Supplemental data

A simple and effective cleavable linker for chemical proteomics applications

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Figure S1

Figure S2

Figure S3

Table S1

Table S2

Supplementary methods:

1 General methods

2 Detailed synthetic methods and compound characterization

Abbreviations:

DMF, dimethyl formamide; EtOAc, ethyl acetate; PE, petroleum ether 40-60; TIS, triisopropyl silane.

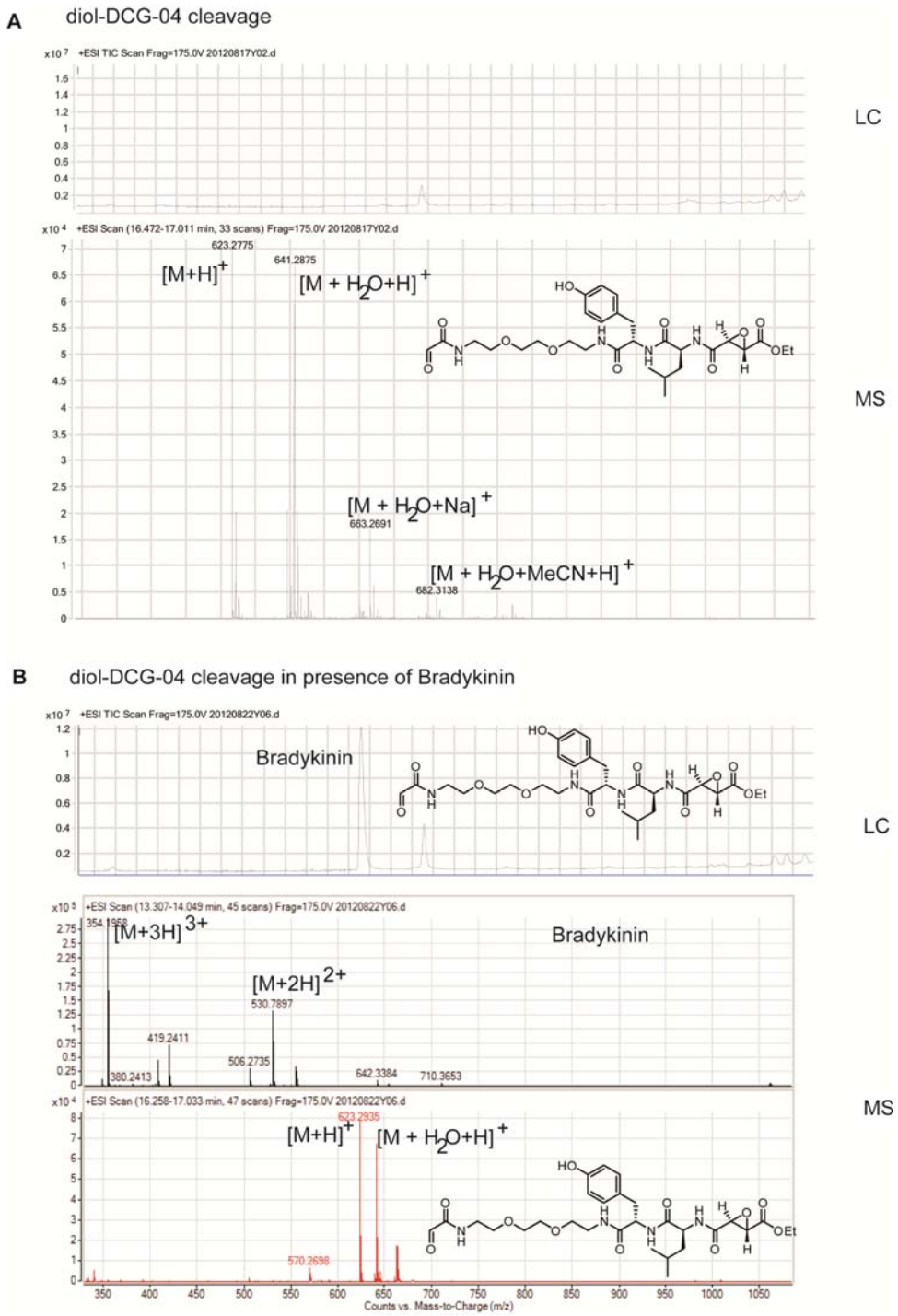


Figure S1. Cleavage of diol-DCG-04. A. diol-DCG-04 was treated with 10 mM $NaIO_4$ and analyzed by LC-MS. B. diol-DCG-04 (10 μM) was treated with 10 mM $NaIO_4$ in the presence of bradykinin (10 μM), purified on a vivapure C18 spin column, and analyzed by LC-MS.

