


 Cite this: *Chem. Commun.*, 2026, 62, 10505

 Received 3rd December 2025,  
 Accepted 29th April 2026

DOI: 10.1039/d5cc06914a

[rsc.li/chemcomm](https://rsc.li/chemcomm)

# Synthesis and characterization of L-3-(pentafluorophosphato-difluoromethyl)-alanine, a structural and functional mimetic of phosphoserine

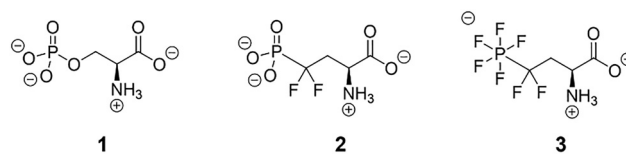
 Anna Magdalena Ambros,<sup>a</sup> Niklas Limberg,<sup>b</sup> Katrin Denzinger,<sup>id</sup><sup>a</sup> Markus Tiemann,<sup>a</sup> Gerhard Wolber,<sup>a</sup> Sebastian Riedel,<sup>id</sup><sup>b</sup> and Jörg Rademann,<sup>id</sup><sup>\*a</sup>

Serine phosphorylation is an essential switch for regulating the interactions and functions of many disease-related proteins. Accordingly, stable phosphoserine mimetics constitute chemical tools to study the effects of these post-translational modifications. In this work, we present the synthesis and characterization of a novel structural analog of phosphoserine, L-3-(pentafluorophosphato-difluoromethyl)-alanine. The hyper-fluorinated amino acid was synthetically accessed in six steps starting from commercially available *N*-Boc-L-serine methyl ester. The protected PF<sub>5</sub> amino acid and peptides derived thereof were inhibitors of the protein phosphoserine phosphatase PPP2CA, demonstrating its activity as functional phosphoserine mimetic.

Protein phosphorylation plays a crucial role in many biological processes, including signal transduction and the regulation of gene expression, cell cycle and metabolism. Aberrant activity of kinases and phosphatases are major drivers of cancer and of other diseases.<sup>1–3</sup> Nearly 90% of all protein phosphorylation events occur on the amino acid serine, making this post-translational modification especially important for studying associated disease states.<sup>4</sup> Since phosphorylation is a dynamic process, there is a need for enzymatically stable modifications mimicking the functional properties of the phosphate group. Methyl phosphonates and difluoromethyl phosphonates are important mimetics of the phosphate residue.<sup>5</sup> While these structures are stable toward phosphatases, they are very polar and carry two negative charges, resulting in poor cell permeability of all molecules containing these residues.<sup>6</sup> Recently, we have introduced 4-(pentafluorophosphato-difluoromethyl)-phenylalanine as a novel phosphotyrosine mimetic, which was shown to bind to protein tyrosine phosphatases with more than 25-fold improved affinity in

comparison to the 4-phosphono-difluoromethyl-analog. The pentafluorophosphate (PF<sub>5</sub>) motif carries one permanent negative charge, which is distributed over a larger surface area than the corresponding phosphonates, resulting in much higher hydrophobicity, an amphiphilic character, and improved cell permeability of the amino acid.<sup>7,8</sup> Following these pioneering findings, we have now investigated the synthesis and biological potency of analogs of phosphoserine **1**, namely 3-(phosphono-difluoromethyl)-Ala **2** (pCF<sub>2</sub>Ala), which has been found to effectively target pSer-binding domains, including disease-related targets like 14-3-3  $\sigma$ ,<sup>9,10</sup> Pin1,<sup>11</sup> and BRCA1,<sup>12</sup> and the novel compound, 3-(pentafluorophosphato-difluoromethyl)-alanine **3** (PF<sub>5</sub>CF<sub>2</sub>Ala) (Fig. 1).

