Bioorganic & Medicinal Chemistry 19 (2011) 1823–1838

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Design, synthesis and in vitro characterization of novel hybrid peptidomimetic inhibitors of STAT3 protein

Vijay M. Shahani [a,‡], Peibin Yue [b,‡], Steven Fletcher [a], Sumaiya Sharmeen [c], Mahadeo A. Sukhai [c], Diana P. Luu [a], Xiaolei Zhang [b], Hong Sun [c], Wei Zhao [b], Aaron D. Schimmer [c,†], James Turkson [b,*], Patrick T. Gunning [a,*]

[a] Department of Chemistry, University of Toronto, Mississauga, ON, Canada L5L 1C6
[b] Department of Molecular Biology and Microbiology, Burnett College of Biomedical Science, University of Central Florida, Orlando, FL 32826, USA
[c] Ontario Cancer Institute/Princess Margaret Hospital, 610 University Avenue, Toronto, ON, Canada M5G 2M9

ARTICLE INFO

Article history:
Received 25 October 2010
Revised 1 December 2010
Accepted 2 December 2010
Available online 9 December 2010

Keywords:
STAT3
Peptidomimetics
SH2 domain
Molecular recognition
Anti-cancer drugs
Protein–protein interactions

ABSTRACT

Aberrant activation of oncogenic signal transducer and activator of transcription 3 (STAT3) protein signaling pathways has been extensively implicated in human cancers. Given STAT3's prominent dysregulatory role in malignant transformation and tumorigenesis, there has been a significant effort to discover STAT3-specific inhibitors as chemical probes for defining the aberrant STAT3-mediated molecular events that support the malignant phenotype. To identify novel, STAT3-selective inhibitors suitable for interrogating STAT3 signaling in tumor cells, we explored the design of hybrid molecules by conjugating a known STAT3 inhibitory peptidomimetic, ISS610 to the high-affinity STAT3-binding peptide motif derived from the ILR/gp-130. Several hybrid molecules were examined in in vitro biophysical and biochemical studies for inhibitory potency against STAT3. Lead inhibitor **14aa** was shown to strongly bind to STAT3 (K_D = 900 nM), disrupt STAT3:phosphopeptide complexes (K_i = 5 μM) and suppress STAT3 activity in in vitro DNA binding activity/electrophoretic mobility shift assay (EMSA). Moreover, lead STAT3 inhibitor **14aa** induced a time-dependent inhibition of constitutive STAT3 activation in v-Src transformed mouse fibroblasts (NIH3T3/v-Src), with 80% suppression of constitutively-active STAT3 at 6 h following treatment of NIH3T3/v-Src. However, STAT3 activity recovered at 24 h after treatment of cells, suggesting potential degradation of the compound. Results further showed a suppression of aberrant STAT3 activity in NIH3T3/v-Src by the treatment with compound **14aa-OH**, which is the non-pTyr version of compound **14aa**. The effect of compounds **14aa** and **14aa-OH** are accompanied by a moderate loss of cell viability.

1. Introduction

STAT3 is a cytosolic transcription factor that becomes activated upon stimulation of cytokine or growth factor receptors.[1] Receptor activation leads to intracellular phosphorylation of STAT3 via receptor associated Janus kinases (JAKs) and, as a result, forms a STAT3–STAT3 protein complex.[2] STAT3 homodimers associate through reciprocal phosphotyrosine–SH2 domain interactions. In the nucleus, the transcriptionally active protein complex binds to specific DNA response elements and elicits a transcriptional response. Typically, STAT3 signaling is transient and responsive to physiological cues. However, dysregulated STAT3 activity results in the uncontrolled expression of genes involved in cell growth, survival and angiogenesis. Moreover, STAT3-mediated up-regulation of anti-apoptotic and cell survival genes provides an underlying mechanism for apoptotic resistance in many cancer cells.[3–7] Since most currently available chemotherapy options aim to initiate apoptosis, cancer cells have an intrinsic resistance to current treatment strategies. Therefore, therapeutics disrupting STAT3-mediated anti-apoptotic gene expression patterns hold significant promise as stand-alone or adjuvant therapeutics.

We herein report a novel family of hybrid peptidomimetic Stat3 inhibitors. The present hybrid inhibitors bind to STAT3's SH2 domain with a high affinity, disrupt STAT3:phosphopeptide complexation and consequently, inhibit STAT3–STAT3 protein dimerization. Lead inhibitor **14aa** exhibited biological activity and inhibited the viability of human breast and prostate cancer.

* Corresponding authors. Fax: +1 407 384 2062 (J.T.), +1 905 828 5425 (P.T.G.).
E-mail addresses: aaron.schimmer@utoronto.ca (A.D. Schimmer), jturkson@ucf.edu (J. Turkson), patrick.gunning@utoronto.ca (P.T. Gunning).
† Fax: +1 416 946 6546.
‡ These authors contributed equally to this work.

0968-0896/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2010.12.010

2. Results and discussion

2.1. Inhibitor design

Peptidomimetic inhibitors of STAT3 have played important roles in understanding the key binding interactions required for STAT3 recognition,[8–12] and in the development of non-peptidic inhibitors.[13–16] Peptidomimetic-inspired antagonists of STAT3 have been derived from both PpYLKTK, the cognate binding sequence of STAT3, and GpYLPQTV-NH_2, a truncated peptide from the gp130 receptor that is known to bind the STAT3-SH2 domain.[8,17] Given that the GpYLPQTV-NH_2 peptide is known to bind Stat3 with high-affinity (K_D, 150 nM), we hypothesized that a hybrid peptidomimetic inhibitor, incorporating key structural facets from this peptide and other most potent peptidomimetic designs could furnish an improved STAT3 inhibitor. As part of this combinatorial process, we elected to fuse **1** (Fig. 1A),[18] a peptidomimetic inhibitor inspired by the native binding sequence of STAT3, to the C-terminal portion of **2**, a gp130-derived peptidomimetic inhibitor, which was derived and previously evaluated by McMurray and co-workers.[17] Significantly, in the discovery process of **2**, these authors demonstrated that modulating the pY+3 Gln side chain was not tolerated, and that, in particular, the carboxamide hydrogens were critical for inhibitory activity.[11,19] Hence, we were keen to maintain a Gln residue or a Gln mimetic in this position of our peptidomimetics. To this end, and in an effort to achieve more cell-permeable peptidomimetic inhibitors, we elected to substitute the Pro-Gln dipeptide unit with a novel functionalized biphenyl-based amino acid that replicates the positioning of the key glutamine side chain to afford scaffold **3** (Fig. 1). We reasoned that the biphenyl moiety favorably reduced the number of structural conformations, rigidifying the peptidomimetic core, and thus reducing the entropic binding penalty. Moreover, inclusion of the biphenyl amino acid significantly reduces the peptidic character of the inhibitor by replacing two amino acid residues (Pro-Gln) and three peptide bonds with one unnatural amino acid, less susceptible to intracellular peptidases.

GOLD[20] computational docking of inhibitor **14aa** (where R = 4′-carboxyamide, Table 1) in the STAT3 SH2 domain,[21] showed accurate replication of the segment common to both **3** and the-gp130-derived peptidomimetic **2** (Fig. 2A). As part of our structural investigation of the biphenyl motif, we prepared two discrete biphenyl amino acid isomers where the C-terminal benzylcarbamoyl group was attached to the 2 or the 3 position of the 'lower' phenyl ring, and two sub-sets of isomers where the functional group on the 'upper' phenyl ring was located at the 3′ and the 4′ positions (Fig. 1). As illustrated in Fig. 2B, a high-scoring GOLD docked pose of the 3-substituted benzylcarbamoyl isomer **14ba** is overlaid with the docked pose of **14aa** taken from Fig. 2A. As anticipated, due to the rigidity of the 'lower' phenyl ring, the benzylcarbamoyl moiety cannot access the same hydrophobic region believed to be occupied by the same moiety in **2** and **14aa**, although most of the other interactions appear to be maintained. Therefore, in terms of mimicry of the gp130-inspired peptidomimetic **2**, we might expect **14ba** to bind in a different manner to **14aa**.

