SUPPORTING INFORMATION

Selective Formation of Homo- and Heterobivalent Peptidomimetics

Mookda Pattarawarapan,[§+] Samuel Reyes, [§] Zebin Xia, [§] Maria C. Zaccaro,[†] H. Uri Saragovi,[†] and Kevin Burgess*[,§]

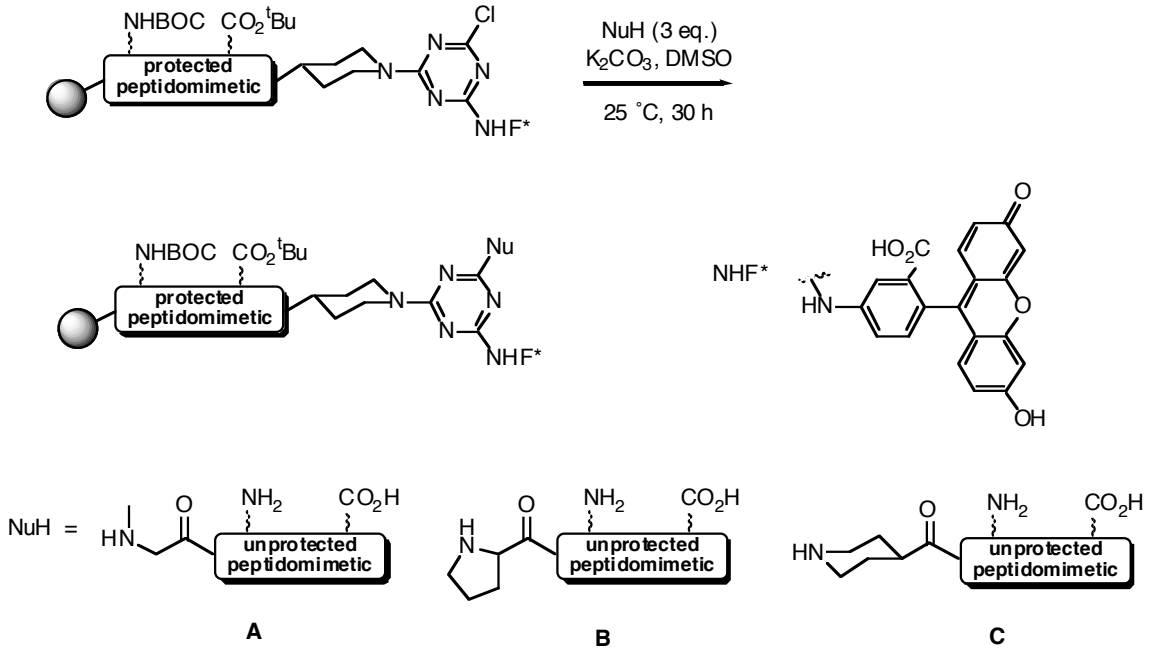
Department of Chemistry, Texas A & M University, Box 30012, College Station, TX 77842-3012, and Department of Pharmacology and Therapeutics, McGill University, Montréal, QC. Canada

[+] Current address: Department of Chemistry, Faculty of Science, Chiang Mai University, Chiang Mai 50200, Thailand
[†] McGill
[§] Texas A & M.

A. Model Studies

Model studies were performed to determine a nucleophile suitable for chemoselective displacement of disubstituted monochlorotriazines. Potassium carbonate was used as base because it is less prone to racemize the peptide than alternatives and it gives an innocuous by-product. DMSO was used because it is a good solvent for the substrates and, unlike DMF, it does not have a secondary amine contaminant that might interfere with the reaction. Exploratory sets of reactions are shown in Scheme S1. Here, test peptides **A**-**C** containing various unprotected nucleophilic groups were reacted with the supported monochlorotriazines. It was found that no reaction occurred with compounds **A** or **B**. However, compound **C** did react to give the desired dimers as proven by MALDI-MS analysis of the cleavage products. It is believed that the secondary amine functionality of *iso*-nipecotic acid (Inp) rather than the primary amine side-chain of lysine is the one that reacts with triazines since other test peptides containing lysine do not react. This inference is supported by subsequent experiments in which the products were more fully characterized.

Scheme S1. Solid-Phase Reaction Illustrating Selective Coupling to Triazine Platform

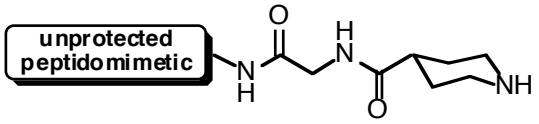


B. Procedure for Preparation of Monomeric Components 2 and 3.

Resin containing side-chain protected cyclic peptidomimetics with the nitro group was swelled in CH_2Cl_2 in a fritted syringe for 30 min and treated with 2 M $SnCl_2.2H_2O$ in DMF for 2 d. After washing with H_2O (3x), DMF (3x), MeOH (3x), DMF (1x), MeOH (1x), CH_2Cl_2 (2x), MeOH (2x) and CH_2Cl_2 (3x) and drying under vacuum for 2 h, the resulting arylamine was treated with FmocGlyCl (3 equiv.), DIEA (5 equiv.) and DMAP (cat.) in CH_2Cl_2 for 1 h. The resin was then filtered and subjected to washing cycle {DMF (3x), MeOH (3x), DMF (1x), MeOH (1x), CH_2Cl_2 (2x), MeOH (2x) and CH_2Cl_2 (3x)}, which will be used throughout the subsequence washing. The Fmoc protecting group was then removed with 20% piperidine in DMF (ca 1.5 ml/100 mg resin, 2 x 10 min). The resin was then washed, after which, FmocInpOH (3 equiv.), DIC (5 equiv.), HOBt (5 equiv.) in DMF were added. After shaking for 3 h, the above washing cycles and Fmoc deprotection was repeated. The resin was then washed, dried and split into two portions. One portion was subjected to cleavage condition by treatment with a mixture of 90% TFA, 5% TIS, and 5% H_2O for 2 h. The resulting crude product was purified via preparative HPLC (Vydac C-18 column, 25 x 2.2 cm, 10 ml/min, 5 - 50 % acetonitrile, *ie* solvent B; solvent A is water) to give peptidomimetics **2**.

The other portion of dry resin was swelled in CH_2Cl_2 for 30 min. After the solvent was drained, 2 equiv. of dichlorotriazinylaminofluorescein (DTAF), DIEA (3 equiv.) in 3:1 mixture of $CHCl_3$/ DMSO were added and the mixture was shaken for 3 h. The resin was then washed and subjected to the above cleavage condition. The resulting crude product was purified via preparative HPLC to give the dye-tagged peptidomimetics 3.

Table S1. Monomers **2** prepared in Scheme 1



2

compounds **2** and **3**	macrocyclic structure[a]	AA[1]	AA[2]
a	**I**	Glu	Lys
b	**I**	Lys	Ser
c	**I**	Ile	Lys
d	**I**	Ile	Arg
e	**II**	Glu	Lys
f	**I**	Ser	Lys
g	**III**	Gly	Lys
h	**III**	Ile	Lys
i	**III**	Ile	Arg
j	**IV**	Lys	Tyr
k	**V**	Ile	Arg
l	**V**	Ile	Lys

[a]Structures as shown for unprotected peptidomimetic in text.