Several examples in the literature describe the asymmetric synthesis<sup>13</sup> of L-3-(phosphono-difluoromethyl)-Ala **2**. Based on the synthetic route by Chen *et al.*,<sup>14</sup> we started from commercially available *N*-Boc protected L-serine methyl ester **4**, that was sulfonated and dehydrated to dehydroalanine **5**, which was then iodinated and converted into phosphonate **7** *via* a cadmium/copper promoted cross coupling step (Scheme 1). Asymmetric hydrogenation using the prochiral catalyst (*S,S*)-Et-DUPHOS-Rh with 20 bar H<sub>2</sub> for three days yielded phosphonate **8-(+)** ([ $\alpha$ ]<sub>D</sub><sup>20</sup> = +7.6° (*c* = 2.1, CHCl<sub>3</sub>)), while hydrogenation with Pd/C and H<sub>2</sub> at ambient pressure yielded the racemic mixture **8-rac** ([ $\alpha$ ]<sub>D</sub><sup>20</sup> = –0.3° (*c* = 1.7, CHCl<sub>3</sub>)). The respective products were analyzed *via* chiral SFC-MS (Fig. S1) which showed baseline separation of the enantiomers for **8-rac** and only one enantiomer within detection limits for **8-(+)**. The Boc protection group of **8-(+)** was replaced by Fmoc to

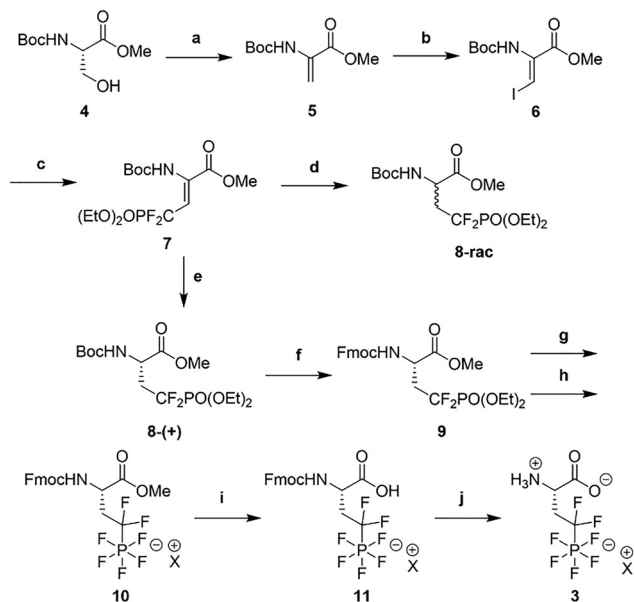


**Fig. 1** Structures of O-phospho-Ser **1** (pSer) and its two mimetics 3-(phosphono-difluoromethyl)-Ala **2** (pCF<sub>2</sub>Ala) and 3-(pentafluorophosphato-difluoromethyl)-alanine **3** (PF<sub>5</sub>CF<sub>2</sub>Ala).

<sup>a</sup> Department of Biology, Chemistry, Pharmacy, Institute of Pharmacy, Freie Universität Berlin, Königin-Luise-Str. 2+4, 14195 Berlin, Germany.  
 E-mail: joerg.rademann@fu-berlin.de

<sup>b</sup> Department of Biology, Chemistry, Pharmacy, Institute of Chemistry, Freie Universität Berlin, Fabeckstraße 34-36, 14195 Berlin, Germany





**Scheme 1** Synthesis of amino acid  $\text{PF}_5\text{CF}_2\text{Ala}$  **3**. Reaction conditions: (a)  $\text{MeSCl}$  (1.3 eq.),  $\text{TEA}$  (1.3 eq.),  $\text{DCM}$ ,  $0^\circ\text{C}$  to rt, overnight;  $\text{DBU}$  (1.2 eq.),  $\text{DCM}$ , rt, 2 h, 86%. (b)  $\text{NIS}$  (1.1 eq.),  $\text{DCM}$ ,  $0^\circ\text{C}$  to rt, overnight;  $\text{TEA}$  (2 eq.), 15 min; 65%. (c)  $\text{Cd}$  (6 eq.),  $\text{BrCF}_2\text{PO}(\text{OEt})_2$  (3.3 eq.),  $\text{DMF}$ , rt, 3 h;  $\text{CuBr}$  (3 eq.),  $\text{DMF}$ , rt, 18 h, 67%. (d)  $\text{H}_2$  (ambient pressure),  $\text{Pd/C}$  (10 wt%), rt, 2 h, 87%. (e)  $\text{H}_2$  (20 bar), (*S,S*)- $\text{Et-DUPHOS-Rh}$  (1.5 mol%), rt, 3 d, 87%. (f)  $\text{TFA}$ :  $\text{DCM}$  (1:1), rt, 3 h;  $\text{Fmoc-OSu}$  (1.2 eq.), aq.  $\text{NaHCO}_3$  (pH = 8–9), rt, 20 h, 90%. (g)  $\text{TMSBr}$  (5 eq.),  $\text{MeCN}$ ,  $60^\circ\text{C}$ , 1.5 h;  $(\text{COCl})_2$  (10 eq.),  $\text{DMF}$  (5 eq.),  $40^\circ\text{C}$ , 1 h;  $\text{TMAF}$  (10 eq.),  $0^\circ\text{C}$  to rt, 1.5 h, 74%. (h)  $\text{HF}$ :pyridine (70:30 wt%) (50 eq.), rt, 2 h, 81%; (i) protease from *B. licheniformis*, rt, 16 h, 89%; (j) 10% piperidine in  $\text{MeCN}$ , rt, 2 h, 86%. After column purification with 10 mM  $\text{NH}_4\text{HCO}_3$  counter ion  $\text{X} = \text{NH}_4$ , after ion exchange with Amberlite™ resin IRC 120 sodium form  $\text{X} = \text{Na}$ .

