Organic & Biomolecular Chemistry

A Fluorescent Electrophile For CLIPS: Self Indicating TrkB Binders

Journal:	*Organic & Biomolecular Chemistry*
Manuscript ID	OB-ART-10-2023-001654.R1
Article Type:	Paper
Date Submitted by the Author:	05-Dec-2023
Complete List of Authors:	Thompson, Tye; Texas A&M University College Station, Chemistry Pewklang, Thitima; Suranaree University of Technology Institute of Science, Chemistry; Piyanuch, Pornthip; Silpakorn University Wanichacheva, Nantanit; Silpakorn University, Department of Chemistry Kamkaew, Anyanee; Suranaree University of Technology, Chemistry Burgess, Kevin; Texas A&M University College Station, Chemistry

A Fluorescent Electrophile For CLIPS: Self Indicating TrkB Binders

Tye Thompson,[1] Thitima Pewklang,[1,2] Pornthip Piyanuch,[3] Nantanit Wanichacheva,[3] Anyanee Kamkaew,[2] and Kevin Burgess*[1]

[1] *Department of Chemistry, Texas A & M University, Box 30012, College Station, TX 77842-3012, USA.*

[2] *School of Chemistry, Institute of Science, Suranaree University of Technology, Nakhon Ratchasima 30000, Thailand.*

[3] *Department of Chemistry, Faculty of Science, Silpakorn University, Nakhon Pathom 73000, Thailand.*

E-mail: burgess@tamu.edu

TITLE RUNNING HEAD: endocyclic fluorescent peptidomimetic

ABSTRACT

Combination of cysteine-containing peptides with electrophiles provides efficient access to *cyclo*-organopeptides. However, there are no routes to *intrinsically fluorescent cyclo*-organopeptides containing robust, brilliant fluorophores emitting at wavelengths longer than cellular autofluorescence. We show such fluorescent *cyclo*-organopeptides can be made via S_NAr reactions of cysteine-containing peptides with a BODIPY system. Seven compounds of this type were prepared to test as probes; six contained peptide sequences corresponding to loop regions in brain-derived neurotrophic factor and neurotrophic factor 4 (BDNF and NT-4) which bind tropomyocin receptor kinase B (TrkB). Cellular assays in serum-free media indicated two of the six key compounds induced survival of HEK293 cells stably transfected with TrkB

whereas a control did not. The two compounds inducing cell survival bound TrkB on those cells (K_d ~40 and 47 nM), illustrating how intrinsically fluorescent *cyclo*-organopeptides can be assayed for quantifiable binding to surface receptors in cell membrane environments.

INTRODUCTION

"*cyclo*-Organopeptides" are comprised of a peptide fragment and an endocyclic organic one,[1, 2] and are used as secondary structure mimics (*eg* stapled helices[3-5] and caps,[6] β-turn analogs[7]) in probes for cell biology and as pharmaceutical leads.[8-12] Incorporation of an organic part confers several advantages over natural cyclic peptides. First, endocyclic organic fragments may impart rigidity, potentially leading to diminished loss of entropy on binding to bio-receptors with complementary conformations, increased affinities and greater selectivities. Second, they can be used to introduce characteristics useful to overcome downstream issues. For instance, endocyclic organics can reduce overall hydrophilicities of peptidic systems, giving favorably longer residence times in blood, and improve cell permeabilities with respect to *intracellular* targets. However, *cyclo*-organopeptides are most suitable for *extracellular* targets where cell permeability is not an issue.

Chemical LInkage of Peptides onto Scaffolds (CLIPS) reactions[13-15] are arguably the most convenient way to construct *cyclo*-organopeptides (Fig 1a). CLIPS reactions are the click reactions of peptidomimetic chemistry because of their chemoselectivities, efficiencies, and tolerance to substrate diversity.

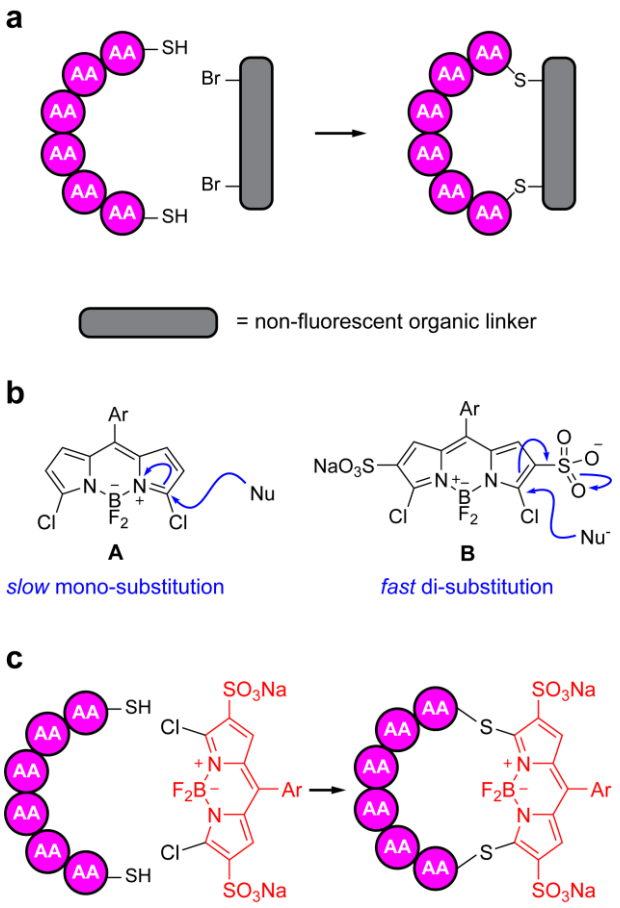


Fig. 1. **a** Typical CLIPS or CLIPS-like construction of cyclic peptides where *S*-containing residues selectively displace a benzylic or aromatic halide. **b** Non-sulfonated BODIPY **A** undergoes S*N*Ar slower and requires harsher conditions less suitable to peptide chemistry than the disulfonated BODIPY **B**. **c** The intrinsically fluorescent *cyclo*-organopeptides synthesized and explored in this paper.