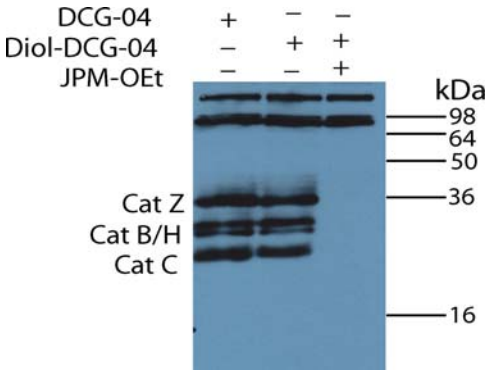


Figure S2. Diol-DCG-04 and DCG-04 labeled cathepsins in rat liver lysate. Rat liver lysate (1 mg/mL total protein, pH 5.5 in 50 mM acetate buffer, 2 mM DTT, 5 mM $MgCl_2$) were treated with diol-DCG-04 and DCG-04 (1µM, 0.5h). Labeled cathepsins in rat liver lysate and the proteins were visualized by streptavidin Western blot. The diol-DCG-04 labeled cathepsins bands are competed by pretreatment with the cysteine protease inhibitor JPM-OEt (50 µM, 0.5h).

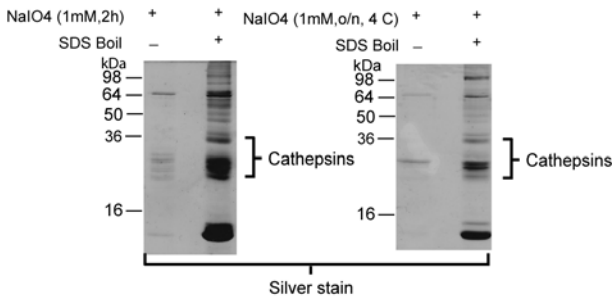


Figure S3. Optimization of protein release. Selective (1 mM $NaIO_4$) release followed by non-selective (SDS sample buffer boil) release of diol-DCG-04 labeled rat liver cathepsins from streptavidin beads detected by silver staining. Both a 2 h (left panel) or overnight (right panel) incubation did not result in satisfactory cleavage, as most proteins are released by the subsequent SDS treatment (right lanes). A 10 mM periodate cleavage turned out to be efficient (see Fig. 3B).

Table S1. The number of total and oxidized methionine residues in all identified peptides of the cathepsin targets in rat liver proteome

	On bead digestion		Chemical cleavage	
Cathepsin	Total Met	Oxidized Met	Total Met	Oxidized Met
Cat B	24	6	18	12
Cat Z	26	3	30	9
Cat C	3	0	3	2
Cat H	6	0	6	1
Cat L	4	0	0	0
Cat J	0	0	0	0

Table S2. Spectral counts of selected cathepsin peptides in oxidized and non-oxidized form from rat liver proteome.

	On bead digestion		Chemical cleavage	
Selected peptides[1]	Non-oxidized	Oxidized	Non-oxidized	Oxidized
Cat B				
HEAGDV**M**GGHAIR	3	2	1	3
SGVYKHEAGDV**M**GGHAIR	3	1	2	2
Cat Z				
M**M**AEIYANGPISCGIMATER	2	1	1	4
Cat C				
NQESCGSCYSFASLG**M**LEAR	2	0	1	1
Cat H				
GI**M**GEDSYPYIGK	2	0	2	1

[1]Oxidized methionines are indicated in bold

Supplemental methods

1 General methods

Unless otherwise noted, all reagents were purchased from commercial suppliers and used without further purification. Reactions were analyzed by thin-layer chromatography on 0.20 mm silica plates with fluorescent indicator. All LC-MS analysis was done with an agilent 6210 LCMS-Systhem using a custom-made C18 column. Flash chromatography was carried out with 230-400 mesh silica gel. Reverse-phase HPLC was conducted on Waters 515 HPLC System using a Waters x-bridge reversed-phase C18 column. Purifications were performed at room temperature and compounds were eluted with increasing concentration of acetonitrile (solvent A: 0.1% TFA in water, solvent B: 0.1% TFA in acetonitrile).