2a: ^{1}H NMR (500 MHz, DMSO-d_6) 12.2 (bs, 1H), 9.78 (s, 1H), 9.64 (s, 1H), 8.49 (m, 1H), 8.38 (m, 1H), 8.34 (s, 1H), 8.20 (m, 1H), 7.89 (s, 1H), 7.68 (m, 4H), 7.57 (bs, 2H), 7.51 (d, *J* = 2.0 Hz, 1H), 7.33 (m, 1H), 6.69 (d, *J* = 9.5 Hz, 1H), 6.25 (bs, 1H), 4.67 (m, 1H), 4.35 (m, 1H), 4.23 (d, *J* = 15 Hz, 1H), 4.15 (m, 1H), 3.81 (m, 2H), 2.88 (m, 2H), 2.77 (m, 2H), 2.37 (t, *J* = 7.0 Hz, 2H), 1.86 (m, 3H), 1.71 (m, 2H), 1.62 (m, 1H), 1.56 (m, 3H), 1.31 (m, 2H); LRMS (MALDI): calc'd for [M+ H]$^+$ 708, found 708; Analytical HPLC: homogeneous single peak, retention time = 9.05 min (5-60 % B in 25 min).

2b: ^{1}H NMR (500 MHz, DMSO-d_6) 9.84 (s, 1H), 9.63 (s, 1H), 8.50 (m, 1H), 8.38 (m, 1H), 8.35 (m, 1H), 8.21 (m, 1H), 7.91 (s, 1H), 7.66 (s, 3H), 7.58 (m, 1H), 7.53 (m, 1H), 7.37 (dd, *J* = 2.0, 8.5 Hz, 1H), 7.31 (s, 1H), 6.75 (d, *J* = 9.5 Hz, 1H), 6.00 (s, 1H), 4.61 (d, *J* = 13 Hz, 1H), 4.33 (m, 1H), 4.17 (m, 2H), 3.81 (m, 2H), 3.75 (m, 1H), 3.56 (m, 1H), 2.88 (m, 2H), 2.78 (m, 2H), 4.17 (m, 2H), 1.70 (m, 2H), 1.57 (m, 2H), 1.42 (m, 2H); LRMS (MALDI): calc'd for [M+ H]$^+$ 666, found 666; Analytical HPLC: homogeneous single peak, retention time = 8.8 min (5-60 % B in 25 min).

2c: ^{1}H NMR (500 MHz, DMSO-d_6) 9.78 (s, 1H), 9.67 (s, 1H), 8.50 (m, 1H), 8.34 (s, 1H), 8.20 (t, *J* = 6 Hz, 1H), 8.11 (d, *J* = 8.5 Hz, 1H), 7.89 (s, 1H), 7.65 (s, 1H), 7.57 (m, 2H), 7.44 (d, *J* = 2.0 Hz, 1H), 7.35 (dd, *J* = 2.0, 9.0 Hz, 1H), 7.31 (s, 1H), 6.67 (d, J = 9.0 Hz, 1H), 6.16 (s, 1H), 4.68 (d, *J* = 15, 5.0 Hz, 1H), 4.36 (m, 1H), 4.22 (d, *J* = 15 Hz, 1H), 3.92 (t, *J* = 9.5 Hz, 2H), 3.80 (m, 2H), 3.25 (m, 2H), 2.89 (m, 2H), 2.78 (m, 2H), 1.84 (m, 4H), 1.71 (m, 2H), 1.58 (m, 4H), 1.34 (m, 2H), 1.24 (m, 1H), 0.88 (m, 6H); LRMS (MALDI): calc'd for [M+ H]$^+$ 692, found 692; Analytical HPLC: homogeneous single peak, retention time = 10.3 min (5-60 % B in 25 min).

2d: ^{1}H NMR (500 MHz, DMSO-d_6) 9.78 (s, 1H), 9.70 (s, 1H), 8.48 (s, 1H), 8.40 (m, 1H), 8.30 (s, 1H), 8.20 (m, 1H), 8.13 (d, *J* = 9.0 Hz, 1H), 7.90 (s, 1H), 7.67 (d, *J* = 7.5 Hz, 1H), 7.58 (s, 2H), 7.50 (m, 1H), 7.43 (m, 1H), 7.29-7.36 (m, 3H), 6.66 (d, *J* = 9.5 Hz, 1H), 6.19 (bs, 1H), 4.68 (d, *J* = 15 Hz, 1H), 4.39 (m, 1H), 4.25 (d, *J* = 15 Hz, 1H), 3.93 (t, *J* = 9.0 Hz, 1H), 3.80 (m, 2H), 3.26 (m, 3H), 3.15 (m, 3H), 2.88 (m, 2H), 1.83 (m, 3H), 1.72 (m, 3H), 1.58 (m, 1H), 1.50 (m, 2H), 1.23 (m, 1H), 0.88 (m, 6H); LRMS (MALDI): calc'd for [M+ H]$^+$ 720, found 720; Analytical HPLC: homogeneous single peak, retention time = 10.1 min (5-60 % B in 25 min).

2e: ^{1}H NMR (500 MHz, DMSO-d_6) 12.1 (s, 1H), 10.0 (s, 1H), 8.78 (d, *J* = 6 Hz, 1H), 8.28 (t, *J* = 6 Hz, 1H0, 8.19 (d, *J* = 8.5 Hz, 1H), 7.71 (s, 3H), 7.65 (s, 1H), 7.60 (d, *J* = 8 Hz, 1H), 7.36 (d, *J* = 7.5 Hz, 1H), 7.28 (s, 1H), 7.18 (s, 1H), 7.11 (d, *J* = 7.5 Hz, 1H), 6.56 (s, 1H), 4.41 (m, 1H), 4.26 (m, 2H), 4.13 (m, 1H), 3.04-3.95 (m, 2H), 2.92 (t, *J* = 11 Hz, 2H), 2.75 (bm, 2H), 2.55 (m, 2H), 2.40 (t, *J* = 7 Hz, 2H), 2.19 (m, 1H), 1.91 (m, 6H), 1.75 (m, 3H), 1.54 (m, 3H),

1.28 (m, 2H); LRMS (MALDI): calc'd for [M+ H]$^+$ 661, found 661; Analytical HPLC: homogeneous single peak, retention time = 6.7 min (5-60 % B in 25 min).

2f: ^{1}H NMR (500 MHz, DMSO-d_6) 9.85 (s, 1H), 9.56 (s, 1H), 8.60 (s, 1H), 8.43 (m, 1H), 8.41 (s, 1H), 8.33 (s, 1H), 8.23 (t, *J* = 6 Hz, 1H), 8.14 (d, *J* = 8 Hz, 1H), 7.92 (s, 1H), 7.83 (d, *J* = 7 Hz, 1H), 7.69 (bs, 3H), 7.63 (d, *J* = 2 Hz, 1H), 7.59 (m, 2H), 7.33 (bs, 2H), 6.79 (d, *J* = 9 Hz, 1H), 6.16 (bs, 1H), 4.64 (d, *J* = 14.5 Hz, 1H), 4.39 (m, 1H), 4.25 (m, 1H), 3.86 (m, 2H), 3.40 (m, 2H), 3.28 (m, 2H), 2.79 (m, 2H), 1.87 (m, 2H), 1.81 (m, 1H), 1.74 (m, 2H), 1.63 (m, 1H), 1.55 (m, 2H), 1.32 (m, 1H); LRMS (MALDI): calc'd for [M+ H]$^+$ 666, found 666; Analytical HPLC: homogeneous single peak, retention time = 8.4 min (5-60 % B in 25 min).