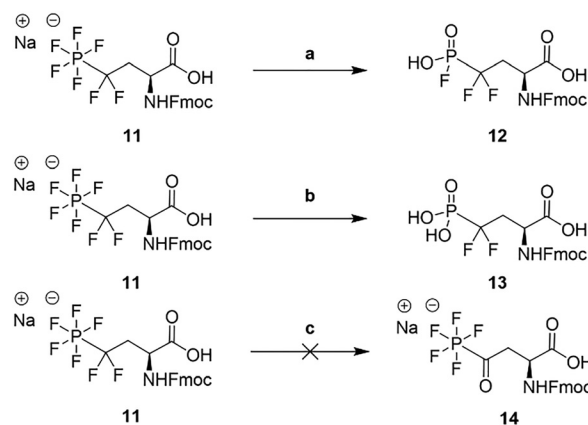
ensure chemical stability under acidic fluorination conditions and to obtain a product suitable for solid-phase peptide synthesis (SPPS). Next, the pentafluorination of the *N*-Fmoc-protected derivative **9** was investigated (Scheme 1). Previously, we synthesized the  $\text{PF}_5$ -group in a three-step one pot reaction using tetramethyl ammonium fluoride (TMAF) as fluorinating agent after activation with trimethylsilyl bromide and oxalyl chloride.<sup>7</sup> TMAF is a hygroscopic salt that required extensive drying prior to the reaction, since already small traces of water led to low yields in the reaction. Another drawback of the previous method was the use of saturated sodium hydrogen carbonate solution for quenching excess HF which led to large amounts of salt by-product. Therefore, we aimed to optimize our protocol. After testing different conditions, we successfully employed Olah's reagent, an  $\text{HF}$ :pyridine complex (70:30 wt%), as fluorinating agent. 50 equivalents of Olah's reagent neat reacted different phosphonate esters smoothly to their corresponding  $\text{PF}_5$ -derivatives in short reaction times.<sup>8</sup> Trimethylsilyl methyl ether was utilized as new quenching agent, furnishing trimethylsilyl fluoride as volatile side product that can easily be removed under reduced pressure. We tested both protocols with structure **9** and were able to obtain the pentafluorinated molecule **10** using the TMAF protocol with 74% yield and the new method using  $\text{HF}$ :pyridine with 81% yield.

The fully deprotected amino acid **3** was obtained by Fmoc-deprotection of **11** in 86% yield (Scheme 1). Due to the amphiphilic