We saw unrealized potential in *cyclo*-organopeptides where the organic part is a fluorescent dye. *Intrinsically labeled* compounds of this type would be ideal for early-stage assays involving fluorescence detection and could evolve to optimized diagnostic probes without excessive modification. We hypothesized intrinsically labeled *cyclo*-organopeptides might be especially useful as probes for cell surface receptors. The problem is there has been surprisingly little research on generation of small molecule libraries containing intrinsic fluorescent probes.[27]

Fluorescent **I**soindole **C**rosslin**k**ing (**FIIC**k) chemistry[16-22] simultaneously involves fluor construction and cyclization to *cyclo*-organopeptides. This innovation was a milestone, but it has downstream limitations insofar as isoindoles are so delicate that shelf half-lives and aberrant

reactions in cell media are concerns. Further, UV and fluorescence properties of isoindoles are unsuitable for some detection methods. Consequently, the **FlIC**k pioneers added extra steps to modify the fluorophore components for enhanced stabilities and photophysical properties, but this means the systems are larger and less accessible in a library format.

In other, less cited, work, a bimane-based linker was used to cyclize linear peptides to *cyclo*-organo forms.[23-25] This innovation was the first CLIPs reaction to give an intrinsic label. Unfortunately, the bimane excitation (maximal at ~380nm) overlaps with cellular autofluorescence. There are other intrinsically fluorescent *cyclo*-organopeptides,[26, 27] but not for libraries.

We saw an opportunity to develop S_NAr reactions of the dichloro-BODIPY framework **A** (Fig 1b), as originally reported from our labs,[28] which are compatible with formation of *cyclo*-organopeptides. Parent BODIPY **A** is not ideal for formation of *cyclo*-organopeptides because the elevated temperatures and organic solvents required[29-31] are not conducive to selective reactions with unprotected and/or temperature sensitive peptides. Our hypothesis was BODIPY **B**[28] (Fig 1b) would have enhanced reactivities towards nucleophiles because of the electron-withdrawing effects of the sulfonate functionalities, hence selective *S_NAr* reactions could be used to form intrinsically fluorescent *cyclo*-organopeptides under mild conditions (Fig 1c). Other, more hydrophobic electron withdrawing groups on the 2- and 6-positions of the BODIPY could potentially be used to enhance cell permeability, though for this study we strove to validate our system on the cell surface receptor tropomyocin receptor kinase B (TrkB)[32-34] as a proof-of-concept. Another goal was quantifiable direct binding assays for these compounds associating with TrkB receptors folded in natural cell surface environments.

RESULTS AND DISCUSSION

Model Studies Amino acid-derived *O*, *N*, and *S*-nucleophiles were reacted with BODIPY **1** (**B** where Ar = 4-MeO-C_6H_4-) in DMF (diisopropylethylamine {DIPEA}, 25 °C, up to 24 h; Figure S1).[29] *N*-Acetyl cysteine produced the highest conversion (44%) to a mono-substituted product, **2a** (Table S1), while the other five nucleophiles gave less (15 - 27%, **2b - f**). That data formed the basis for comparison of similar reactions in aqueous media compatible with unprotected amino acids. Encouragingly, the di-substituted product **3a** formed rapidly with excellent conversion in $NaHCO_{3\,(aq)}$ (0.1 M, pH 8, 25 °C).

Other amino acid-derived nucleophiles were reacted under the same conditions to explore selectivity. Substitution using *N*-α-acetyl lysine proceeded rapidly, but only the mono-substituted product **2b** was formed, even after 25 h at 75 °C in an unfruitful attempt to force formation of the disubstituted BODIPY **3b**. Other nitrogen nucleophiles also gave only mono-substitution at 25°C (*N*-ε-acetyl lysine, proline, and piperidine; **2c**, **2d**, and **2g** respectively). These observations indicated there could be significant and useful chemoselectivity in favor of di-substitution with *S*-nucleophiles over *N*- and *O*-ones in S_NAr reactions of **1** (Fig 2 and Table S1).

Reactions under dilute conditions were monitored via UV-Vis spectroscopy to compare reaction rates between cysteine and lysine nucleophiles (Fig 2b). Formation of the di-substituted product **3a** from Cys occurred ~60 x faster than those to give the mono-substituted Lys derivative **2b** ($t_{1/2}$ 6.2 and 390 min, respectively; Fig 2c). Direct competition between the two nucleophiles in one pot gave near 100% **3a** while **2b** was undetectable (Fig 2d).