2.2. Synthetic protocols

The synthesis of the peptidomimetic inhibitor **14aa** is depicted in Scheme 1, and serves as a representative synthesis for the family of peptidomimetics described in this work. Oxidation of the methyl group of 2-bromo-1-methyl-4-nitrobenzene (**3**) was achieved with $KMnO_4$ in refluxing pyridine/water to afford the corresponding carboxylic acid species (**4**), which was subsequently condensed with benzylamine to furnish amide **5**. Chemoselective reduction of the nitro group was effected with $SnCl_2$ to afford aniline **6** in almost quantitative yield. The poorly nucleophilic amino group in **6** demanded pre-activation of the carboxylic acid of *N*-Boc-Leu-OH as its mixed anhydride with isobutyl chloroformate, since attempted condensations employing HBTU and EDCI proved unsuccessful. *N*-Boc-protected analogue of **7** was thus afforded in an excellent yield of 89%, and was quantitatively Boc-deprotected to furnish **7** as its HCl salt. Meanwhile, in this convergent synthetic strategy,

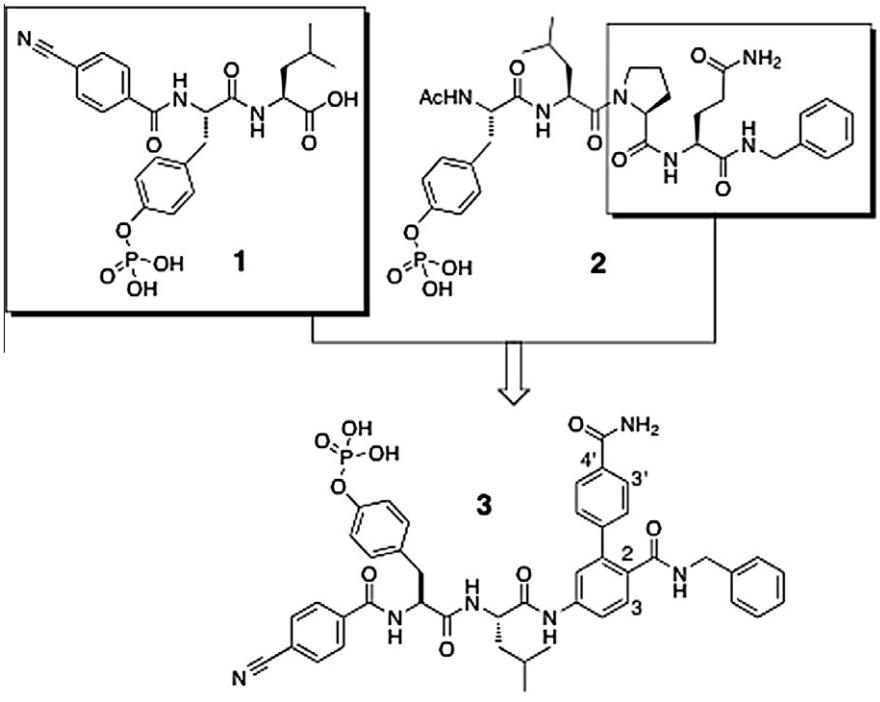
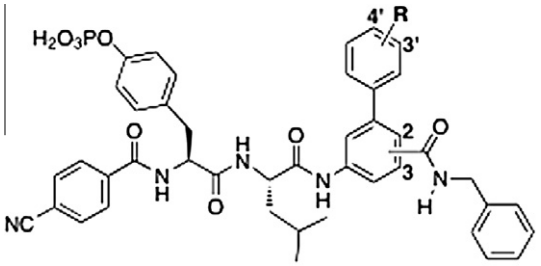


Figure 1. Development of STAT3 hybrid peptidomimetic **3** derived from peptidomimetic inhibitors **1** and **2**.

Table 1
IC_{50} inhibitory potencies of novel hybrid peptidomimetic family



Inhibitor	**R** Group	Benzyl amide	FP K_i (μM)	EMSA IC_{50} (μM)
14aa	4′-Amide	2-Benzylcarbamoyl	5 ± 1	73.1 ± 6
14ab	4′-Cyano	2-Benzylcarbamoyl	11 ± 4	33 ± 2
14ac	4′-Ester	2-Benzylcarbamoyl	26 ± 5	>200
14ad	3′-Amide	2-Benzylcarbamoyl	15 ± 2	62 ± 2
14ae	3′-Cyano	2-Benzylcarbamoyl	13 ± 1	64 ± 6
14af	3′-Ester	2-Benzylcarbamoyl	10 ± 2	40 ± 2
14ba	4′-Amide	3-Benzylcarbamoyl	9 ± 2	5 ± 1
14bb	4′-Cyano	3-Benzylcarbamoyl	36 ± 8	60 ± 2
14bc	4′-Ester	3-Benzylcarbamoyl	25 ± 6	112 ± 12
14bd	3′-Amide	3-Benzylcarbamoyl	38 ± 16	188 ± 48
14be	3′-Cyano	3-Benzylcarbamoyl	18 ± 3	92 ± 10
14bf	3′-Ester	3-Benzylcarbamoyl	23 ± 2	66 ± 1

the 4-cyanobenzoyl-tyrosyl dipeptide unit of the target molecule was prepared in parallel. Specifically, the carboxylic acid of tyrosine (**8**) was esterified with benzyl alcohol in excellent yield under acid-catalyzed conditions. Subsequent condensation with *p*-cyanobenzoic acid, once more enlisting the mixed anhydride method, furnished the dipeptide **9** in 84% yield. Subsequently, debenzylation of **9** with hydrogen gas over catalytic palladium on carbon (10% Pd/C) delivered carboxylic acid **10**. Condensation of amine **7** with acid **10** was accomplished using coupling agent HBTU to afford **11** in 63% yield. Next, microwave-assisted Suzuki coupling of aryl bromide **11** to (4-carbamoylphenyl) boronic acid using $Pd(PPh_3)_4$ and K_2CO_3 gave the biphenyl species **12** in 57% yield. The tyrosyl phenol OH group was then phosphorylated using bis(dimethylamino)-phosphoramidic chloride to give **13** in 42% yield, and then hydrolyzed with aqueous trifluoroacetic acid to furnish phosphoric acid **14aa** in quantitative yield.

2.3. Evaluation of STAT3-phosphopeptide inhibition by fluorescence polarization (FP) assay

We first investigated inhibitor binding potency for the STAT3-SH2 domain using a fluorescence polarization (FP) assay developed by Berg and Schust (Table 1, column 4).[22] In this assay, inhibitor-mediated displacement of an N-terminus 5-carboxyfluorescein-labeled (F*) gp130 phosphopeptide from the STAT3-SH2 domain results in reduced polarization of the emitted fluorescence and allows binding constants to be calculated. Library screening identified **14aa** and **14ba** (**14aa**: R^1 = 4′-$CONH_2$; R^2 = 2-benzylcarbamoyl, and; **14ba**: R^1 = 4′-$CONH_2$; R^2 = 3-benzylcarbamoyl) as the most potent inhibitors (**14aa**: K_i = 5 μM; **14ba**: K_i = 9 μM, full FP data shown in Supplementary data). Given the importance of the Gln side chain in McMurray's peptidomimetic (**2**), and considering our docking studies (Fig. 2), it is possible that the biphenyl carrying the *para*-carboxamide in peptidomimetics **14aa** and **14ba** is operating as a functional mimetic of the Pro-Gln dipeptide unit in **2**, as we had hoped. Interestingly, **14ba**, the 3-benzylcarbamoyl isomer of **14aa**, was only half as potent as **14aa**. Moreover, as can be seen from the data in Table 1, the 3-benzylcarbamoyl isomer of each series shows slightly lower activity than the corresponding 2-isomer in almost every case. McMurray has previously