2 Synthesis

Synthesis of cleavable building block

The synthesis of the cleavable building block **1** follows the procedure as outlined in Figure S4.

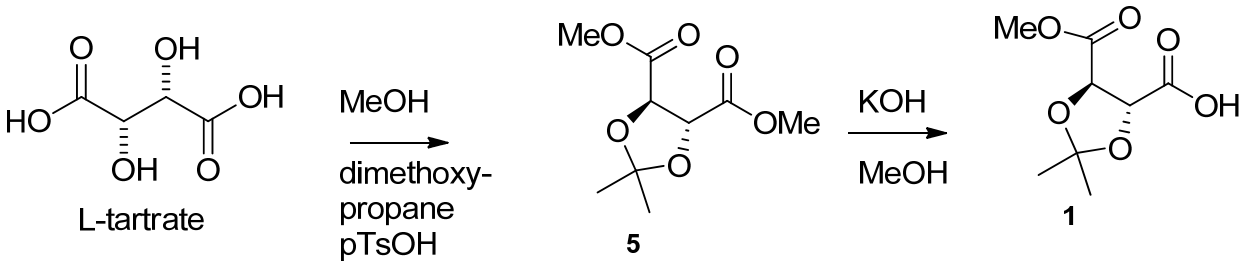


Figure S4. Synthetic scheme for **1**

Dimethyl 2,3-*O*-isopropylidene-L-tartrate (5)

L –tartaric acid (10.1 g) was added to a solution of methanol (4 ml) and *p*-toluenesulfonic acid (40 mg) and 2, 2-dimethoxypropane (19 ml). The solution was stirred with reflux at 50 ^{o}C for 2h. Another 10 ml 2,2-dimethoxypropane and dry cyclohexane (40 ml) was added. The solution was hearted to 68 ^{o}C and refluxed for 2 days and the volatile compounds (acetone, methanol) with cyclohexane were slowly removed by simple distillation. Anhydrous potassium carbonate (100

mg) was added to neutralize the reaction. The solvent and unreacted 2,2-dimethoxypropane were removed under reduce pressure. The crude compound was purified by vacuum distillation and isolated as a light yellow oil (10.9 g, 74%). ^{1}H NMR (400 MHz): 4.81 (s, 2H), 3.83 (s, 6H), 1.50 (s, 6H). ^{13}C DEPT NMR (400 MHz): 76.99, 52.83, 26.30.

Methyl 2,3-*O*-isopropylidene-L-tartrate (1)

To a solution of dimethyl 2,3-*O*-isopropylidene-L-tartrate (2.06 g, 9.468 mmol) in CH_3OH (20 ml) was added a solution of KOH (531.24 mg, 9.468 mmol) over 1h. The reaction was stirred overnight and evaporated. The resulted residue was dissolved in Et_2O (30 ml) and washed with 10 ml saturated $NaHCO_3$ solution. The aqueous portion was acidified to pH 3.5 with 3 M HCI and extracted with Et_2O (8 x 20 ml). The combined Et_2O extracts were dried and evaporated to give colorless oil 1.21g (63%). ^{1}H NMR (400 MHz): 4.89 (d, 1H, *J* = 6.0 Hz), 4.83 (d,1H, *J* = 5.5 Hz), 3.85 (s, 3H), 1.53 (s,3H), 1.51 (s, 3H). ^{13}C DEPT NMR (400 MHz): 77.04, 76.46, 53.03, 26.33, 26.29.

Synthesis of the biotin-diol-alkyne

The synthesis of the cleavable alkyne reagent **3** is performed as outlines in Figure S5.

