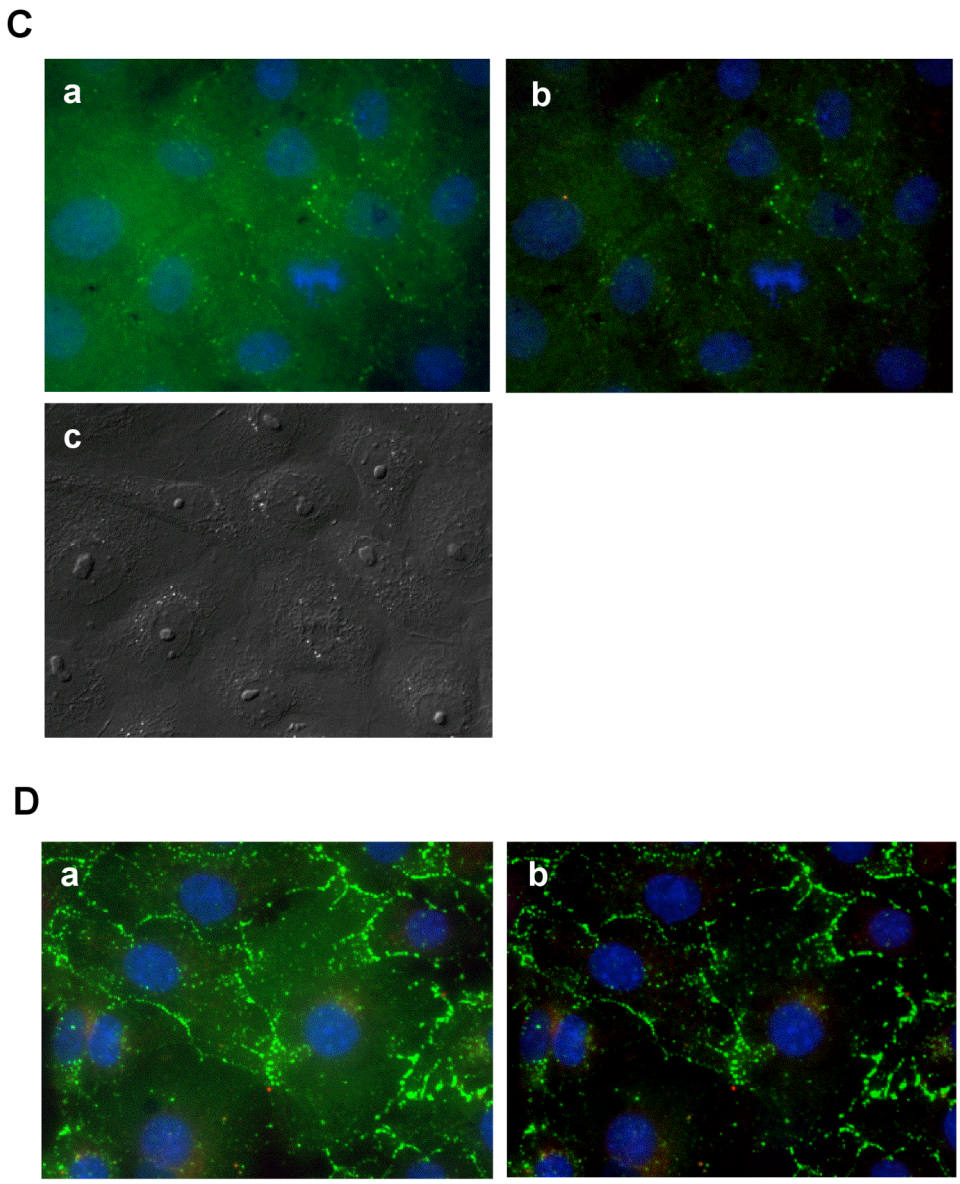
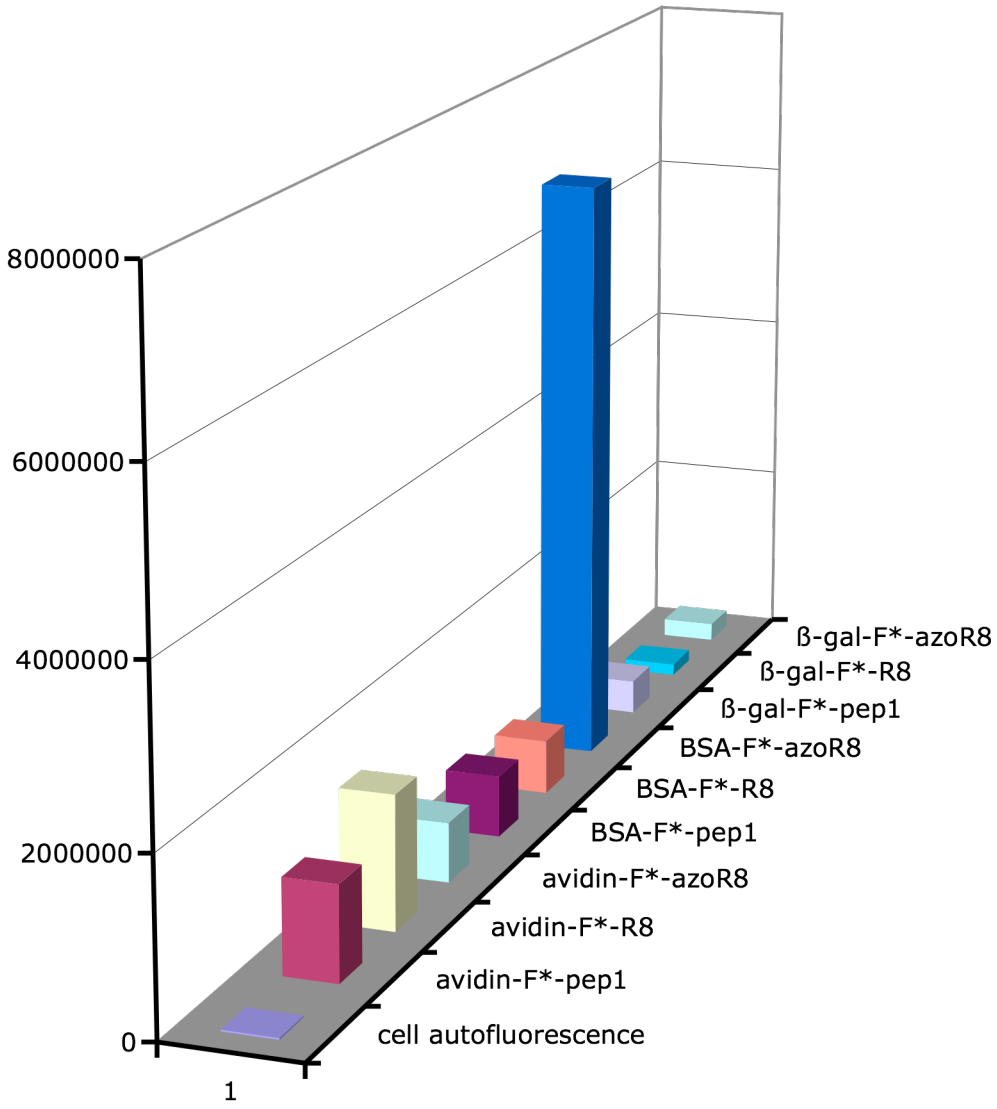
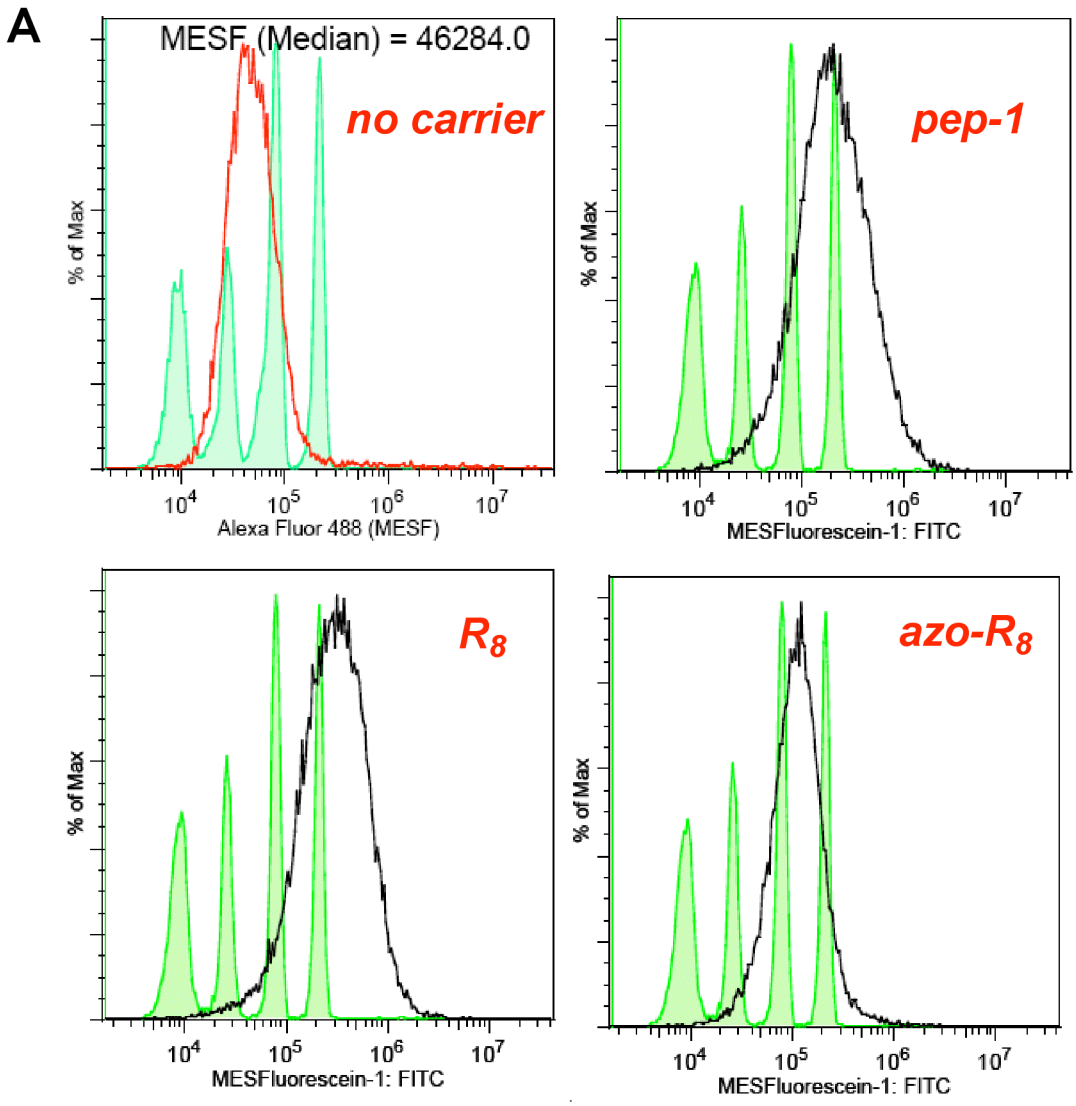


Figure S3. Delivery of avidin-F* in COS-7 cells at 4°C mediated by R_8. **(A)** 0.5 μM of avidin–F*. (**B**) 2 μM avidin–F*, including 3x 5 min heparin wash cycles before imaging. **(C)** 2 μM avidin–F*, but no heparin wash. **(D)** 5 μM avidin–F*, but no heparin wash. Throughout COS-7 cells were incubated for 1 h at 4 °C with R_8 and avidin-F* (10:1.0 mol:mol), then washed 4x with PBS buffer. Images **a** are before deconvolution; **b** are after deconvolution; and, **c** are DIC.

7. Summary of the Flow Cytometry Data Obtained for the Cellular Uptake of avidin-F*, BSA-F* and β-gal-F* at 37°C Mediated by R_8, azoR_8 and Pep-1.





1. Wender, P.A., Jessop, T.C., Pattabiraman, K., Pelkey, E.T., and VanDeusen, C.L. (2001). An Efficient, Scalable Synthesis of the Molecular Transporter Octaarginine via a Segment Doubling Strategy. Org. Lett. *3*, 3229-3232.