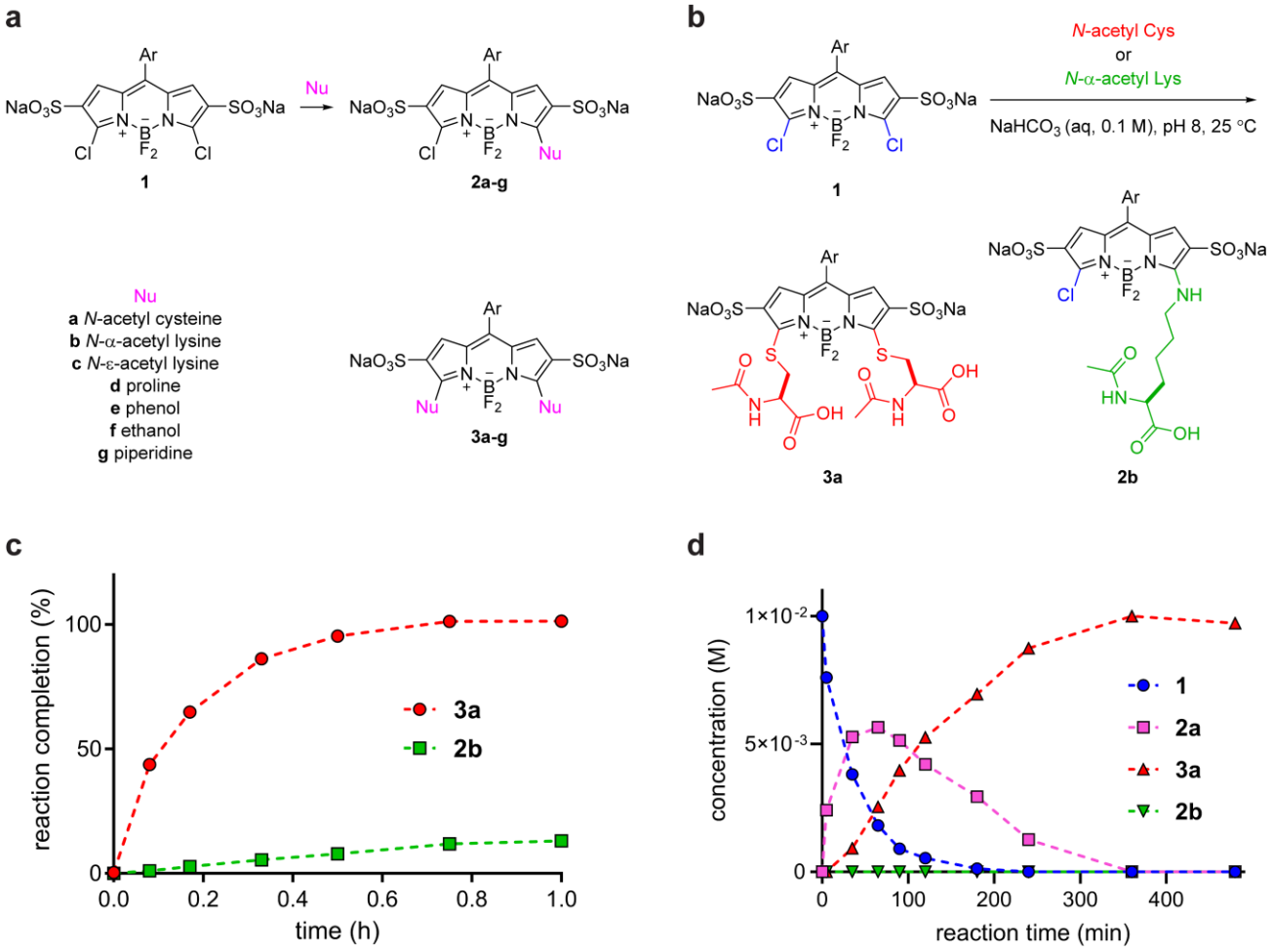
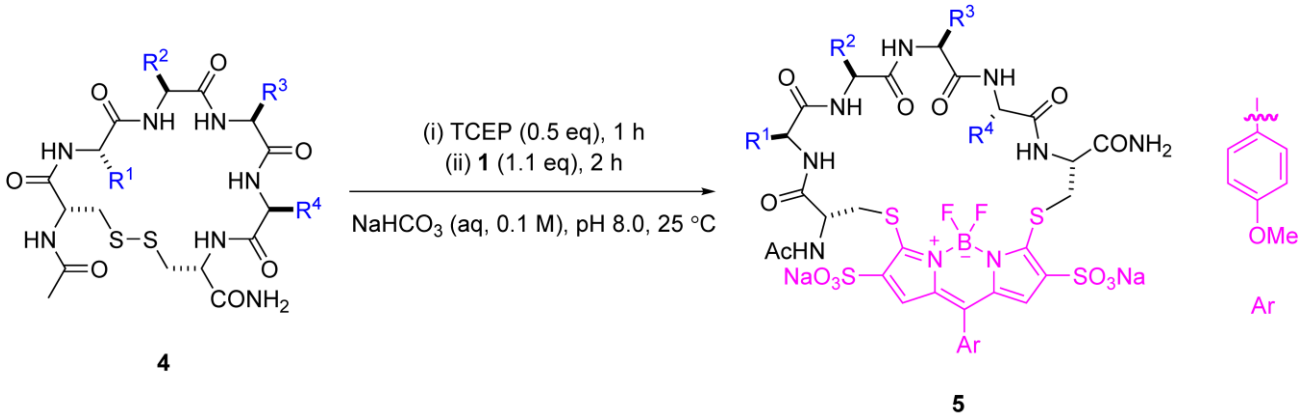


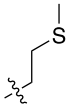
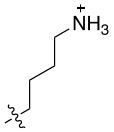
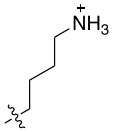
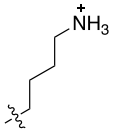
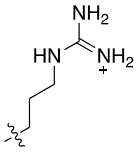
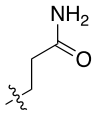
Fig 2. **a** Reaction of BODIPY **1** with all nucleophiles and product naming scheme. **b** Reaction scheme of **1** with two nucleophiles of interest and their respective products. **c** Independent comparison of the rates of product formation between the cysteine and lysine nucleophiles. **d** Reaction mixture rate analysis (evaluated via HPLC).