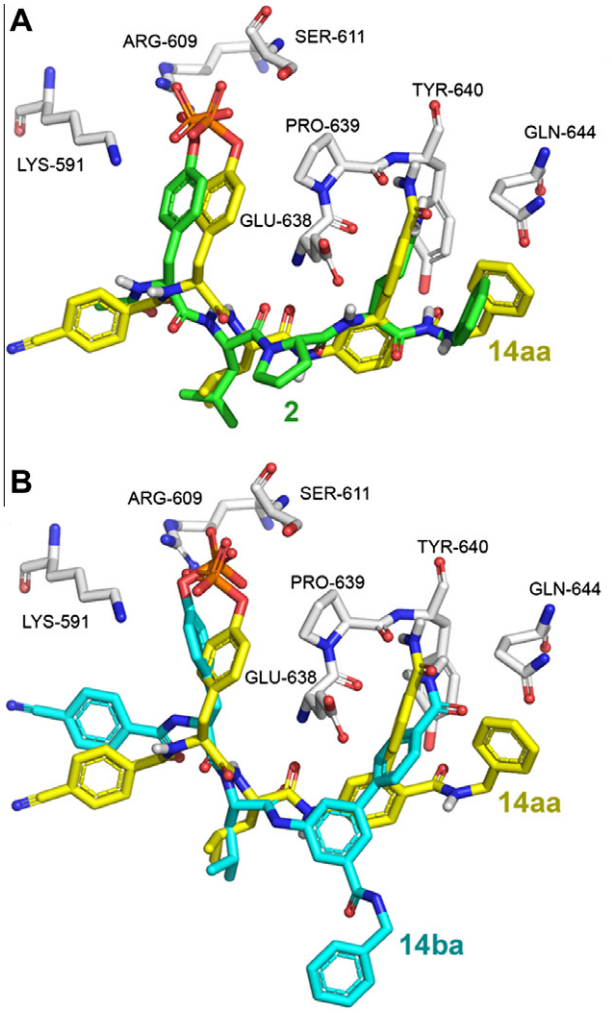
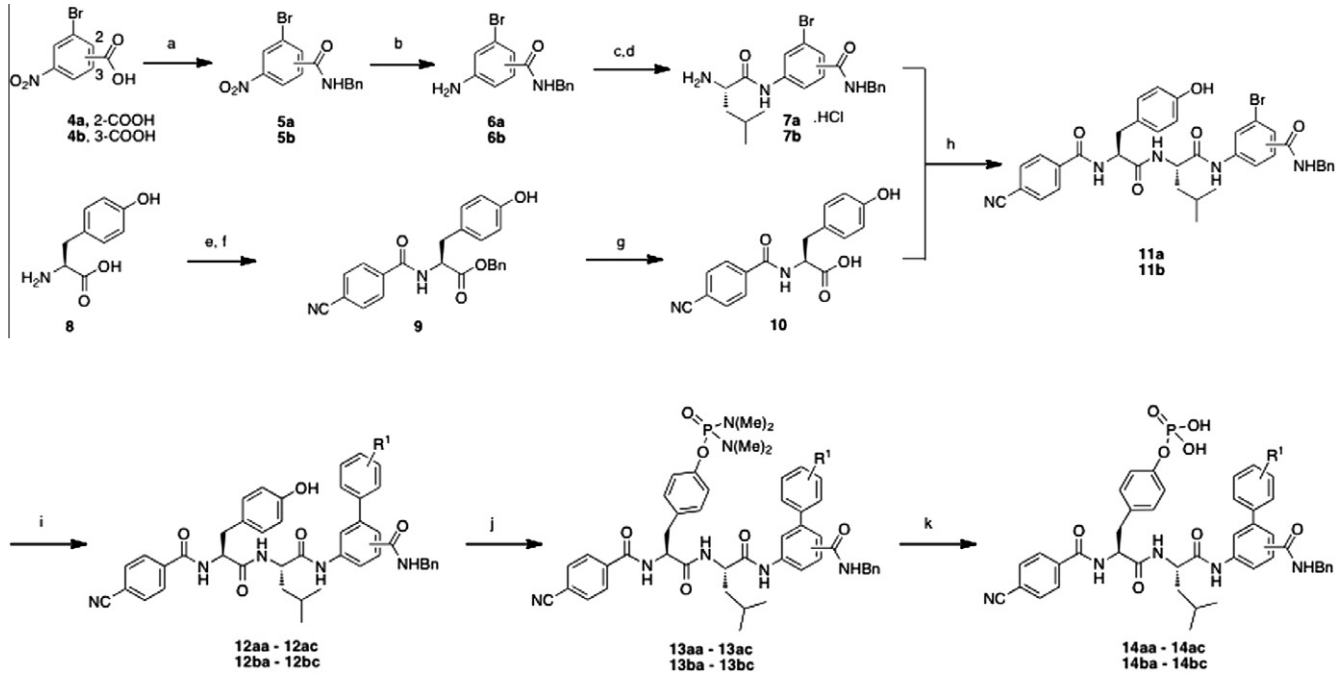


Figure 2. Comparative lowest energy GOLD docking results for (A) **14aa** and peptidomimetic **2** (B) inhibitors **14aa** and **14ba** and bound in the SH2 domain of the STAT3β dimer (PDB: 1BG1).

presented encouraging evidence to suggest that the Leu-Pro peptide bond in the gp130 sequence is *trans* when bound to STAT3. We speculated that the 2-isomer, which would be anticipated to exhibit a larger aryl–aryl twist angle owing to the additional steric hindrance, better mimics the *trans* peptide configuration than does the 3-isomer and consequently elicits moderately higher potency through improved interactions between the carboxamide group of the peptidomimetic and the STAT3-SH2 domain.[11] Moreover, our docking studies demonstrate that the benzylcarbomyl unit in **14aa**, in contrast to that unit in **14ba**, closely mimics that in **2**, suggesting that **14aa** makes different binding contacts with the protein than does **14ba** (Fig. 2A). Docking studies revealed that **14ba** accesses an adjacent hydrophobic sub-domain and makes binding contacts with residues Pro715 and Phe716 (Fig. 2B). To assess for STAT isoform selectivity, **14aa** and **14ba** were subjected to a series of analogous, previously published, FP-based competitive binding experiments for both the STAT1 and STAT5 isoforms (Fig. 3, **14aa** data shown).[23,24] We found that both **14aa** and **14ba** were approximately equipotent against the structurally homologous STAT1 isoform (78% homology), inhibiting STAT1–phosphopeptide complexes with K_i values of 6.3 μM and 16.5 μM, respectively (**14ba** data shown in Supplementary data). Against the structurally dissimilar STAT5 isoform (53% homology), both **14aa** and **14ba** were found to have no effect (data not shown).



Scheme 1. (a) Benzylamine, HBTU, DIPEA, DMF, 25 °C, 4 h, 74%; (b) $SnCl_2$, EtOAc, 70 °C, 2 h, 95%; (c) (i) *N*-Boc-Leu-OH, isobutyl chloroformate, CH_2Cl_2, *N*-methyl morpholine, 25 °C, 10 min; (ii) (**6a**, **6b**) *N*-methyl morpholine, CH_2Cl_2/THF (1:1), 25 °C, 1.5 h, 96%; (d) 4 M HCl, dioxane/MeOH (1:1), 25 °C, 1 h, 99%; (e) benzyl alcohol, *p*-TsOH·H_2O, 110 °C, 24 h; 95%; (f) (i) *p*-cyanobenzoic acid, isobutyl chloroformate, *N*-methyl morpholine, 25 °C, 15 min; (ii) **8**, *N*-methyl morpholine, CH_2Cl_2/THF (1:1), 25 °C, 30 min, 84%; (g) H_2, Pd/C, THF/MeOH (1:1) 25 °C, 1 h, 95%; (h) HBTU, DIPEA, DMF, 25 °C, 4 h, 76%; (i) $ArB(OH)_2$, $Pd(PPh_3)_4$, K_2CO_3, DMF, 170 °C, 15 min, 50%; (j) bis(dimethylamino)phosphoramidic chloride, DMAP, DBU, THF/CH_2Cl_2 (1:1) 25 °C, 16 h, 62%; (k) TFA/H_2O (9:1), 25 °C, 16 h, 99%.

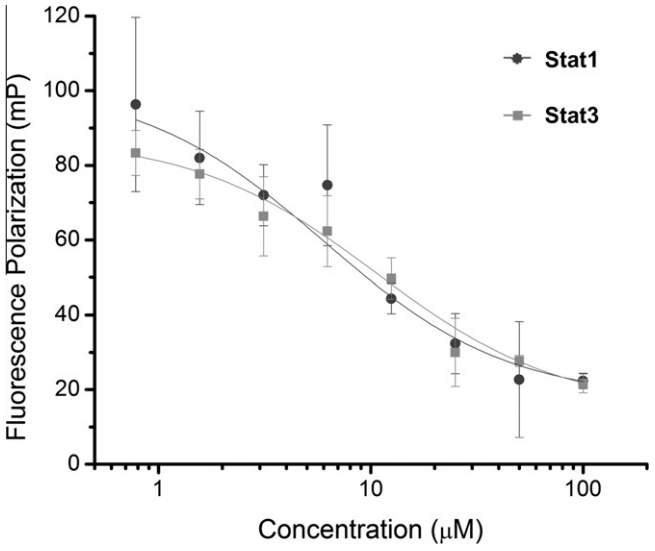


Figure 3. STAT3 versus STAT1 binding potency as assessed by FP assays. For STAT3, FP measured upon titrating the F*pYLPQTV (10 nM) with compound **14aa** in the presence of STAT3 protein (150 nM). For STAT1, FP measured upon titrating the F*GpYDKPHVL-NH_2 (10 nM) with compound **14aa** in the presence of STAT1 protein (80 nM). STAT5 data not shown.