2g: ^{1}H NMR (500 MHz, DMSO-d_6); 10.05(s, 1H), 8.87 (s, 1H), 8.72 (t, J = 5.5, 1H), 8.50 (d, J=8.5, 1H), 8.40 (s, 1H), 8.28 (t, J=4.65, 1H), 7.99 (s, 1H), 7.80-7.61 (m, 7H), 7.44-7.38 (m, 2H), 5.43 (d, J = 12.0, 1H), 5.20 (d, J =12.0, 1H), 4.54-4.47 (m, 1H), 4.01(dd, J=6.5, 6.5, 1H), 3.91-3.83 (m, 3H), 3.33-3.27 (m, 2H), 2.94 (t, J=9.5, 2H), 2.85-2.76 (m, 2H), 2.59-2.52 (m, 1H), 2.01-1.85 (m, 3H), 1.81-1.66 (m, 3H), 1.65-1.51 (m, 3H), 1.48-1.34 (m, 2H). LRMS (MALDI): calc'd for (M+H$^+$) 637, found 637; analytical HPLC: homogeneous single peak, retention time = 11.32 min (2 - 40% B in 30 min).

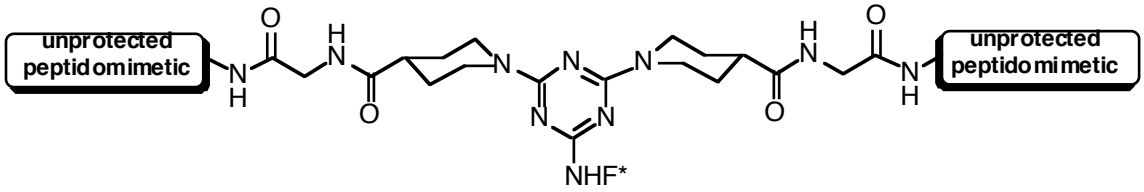
2h: ^{1}H NMR (500 MHz, DMSO-d_6); 10.01(s, 1H), 8.8 (s, 1H), 8.53 (d, J = 8.0, 1H), 8.39 (s, 1H), 8.33 (d, J=9.0), 8.29 (m, 1H), 8.25 (t, J=5.5, 1H), 7.95 (s, 1H), 7.73-7.67 (m, 4H), 7.64 (dd, J=9.0, 2.6, 2H), 7.61(dd, J=8.5, 1.5, 2H), 7.42-7.36 (m, 2H), 5.44 (d, J = 12.0, 1H), 5.26 (d, J =12.0, 1H), 4.45-4.38 (m, 1H), 4.26 (t, J=9.0, 1H), 3.85 (d, J=6.0, 2H), 3.33-3.25 (m, 2H), 2.98-2.87 (m, 2H), 2.84-2.73 (m, 2H), 2.57-2.51 (m, 1H), 1.99-1.84(m, 4H), 1.78-1.68 (m, 3H), 1.62-1.52 (m, 3H), 1.46-1.34 (m, 2H), 1.25-1,15 (m, 1H), 0.95-0.86 (m, 6H). LRMS (MALDI): calc'd for (M+H$^+$) 693, found 693; analytical HPLC: homogeneous single peak, retention time = 14.34 min (2 - 40% B in 30 min).

2i: ^{1}H NMR (500 MHz, DMSO-d_6); 10 (s, 1H), 8.79 (s, 1H), 8.57-8.49 (m, 2H), 8.37 (s, 1H), 8.33 (d, J=9.0, 1H), 8.29-8.22 (m, 2H), 7.96 (s, 1H), 7.72-7.67 (m, 2H), 7.65-7.59 (m, 3H), 7.42-7.36 (m, 3H), 5.42 (d, J = 12.0, 1H), 5.25 (d, J =12.0, 1H), 4.45-4.38 (m, 1H), 4.26 (t, J=9.0, 1H), 3.85 (d, J=6.0, 2H), 3.31-3.26 (m, 2H), 3.18-3.11 (m, 2H), 2.96-2.86 (m, 2H), 2.57-2.51 (m, 1H), 2.05-1.95(m, 1H), 1.95-1.84 (m, 3H), 1.79-1.66 (m, 3H), 1.62-1.48 (m, 3H), 1.25-1,15 (m, 1H), 0.94-0.85 (m, 6H). LRMS (MALDI): calc'd for (M+H$^+$) 721, found 721; analytical HPLC: 93.55%, retention time = 15.423 min (2 - 40% B in 30 min).

3j: ^{1}H NMR (500 MHz, DMSO-d_6) 10.61 (s, 1H), 10.15 (bs, 1H), 9.17 (s, 1H), 8.56 (d, *J* = 8.17 Hz, 1H), 8.45, (bs, 1H), 8.27 (m, 2H), 7.92 (m, 2H), 7.64 (bs, 1H), 7.48 (d *J* = 9.06 Hz, 1H), 7.36 (s, 1H), 7.31 (d, *J* = 9.06 Hz, 1H), 7.24 (d, *J* = 8.49 Hz, 2H), 7.20 (s, 1H), 7.02 (d, *J* = 8.49 Hz, 1H), 6.98 (d *J* = 8.49 Hz, 1H), 6.67 (d, *J* = 2.39 Hz, 2H), 6.64 (bs, 1H), 6.63 (s, 1H), 6.62 (bs, 1H), 6.60 (bs, 1H), 6.56 (m, 1H), 6.54 (m, 1H), 4.59 (m, 1H), 4.53 (d, *J* = 13.34 Hz, 1H), 4.41 (m, 2 H), 4. 14 (q, *J* = 7.40 Hz, 1H), 3.91 (m, 1H), 3.89 (bs, 1H), 3.77 (d, *J* = 12.26 Hz, 1H), 3.04, (m, 1H), 2.94 (m, 1H), 2.88 (m, 2H), 2.80 (m, 1H), 2.76 (m, 1H), 2.72 (m, 2H), 2.63 (m, 2H), 2.61 (m, 2H), 2.54 (m, 2H), 2.36 (m, 1H), 1.55 (m, 2H), 1.47 (m, 2H), 1.24 (m, 2H); LRMS (MALDI): calc'd for [M+ H]$^+$ 1170, found 1170; Analytical HPLC: homogeneous single peak, retention time = 10.20 min (8-70 % B in 30 min).