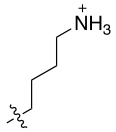
Syntheses Of Loop Mimics. Chemoselectivities in the reaction of Fig 1c were examined for unprotected, multifunctional peptides. Peptides of the type Cys-AA$^{1-4}$-Cys were prepared via manual solid phase syntheses on TentaGel S-Ram (standard loading, Rink amide linker). These were simultaneously cleaved from resin and globally deprotected (95% TFA, 2.5% H_2O, and 2.5% $^{i}Pr_3SiCl$). Some oxidation to disulfide linked products was not conveniently avoidable in this process; the cleavage products shown in Table 1 are represented as disulfides **4** for clarity, but they were mixtures with the reduced form. These crude products were reduced *in situ* (using TCEP, tricarboxyethyl phosphine) then reacted with BODIPY **1** at 25°C in $NaHCO_{3\ (aq)}$.

Analytical HPLC analyses of the crude products indicated formation of one predominant product under these conditions. *cyclo*-Organopeptides **5a** - **g** were isolated in 2 -10 mg amounts after preparative HPLC and were >95% pure (anal HPLC 280 nm UV detection, HR-ESI, 1 and 2D NMR; Table 1 and SI).

Table 1. Cyclization of peptides **4** with dye **1** to form the indicated *cyclo*-organopeptides **5**.



	sequence C-(AA$^{1-4}$)-C	R^1	R^2	R^3	R^4	neurotrophin[1]	loop
5a	-DMSG-					BDNF	1
5b	-VSKG-					BDNF	2
5c	-DSKK-					BDNF	3
5d	-DLRG-					NT-4	1
5e	-AGGS-					NT-4	2
5f	-DAQG-					NT-4	3

5g (control)[2]	-DIKG-					NGF	1

[1]All these sequences correspond to neurotrophins found in humans, *eg* hBDNF. [2]This sequence is based on loop 1 of hNGF, a neurotrophin that binds TrkA, thus is a partial control.

Neurotrophins are highly homologous cytokines which bind the tropomyosin kinase receptors, Trk.[35, 36] Crystallographic data for these interactions is limited due to the usual difficulties crystallizing complete cell surface receptors, especially complexed with their neurotrophin ligands.[37, 38] Loops highlighted in Fig 3 are hot-spots for Trk binding, based on evidence from site-directed mutagenesis, chimeric proteins, and some crystallographic data.[37, 39-45] Consequently, we[46-49] and others[50-52] have made “dark” (non-fluorescent) loop mimics that effect on Trk-expressing cells.[41, 53-55]

Peptide sequences in the fluorescent loop mimics in Table 1 correspond to the loop regions of brain-derived neurotrophic factor and neurotrophic factor-4 (hBDNF and hNT-4, Fig 3). These are extracellular neurotrophins which preferentially bind TrkB over A and C.[56] Compound **5g** in Table 1 contains a sequence corresponding to a loop in nerve growth factor (NGF, a TrkA ligand) to be used as a negative control as it does not correspond to the loops in BDNF and NT-4. We refer to **5g** as a *partial* negative control because the neurotrophins can cross bind Trk receptors with diminished affinities.[35, 36]

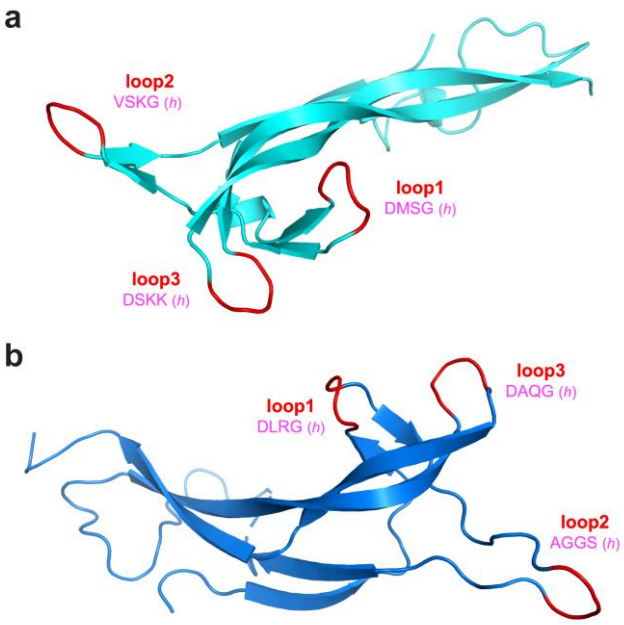


Fig 3. Hot-loop regions in: **a** BDNF; and **b** NT-4.

Fluorescence and UV absorption spectra collected for loop mimics in Table 1 were similar to that of compound **3a** prepared in the model study to assess displacement of chloride by *N*-AcCys (Fig S1). There is a close correspondence of spectroscopic parameters among the loop mimics due to the conservation of the di-substituted BODIPY fluorophore structure, where the average UV absorbance maxima were centered on 545 nm (in the range 543 - 547 nm) and their average fluorescence maxima in water occurred at 574 nm (in the range 572 - 577 nm; see Fig S1).