character of the pentafluorinated amino acid derivatives extraction was not possible. All  $\text{PF}_5$ -molecules were purified *via* reversed-phase column chromatography using a C18 column with a 10 mM  $\text{NH}_4\text{HCO}_3$  buffer. Ion exchange was performed on the Amberlite™ IRC120 Na resin to yield the corresponding sodium salts.  $^{19}\text{F}$  NMR analysis confirmed the expected octahedral structure of the  $\text{PF}_5$ -group, showing a doublet–quintet splitting of the axial fluorine and a doublet–doublet–triplet splitting of the four equatorial fluorine atoms, as was already observed for the amino acid  $\text{PF}_5\text{CF}_2\text{Phe}$ .<sup>7</sup> Stability studies of structure **11** showed no decomposition under physiological conditions, and in a pH range of 3–12 for 48 hours. Under strong acidic conditions, hydrolysis to monofluorophosphonate **12** and phosphonate **13** were observed (Scheme 2 and Fig. S2). Little to no decomposition was found after incubation with silicon-based compounds trimethylsilyl bromide and hexamethyldisilazane. In contrast to the amino acid  $\text{PF}_5\text{CF}_2\text{Phe}$ , no hydrolysis of the  $\text{CF}_2$ -group to the carbonyl was observed, which might be explained by the absence of the benzylic position in  $\text{PF}_5\text{CF}_2\text{Ala}$  (Scheme 2 and Table S3).

The new chemical structure was employed for SPPS after deprotection of the methyl ester **10** using a protease from *B. licheniformis* as described previously furnishing *N*-Fmoc-(3- $\text{PF}_5\text{CF}_2$ )-Ala **11**.<sup>7</sup> Cleavage of synthesized  $\text{PF}_5\text{CF}_2\text{Ala}$ -peptides from the resin was performed with  $\text{HF}$ :pyridine and 10% anisole for one hour at room temperature. Under these conditions, the peptides were entirely stable, and no hydrolysis of the  $\text{PF}_5$ -moiety was observed.

14-3-3  $\sigma$  is a regulatory protein binding phosphoserine- and threonine containing peptides and proteins.<sup>15</sup> To investigate the potency of  $\text{PF}_5\text{CF}_2\text{Ala}$  to mimic pSer in a biological context, we synthesized a 14-3-3  $\sigma$  binding heptapeptide, Ac-RFRpSYPP-NH<sub>2</sub> **15**.<sup>16</sup> For comparison, the analogous heptapeptide **16** was prepared, in which the native phosphoserine residue (pS) was replaced by pentafluorinated amino acid  $\text{PF}_5\text{CF}_2\text{Ala}$ . A noticeable shift of retention time on a C18 column was observed for the two peptides, demonstrating higher lipophilicity of  $\text{PF}_5\text{CF}_2\text{Ala}$  compared to native pS (Fig. S4). Both peptides were tested in a fluorescence



**Scheme 2** Decomposition of  $\text{PF}_5\text{CF}_2\text{Ala}$ . (a) Full hydrolysis of **11** to monofluorophosphonate **12** was observed with  $\text{TFA}$ : $\text{MeCN}$  (50:50) after 1 h; (b) **11** was fully hydrolyzed to the free phosphonate **13** under strong acidic aqueous conditions ( $\text{TFA}$ : $\text{H}_2\text{O}$ , 50:50) in 24 h; (c)  $\text{HF}$ :pyridine with 1%  $\text{H}_2\text{O}$  did not provide the  $\text{CF}_2$ -hydrolyzed carbonyl structure **14** as observed for benzylic  $\text{PF}_5\text{CF}_2$  structures.<sup>7</sup>



polarization assay using in-house expressed 14-3-3  $\sigma$  protein and the 5-carboxytetramethylrhodamine (TMR) labeled peptide 5-TMR-GGRLSH-pS-LPG-NH<sub>2</sub> (commercial) as fluorescent probe. While the native heptapeptide **15** exhibited an IC<sub>50</sub> of 3.9  $\mu$ M (Fig. S6), no binding of the PF<sub>5</sub>CF<sub>2</sub>Ala peptide **16** was observed up to 1 mM (Fig. S7). Looking at co-crystallized structures of 14-3-3  $\sigma$ <sup>17,18</sup> it is apparent that the phosphoserine binding site is highly water accessible. Thus, it does not provide a positively charged, deeper amphiphilic pocket as in the protein tyrosine phosphatases, which enabled the desolvation of the pentafluorinated moiety upon binding.<sup>7,8</sup> These different properties might explain, why the PF<sub>5</sub>-peptide **16** did not show interactions with 14-3-3  $\sigma$ . To challenge this hypothesis, we further tested the amino acid against the catalytic site of the protein serine phosphatase PPP2CA, which is characterized by a deeper, more hydrophobic binding pocket.<sup>19</sup> While the free amino acid **3** (Na<sup>+</sup>) did not show significant inhibition of PPP2CA (Fig. S8), the protected PF<sub>5</sub>-amino acid **10** (Na<sup>+</sup>) showed concentration-dependent inhibition of PPP2CA with full inhibition at 2 mM and an IC<sub>50</sub> of 151  $\mu$ M (Fig. 2A). For comparison, **10** was converted to the established pSer mimetic *N*-Fmoc-3-(phosphono-difluoromethyl)alanine methyl ester Fmoc-pCF<sub>2</sub>Ala-OMe **17** by treatment with TFA in water/acetonitrile and tested with PPP2CA. Incubation with structure **17** also led to a concentration-dependent inhibition of the phosphatase, however, at 2 mM 18% residual enzyme activity was observed, the IC<sub>50</sub> was