2k: ^{1}H NMR (500 MHz, DMSO-d_6) 10.17 (s, 1H), 8.58 (m, 1H), 8.41 (d, *J* = 9.33 Hz, 1H), 8.30 (t, *J* = 5.50 Hz, 1H), 8.24 (d, *J* = 8.62 Hz, 1H), 8.13 (d, *J* = 7.66 Hz, 1H), 7.84 (s, 1H), 7.78 (t, *J* = 5.08 Hz, 1H), 7.75 (s, 1H), 7.59 (m, 2H), 7.43 (s, 1H), 7.27 (s, 1H), 4.55 (q, *J* = 7.55 Hz, 1H), 4.22 (t, *J* = 9.56 Hz, 1H), 4.17 (m, 1H), 3.98 (dd, *J* = 6.66, 9.76 Hz, 1H), 3.87 (m, 3H), 3.75 (d, *J* = 12.48 Hz, 2H), 3.56 (dd, *J* = 3.49, 13.31 Hz, 1H), 3.50 (m, 1H), 3.29 (d, *J* = 11.19 Hz, 2H), 3.11 (m, 2H), 2.92 (q, *J* = 11.21 Hz, 2H), 2.75 (m, 1H), 2.54 (m, 1H), 2.33 (m, 1H), 1.89 (m. 3H), 1.82 (m, 1H), 1.73 (m, 2H), 1.64 (m, 1H), 1.46 (m, 3H), 1.14 (m, 1H), 0.89 (d, *J* = 6.84 Hz, 3H), 0.85 (t, *J* = 7.41 Hz, 3H); LRMS (MALDI): calc'd for [M+ H]$^+$ 746, found 746; Analytical HPLC: homogeneous single peak, retention time = 11.51 min (8-50 % B in 30 min).

2l: ^{1}H NMR (500 MHz, DMSO-d_6) 10.18 (s, 1H), 8.60 (m, 1H), 8.40 (d, *J* = 9.24 Hz, 1H), 8.31 (t, *J* = 6.01 Hz, 1H), 8.26 (d, *J* = 8.32 Hz, 1H), 8.11 (d, *J* = 8.32 Hz, 1H), 7.85 (s, 1H), 7.76 (m, 1H), 7.70 (bs, 2H), 7.59 (s, 1H), 7.44 (s, 1H), 7.28 (s, 1H), 4.46 (q, *J* = 7.79 Hz, 1H), 4.20 (m, 2H), 3.98 (dd, *J* = 7.42, 10.01 Hz, 1H), 3.88 (m, 3H), 3.76 (m, 3H), 3.58 (dd, *J* = 3.68, 13.78 Hz, 1H), 3.51 (m, 1H), 3.30 (d, *J* = 11.94 Hz, 2H), 2.93 (m, 2H), 2.76 (m, 2H), 2.55 (m, 1H), 2.33 (m, 1H), 1.90 (m, 2H), 1.82 (m, 1H), 1.74 (m, 2H), 1.63 (m, 1H), 1.53 (m, 3H), 1.32 (m, 2H), 1.15 (m, 1H), 0.90 (d, *J* = 6.63 Hz, 3H), 0.85 (t, *J* = 7.46 Hz, 3H); LRMS (MALDI): calc'd for [M+ H]$^+$ 718, found 718; Analytical HPLC: homogeneous single peak, retention time = 10.91 min (8-50 % B in 30 min).



4

C. Procedure for Preparation of Dimers 4

Culture glass tubes (6 x 50 mm, VWR Scientific Products) were used as a reaction vessel. Stock solutions of all reagents in DMSO were prepared; 0.030 M of compound **3** and 0.033 M of compound **2**. Solid K_2CO_3 (*ca* 1 mg) was first added to each tube, followed by 45 μL of a stock solution of **2** and 45 μL of one of **3**. The reaction vessels were sealed with parafilm, gently shaken by hand and sonicated for 10 min. The mixtures were left at 25 ˚C without stirring for 24 h. The solutions in each vessel were transferred into 5 mL sample vials using a micropipet and then lyophilized to remove DMSO. The solid materials were then washed with H_2O by adding *ca* 0.5 mL of H_2O into each sample and sonicating for 5 min. The solution was left to stand for 30 min then the supernatant was carefully decanted away. The solid products were re-dissolved in 1:1 mixture of H_2O/ CH_3CN, then lyophilized again to give the final products **4**. All crude products were analyzed by analytical HPLC and MALDI-MS. Further spectral data were obtained for ten illustrative compounds, which were purified by preparative HPLC.

4aa: ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.46 (m, 2H), 8.21 (m, 2H), 8.03 (m, 3H), 7.92 (m, 3H), 7.58 (m, 6H), 7.31 (m, 2H), 7.15 (m, 1H), 6.92 (m, 1H), 6.64 (m, 3H), 6.56 (m, 2H), 6.09 (m, 2H), 4.67 (m, 2H), 4.53 (m, 2H), 4.46 (m, 2H), 4.21 (m, 4H), 2.21 (m, 2H), 2.11 (m, 2H), 1.95 (m, 4H), 1.80 (m, 6H), 1.51 (m, 8H), 1.33 (m, 4H); LRMS (MALDI): calc'd for [M+ H]$^+$ 1838, found 1838; Analytical HPLC: homogeneous single peak, retention time = 12.2 min (5-60 % B in 25 min).

4ab: ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.1 (bs, 3H), 9.84 (s, 1H), 9.78 (s, 1H), 9.64 (s, 2H), 9.60 (s, 1H), 8.60 (s, 2H), 8.41 (s, 1H), 8.37 (s, 1H), 8.24 (m, 1H), 8.16 (m, 2H), 7.93 (m, 4H), 7.74 (m, 2H), 7.62 (m, 14H), 7.38 (m, 2H), 7.33 (m, 2H), 7.16 (m, 1H), 6.78 (d, J = 8.5 Hz, 1H), 6.73 (d, J = 8.5 Hz, 1H), 6.67 (bs, 2H), 6.62 (m, 2H), 6.56 (m, 2H), 6.28 (bs, 1H), 6.03 (bs, 1H), 4.67 (bm, 6H), 4.36 (m, 3H), 4.21 (m, 5H), 2.95 (m, 6H), 2.80 (m, 5H), 2.55 (m, 4H), 2.40 (m, 2H), 1.91 (m, 2H), 1.86 (m, 6H), 1.71 (m, 4H), 1.58 (m, 8H), 1.49 (m, 3H), 1.33 (m, 2H); LRMS (MALDI): calc'd for [M+ H]$^+$ 1796, found 1796; Analytical HPLC: homogeneous single peak, retention time = 13.2 min (5-60 % B in 25 min).

4ac: ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.1 (bs, 2H), 9.77 (m, 2H), 9.68 (s, 1H), 9.63 (s, 1H), 9.59 (s, 1H), 8.57 (s 1H), 8.36 (m, 2H), 8.22 (m, 1H), 8.13 (m, 3H), 7.91 (m, 3H), 7.72 (m, 1H), 7.65 (m, 6H), 7.59 (m, 4H), 7.53 (s, 1H), 7.47 (s, 1H), 7.38 (m, 1H), 7.32 (bs, 2H), 7.14 (d, *J* = 8.5 Hz, 1H), 6.70 (m, 3H), 6.62 (m, 2H), 6.55 (m, 2H), 6.24 (bs, 1H), 6.16 (bs, 1H), 4.66 (m, 6H), 4.38 (m, 2H), 4.24 (m, 2H), 4.17 (m, 2H), 3.94 (m, 2H), 3.83 (b, 4H), 2.94 (m, 4H), 2.80 (m, 4H), 1.92 (m, 6H), 1.81 (m, 6H), 1.66 (m, 2H), 1.58 (m, 4H), 1.52 (m, 4H), 1.34 (m, 4H), 1.25 (m, 3H), 0.90 (m, 6H); LRMS (MALDI): calc'd for [M+ H]$^+$ 1822, found 1822; Analytical HPLC: homogeneous single peak, retention time = 13.2 min (5-60 % B in 25 min).

