Supporting Information

Rapid Discovery of Death Ligands with One-Bead Two-Compound Combinatorial Library Methods

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Synthesis of biotinylated ligands:

Rink amide MBHA resin (0.2g, loading: 0.59mmole/g) was swollen in DMF for 3 h prior to Fmoc-deprotection with 20% piperidine in DMF. Fmoc-Lys(Alloc)-OH (3 equiv., 0.35 mmol) was dissolved in a solution of HOBt (3 equiv., 0.35 mmol) and DIC (3 equiv., 0.35 mmol) in DMF and added into the beads. The coupling reaction was carried out at room temperature for 2 h. After filtration, the beads were washed with DMF, MeOH and DCM for 3 times, respectively. The resin was treated twice with $Pd(PPh_3)_4$ (0.2 equiv, 0.024 mmole) in the presence of excessive $PhSiH_3$ (20 equiv, 2.36 mmole) in DCM for 30 min to remove Alloc protecting group on the side chain of lysine. After thoroughly washing the resin with DCM, DMF, MeOH and DMF three times each, the resin was treated with a solution of biotin (5 equiv, 0.59 mmol), DIPEA (10 equiv., 1.18 mmol) and HBTU (5 equiv, 0.59 mmol) in DMF overnight. After filtration, the beads were washed with DMF, MeOH, and DCM 3 times each. After Fmoc deprotection, Fmoc-Ebes-OH (a linear hydrophilic linker, see ref. 1) was coupled to the α-amino group of the lysine. LLP2A or peptides were

constructed after coupling of linker using standard Fmoc chemistry and DIC/HOBt as coupling agents. After the coupling of the last amino acid, the N-terminus Fmoc protection group was removed by 20% piperidine in DMF. The resin was thoroughly washed with DMF, MeOH, DMF and DCM, then dried under vacuum. To the dried resin was added 4 mL of cleavage mixture (95% TFA: 2.5% water: 2.5% TIS), the cleavage reaction was conducted at room temperature for 2 h. The cleavage solution was collected and concentrated. The crude product was precipitated with cold diethyl ether and lyophilized. The crude peptide was analyzed and purified by RP-HPLC and characterized by ESIMS.

Table S1. Analytical data of biotinylated compounds

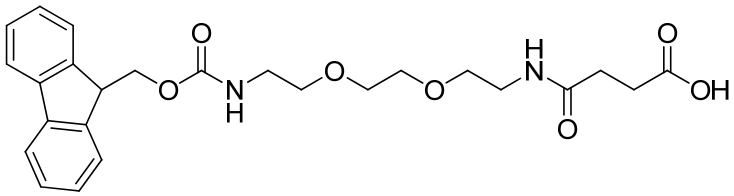
Compd	MS (calcd./found $[M+1]^+$)	Purity (RP-HPLC)
LLP2A-biotin	1394.71/1395.75	99%
EQAHEL-biotin	1308.65/1309.82	99%
HGSYWQ-biotin	1359.64/1360.83	99%

Table S2. Amino acids used in the synthesis of OBTC library 1

X_1-X_6 (library 1)
Arg, Lys, His, Asp, Glu, Phe, Ala, Leu, Met, Ile, Trp, Pro, Val, Gly, Gln, Asn, Ser, Tyr, Thr

S3. Hydrophilic Linkers:

N-Fmoc protected polyoxyethylene-based amino acid type linkers were used for peptide derivatization in solid phase synthesis. The graphical structure of the linker is shown below. The linkers can be stored at 4 °C for 2 years without significant decomposition.



Synthesis of 2, 2'-ethylenedioxy bis(ethylamine) monosuccinamide (Fmoc-Ebes-OH):

2,2'-(ethhylenedioxy)-bis(ethylamine) (50mmol, 7.4g) was dissolved in 250ml acetonitril (ACN). A solution of succinic anhydride (50mmol, 5.0g) in 125mL ACN was added dropwise into the above solution under vigorous magnetic stirring over 1h. The stirring was stopped after 3h. After the waxy product settled down, the liquid phase was decanted and discarded. The waxy product was redissolved in 500 mL 50% ACN/water and chilled in an ice bath for 30 min. A solution of Fmoc-OSu (65mmol, 21.9g) in 300 ml ACN was added dropwise under vigorous magnetic stirring over 4 h. The pH of reaction mixture was maintained at 8-9 by adding $NaHCO_3$ solution throughout the reaction. The reaction was allowed to proceed overnight at room temperature. The organic solvents were removed under vacuum. The aqueous phase was washed with ethyl acetate (150mL x 7) and then acidified with 1M HCl to pH 2 and extracted with ethyl acetate (250mL x 3). The combined organic phase was

washed with brine (3 times), dried over anhydrous $MgSO_4$ and then concentrated to get an oily product. Oily product was allowed to stand at 4 degree for a few days to be solidified.

The final product was checked by RP-HPLC (purity >97%) and ESI-MS (Calcd./found$[M+1]^+$ 470.21/471.48). More details for linker synthesis, biotinylation and fluorescent labeling of peptides in solid phase can be found in the following reference.

Reference

1. Song A, Wang X, Zhang J, Marik J, Lebrilla CB, Lam KS. Synthesis of hydrophilic and flexible linkers for peptide derivatization in solid phase. Bioorg Med Chem Lett 2004 Jan 5;14(1):161-5.