2.4. Inhibition of STAT3–DNA binding activity (EMSA analysis) in nuclear extracts

Next, we examined inhibitor-mediated disruption of STAT3–STAT3:DNA complexation in nuclear extracts obtained from NIH3T3/vSrc transformed cells harboring constitutively activated STAT3 signaling.[4,25] The nuclear extracts were treated with increasing concentrations of inhibitors and incubated with the radiolabeled STAT3-specific hSIE oligonucleotide probe, followed by electrophoretic mobility shift assay (EMSA) analysis and densitometry quantification (Table 1, column 5). It was found that STAT3–STAT3:DNA binding was thus suppressed in a dose-dependent manner. Most notably, **14ba** showed excellent disruption of STAT3 dimerization (IC_{50} = 5 ± 1 μM). In contrast, **14aa** showed >50 μM activity in the EMSA cf. 5 μM in the FP assay. In general, we observed lower inhibitory activity in the EMSA assay, most likely due to the presence of significantly more protein targets, including the entire STAT family and in particular, STAT1. Given the relative activities observed for STAT3 cf. STAT1 in the FP assay, this was not an unsurprising result.

It has long been known that the inhibitory activities of SH2 domain binding pTyr-peptide probes have a key requirement for the phosphate ester. To verify that the pTyr moiety was essential for the activity of the hybrid peptides against STAT3–DNA binding activity, we investigated whether the dephosphorylated phenolic species retained activity in the DNA binding assay using the dephosphorylated analogs of **14aa** and **14ba**, **14aa-OH** and **14ba-OH**, respectively. Interestingly, both **14aa-OH** and **14ba-OH** inhibited STAT3–STAT3–DNA binding activity, with IC_{50} values of 103 ± 11 μM and 190 ± 8 μM, respectively (Supplementary data). This result was not too unsurprising, given that Dourlat et al. reported a similar phenomenon with the STAT3-binding sequence, AYRNRpYRRQYRY, wherein the corresponding non-phosphorylated sequence was found to be equipotent and effectively retained Stat3 inhibitory activity.[26] Poor cell permeability and dephosphorylation by intracellular and/or cell surface phosphatases/esterasase of phosphate ester containing inhibitors have led to the development of a number of successful protecting group/prodrug strategies, including difluorophosphonates and POM protecting group strategies.[27] However, phosphate group modulation was beyond the scope of this study. Based on the present findings, it is conceivable that the non-phospho-counterparts could similarly inhibit STAT3 and STAT3-mediated events in tumor cells. However, studies are needed to further characterize how the non-phospho-counterparts

promote Stat3 inhibition and how well it would induce STAT3-depedent anti-tumor cell effects compared to the phospho-counterparts. To further investigate **14ba** and **14aa**'s binding to STAT3, and to complement the EMSA and FP analysis, we conducted surface plasmon resonance (SPR) binding experiments with full-length STAT3 protein to determine directly the binding affinity of peptidomimetics for STAT3's SH2 domain.

2.5. SPR analysis of inhibitor-STAT3 binding

SPR binding experiments with lead inhibitors **14ba** and **14aa** were conducted against His-tagged STAT3 protein immobilized on a Ni-NTA sensor chip on a Biacore instrument as previously reported.[28] SensiQ and its analysis software Qdat (ICX Technologies) were utilized to analyze the interaction between peptidomimetic and STAT3 protein. Various concentrations of **14ba** and **14aa**, in running buffer (1 × PBS, 0.5% DMSO) were passed over the sensor chip to produce response signals. Association and dissociation measurements were recorded and the binding affinities determined. The results showed that **14ba** bound to STAT3 with a lower dissociation constant (K_D) than the high-affinity STAT3 binding sequence GpYLPQTV-NH_2, (**14ba**, K_D = 205 nM (Fig. 4) cf. GpYLPQTV-NH_2, K_D = 24nM).[28] In addition, **14aa** was shown to bind to STAT3 protein with good affinity (K_D = 900 nM (Fig. 4)). Thus taken in conjunction with the FP and EMSA data, we concluded that inhibitor **14ba** and **14aa** are potent in vitro STAT3 binders and inhibitors of STAT3 complexation events. Moreover, SPR analysis of the non-phosphorylated analogs, **14aa-OH** and **14ba-OH** were performed to determine the binding constants and corroborate the EMSA analysis. Encouragingly, both phenolic inhibitors **14aa-OH** and **14ba-OH** showed binding affinity for STAT3, with K_D values of 12 μM and 17 μM, respectively (Supplementary data). These data showed that the dephosphorylated metabolites bind to the STAT3 protein surface. However, we cannot specify the location of binding by SPR, only that **14aa-OH** and **14ba-OH** bind STAT3. To determine if **14aa-OH** or **14ba-OH** bind to STAT3's SH2 domain, we repeated the STAT3 FP assay, which is an excellent indicator of SH2 domain binding. Most interestingly, we found that neither peptidomimetic inhibited the STAT3–phosphopeptide complex (data not shown). Thus, in addition to the phosphorylated inhibitors acting as STAT3 SH2 domain inhibitors, it is conceivable that the dephosphorylated metabolites may also act as STAT3 inhibitors via a different mode of inhibition.

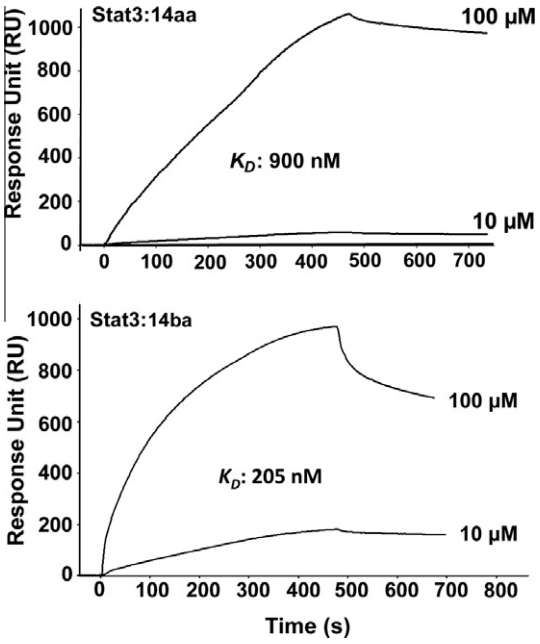


Figure 4. SPR analysis of the binding of STAT3 protein to inhibitors **14aa** and **14ba**.

2.6. Permeability and efflux analysis of STAT3 inhibitors 14aa, 14aa-OH, 14ba and 14ba-OH

Given the pharmacokinetic drawbacks associated for phosphate-containing inhibitors, we examined cell permeability and efflux in Caco-2 human epithelial cells, routinely used to mimic the small intestinal mucosa.[29] Experiments were performed at the Ontario Institute for Cancer Research as described in the experimental section. Permeability was classified based on A–B apparent permeability rate coefficient (Papp) values (for absorptive transport) as: low (Papp <2), medium (Papp 2–10) or high (Papp >10) × 10^{-6} cm/s. In summary, as assessed by a narrow window mass extraction LC/MS analysis (Waters Xevo quadrupole time-of-flight MS), both **14aa** and **14ba** were predominantly dephosphorylated at the end of the 90-min permeability assay (1–2% post assay recovery) and showed negligible cell penetration (Papp(A–B) values of <0.5). Dephosphorylated derivatives, **14aa-OH** and **14ba-OH** exhibited low cell permeability (Papp(A–B) values of >0.5), but were significantly more metabolically stable (52% and 36% post-assay recovery).