4ad: ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.1 (bs, 2H), 9.77 (m, 1H), 9.71 (s, 1H), 9.63 (s, 1H), 9.59 (s, 1H), 8.57 (s, 1H), 8.37 (s, 1H), 8.33 (s, 1H), 8.23 (m, 1H), 8.15 (bm, 2H), 7.93 (m, 2H), 7.74 (m, 1H), 7.66 (m, 3H), 7.59 (m, 3H), 7.51 (m, 2H), 7.47 (bs, 1H), 7.38 (m, 3H), 7.32 (bs, 3H), 7.15 (d, *J* = 8.5 Hz, 1H), 6.73 (d, *J* = 9.0 Hz, 1H), 6.68 (m, 2H), 6.62 (m, 2H), 6.56 (m, 2H), 6.24 (bs, 1H), 4.67 (m, 5H), 4.38 (m, 2H), 4.28 (m, 2H), 4.17 (m, 2H), 3.17 (m, 2H), 2.94 (m, 4H), 2.80 (m, 2H), 2.57 (m, 4H), 2.40 (m, 2H), 1.91 (m, 2H), 1.80 (m, 5H), 1.68 (m, 2H), 1.55 (m, 9H), 1.35 (m, 2H), 1.24 (m, 4H), 0.90 (m, 6H); LRMS (MALDI): calc'd for [M+ H]$^+$ 1850, found 1850; Analytical HPLC: homogeneous single peak, retention time = 13.4 min (5-60 % B in 25 min).

4bb: ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.1 (bs, 1H), 9.83 (s, 1H), 9.63 (s, 1H), 9.59 (s, 1H), 8.60 (s, 1H), 8.41 (bs, 3H), 8.16 (t, *J* = 6 Hz, 2Hz), 7.91 (bs, 3H), 7.75-7.63 (m, 14H0, 7.37 (d, J = 8.5 Hz, 2H), 6.77 (d, *J* = 9.5 Hz, 2H), 6.67 (m, 2H), 6.62 (d, *J* = 9.5 Hz, 2H), 6.55 (dd, *J* = 1.5, 8.5 Hz, 2H), 6.02 (s, 2H), 4.65 (m, 4H), 4.34 (m, 2H), 4.19 (m, 4H), 2.94 (m, 4H), 2.79 (m, 4H), 2.54 (m, 4H), 1.80 (m, 4H), 1.71 (m, 4H), 1.59 (m, 4H), 1.50 (m, 8H); LRMS (MALDI): calc'd for [M+ H]$^+$ 1754, found 1754; Analytical HPLC: homogeneous single peak, retention time = 12.0 min (5-60 % B in 25 min).

4bc: ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.1 (bs, 2H), 9.82 (s, 1H), 9.72 (s, 1H), 9.68 (s, 1H), 9.64 (s, 1H), 9.59 (s, 2H), 8.59 (s, 1H), 8.36 (s, 1H), 8.14 (m, 3H), 7.92 (m, 3H), 7.56 (m, 1H), 7.38 (s, 1H), 7.36 (m, 1H), 7.32 (s, 2H), 7.14

(d, *J* = 8.5 Hz, 1H), 6.78 (d, *J* = 8.5 Hz, 1H), 6.69 (d, *J* = 8.0 Hz, 1H), 6.67 (m, 1H), 6.62 (m, 2H), 6.55 (m, 3H), 6.16 (bs, 1H), 6.00 (bs, 1H), 5.06 (bs, 1H), 4.65 (m, 6H), 4.38 (m, 3H), 4.19 (m, 4H), 3.95 (m, 2H), 2.94 (m, 4H), 2.79 (m, 4H), 1.80 (m, 5H), 1.70 (m, 2H), 1.58 (m, 10H), 1.51 (m, 2H), 1.34 (m, 2H), 1.26 (m, 2H), 1.16 (m, 2H), 0.90 (m, 6H); LRMS (MALDI): calc'd for $[M+H]^+$ 1780, found 1780; Analytical HPLC: homogeneous single peak, retention time = 13.3 min (5-60 % B in 25 min).

4bd: ^{1}H NMR (500 MHz, DMSO-d_6) □10.1 (bs, 2H), 9.83 (s, 1H), 9.76 (s, 1H), 9.71 (s, 1H), 9.63 (s, 1H), 9.59 (s, 1H), 8.59 (s, 1H), 8.41 (m, 2H), 8.33 (s, 1H), 8.15 (m, 3H), 7.92 (m, 3H), 7.67 (m, 4H), 7.58 (m, 7H), 7.47 (s, 1H), 7.38 (m, 2H), 7.32 (m, 3H), 7.15 (d, *J* = 8.0 Hz, 1H), 6.78 (d, *J* = 8.0 Hz, 1H), 6.68 (m, 3H), 6.62 (m, 3H), 6.56 (m, 3H), 6.16 (bs, 1H), 6.00 (bs, 1H), 5.86 (bs, 1H), 4.66 (m, 8H), 4.40 (m, 2H), 4.35 (m, 2H), 4.27 (m, 1H), 4.20 (m, 4H), 3.15 (m, 3H), 2.94 (m, 6H), 2.79 (m, 4H), 1.81 (m, 6H), 1.70 (m, 4H), 1.58 (m, 4H), 1.51 (m, 8H), 1.25 (m, 2H), 0.89 (m, 6H); LRMS (MALDI): calc'd for $[M+H]^+$ 1808, found 1808; Analytical HPLC: homogeneous single peak, retention time = 13.2 min (5-60 % B in 25 min).