approximated to 212  $\mu$ M (Fig. 2A). To further validate the potential of these mimetics, we incorporated PF<sub>5</sub>CF<sub>2</sub>Ala into the peptide sequence Ac-SPQPPpSRFQ-NH<sub>2</sub> obtained from the heatmap analysis of a phosphoserine-peptide library with PPP2CA<sup>20</sup> furnishing peptide **18**. The PO<sub>3</sub>CF<sub>2</sub>-analog **19** was synthesized from **18** using TFA in water/acetonitrile. The pentafluorinated peptide **18** again showed a noticeable prolongation of the retention time on the C18-column compared to the less hydrophobic PO<sub>3</sub>CF<sub>2</sub>-peptide **19** (Fig. S9). While previous studies suggested that difluoromethyl-based pSer peptide mimetics lack sufficient affinity for metal-dependent phosphatases in the cellular context of holoenzymes,<sup>21</sup> our synthesized peptides exhibited a concentration-dependent inhibition of PPP2CA with IC<sub>50</sub> values of 104 and 83  $\mu$ M, respectively (Fig. 2B). These results confirm the potency of PF<sub>5</sub>CF<sub>2</sub>Ala **3** as functional phosphoserine mimetic showing increased affinity when incorporated into peptide **18** and similar activity compared to the traditional mimetic PO<sub>3</sub>CF<sub>2</sub>Ala **2** and the derived peptide **19**. We further synthesized and tested analogs of the reported phosphoserine peptide substrate TPAPPpSAAAK (*K*<sub>M</sub> > 500  $\mu$ M)<sup>20</sup> with PF<sub>5</sub>CF<sub>2</sub>Ala (**20**) and PO<sub>3</sub>CF<sub>2</sub>Ala (**21**), which showed similar affinities as the substrate (Fig. S10, S11) and bound substantially weaker than peptides **18** and **19**.

In molecular docking studies, structures **10** and **17** shared similar binding modes in the catalytic center of PPP2CA (Fig. 3) in accordance with assay data. The phosphonate and PF<sub>5</sub>-group