2.7. Inhibition of intracellular Stat3 activation

Despite the poor cell permeability results observed in Caco-2 cell permeability studies, we reasoned that the same effects might not be replicated in different cellular assays. Moreover, several Stat3-targeting groups have demonstrated cellular efficacy with phosphorylated compounds in whole cells.[12,15,16] Thus, we evaluated **14aa**, **14ba**, **14aa-OH** and **14ba-OH** in v-Src transformed mouse fibroblasts (NIH3T3/vSrc) for suppression of STAT3–STAT3:DNA binding activity. After 6 h and 48 h of treatment with inhibitors (100 μM), nuclear extracts were prepared from cells and subjected to STAT3–DNA binding assay in vitro using the radiolabeled hSIE probe and analyzed by EMSA (Fig. 5). We found that both **14aa** and **14ba** suppressed STAT3–DNA binding activity after 6 h (**14aa** >80% cf. 14ba ~50%). However, after 24 h, phosphorylated STAT3 activity was partially recovered, suggesting that both inhibitors exert a temporal suppression of STAT3 dimerization. Interestingly, the dephosphorylated inhibitor, **14aa-OH** was found to suppress STAT3–DNA binding activity with

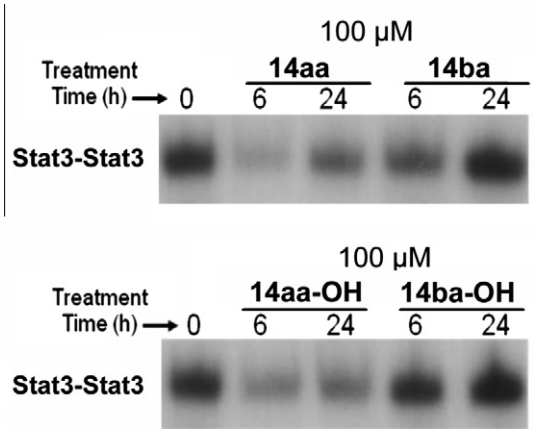


Figure 5. v-Src transformed mouse fibroblasts (NIH3T3/v-Src) treated or untreated with 100 μM **14aa**, **14aa-OH**, **14ba** and **14ba-OH** for 6 h and 24 h and assayed for STAT3–DNA binding via EMSA analysis of nuclear extracts.

almost the same potency as **14aa**, suggesting that **14aa** may well be dephosphorylated to **14aa-OH**. Alternatively, internalized **14aa-OH** is phosphorylated by intracellular kinases to **14aa** and elicits temporal suppression of STAT3–DNA binding. **14ba-OH** showed negligible disruption of STAT3–DNA binding activity at both 6 and 24 h of treatment.

2.8. Tumor whole cell studies

In an effort to evaluate the cellular efficacy of our peptidomimetics in whole cell tumor models, we elected to screen STAT3 inhibitors **14aa** and **14ba** against a series of cancer cell lines known to harbor constitutively activated STAT3. Specifically, prostate cancer cells (DU145),[30] pancreatic cancer cells (Panc-1),[31] NIH3T3/vSrc fibroblasts and breast cancer (MDA468) cells[32] were treated with a range of inhibitor concentrations and incubated for 24 h. Promyelocytic leukemia (HL-60) cells that do not harbor aberrant STAT3 signaling were also treated as a control experiment. Disruption of cell growth and viability were measured by a CyQuant cell viability assay (Invitrogen). From this screen, only **14aa** was shown to suppress cell viability as revealed in Figure 6 (data for **14ba** not shown). At 50 μM concentrations of **14aa**, there was an approximately 50% suppression in cell viability in all the cell lines investigated with the exception of HL60 cells, where the IC_{50} value was >200 μM. Compared to the cellular activities of our previous STAT3 peptidomimetics (ISS610 (**1**) and ISS840)[9], which required mM dosages, the present data indicates significantly improved cellular activities of the hybrid peptidomimetics. Corroborating the whole cell STAT3–DNA binding EMSA data, **14ba** showed only limited anti-cancer activity at lower concentrations.

3. Conclusion

We have identified a series of novel STAT3-targeting peptidomimetic scaffolds incorporating an unnatural biphenyl-based amino acid residue. These hybrid Stat3 peptidomimetic inhibitors combine strong features of high-affinity binding to and improved potency against Stat3. While lead compound **14aa** binds to STAT3 protein, disrupts phosphopeptide–STAT3 protein complexes, inhibits STAT3–STAT3 protein–protein interactions in both nuclear extracts and in whole cells, limited tumor cell cytotoxicity was observed, likely a result of poor cell permeability and metabolic stability. We are currently exploring the application of phosphotyrosine bioisoteres as well as prodrug strategies to improve the whole cell efficacy of lead peptidomimetics. These results shall be reported in due course.

4. Experimental

4.1. Chemical methods

Anhydrous solvents methanol, DMSO, CH_2Cl_2, THF and DMF were purchased from Sigma–Aldrich and used directly from Sure-Seal bottles. Molecular sieves were activated by heating to 300 °C under vacuum overnight. All reactions were performed under an atmosphere of dry nitrogen in oven-dried glassware and were monitored for completeness by thin-layer chromatography (TLC) using silica gel (visualized by UV light, or developed by treatment with $KMnO_4$ stain or phosphomolybdic acid stain). Low-resolution mass spectrometry (LRMS) was obtained using a Waters Micromass ZQ with an ESI source and samples in methanol. An AB/Sciex QStar mass spectrometer with an ESI source and accurate mass capabilities was used with samples dissolved in methanol to produce high-resolution mass spectra. ^1H and ^{13}C NMR spectra were recorded on Bruker 400 MHz and a Varian 500 MHz spectrometers in either $CDCl_3$, CD_3OD or DMSO-d_6. Chemical shifts (δ) are reported in parts per million after calibration to residual isotopic solvent. Coupling constants (J) are reported in hertz. Before biological testing, inhibitor purity was evaluated by reversed-phase HPLC (rpHPLC). Analysis by rpHPLC was performed using a Microsorb-MV 300 A C18 250 mm × 4.6 mm column run at 1 mL/min, and using gradient mixtures of (A) water with 0.1 M CH_3COONH_4 and (B) methanol. Ligand purity was confirmed using linear gradients from 75% A and 25% B to 100% B after an initial 2 min period of 100% A. The linear gradient consisted of a changing solvent composition of either (I) 4.7% per minute and UV detection at 254 nm or (II) 1.4% per minute and detection at 214 nm, each ending with 5 min of 100% B. For reporting HPLC data, percentage purity is given in parentheses after the retention time for each condition. All biologically evaluated compounds are >95% chemical purity as measured by HPLC. The HPLC traces for all tested compounds are provided in Supplementary data.

4.1.1. General procedure A (*N*-benzylation of carboxylic acids)

To a stirring solution of the relevant acid (1.8 g, 6.5 mmol) in anhydrous CH_2Cl_2 (0.1 M) was added $(COCl)_2$ (0.85 mL, 9.8 mmol) and catalytic DMF under an inert N_2 atmosphere at rt. The reaction was completed after 10 min as assessed by TLC. The product was concentrated under reduced pressure and the resulting residue dissolved in anhydrous CH_2Cl_2 (0.1 M), followed by the step-wise addition of DIPEA (5 equiv) and benzylamine (1.07 mL, 9.8 mmol). The reaction was complete after 15 min as judged by TLC. The solution was diluted with CH_2Cl_2 and the organics washed

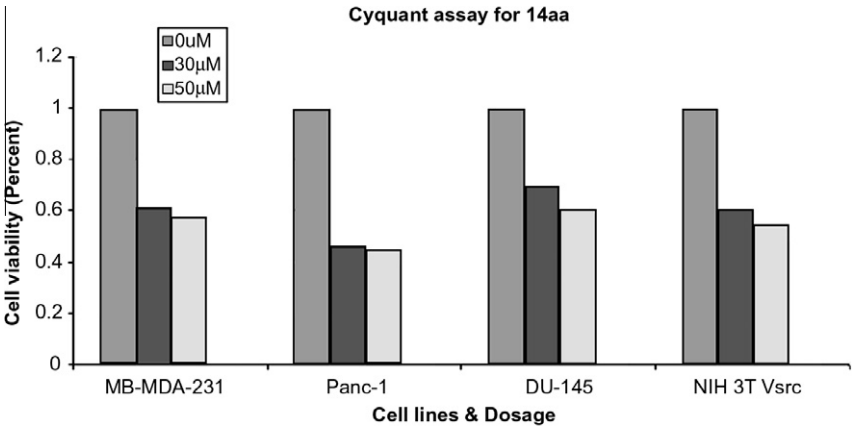


Figure 6. Human breast (MDA-MB-231), pancreatic (Panc-1), prostate (DU-145) cancer cells, and v-Src transformed mouse fibroblasts (NIH3T3/v-Src) were treated or untreated with 30–50 μM **14aa** for 24 h and assayed for viability using a CyQuant cell proliferation kit.

consecutively with 0.1 M HCl, saturated $NaHCO_3$, and brine solution. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The resultant product was purified by flash column chromatography.

4.1.2. General procedure B (SnCl mediated nitro group reduction)

To a stirring solution of the appropriate nitro compound (1.7 g, 5.0 mmol) in EtOAc (0.1 M) was added SnCl dihydrate (5.7 g, 25.0 mmol) in one portion. The resultant solution was refluxed for 2 h at 70 °C before quenching with saturated $NaHCO_3$. The aqueous layer was extracted using EtOAc and the combined organics were dried over anhydrous $Na_2SO_4$and concentrated under reduced pressure to furnish the product.