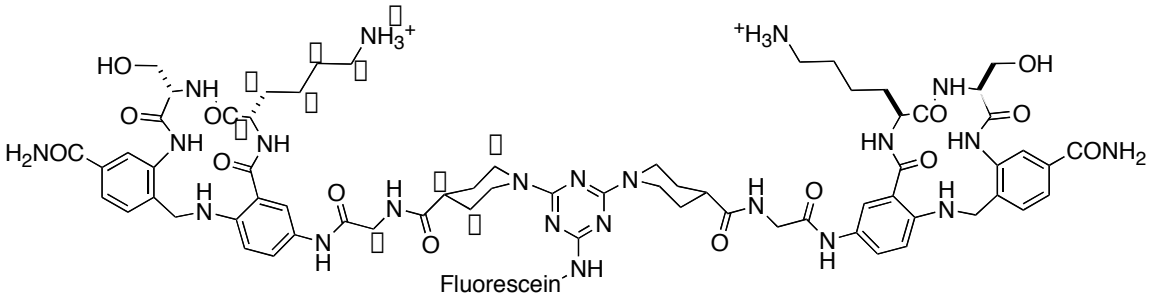
4cc: ^{1}H NMR (500 MHz, DMSO-d_6) □10.1 (bs, 2H), 9.76 (s, 1H), 9.68 (s, 2H), 9.59 (s, 1H), 8.57 (s, 1H), 8.36 (s, 2H), 8.25 (s, 2H), 8.14 (m, 3H), 7.92 (m, 3H), 7.67 (m, 6H), 7.59 (bs, 4H), 7.47 (s, 2H), 7.37 (d, *J* = 9.5 Hz, 2H), 7.32 (s, 2H), 7.14 (d, *J* = 8.5 Hz, 1H), 6.68 (m, 3H), 6.62 (m, 2H), 6.56 (m, 3H), 6.15 (bs, 2H), 4.66 (m, 6H), 4.38 (m, 2H), 4.24 (d, *J* = 13.5 Hz, 2H), 3.94 (t, *J* = 9 Hz, 2H), 3.82 (m, 4H), 2.93 (m, 6H), 2.79 (m, 4H), 1.80 (m, 8 H), 1.58 (m, 2H), 1.34 (m, 2H), 1.24 (s, 4H), 0.88 (m, 12H); LRMS (MALDI): calc'd for $[M+H]^+$ 1807, found 1807; Analytical HPLC: homogeneous single peak, retention time = 14.1 min (5-60 % B in 25 min).

4cd: ^{1}H NMR (500 MHz, DMSO-d_6) □10.1 (bs, 2H), 9.76 (s, 1H), 9.72 (s, 2H), 9.68 (s, 1H), 9.59 (s, 1H), 8.58 (s, 1H), 8.33 (s, 1H), 8.14 (m, 4H), 7.92 (m, 4H), 7.67 (m, 5H), 7.59 (m, 5H), 7.52 (m, 2H), 7.47 (m, 2H), 7.37 (m, 2H), 7.32 (m, 4H), 7.14 (m, 2H), 6.69 (m, 4H), 6.62 (m, 3H), 6.55 (b, 2H), 4.67 (m, 8H), 4.40 (m, 4H), 4.24 (m, 4H), 3.96 (m, 4H), 3.16 (m, 4H), 2.95 (m, 6H), 2.81 (m, 4H), 1.81 (m, 10 H), 1.54 (m, 8H), 1.37 (m, 2H), 1.26 (m, 10H), 0.88 (m, 16H); LRMS (MALDI): calc'd for $[M+H]^+$ 1834, found 1834; Analytical HPLC: homogeneous single peak, retention time = 14.2 min (5-60 % B in 25 min).

4dd: ^{1}H NMR (500 MHz, DMSO-d_6) □10.1 (bs, 2H), 9.75 (s, 1H), 9.71 (s, 1H), 9.58 (s, 1H), 8.58 (s, 1H), 8.32 (s, 2H), 8.14 (m, 3H), 7.92 (m, 3H), 7.68 (d, *J* = 7 Hz, 2H), 7.60 (s, 3H), 7.49 (t, *J* = 6 Hz, 2H), 7.47 (s, 2H), 7.36 (m, 2H), 7.31 (s, 2H), 7.14 (d, *J* = 8 Hz, 2H), 6.67 (m, 4H), 6.62 (m, 2H), 6.52 (m, 2H), 6.21 (s, 2H), 4.66 (m, 2H), 4.42 (m, 2H), 4.25 (d, *J* = 14 Hz, 2H), 3.94 (t, *J* = 8.5 Hz, 2H), 3.82 (m, 2H), 3.17 (m, 4H), 2.94 (m, 4H), 1.80 (m, 8H), 1.71 (m, 2H), 1.54 (m, 10H), 1.24 (s, 6H), 0.90 (m, 12H); LRMS (MALDI): calc'd for $[M+H]^+$ 1862, found 1862; Analytical HPLC: homogeneous single peak, retention time = 14.5 min (5-60 % B in 25 min).

To provide further evidence that the monochlorotriazine derivatives **3** selectively couple to the amine of the Inp residue over other reactive sites in fragments **2**, TOCSY spectra of the dimer **4bb** and its monomeric constituent **2b** were analyzed. Absence of the InpN<u>H</u> protons and the change in piperidine ring hydrogens chemical shifts in compound **4bb** (Figure S1), relative to those observed in compound **2b** (Figure 5.8), confirmed our assumption that the amino group of the Inp residue is the only reactive nucleophile under the reaction condition used. It is noted that the two signals of the InpN<u>H</u> in compound **2b** are due to its protonatation after TFA cleavage.

a



4bb

b

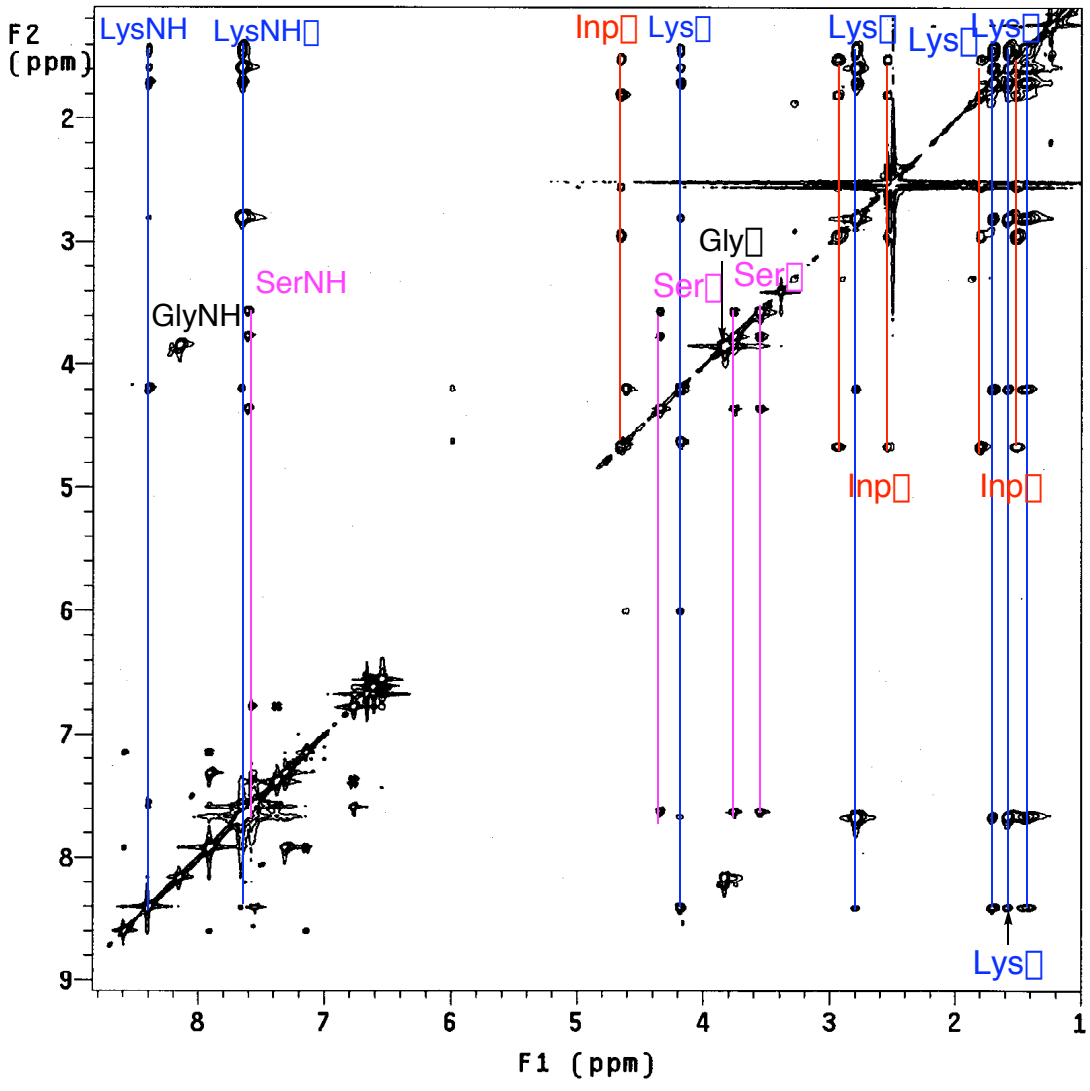
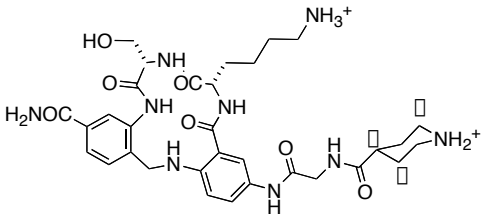