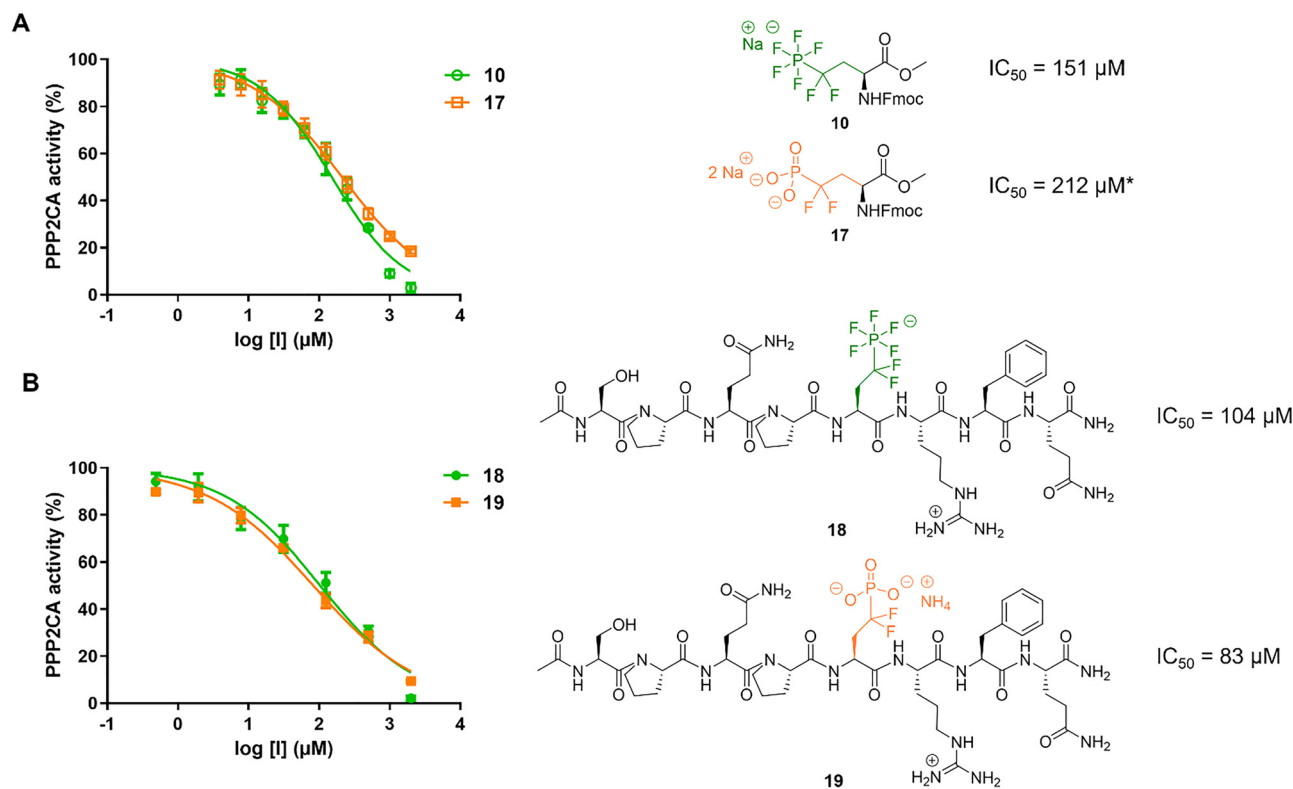
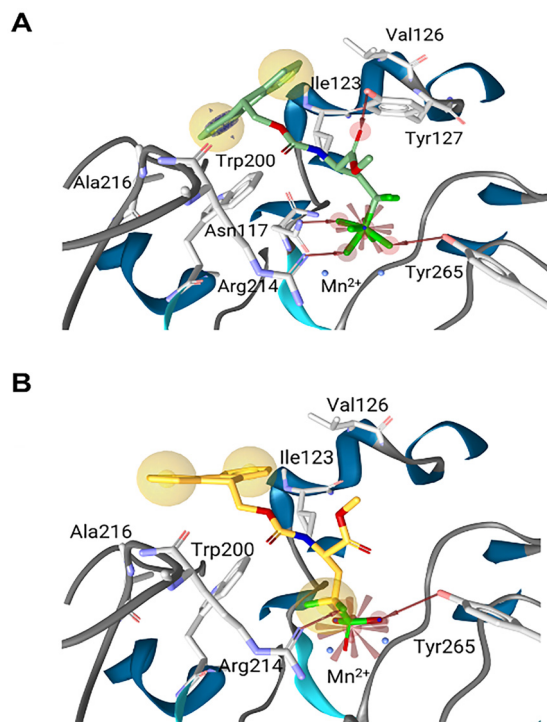


Fig. 2 Biological activity against protein serine phosphatase PPP2CA. (A) Pentafluorinated amino acid **10** and free phosphonate **17** exhibited concentration-dependent inhibition of the phosphatase, with **10** leading to full inhibition at 2 mM concentration and an IC<sub>50</sub> of 151  $\mu$ M. Incubation with the free phosphonate **17** led to 18% residual enzyme activity at 2 mM and showed an IC<sub>50</sub> of 212  $\mu$ M (\* approximated with a normalized response function). (B) Pentafluorinated peptide **18** and phosphonate peptide **19** showed improved activity compared to the protected amino acids, with IC<sub>50</sub> values of 104 and 83  $\mu$ M, respectively.