4.1.3. General procedure C (peptide couplings)

4.1.3.1. Method A. Under a N_2 atmosphere, the relevant carboxylic acid (1.5 equiv) (1.6 g, 6.5 mmol) was added to a stirring solution of NMM (0.74 mL, 5.6 mmol) in anhydrous THF (0.1 M). Isobutylchloroformate (0.61 mL, 5.6 equiv) was added in one portion and the solution was allowed to stir at rt. After 15 min, the appropriate amine (1.3 g, 4.3 mmol) was added drop-wise in a solution of THF containing NMM (0.52 mL, 4.8 mmol). The reaction mixture was left to stir overnight, concentrated and redissolved in distilled water. The water layer was then extracted with CH_2Cl_2, and the combined organic layers washed with saturated $NaHCO_3$solution, distilled water, and brine, dried over anhydrous Na_2SO_4 and concentrated.

4.1.3.2. Method B. The required carboxylic acid (340 mg, 0.74 mmol) was added in one portion to a solution of HBTU (334 mg, 0.88 mmol) and DIPEA (0.18 mL, 1.0 mmol) in DMF (0.1 M), and the resulting solution stirred at room temperature for 10 min. The required amine was then dissolved in a solution of DIPEA (0.18 mL, 1.0 mmol) in DMF (0.1 M) and added to the activated acid in one portion. The resulting solution was stirred for 4 h, then diluted with EtOAc (0.1 M) and washed successively with equal volumes of: 2 M HCl, saturated bicarbonate and brine. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated.

4.1.4. General procedure D (TFA mediated Boc deprotection)

To a stirred solution of Boc protected amine (300 mg, 0.44 mmol) in CH_2Cl_2 (2.2 mL) under N_2 was added trifluoroacetic acid (2.2 mL) and allowed to stir at room temperature for 3 h. The reaction was monitored via TLC and stopped upon consumption of starting material. The solution was concentrated and purified via silica gel chromatography to yield pure amine.

4.1.5. General procedure E (*O*-benzylation of tyrosine)

To a stirring solution of L-tyrosine (2.0 g, 13.8 mmol) in toluene, was added TsOH-monohydrate (3.2 g, 15.2 mmol) and benzyl alcohol (28.6 mL, 276 mmol). The solution was refluxed at 110 °C for 16 h before removing the solvent under reduced pressure. The concentrates were dissolved in diethyl ether and refrigerated. Product was precipitated out of solution, vacuumed filtered and subsequently washed with cold ether to furnish pure product. The product was carried over to the next step without further purification.

4.1.6. General procedure F (hydrogenolysis of benzyl ester)

The required benzyl ester (1.6 g, 4.0 mmol) was dissolved in a stirred solution of MeOH/EtOAc (1:1) and degassed thoroughly before the addition of Pd/C 10% (10 mg/mmol). H_2 gas was then bubbled through the solution for 5 min before the solution was put under an atmosphere of H_2 gas and stirred continuously for 3 h. The hydrogen gas was excluded from the reaction vessel and the reaction mixture filtered to remove the Pd/C through glass fiber paper. The solution was then concentrated to give pure product.

4.1.7. General procedure G (Suzuki cross-couplings)

A mixture of arylbromide (142 mg, 0.2 mmol), boronic acid (36 mg, 0.22 mmol), K_2CO_3 (69 mg, 0.5 mmol) and $Pd(PPh_3)_4$ (34 mg, 0.03 mmol) was suspended in DMF (0.1 M) in a sealed tube vessel and irradiated in a Biotage Initiator microwave reactor (17 min, 170 °C). After cooling to rt, the reaction was diluted with water and repeatedly extracted with CH_2Cl_2. The combined organic extracts were washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure.

4.1.8. General procedure H (phosphorylation using bis(dimethylamino)-phosphoramidic chloride)

To a stirring solution of phenol (80 mg, 0.1 mmol) and DMAP (24.4 mg, 0.2 mmol) in CH_2Cl_2/THF (0.1 M), was added DBU (22.4 μL, 0.15 mmol) in one portion, followed by bis(dimethylamino)-phosphoramidic chloride (18.8 μL, 0.13 mmol). The reaction was left to stir under an inert N_2 atmosphere at rt for 6 h. The reaction was quenched with distilled water, the product extracted using EtOAc, and the combined organics washed with a 1:1 mixture of distilled water:NaH_2PO_4, distilled water, and brine. The organics were dried over anhydrous Na_2SO_4, and concentrated under vacuum.

4.1.9. General procedure I (TFA mediated phosphoramidate hydrolysis)

Phosphoramidate (45 mg, 0.051 mmol) was added to a 9:1 mixture of TFA/H_2O at room temperature. The reaction mixture was left for 16 h. Complete transformation into the product was confirmed by TLC. Reaction mixtures were co-evaporated with MeOH to near dryness, then diluted with a mixture of HPLC grade water/acetonitrile (6:1) and lyophilized.

4.2. Detailed synthetic procedures for all compounds

4.2.1. *N*-Benzyl-2-bromo-4-nitrobenzamide (5a)

Reaction of **4a** (1.8 g, 7.3 mmol) according to procedure **A**, and purified by flash column chromatography (49:1 CH_2Cl_2/EtOAc) to furnish **5a** as a white solid (1.79, 5.3 mmol, 73%): δ_H (400 MHz, DMSO-d_6), 4.48 (d, J = 5.9 Hz, 2H, CH_2Ph), 7.26–7.30 (m, 1H, CH (Ar)), 7.34–7.40 (m, 4H, 4 CH (Ar)), 7.71 (d, J = 8.5 Hz, 1H, 1 CH (Ar)), 8.28 (dd, J = 8.3 Hz and 2.2 Hz, 1H, CH (Ar)), 8.47 (d, J = 2.2 Hz, 1H, CH, (Ar)), 9.22 (t, J = 5.9 Hz, 1H, NH): δ_C (100 MHz, DMSO-d_6) 42.5, 119.5, 122.8, 127.0, 127.4, 127.5, 128.4, 129.8, 138.7, 144.8, 148.1, 166.0; LRMS (MS ES), calcd for $C_{14}H_{11}BrN_2O_3$ [M+H] m/z = 335.01, found 335.08.

4.2.2. *N*-Benzyl-3-bromo-5-nitrobenzamide (5b)

Reaction of **4b** (1.8 g, 7.3 mmol) according to procedure **A**, and purified by flash column chromatography (49:1 CH_2Cl_2/EtOAc) to furnish **5b** as a white solid (1.81 g, 5.4 mmol, 74%): δ_H (400 MHz, DMSO-d_6) 4.51 (d, J = 5.8 Hz, 2H, CH_2), 7.26 (m, 1H, CH (Ar)), 7.34 (m, 4H, 4 CH (Ar)), 8.51 (t, J = 1.5 Hz, 1H, CH (Ar)), 8.55 (t, J = 1.9 Hz, 1H, CH (Ar)), 8.70 (t, J = 1.8 Hz, 1H, CH (Ar)) 9.49 (t, J = 5.8 Hz, 1H, NH): δ_C (100 MHz, DMSO-d_6). 43.1, 121.4, 122.2, 127.0, 127.5, 128.4, 128.6, 136.1, 137.1, 138.9, 148.7, 162.7; LRMS (MS ES), calcd for $C_{14}H_{11}BrN_2O_3Na$ [M+Na] m/z = 357.00, found 357.13.

4.2.3. 4-Amino-*N*-benzyl-2-bromobenzamide (6a)

Reaction of **5a** (1.7 g, 5.1 mmol) according to procedure **B**, and purified by flash column chromatography (7:2 CH_2Cl_2/EtOAc) to furnish **6a** as a white solid (1.5 g, 4.9 mmol, 96%): δ_H (400 MHz, CD_3Cl) 4.64 (d, J = 6.0 Hz, 2H, CH_2Ph), 5.67 (br s, 2H, NH_2), 6.54 (dd, J = 8.3 and 2.0 Hz, 1H, CH (Ar)), 6.80 (d, J = 2.0 Hz, 1H, CH

(Ar)), 7.15 (d, J = 8.3 Hz, 1H, 1 CH (Ar)), 7.23 (m, 1H, CH (Ar)), 7.33 (m, 4H, 4 CH (Ar)), 8.58 (t, J = 6.0 Hz, 1H, N*H*CH_2); δ_C (100 MHz, DMSO-d_6) 42.5, 112.0, 116.9, 120.2, 124.9, 126.7, 127.2, 128.2, 130.2, 139.6, 151.1, 167.5; LRMS (MS ES), calcd for $C_{14}H_{13}BrN_2O$ [M+H] m/z = 305.03, found 305.18.