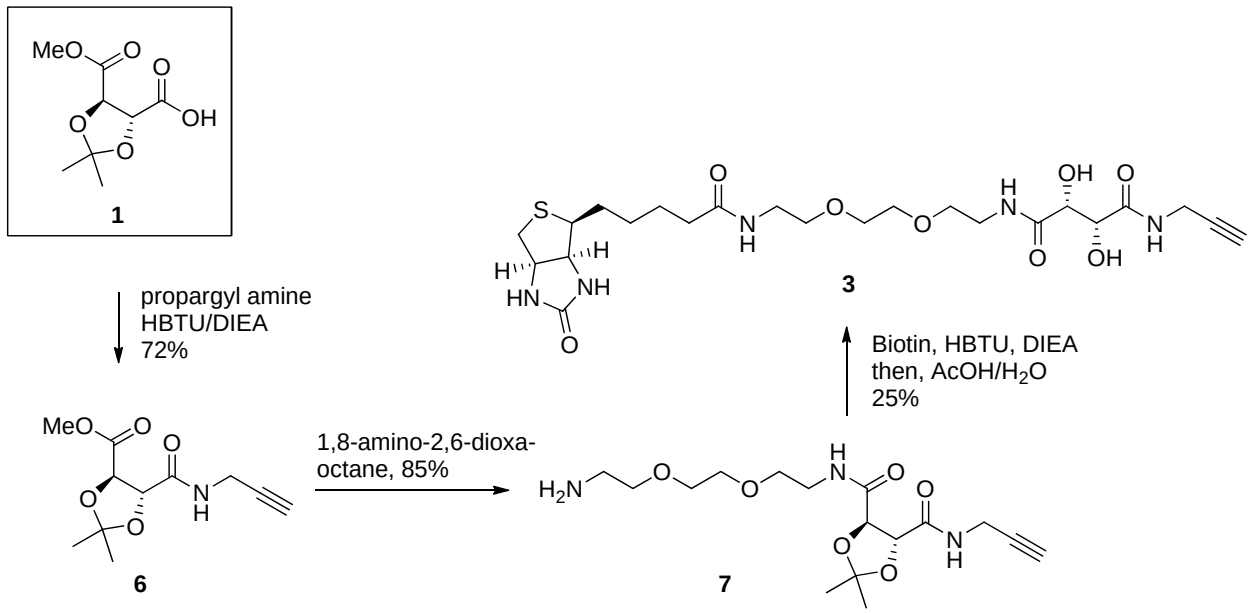


Figure S5. Synthesis of reagent **3**.

Synthesis of 6

To a solution of methyl 2,3-*O*-isopropylidene-L-tartrate (**1**, 448 mg, 2.2 mmol) in DMF (10 ml) was added propargylamine-hydrochlorid (183 mg, 2 mmol), HBTU (758 mg, 2 mmol) and DIEA (690 µl, 4 mmol), The reaction was stirred overnight and evaporated. The resulted residue was dissolved in DCM and washed with saturated $NaHCO_3$, 1 M $KHSO_4$ and brine. The organic layer was dried on $MgSO_4$ and concentrated under reduced pressure. Silica column chromatograph (10–40% EtOAc in PE) afforded the title compound (347.4 mg, 72%). ESI-MS: $[M+H]^+$ calculated for $C_{11}H_{15}NO_5$ 242.0950, found 242.1047. ^{1}H NMR (400 MHz): 6.7 (bs, 1H), 4.79 (d, 1H, *J* = 5.0 Hz), 4.76 (d, 1H, *J* = 5.0 Hz), 4.17-4.04 (m, 2H), 3.83 (s, 3H), 2.27 (t, 1H, *J* = 2.4 Hz), 1.51 (s, 3H), 1.49 (s, 3H). ^{13}C DEPT NMR (400 MHz): 77.68, 77.30, 52.89, 38.62, 28.88, 26.60, 26.22.

Synthesis of 7

To a solution of **6** (183 mg, 0.76 mmol) in toluene (1 ml) was added 1,8-diamino-3,6-dioxaoctane (555 µl, 3.8 mmol). The reaction was stirred at 80^{o}C overnight. The solvents were evaporated in vacuo, and the product was purified to homogeneity by silica gel chromatography (MeOH/DCM/NH_4OH 0/10/0.1 to 1/10/0.1) to yield compound **7** (231mg, 0.646 mmol, 85%). ESI-MS: $[M+H]^+$ calculated for $C_{16}H_{27}N_3O_6$ 358.1900, found 358.2207. ^{1}H NMR (400 MHz): 7.40 (bs, 1H), 7.22 (bs, 1H), 4.61 (d, 1H, *J* = 6.8 Hz), 4.58 (d, 1H, *J* = 7.2 Hz), 4.17-4.05 (m, 2H), 3.65- 3.46 (m, 10H), 2.88 (t, 2h, *J* = 5.2 Hz), 2.25 (t, 2H, *J* = 2.4 Hz), 1.54 (bs, 2H), 1.51 (s, 3H), 1.49 (s, 3H). ^{13}C DEPT NMR (400 MHz): 77.46, 77.29, 73.48, 70.46, 70.26, 69.55, 41.75, 39.04, 28.98, 26.22, 26.07.