Figure S1. **a** Structure of compound **4bb**, **b** TOCSY spectrum of compound **4bb**.

a



b

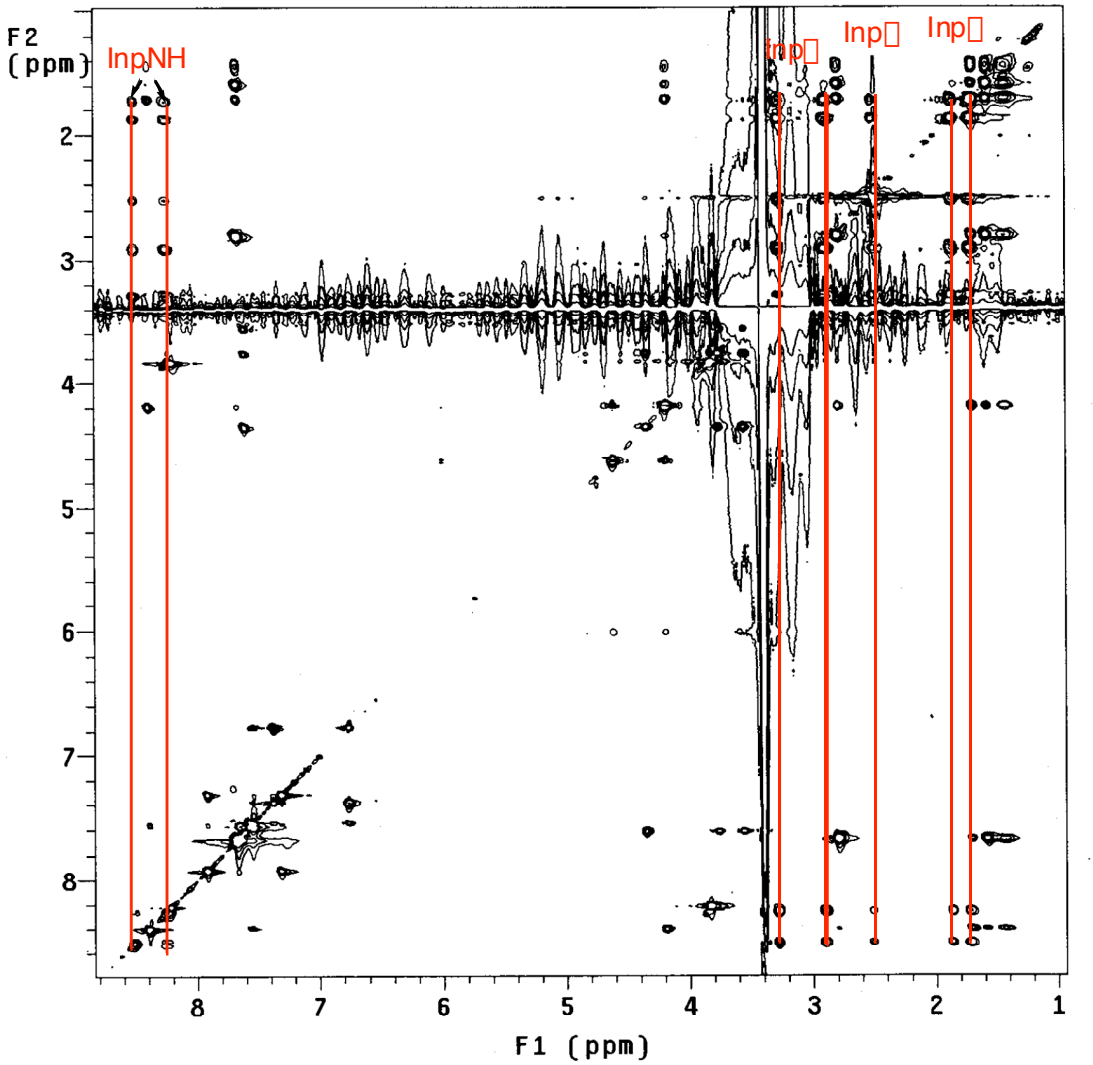


Figure S2. **a** Structure of compound **2b**; **b** TOCSY spectrum of compound **2b**.

D. Procedure for FACS Assays

Cells (2x105) in FACScan binding buffer (PBS, 0.5% BSA, and 0.1% NaN3) were exposed to compounds at 4 °C for 30 min. Cells used were wild type NIH3T3 that do not express any of the Trk receptors, or wild type NIH3T3 cells stably transfected with TrkA cDNA (NIH-TrkA cells) or TrkC cDNA (NIH-TrkC cells). Standardization of binding by FITC-tagged compounds was done using FITC-tagged positive controls anti-TrkA mAb 5C3, or anti-TrkC mAb 2B7 (each at saturating concentrations, ~7 nM). Data were acquired on a FACScan (Becton Dickinson, CA) and mean channel fluorescence of bell-shaped histograms were analyzed using the LYSIS II program. Data were averaged relative to positive control. In each case fluorescent peptidomimetic binding to wild type cells (that do not express Trk receptors) were studied and considered to be background, n=3 ± sd.

In Figure 2, % binding data ± standard deviation obtained from direct binding studies with FITC tagged compounds (25 μM) or FITC-tagged mAbs (saturating concentrations, 7 nM). Data are standardized relative to positive control mAbs = 100%. n=3-5 ± sd. The fluorescence staining observed is a measure of the affinity of the fluorescently labeled peptidomimetics for cells that over-express the TrkA or the TrkC receptors. These data are not directly proportional to binding constants since other parameters, particularly dissociation rates k_{off}, play a role. However, to be observed in this assay the ligands must have K_d values of less than approximately 1 μM. The mAb's used as positive controls have K_d values of 2 – 8 nM.