**Fig. 3** Docking poses of structures **10** (A) and **17** (B) in the catalytic center of PPP2CA (PDB: 2IE4), respectively. The amino acids showed similar binding modes with the charged phosphonate group interacting with amino acids Arg214, Asn117 and Tyr265 in close proximity to the two  $Mn^{2+}$  ions, suggesting potential charge interactions with the metals. The lipophilic Fmoc group bound in a hydrophobic subpocket showing interactions with Ile123, Val126 and, in the case of structure 10,  $\pi$ -stacking with Trp200.

bound close to the two catalytic  $Mn^{2+}$  ions suggesting potential charge interactions. Further interactions were observed with the surrounding hydrophilic amino acids Tyr265, Arg214 and Asn117. The Fmoc group was placed in a hydrophobic subarea of the binding pocket, potentially stabilizing the overall structures in this favorable position and contributing to the binding. Structure **10** formed two additional interactions and displayed an overall better fit in the binding pocket, potentially explaining the slightly enhanced activity compared to structure **17**.

In summary, we have established a synthetic protocol to access a novel, hyperfluorinated structural mimetic of phosphoserine, 1-3-(pentafluorophosphato-difluoromethyl)-alanine ( $PF_5CF_2Ala$ ) **3**, and incorporated it successfully in several phosphopeptide mimetics. We have demonstrated that  $PF_5CF_2Ala$  can serve as a functional biomimetic of pSer depending on the structure and accessibility of the phosphoserine binding pocket. Presumably due to the amphiphilic character of the  $PF_5$ -residue, the deeper, less solvent-exposed and more hydrophobic pocket of PPP2CA was preferred over the water-accessible binding site of 14-3-3  $\sigma$ . This hypothesis will be challenged in further studies of alternative target proteins.

## Conflicts of interest

There are no conflicts to declare.

## Data availability

The data supporting this article have been included as part of the supplementary information (SI), which contains descriptions of all chemical, biochemical, and computational methods, results of chiral SFC-MS, stability testing, protein binding assay and NMR spectroscopy. The authors have cited additional references within the supplementary information. See DOI: <https://doi.org/10.1039/d5cc06914a>.

## Acknowledgements

The authors acknowledge funding by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) –Project-ID 387284271 – SFB 1349 (fluorine-specific interactions, projects A3, A5, and B3). A. M. A. received an Elsa-Neumann graduate fellowship from the state of Berlin. The work was supported by the DFG-funded core facility BioSupraMol and the IT Department of Freie Universität Berlin (FUBIT). The authors greatly thank the late Lukas Bruno Lassak (1992–2025) for his important intellectual contributions to the development of the new synthesis route of pentafluorophosphates. The authors thank David Reiter for his technical assistance and support with the peptide synthesizer and Christoph Arkona for the expression of the 14-3-3 protein.