4.2.4. 3-Amino-*N*-benzyl-5-bromobenzamide (6b)

Reaction of **5b** (1.7 g, 5.1 mmol) according to procedure **B**, and purified by flash column chromatography (7:2 CH_2Cl_2/EtOAc) to furnish **6b** as a white solid (1.5 g, 4.8 mmol, 95%). δ_H (400 MHz, DMSO-d_6) 4.42 (d, J = 6.0 Hz, 2H, CH_2Ph), 5.61 (s, 2H, NH_2), 6.87 (t, J = 1.9 Hz, 1H, CH (Ar)), 7.05 (t, J = 1.8 Hz, 1H, (Ar)), 7.14 (t, J = 1.5 Hz, 1H, ArBr), 7.23 (m, 1H, CH (Ar)), 7.30 (m, 4H, 4 CH (Ar)), 8.94 (t, J = 6.0 Hz, 1H, CON*H*); δ_C (100 MHz, DMSO-d_6) 42.6, 112.1, 116.3, 118.1, 121.9, 126.7, 127.2, 128.3, 137.2, 139.6, 150.5, 165.5; LRMS (MS ES), calcd for $C_{14}H_{13}BrN_2ONa$ [M+Na] m/z = 327.02, found 327.21.

4.2.5. (*S*)-4-(2-Amino-4-methylpentanamido)-*N*-benzyl-2-bromobenzamide (7a)

Reaction of **6a** (1.3 g, 4.3 mmol) according to procedure **C** (Method A) followed by Boc deprotection according to procedure **D** furnished **7a** as a white solid (1.7 g, 4.1 mmol, 97%): δ_H (400 MHz, DMSO-d_6) 0.91–0.94 (m, 6H, 2 CH_3 (Leu)), 1.66–1.77 (m, 3H, C*H*CH_2 (Leu)), 4.12 (s, 1H, C*H*(Leu)), 4.43 (d, J = 6.0 Hz, 2H, CH_2Ph) 7.25–7.26 (m, 1H, CH (Ar)), 7.31–7.38 (m, 4H, 4 CH (Ar)), 7.43 (d, J = 8.4 Hz, 1H, CH (Ar)), 7.71 (dd, J = 8.4 and 1.9 Hz, 1H, CH (Ar)), 8.09 (d, J = 1.9 Hz, 1H, CH (Ar)), 8.50 (br s, 3H, NH_3), 8.93 (t, J = 6.0 Hz, 1H, NHBn), 11.5 (s, 1H, NHAr); δ_C (100 MHz, DMSO-d_6) 22.2, 22.6, 23.7, 42.5, 51.7, 118.1, 119.1, 122.9, 126.8, 127.3, 128.3, 129.4, 134.1, 139.1, 140.1, 166.9, 168.5; LRMS (MS ES), calcd for $C_{20}H_{25}BrN_3O_2$ [M+H] m/z = 418.11, found 418.20.

4.2.6. (*S*)-3-(2-Amino-4-methylpentanamido)-*N*-benzyl-5-bromobenzamide (7b)

Reaction of **6b** (1.3 g, 4.3 mmol) according to procedure **C** (Method A) followed by Boc deprotection according to procedure **D** furnished **7b** as a white solid (1.7 g, 4.2 mmol, 98%): δ_H (400 MHz, DMSO-d_6) 0.91–0.94 (m, 6H, 2 CH3 (Leu)), 1.66–1.73 (m, 3H, CHCH2 (Leu)), 4.09–4.10 (m, 1H, CH (Leu)), 4.43 (d, J = 6.0 Hz, 2H, CH2Ph) 7.21–7.27 (m, 1H, CH (Ar)), 7.30–7.32 (m, 4H, 4 CH (Ar)), 7.86–7.87 (m, 1H, CH (Ar)), 8.13–8.14 (m, 1H, CH (Ar)), 8.16–8.17 (m, 1H, CH (Ar)), 8.52 (br s, 3H, NH3), 9.24–9.26 (t, J = 6.0 Hz, 1H, NHBn), 11.43 (s, 1H, NHAr); δ_C (100 MHz, DMSO-d_6) 22.2, 22.6, 23.8, 42.8, 51.7, 118.0, 121.5, 124.2, 124.9, 126.8, 127.3, 128.3, 137.0, 139.3, 139.9, 164.5, 168.5; LRMS (MS ES), calcd for $C_{20}H_{25}BrN_3O_2$ [M+H] m/z = 418.11, found 418.28.

4.2.7. (*S*)-Benzyl 2-(4-cyanobenzamido)-3-(4-hydroxyphenyl)-propanoate (9)

Reaction of **8** (1.0 g, 5.5 mmol) according to procedure **E** yielded the benzyl ester (not isolated) that was immediately coupled to 4-cyanobenzoic acid according to procedure **A** (method A) to furnish **9** as a white solid (1.7 g, 4.2 mmol, 84%): δ_H (400 MHz, DMSO-d_6) 2.97–3.11 (m, 2H, CH_2Ar(Tyr)), 4.61–4.67 (m, 1H, C*H* (Tyr)), 5.12 (s, 2H, CH_2Ph), 6.65 (d, J = 8.44 Hz, 2H (Ar)), 7.08 (d, J = 8.4 Hz, 2H, 2 CH (Ar)), 7.27–7.37 (m, 5H, 5 CH (Ar)), 7.93–7.98 (m, 4H, *Ar*-CN), 9.14 (d, J = 7.6 Hz, 1H, N*H*), 9.27 (s, 1H, O*H*); δ_C (100 MHz, DMSO-d_6) 35.5, 55.0, 66.1, 113.9, 115.1, 118.3, 127.4, 127.8, 128.1, 128.3, 128.4, 130.1, 135.9, 137.7, 156.1, 165.3, 171.4; LRMS (MS ES), calcd for $C_{24}H_{20}N_2O_4Na$ [M+Na] m/z = 423.14, found 423.23.

4.2.8(*S*)-2-(4-Cyanobenzamido)-3-(4-hydroxyphenyl)propanoic acid (10)

Reaction of **9** (1.6 g, 4.0 mmol) according to procedure **F** yielded the carboxylate **10** as a white solid in quantitative yield (1.2 g, 4.0 mmol, 99%): δ_H (400 MHz, DMSO-d_6) 2.90–2.96 (m, 1H, CH, CH_2 (Tyr)), 3.06–3.11 (m, 1H, CH_2 (Tyr)), 4.51–4.57 (m, 1H, C*H* (Tyr)), 6.34 (d, J = 8.4 Hz, 2H, 2 CH (Ar)), 7.09 (d, J = 8.4 Hz, 2H, 2 CH (Ar)), 7.94 (s, 4H, *Ar*-CN), 8.92 (d, J = 8.1 Hz, 1H, N*H*); δ_C (100 MHz, DMSO-d_6) 35.6, 54.8, 113.8, 115.0, 118.4, 128.1, 128.2, 130.0, 132.4, 138.0, 155.9, 165.0, 173.1; LRMS (MS ES), calcd for $C_{17}H_{13}N_2O_4$ [M−H] m/z = 309.10, found 309.12.