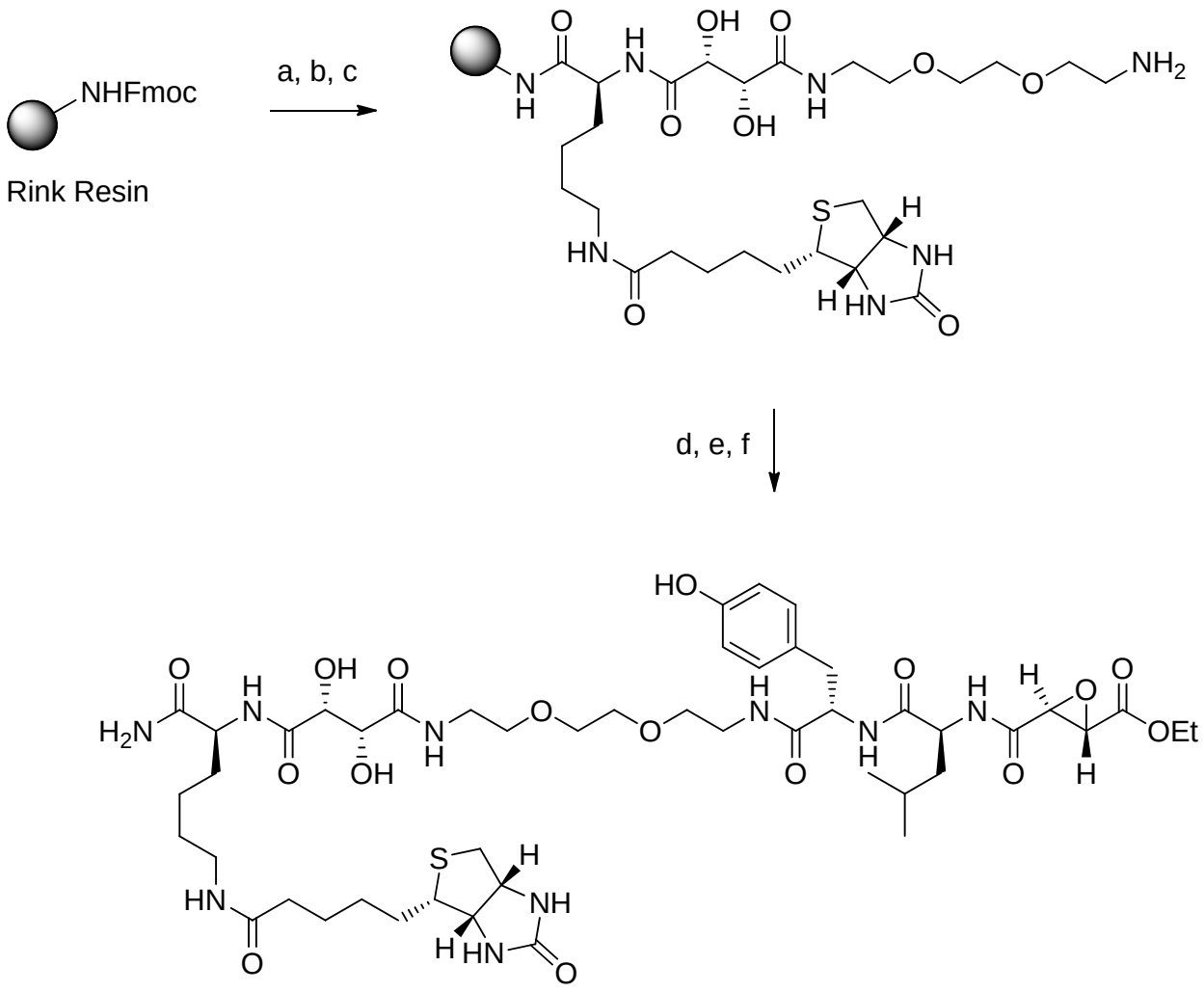
Synthesis of cleavable alkyne-biotin reagent 3

To a solution of **7** in DMF (728 µl) was added biotin (39.2 mg), HBTU (60.9 mg) and DIEA (27.6 µl), and the reaction was stirred overnight. The solvents were evaporated *in vacuo*, 90% AcOH in water (3 ml) was added, and the mixture was stirred at 80 ^{o}C overnight. Solvents were evaporated *in vacuo* and the product was purified by HPLC to yield 20 mg (25%) of the title compound. ESI-MS: $[M+H]^+$ calculated for $C_{26}H_{41}N_5O_8S$ 544.2363, found 544..2407; ^{1}H NMR (400 MHz): 8.06 (t, 1H, *J* = 5.6 Hz), 7.83 (t, 1H, *J* = 5.6 Hz), 7.63 (t, 1H, *J* = 6.0 Hz), 6.41 (bs, 2H), 4.30 (dd, 1H, *J* = 7.6 Hz, *J* = 5.2 Hz), 4.24 (dd, 2H, *J* = 8.8 Hz, *J* = 1.6 Hz), 4.13 (dd, 1H, *J*