## References

- 1 F. Ardito, M. Giuliani, D. Perrone, G. Troiano and L. Lo Muzio, *Int. J. Mol. Med.*, 2017, **40**, 271–280.
- 2 K. S. Bhullar, N. O. Lagarón, E. M. McGowan, I. Parmar, A. Jha, B. P. Hubbard and H. P. V. Rupasinghe, *Mol. Cancer*, 2018, **17**, 48.
- 3 A. Turdo, C. D'Accardo, A. Glaviano, G. Porcelli, C. Colarossi, L. Colarossi, M. Mare, N. Faldetta, C. Modica, G. Pistone, M. R. Bongiorno, M. Todaro and G. Stassi, *Front. Cell. Dev. Biol.*, 2021, **9**, 690306.
- 4 D. Ochoa, A. F. Jamuczak, C. Viétez, M. Gehre, M. Soucheray, A. Mateus, A. A. Kleefeldt, A. Hill, L. Garcia-Alonso, F. Stein, N. J. Krogan, M. M. Savitski, D. L. Swaney, J. A. Vizcaino, K.-M. Noh and P. Beltrao, *Nat. Biotechnol.*, 2020, **38**, 365–373.
- 5 L. Azéma, R. Baron and S. Ladame, *Curr. Enzyme Inhib.*, 2006, **2**, 61–72.
- 6 A. J. Wiemer and D. F. Wiemer, *Top. Curr. Chem.*, 2015, **360**, 115–160.
- 7 (a) M. Accorsi, M. Tiemann, L. Wehrhan, L. M. Finn, R. Cruz, M. Rautenberg, F. Emmerling, J. Heberle, B. G. Keller and J. Rademann, *Angew. Chem., Int. Ed.*, 2022, **61**, e202203579; (b) S. Wagner, M. Accorsi and J. Rademann, *Chem. – Eur. J.*, 2017, **23**, 15387–15395.
- 8 A. M. Ambros, H. Qianzhu, M. Tiemann, E. Habel, K. Denzinger, H. Zupan, M. Accorsi, B. G. Keller, G. Wolber, T. Huber and J. Rademann, *Angew. Chem., Int. Ed.*, 2026, **63**, e18789.
- 9 W. Zheng, D. Schwarzer, A. Lebeau, J. L. Weller, D. C. Klein and P. A. Cole, *J. Biol. Chem.*, 2005, **280**, 10462–10467.
- 10 A. Arrendale, K. Kim, J. Y. Choi, W. Li, R. L. Geahlen and R. F. Borch, *Chem. Biol.*, 2012, **19**, 764–771.
- 11 M. K. Tarrant, H.-S. Rho, Z. Xie, Y. L. Jiang, C. Gross, J. C. Culhane, G. Yan, J. Qian, Y. Ichikawa, T. Matsuoka, N. Zachara, F. A. Etzkorn, G. W. Hart, J. S. Jeong, S. Backshaw, H. Zhu and P. A. Cole, *Nat. Chem. Biol.*, 2012, **8**, 262–269.
- 12 Z. Na, S. Pan, M. Uttamchandani and S. Q. Yao, *Angew. Chem., Int. Ed.*, 2014, **53**, 8421–8426.
- 13 (a) D. B. Berkowitz, Q. Shen and J. H. Maeng, *Tetrahedron Lett.*, 1994, **35**, 6445–6448; (b) A. Otaka, K. Miyoshi, T. R. Burke, P. P. Roller, H. Kubota, H. Tamamura and N. Fujii, *Tetrahedron Lett.*, 1995, **36**, 927–930; (c) T. Yokomatsu, M. Sato and S. Shibuya, *Tetrahedron Asymm.*, 1996, **7**, 2743–2754; (d) A. M. Kawamoto and M. M. Campbell, *J. Fluorine Chem.*, 1997, **81**, 181–186; (e) A. Otaka, E. Mitsuyama, T. Kinoshita, H. Tamamura and N. J. Fujii, *Org. Chem.*, 2000, **65**, 4888–4899.



- 14 H.-X. Chen, J. Kang, R. Chang, Y. L. Zhang, H. Z. Duan, Y.-M. Li and Y. X. Chen, *Org. Lett.*, 2018, **20**, 3278–3281.
- 15 H. Fu, R. R. Subramanian and S. C. Masters, *Annu. Rev. Pharmacol. Toxicol.*, 2000, **40**, 617–647.
- 16 L. M. Stevers, E. Sijbesma, M. Botta, C. MacKintosh, T. Obsil, I. Landrieu, Y. Cau, A. J. Wilson, A. Karawajczyk, J. Eickhoff, J. Davis, M. Hann, G. O'Mahony, R. G. Doveston, L. Brunsveld and C. Ottmann, *J. Med. Chem.*, 2017, **61**, 3755–3778.
- 17 E. Wilker, R. A. Grant, S. C. Artim and M. B. Yaffe, *J. Biol. Chem.*, 2005, **280**, 18891–18898.
- 18 K. V. Tugaeva, J. Titterington, D. V. Sotnikov, E. G. Maksimov, A. A. Antson and N. N. Sluchanko, *FEBS J.*, 2020, **287**, 3944–3966.
- 19 Y. Xing, Y. Xu, Y. Chen, P. D. Jeffrey, Y. Chao, Z. Lin, Z. Li, S. Strack, J. B. Stock and Y. Shi, *Cell*, 2006, **127**, 341–353.
- 20 B. Hoermann, T. Kokot, D. Helm, S. Heinzlmeir, J. E. Chojnacki, T. Schubert, C. Ludwig, A. Berteotti, N. Kurzawa, B. Kuster, M. M. Savitski and M. Köhn, *Nat. Commun.*, 2020, **11**, 3583.
- 21 L. Scheinost, C. Ludwig, N. Höfflin, A. A. Taskin, A. Marada, F.-N. Vögtle, C. Meisinger and M. Köhn, *FEBS J.*, 2025, **293**, 271–294.