Cell Survival Assays. The next phase was to test the loop mimics in biological assays to detect TrkB-related activities. Cells grown in serum-free media typically starve and undergo apoptosis. Those expressing TrkB can be rescued from cell death by adding BDNF, NT-4, or a small-molecule agonist. Cell survival assays of this kind can be used to probe the cellular effects of synthetic loop mimics.

All compounds shown in Table 1 were tested using HEK293 cells stably transfected with TrkB. Loop mimics **5a** - **g** were incubated with these cells in serum free media (SFM), and degree of

survival was quantified via flow cytometry, normalized to the maximum survival imparted by 1.0 nM BDNF. Partial agonists are anticipated to enhance cell survival in the presence of suboptimal levels of BDNF (0.6 nM) and true agonists to increase survival without supplemental neurotrophin.

High levels of cell survival imparted by **5a** and **5b** (Fig 4a, blue bars) indicate these are true agonists of TrkB. A decreased response under partial agonism conditions (Fig 4a, red) relative to true agonism indicates competition between BDNF and the loop mimics, reducing their efficacy. These compounds also show increased survival relative to our previously reported **pan**[41, 54] (a "pan-Trk" agonist) and **D3**[41, 54] (a TrkA partial agonist, used as a partial negative control here) under true agonism conditions (Fig 4b), though **pan** outperforms both under partial agonism conditions (Fig 4c). Dose-dependent cell survival experiments for **5a** and **5b** (Fig 4d - e, respectively) were consistent with the initial screen showing slightly higher efficacy for **5a** over **5b** (EC_{50} = 0.4 and 0.9 μM). The EC_{50} is higher under partial agonism conditions (1.8 and 2.8 μM), illustrating that BDNF and the compounds are competing for binding to TrkB, ultimately leading to reduction in activity. These observations were seen for all points in the dose response curves.