= 7.6 Hz, *J* = 4.4 Hz), 3.96-3.56 (m, 2H + DMSO), 3.51 (m, 4H), 3.44(t, 2H, *J* = 6.0 Hz), 3.40 (t, 2H, *J* = 6.0 Hz), 3.1-3.1 (m, 5H), 3.05 (d, 1H, *J* = 2.4 Hz), 2.82 (dd, 1H, *J* = 5.2 Hz, *J* = 12.4 Hz), 2.58 (d, 1H, *J* = 12.4 Hz), 2.07 (t, 2H, *J* = 7.2 Hz), 1.65-1.25 (m, 6H).

Synthesis of diol-DCG-04

Solid phase peptide synthesis was performed on Rink resin as shown in Figure S6, using Fmoc-amino acid/DIC/HOBt (3eq/3eq/3eq with respect to the loading reported by the supplier; final concentration ~ 0.3 M) in DMF for 3-5h at room temperature. After the coupling of methyl 2,3-O-isopropylidene-L-tartrate under the same conditions as for amino acids, 8-diamino-3,6-dioxaoctane (1 ml) was added and the resin was shaken overnight. Next, the tyrosine and leucine building blocks were introduced and finally, the epoxysuccinate warhead (1.5 eq) was coupled with DIC/HOBt overnight. The probes were cleaved by incubation with a solution of TFA: TIS: H_2O (90%: 2.5%: 7.5%) for 3 hours. The peptide was precipitated in cold diethyl ether and purified by HPLC yielding a white solid (17%). ESI-MS: $[M+H]^+$ calculated for $C_{47}H_{73}N_9O_{16}S$ 1052.4896, found 1052.4897. ^{1}H NMR (400 MHz): 8.52 (d, 1H, *J* = 4.4 Hz), 8.08 (d, 1H, *J* = 8.0 Hz), 7.9 (t, 1H, *J* = 5.6 Hz), 7.73 (t, 1H, *J* = 5.6 Hz), 7.68 (d, 1h, *J* = 8.0 Hz), 7.65 (t, 1H, *J* = 6.0 Hz), 7.35 (s, 1H), 7.16 (s, 1H), 6.97 (d, 2H, *J* = 8.4 Hz), 6.61 (d, 2H, *J* = 8.4 Hz), 6.42 (bs, 2H), 4.39 -4.27 (m, 4H), 4.24-4.11 (m, 5H), 3.71 (d, 1H, *J* = 1.6 Hz), 3.59 (d, 1H, *J* = 2.0 Hz), 3.54-3.46 (m, 4H), 3.43 (t, 2H, *J* = 6.0 Hz), 3.38- 3.07 (m, 7H), 3.01- 2.94 (m, 2H), 2.82(dd, 2H, *J* = 5.2 Hz, *J* = 12.8 Hz), 2.71-2.65 (m, 1H), 2.57(d,1H, *J* = 12.4 Hz), 2.035 (t, 2H, *J* = 7.2 Hz), 1.75-1.28 (m, 15H), 1.23 (t, 3H, *J* = 7.2 Hz), 0.86 (d, 3H, *J* = 6.4 Hz), 0.822 (d, 3H, *J* = 6.4 Hz).



2 diol-DCG-04

Figure S6. Synthesis of diol-DCG-04. a) 20% piperidine in DMF, then Fmoc-Lys(biotin)-OH, DIC, HOBt; b) 20% piperidine in DMF, then building block **1**, DIC, HOBt; c) 1, 8-diamino-3,6-dioxaoctane, overnight; d) Fmoc-Tyr-OH, DIC, HOBt; e) 20% piperidine in DMF, Fmoc-Leu-OH, DIC, HOBt; f) 20% piperidine in DMF, ethyl (2S, 3S) epoxysuccinate, DIC, HOBt; then TFA: TIS: H_2O (90%: 2.5%: 7.5%) for 3 hours, 17% after HPLC purification.