E. Complete Data From FACS Assays

	TrkC Binding	TrkA Binding
mAbs	100 ± 0	100 ± 0
Monomers		
a	**31 ± 2**	10 ± 7
b	-1 ± 8	12 ± 7
c	**39 ± 12**	5 ± 3
d	4 ± 8	3 ± 5
e	15 ±22	12 ± 13
f	16 ± 5	2 ± 2
g	**28 ± 6**	7 ± 5
h	**41 ± 8**	12 ± 8
i	14 ± 3	5 ± 2
j	12 ± 6	2 ± 1
k	23 ± 16	4 ± 2
l	15 ± 23	4 ± 4
Homobivalents		
aa	**76** ± 22	**9** ± 1
bb	18 ± 13	3 ± 2
cc	**23 ± 3**	4 ± 5
dd	43 ± 50	4 ± 5
ee	15 ± 8	**63** ± **11**
ff	0 ± 3	-1 ± 1
gg	3 ± 4	1 ± 3
hh	2 ± 2	0 ± 3
ii	2 ± 2	0 ± 2
jj	5 ± 2	0 ± 1
kk	7 ± 15	4 ± 9
ll	23 ± 15	-4 ± 4

	TrkC Binding	TrkA Binding
mAbs	100 ± 0	100 ± 0
	Heterobivalent	
ac	34 ± 19	7 ± 4
bc	26 ± 25	4 ± 5
ad	**30** ± 2	5 ± 4
bd	26 ± 11	7 ± 7
cd	**30** ± 8	3 ± 6
ae	**47 ± 9**	12 ± 5
be	**15 ± 3**	2 ± 4
ce	10 ± 1	2 ± 2
de	12 ± 0.5	3 ± 4
af	9 ± 4	-1 ± 1
bf	4 ± 0.5	0 ± 1
cf	-1 ± 1	0 ± 1
df	1 ± 1	0 ± 1
ef	17 ± 5	-6 ± 8
ag	5 ± 4	-1 ± 1
bg	2 ± 3	0 ± 1
cg	13 ± 14	-4 ± 2
dg	3 ± 9	7 ± 1
eg	10 ± 4	1 ± 6
fg	2 ± 5	-2 ± 2
ah	12 ± 9	0 ± 1
bh	5 ± 4	0 ± 1
ch	5 ± 7	-1 ± 2
dh	9 ± 8	0 ± 2
eh	12 ± 3	-1 ± 6
fh	9 ± 10	-3 ± 3
gh	6 ± 4	4 ± 6
ai	1 ± 2	4 ± 2
bi	1 ± 1	2 ± 1
ci	0 ± 1	0 ± 1
di	-2 ± 2	2 ± 1
ei	**20 ± 0.5**	2 ± 12
fi	2 ± 1	3 ± 1
gi	2 ± 1	0 ± 2
hi	0 ±2	1 ± 2
aj	4 ± 10	0 ± 7
bj	0 ± 3	-1 ± 2
cj	0 ± 2	0 ± 2
dj	0 ± 2	0 ± 1
ej	11 ± 14	-6 ± 8
fj	0 ± 2	0 ± 1
gj	0 ± 2	0 ± 2
hj	1 ± 4	0 ± 3
ij	3 ± 1	1 ± 1

	TrkC Binding	TrkA Binding
mAbs	100 ± 0	100 ± 0
ak	7 ± 10	-2 ± 1
bk	8 ± 5	1 ± 1
ck	4 ± 1	1 ± 1
dk	6 ± 4	2 ± 3
ek	**64 ±20**	3 ± 9
fk	12 ± 4	4 ± 3
gk	8 ± 1	1 ± 1
hk	**31 ± 14**	6 ± 6
ik	**51 ± 12**	5 ± 2
jk	6 ± 2	0 ± 2
al	**34 ± 18**	-3 ± 1
bl	**14 ± 1**	3 ± 1
cl	11 ± 5	10 ± 7
dl	**28 ± 10**	**20** ± 2
el	**41 ± 8**	4 ± 3
fl	13 ± 3	1 ± 1
gl	**31 ± 8**	3 ± 3
hl	**32** ± 10	5 ± 5
il	**54** ± 5	4 ± 9
jl	15 ± 7	-5 ± 2
kl	**33** ± 9	3 ± 4

E. Further Discussion of Data From FACS Assays

Full consideration of these data is reserved for a publication in a more biologically oriented journal. The following are some initial observations. We identified 4 monomers, 3 homodimers, and 16 heterodimers (23 compounds out of a library of 88 compounds) with significant ($p \leq 0.01$) binding towards a specific receptor. Monomers **a**, **c**, **g**, and **h** had significant TrkC binding ($p \leq 0.01$). Binding was selective because no significant TrkA binding was seen for any of the monomers.

Homodimer **cc**. had significant and selective TrkC binding. However, there was no statistical difference between the TrkC binding by monomer **c** and homodimer **cc**. This suggests that ligand homodimerization did not result in efficient bivalent binding to dimeric receptors. There are two explanations for this observation. First, the **cc** homobivalent compound may target receptor hot-spots at a distance greater than that spanned by the linker (~20 Å). Alternatively, it is possible that the linker interferes with binding efficiency. We favor the latter possibility because homodimers of **g** and **h** did not bind TrkC while their corresponding monomers do, suggesting steric interference.

Homodimer **aa** had statistically significant TrkC and TrkA binding, but binding to TrkC was more robust. TrkC binding by the **aa** homodimer was significantly improved compared to the **a** monomer. TrkA binding by the **aa** homodimer was not statistically better compared to the **a** monomer.

Homodimer **ee** had significant and selective TrkA binding. Homodimerization of monomer **e** (and to some extent also the homodimerization of monomer **a**) affords the ability to detect robust binding to TrkA in the FACscan assay. It is likely that this is the consequence of an overall increased affinity due to a decrease in the off rate of the **ee** ligand binding as a dimer.

All the heterodimers shown in Figure 2 had significant binding to TrkC, namely, **cd**, **ad, ae**, **be**, **ei**, **ek**, **hk**. **ik**, **al**, **bl**, **dl**, **el**, **gl**, **hl**, **il**, and **kl**. These heterodimers (except **dl**) were selective for TrkC and they did not bind TrkA. All the other heterodimers did not bind either TrkC or TrkA significantly. It was anticipated that any heterodimer ligand containing **a**, **c**, **g**, and **h** would bind TrkC because these ligands do bind as monomers. Given that this is not the case it is clear that linker can have an impact. However, for TrkC we did not detect improved intra-receptor binding with heterodimeric ligands, as would be expected if, for example, ligands **a** and **c** had different but topographically close epitopes on TrkC. One example where a heterodimer improved intra-receptor binding was for **dl** binding to TrkA. Each monomer **d** and **l** did not have any binding (5±3 % and 4±5 % respectively), whereas heteromer **dl** had a substantial and significant 20 ± 2.5 % binding to TrkA.