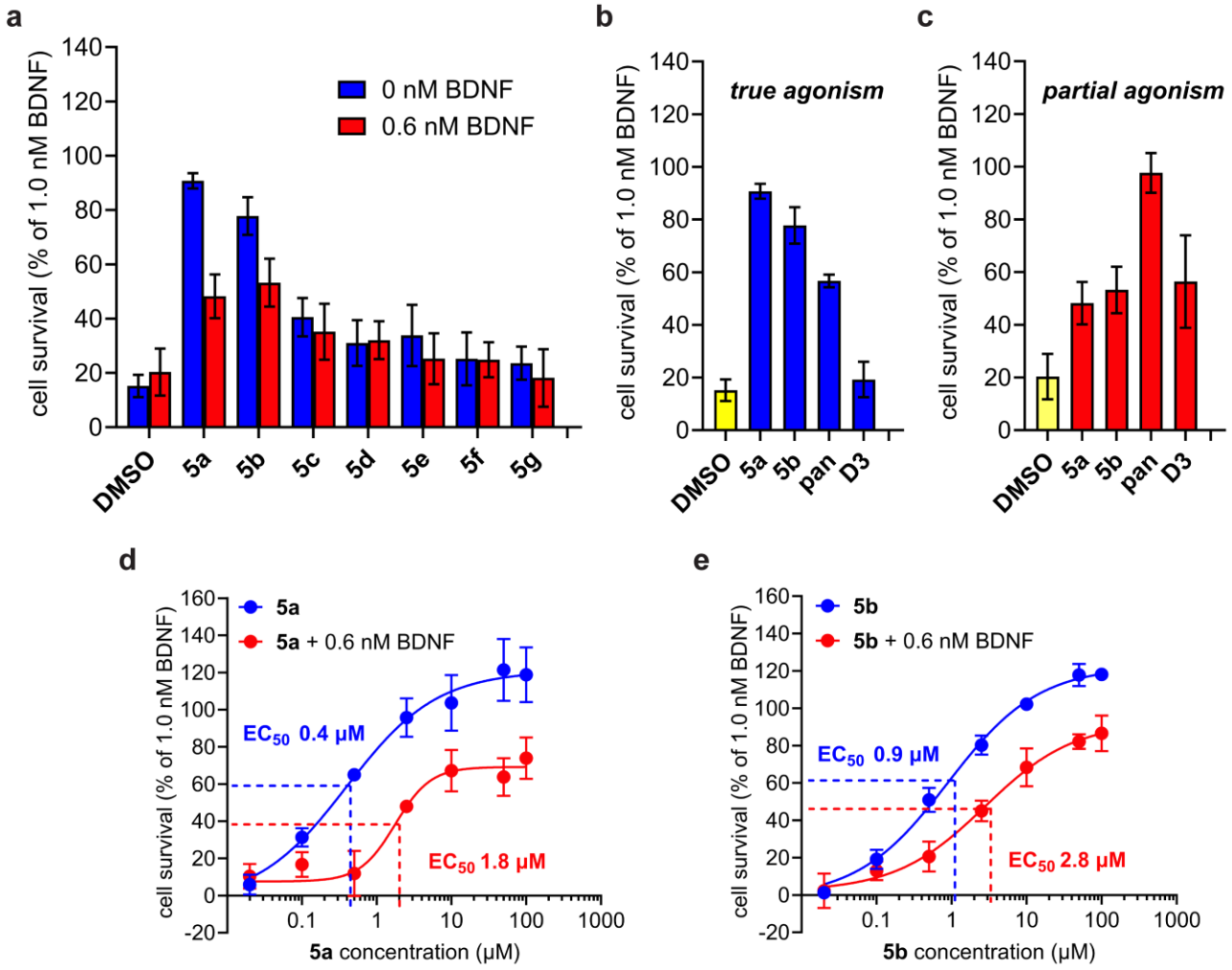


Fig 4. **a** Screen of all synthesized compounds (50 μM) for TrkB-induced cell survival in HEK293-TrkB cells, normalized to survival imparted by BDNF. **b** and **c** Comparison between the two best compounds and other reported compounds in true (**b**) and partial (**c**) agonism experiments. **d** and **e** Comparison of cell survival dose response between **5a** (**d**) and **5b** (**e**) in true agonism (blue) and partial agonism (red) assays.

Trk B Binding Affinities. Intrinsic fluorescence of **5a** and **5b** facilitates determination of K_d values for relative binding to the cell surface[57] on TrkB-expressing cells via observation of cell surface fluorescence after washing and correction for non-specific binding (Fig 5). It is worth nothing that these K_d values are dependent on the level of expression of the TrkB receptor. Controls in these experiments are: (i) competition with the native neurotrophin BDNF (red line); and (ii) repetition of the experiment using the parental cell line which does not express Trk receptors (HEK293 in this case; black line). Expected outcomes for these control experiments, diminished and negligible fluorescence, respectively, were observed. Both **5a** and **5b** showed significant binding. The more potent compound in cell survival assays, **5a**, also bound TrkB-expressing cells with slightly higher affinity (**5a**, K_d ~40; and **5b**, ~47 nM). Consistent with cell survival, incubation with 0.6 nM BDNF in addition to the compound inhibited most of the binding. Incubation of the compounds with the parent cell line demonstrated low levels of non-specific binding to the cell surface.

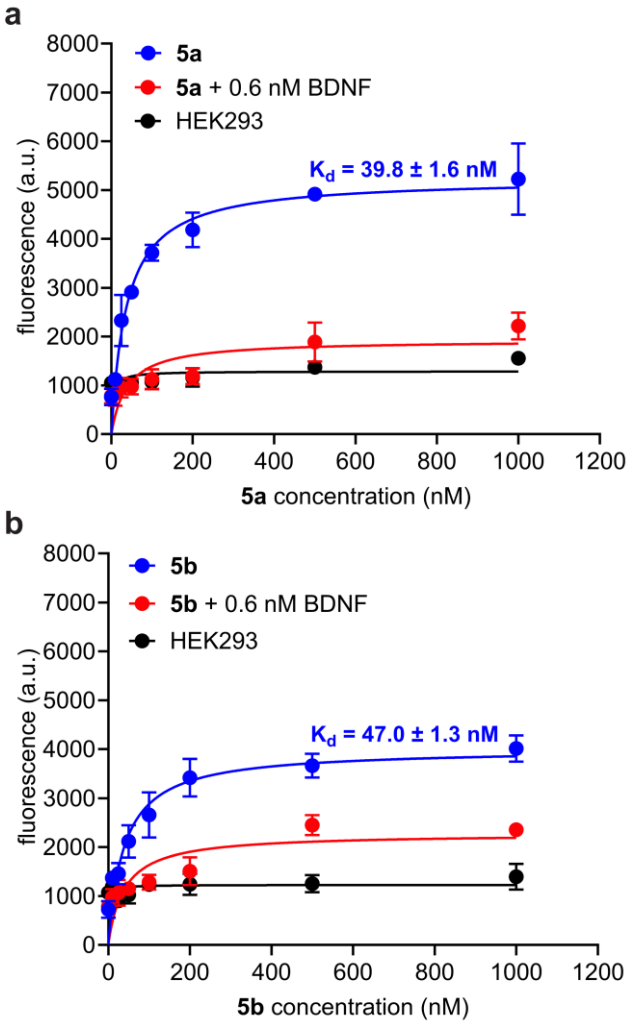


Fig 5. Observed fluorescence of **5a** (**a**) and **5b** (**b**) binding to HEK293-TrkB cells by themselves (blue) and in competition with BDNF (red), or to non-TrkB expressing HEK293 control cell line (black).

CONCLUSIONS

Methodologies presented here enable syntheses of intrinsically fluorescent *cyclo*-organopeptides where the dye fluoresces at around 550 nm, *ie* at a longer wavelength than most autofluorescence in cells. Those methodologies leverage the synthetic advantages of CLIPS chemistry applied to a unique BODIPY-based dielectrophile.

Compounds **5a** and **b** are the first intrinsically fluorescent probes for TrkB. Evidence for TrkB binding was derived from experiments to measure K_d, which also showed the competing effect of the parent neurotrophin BDNF. These fluorescent probes do not merely bind TrkB, but they

elicit cell survival responses without sub-optimal BDNF, *ie* true agonism. These observations open opportunities for using them to simultaneously detect TrkB and the effects of activating that receptor.

ASSOCIATED CONTENT

Electronic Supplementary Information (ESI) available. Protocols for compound syntheses and characterization. Fluorescent binding and cell survival assays.

AUTHOR INFORMATION

Corresponding Author

burgess@tamu.edu

Notes

The authors declare no competing financial interests. The study was designed by KB, syntheses of the compounds were performed by TP, TT; TP performed the cell survival assays and K_d determinations. PP assisted in development of a practical route to the fluorescent BODIPY-dichloride.

ACKNOWLEDGEMENTS

Financial support for this project was provided by R21NS130471-01A1 and NIH R01EY029645, and Texas A&M University T3-Grants Program (246292-00000). NMR instrumentation at Texas A&M University was supported by a grant from the National Science Foundation (DBI-9970232) and the Texas A&M University System.

P. Piyanuch acknowledges the Royal Golden Jubilee Ph.D. Program (Grant no. PHD/0079/2558) for funding.

Thank you to DPST Scholarship (Thailand) for sponsoring Thitima Pewklang's short internship at Texas A&M University.

REFERENCE

1. C. Bechtler and C. Lamers, *RSC Med. Chem.*, 2021, **12**, 1325-1351.

2. K. Bozovičar and T. Bratkovič, *Int. J. Mol. Sci.*, 2021, **22**, 1611-1636.

3. T. K. Sawyer, A. W. Partridge, H. Y. K. Kaan, Y.-C. Juang, S. Lim, C. Johannes, T. Y. Yuen, C. Verma, S. Kannan, P. Aronica, Y. S. Tan, B. Sherborne, S. Ha, J. Hochman, S. Chen, L. Surdi, A. Peier, B. Sauvagnat, P. J. Dandliker, C. J. Brown, S. Ng, F. Ferrer and D. P. Lane, *Bioorg. Med. Chem.*, 2018, **26**, 2807-2815.

4. X. Li, Y. Zou and H.-G. Hu, *Chinese Chem. Lett.*, 2018, **29**, 1088-1092.

5. R. A. Hillman, J. W. Nadraws and M. A. Bertucci, *Current Topics in Med. Chem.*, 2018, **18**, 611-624.

6. A. Patgiri, A. L. Jochim and P. S. Arora, *Acc. Chem. Res.*, 2008, **41**, 1289-1300.

7. M. Pelay-Gimeno, A. Glas, O. Koch and T. N. Grossmann, *Angew. Chem., Int. Ed.*, 2015, **54**, 8896-8927.

8. E. M. Driggers, S. P. Hale, J. Lee and N. K. Terrett, *Nature Rev. Drug Discovery*, 2008, **7**, 608-624.

9. E. Valeur, S. M. Gueret, H. Adihou, R. Gopalakrishnan, M. Lemurell, H. Waldmann, T. N. Grossmann and A. T. Plowright, *Angew. Chem., Int. Ed.*, 2017, **56**, 10294-10323.

10. K. T. Mortensen, T. J. Osberger, T. A. King, H. F. Sore and D. R. Spring, *Chem. Rev.*, 2019, **119**, 10288-10317.

11. I. V. Smolyar, A. K. Yudin and V. G. Nenajdenko, *Chem. Rev.*, 2019, **119**, 10032-10240.

12. R. Jwad, D. Weissberger and L. Hunter, *Chem. Rev.*, 2020, **120**, 9743-9789.

13. P. Timmerman, J. Beld, W. C. Puijk and R. H. Meloen, *Chembiochem : a European journal of chemical biology*, 2005, **6**, 821-824.

14. D. Bernhagen, V. Jungbluth, N. Gisbert Quilis, J. Dostalek, P. B. White, K. Jalink and P. Timmerman, *ACS Comb. Sci.*, 2019, **21**, 598-607.

15. G. J. J. Richelle, S. Ori, H. Hiemstra, J. H. van Maarseveen and P. Timmerman, *Angew. Chem., Int. Ed.*, 2018, **57**, 501-505.

16. M. Todorovic, K. D. Schwab, J. Zeisler, C. Zhang, F. Benard and D. M. Perrin, *Angew. Chem., Int. Ed.*, 2019, **58**, 14120-14124.

17. M. Todorovic and D. M. Perrin, in *Methods in Enzymology*, ed. D. M. Chenoweth, Academic Press, 2020, vol. 639, pp. 313-332.

18. Q. Zhang, Y. Zhang and X. Li, in *Methods in Enzymology*, ed. D. M. Chenoweth, Academic Press, 2020, vol. 639, pp. 237-261.

19. J. C. H. Chu, C. Shao, S. Y. Y. Ha, W.-P. Fong, C. T. T. Wong and D. K. P. Ng, *Biomaterials Science*, 2021, **9**, 7832-7837.

20. T. Wei, D. Li, Y. Zhang, Y. Tang, H. Zhou, H. Liu and X. Li, *Small Methods*, 2022, **6**, 2201164.

21. B. Li, L. Wang, X. Chen, X. Chu, H. Tang, J. Zhang, G. He, L. Li and G. Chen, *Nature Commun.*, 2022, **13**, 311.

22. Y. Bao, M. Xing, N. Matthew, X. Chen, X. Wang and X. Lu, *Org. Lett.*, 2023, DOI: 10.1021/acs.orglett.3c01817.

23. A. J. Horsfall, B. A. Vandborg, Z. Kikhtyak, D. B. Scanlon, W. D. Tilley, T. E. Hickey, J. B. Bruning and A. D. Abell, *RSC Chem. Biol.*, 2021, **2**, 1499-1508.

24. A. J. Horsfall, K. R. Dunning, K. L. Keeling, D. B. Scanlon, K. L. Wegener and A. D. Abell, *Chembiochem*, 2020, **21**, 3423-3432.

25. A. J. Horsfall and A. D. Abell, *Aust. J. Chem.*, 2021, **74**, 686-687.

26. L. Mendive-Tapia, J. Wang and M. Vendrell, *Peptide Science*, 2021, **113**, e24181.

27. Y. Wu, H.-F. Chau, W. Thor, K. H. Y. Chan, X. Ma, W.-L. Chan, N. J. Long and K.-L. Wong, *Angew. Chem. Int. Ed.*, 2021, **60**, 20301-20307.

28. L. Li, B. Nguyen and K. Burgess, *Bioorg. Med. Chem. Lett*, 2008, **18**, 3112-3116.

29. T. Rohand, M. Baruah, W. Qin, N. Boens and W. Dehaen, *Chem. Commun.*, 2006, 266-268.

30. N. Boens, V. Leen and W. Dehaen, *Chem. Soc. Rev.*, 2012, **41**, 1130-1172.

31. N. Boens, B. Verbelen and W. Dehaen, *Eur. J. Org. Chem.*, 2015, **2015**, 6577-6595.

32. P. Pankiewicz, M. Szybiński, K. Kisielewska, F. Gołębiowski, P. Krzemiński, I. Rutkowska-Włodarczyk, R. Moszczyński-Pętkowski, L. Gurba-Bryśkiewicz, M. Delis, K. Mulewski, D. Smuga, J. Dominowski, A. Janusz, M. Górka, K. Abramski, A. Napiórkowska, M. Nowotny, K. Dubiel, K. Kalita, M. Wieczorek, J. Pieczykolan and M. Matłoka, *Pharmaceuticals*, 2021, **14**, 704.

33. O. Obianyo and K. Ye, *Biochim. Biophysica Acta (BBA) - Proteins and Proteomics*, 2013, **1834**, 2213-2218.

34. U. M. Boltaev, Y.; Tolibzoda, F.; Jacques, T,; Gassaway, M.; Xu, Q.; Wagner, F.; Zhang, Y.-L.; Palmer, M.; Holson, E.; Sames, D., *Science Signaling*, 2017, **10**, eaal1670.

35. C. F. Ibanez, *J. Neurobiol.*, 1994, **25**, 1349-1361.

36. C. F. Ibanez, *TINS*, 1998, **21**, 438-444.

37. M. Pattarawarapan and K. Burgess, *J. Med. Chem.*, 2003, **46**, 5277-5291.

38. T. Wehrman, X. He, B. Raab, A. Dukipatti, H. Blau and K. C. Garcia, *Neuron*, 2007, **53**, 25-38.

39. L. LeSauteur, L. Wei, B. Gibbs and H. U. Saragovi, *J. Biol. Chem.*, 1995, **270**, 6564-6569.

40. L. LeSauteur, N. K. V. Cheung, R. Lisbona and H. U. Saragovi, *Nature Biotech.*, 1996, **14**, 1120-1122.

41. S. Maliartchouk, Y. Feng, L. Ivanisevic, T. Debeir, A. C. Cuello, K. Burgess and H. U. Saragovi, *Mol. Pharmacol.*, 2000, **57**, 385-391.

42. F. M. Longo, M. Manthorpe, Y. M. Xie and S. Varon, *J. Neurosci. Res.*, 1997, **48**, 1-17.

43. Y. Xie, M. A. Tisi, T. T. Yeo and F. M. Longo, *J. Biol. Chem.*, 2000, **275**, 29868-29874.

44. Y. Xie and F. M. Longo, *Prog. Brain Res.*, 2000, **128**, 333-347.

45. P. D. O'Leary and R. A. Hughes, *J. Biol. Chem.*, 2003, **278**, 25738-25744.

46. Y. Feng, Z. Wang, S. Jin and K. Burgess, *J. Am. Chem. Soc.*, 1998, **120**, 10768-10769.

47. Y. Feng and K. Burgess, *Chem. Eur. J.*, 1999, **5**, 3261-3272.

48. Y. Feng, M. Pattarawarapan, Z. Wang and K. Burgess, *Org. Lett.*, 1999, **1**, 121-124.

49. H. U. Saragovi and K. Burgess, *Exp. Opin. Ther. Patents*, 1999, **9**, 737-751.

50. L. F. Jessica, J. W. Rhiannon, N. Jacqueline, M. L. N. Eleanor, M. K. J. Christine, R. P. Alexa, B. Melissa, T. H. N. Huynh, E. N. Susan, A. H. Richard, G. G. David, X. Junhua and S. M. Simon, *J. Neurosci.*, 2018, **38**, 7088.

51. A. W. Wong, L. Giuffrida, R. Wood, H. Peckham, D. Gonsalvez, S. S. Murray, R. A. Hughes and J. Xiao, *Mol. Cell. Neurosci.*, 2014, **63**, 132-140.

52. F. M. Longo and S. M. Massa, *Nature Reviews Drug Discovery*, 2013, **12**, 507-525.

53. K. Burgess and H. U. Saragovi, US 6,881,719 B2, 2001.

54. Z. Yu, S. Joy, T. Mi, G. Yazdanpanah, K. Burgess and C. S. de Paiva, *Frontiers in Medicine*, 2022, **9**.

55. Z. Yu, G. Yazdanpanah, T. S. Thompson, K. Burgess and C. S. De Paiva, *Invest. ophthal. visual sci.*, 2022, **63**, 1979 – A0309-1979 – A0309.

56. R. A. Segal, *Annu. Rev. Neurosci.*, 2003, **26**, 299-330.

57. C. Wayua and P. S. Low, *Mol. Pharm.*, 2014, **11**, 468-476.