4.2.9. *N*-Benzyl-2-bromo-4-((*S*)-2-((*S*)-2-(4-cyanobenzamido)-3-(4-hydroxyphenyl)propanamido)-4-methylpentanamido)-benzamide (11a)

Compound **10** (687 mg, 2.2 mmol) was coupled to **7a** (840 mg, 2.0 mmol), according to procedure **A** (method B) to furnish **11a**, and then purified by flash chromatography (96.8:2.8:0.4 CH_2Cl_2/MeOH/NH_4OH) to obtain a yellow solid (896 mg, 1.3 mmol, 63%): δ_H (400 MHz, DMSO-d_6) 0.91 (dd, J = 17.0 and 6.4 Hz, 6H, 2 CH_3 (Leu)), 1.52–1.71 (m, 3H, CH_2C*H* (Leu)), 2.82–2.88 (m, 1H, CH, CH_2 (Tyr)), 3.02–3.06 (m, 1H, CH, CH_2 (Tyr)), 4.43–4.47 (m, 3H, C*H*(Leu) and NHCH_2Ph), 4.66–4.71 (m, 1H, C*H* (Tyr)), 6.63 (d, J = 8.4 Hz, 2H, *Ar*-OH (Tyr)), 7.15 (d, J = 8.4 Hz, 2H, 2 CH (Ar, Tyr)), 7.23–7.27 (m, 1H, CH (Ar)), 7.32–7.42 (m, 5H, 5 CH (Ar)), 7.59 (d, J = 8.3 Hz, 1H, CH (Ar)), 7.92–7.97 (m, 4H, 4 CH (*Ar*-CN)), 8.03 (d, J = 2.0 Hz, 1H, CH (Ar)), 8.37 (d, J = 8.0 Hz, 1H, CON*H*), 8.80 (d, J = 8.4 Hz, 1H, CON*H*), 8.87 (t, J = 6.0 Hz, 1H, N*H*Bn), 9.15 (s, 1H, O*H* (Tyr)), 10.24 (s, 1H, N*H*Ar); δ_C (100 MHz, DMSO-d_6) 21.6, 23.0, 24.3, 36.2, 40.5, 42.5, 52.3, 55.3, 113.7, 114.9, 117.8, 118.3, 119.1, 122.6, 126.8, 127.2, 128.2, 128.2, 128.3, 129.4, 130.1, 132.4, 133.5, 138.0, 139.2, 140.6, 155.7, 165.0, 167.0, 171.4, 171.6; LRMS (MS ES), calcd for $C_{37}H_{36}BrN_5O_5$ [M+H] m/z = 710.20, found 710.22.

4.2.10. *N*-Benzyl-3-bromo-5-((*S*)-2-((*S*)-2-(4-cyanobenzamido)-3-(4-hydroxyphenyl)propanamido)-4-methylpentanamido)-benzamide (11b)

Compound **10** (512 mg, 1.6 mmol) was coupled to **7b** (630 mg, 1.5 mmol) according to procedure **A** (method B) to furnish **11b**, and then purified by flash chromatography (3:1 CH_2Cl_2:(92:7:1 CH_2Cl_2/MeOH/NH_4OH)) to obtain a white solid (672 mg, 0.9 mmol, 50%): δ_H (400 MHz, DMSO-d_6) 0.91 (dd, J = 17.2 and 6.2 Hz, 6H, 2 CH_3 (Leu)), 1.53–1.69 (m, 3H, CH_2C*H* (Leu)), 2.80–2.89 (m, 1H, CH, CH_2 (Tyr)), 3.02–3.05 (m, 1H, CH, CH_2 (Tyr)), 4.43–4.47 (m, 3H, C*H*(Leu) and NHCH_2Ph), 4.65–4.71 (m, 1H, C*H* (Tyr)), 6.61 (d, J = 8.4 Hz, 2H, Ar-OH (Tyr)), 7.14 (d, J = 8.4 Hz, 2H, 2 CH (*Ar*-OH, Tyr)), 7.23–7.26 (m, 1H, CH (Ar)), 7.29–7.35 (m, 4H, 4 CH (Ar)), 7.77 (t, J = 1.5 Hz, 1H, CH (Ar)), 7.90–7.96 (m, 4H, 4 CH (*Ar*-CN)), 8.01 (t, J = 1.5 Hz, 1H, CH (Ar)), 8.14 (t, J = 2.0 Hz, 1H, CH (Ar)), 8.37 (d, J = 7.1 Hz, 1H, CON*H*), 8.79 (d, J = 8.3 Hz, 1H, CON*H*) 9.13–9.17 (m, 2H, N*H*Bn and Ar-O*H* (Tyr)), 10.36 (s, 1H, N*H*ArBr); δ_C (100 MHz, DMSO-d_6) 21.6, 23.0, 24.3, 36.2, 40.7, 42.8, 52.3, 55.3, 113.7, 114.9, 117.7, 118.3, 121.5, 123.9, 124.2, 126.8, 127.3, 128.2, 128.2, 128.3, 130.1, 132.4, 136.9, 138.0, 139.3, 140.5, 155.7, 164.6, 165.0, 171.5, 171.6; LRMS (MS ES), calcd for $C_{37}H_{36}BrN_5O_5Na$ [M+Na] m/z = 732.19, found 732.08.

4.2.11. (N^2-Benzyl-5-((*S*)-2-((*S*)-2-(4-cyanobenzamido)-3-(4-hydroxyphenyl)propanamido)-4-methylpentanamido)-biphenyl-2,4′-dicarboxamide (12aa)

Compound **11a** (142 mg, 0.2 mmol) was coupled to 4-aminocarbonylphenyl boronic acid according to general procedure **G**. Crude material was purified by flash chromatography (92:7:1 CH_2Cl_2/MeOH/NH_4OH) and yield final product **12aa** as white solid (86 mg, 0.11 mmol, 57%): δ_H (400 MHz, DMSO-d_6) 0.91 (dd, J = 16.2 and 6.4 Hz, 6H, 2 CH_3 (Leu)), 1.52–1.71 (m, 3H, CH_2C*H* (Leu)), 2.82–2.89 (m, 1H, CH, CH_2 (Tyr)), 3.01–3.05 (m, 1H, CH, CH_2 (Tyr)), 4.27–4.28 (m, 2H, CH_2Ph), 4.45–4.50 (m, 1H, CH (Leu)), 4.65- 4.71 (m, 1H, CH (Tyr)), 6.61 (d, J = 8.4 Hz, 2H, 2 CH (*Ar*)), 7.02 (d, J = 6.4 Hz, 2H, 2 CH (Ar)), 7.14 (d, J = 8.4 Hz, 2H,

Ar-OH (Tyr)), 7.17–7.25 (m, 3H, 3 CH (Ar)), 7.37–7.47 (m, 4H, 4 CH (Ar)), 7.67–7.69 (m, 2H, 2 CONH_2), 7.86–7.95 (m, 6H, 6 CH (Ar-CN and CH (Ar)), 8.03 (s, 1H, CH (Ar)), 8.35 (d, *J* = 7.7 Hz, 1H, CON*H*), 8.61 (t, *J* = 6.0 Hz, 1H, N*H*Bn), 8.80 (d, *J* = 8.4 Hz, 1H, CON*H*), 9.16 (s, 1H, Ar-O*H* (Tyr)), 10.23 (s, 1H, N*H*Ar); δ_C (100 MHz, DMSO-d_6) 21.6, 23.0, 24.3, 36.2, 40.7, 42.4, 52.2, 55.3, 113.7, 114.9, 117.8, 118.3, 120.24, 126.6, 127.1, 127.4, 128.1, 128.2, 128.2, 129.6, 130.0, 132.0, 132.4, 133.0, 138.0, 139.2, 139.4, 139.7, 143.2, 155.7, 165.0, 167.6, 168.6, 171.4, 171.4; LRMS (MS ES), calcdfor $C_{44}H_{42}N_6O_6$ [M+Na] *m/z* = 773.31, found 773.33.

4.2.12. *N*-Benzyl-4′-cyano-5-((*S*)-2-((*S*)-2-(4-cyanobenzamido)-3-(4-hydroxyphenyl)propanamido)-4-methylpentanamido)-[1,1′-biphenyl]-2-carboxamide (12ab)

Compound **11a** (142 mg, 0.2 mmol) was coupled to 4-cyanophenylboronic-acid according to general procedure **G**. Crude material was purified by flash chromatography (2:1 CH_2Cl_2:(92:7:1 CH_2Cl_2/MeOH/NH_4OH)) and yield final product **12ab** as white solid (81 mg, 0.11 mmol, 55%): δ_H (400 MHz, DMSO-d_6)) 0.91 (dd, *J* = 16.4 and 6.4 Hz, 6H, 2 CH_3 (Leu)), 1.59–1.71 (m, 3H, CH_2C*H*(Leu)), 2.81–2.89 (m, 1H, CH_2 (Tyr)), 3.00–3.05 (m, 1H, CH, CH_2 (Tyr)), 4.26 (d, *J* = 6.0 Hz, 2H, CH_2Ph), 4.43–4.50 (m, 1H, C*H* (Leu)), 4.65–4.71 (m, 1H, C*H* (Tyr)), 6.61 (d, *J* = 8.5 Hz, 2H, *Ar*-OH (Tyr)), 7.06–7.08 (m, 2H, 2 CH (Ar)), 7.13 (d, *J* = 8.5 Hz, 2H, *Ar*-OH (Tyr)), 7.23–7.29 (m, 3H, 3 CH (Ar)), 7.45 (d, *J* = 8.4 Hz, 2H, 2 CH (Ar)), 7.49–7.20 (m, 1H, CH (Ar)), 7.69–7.71 (m, 2H, 2 CH (Ar)), 7.76–7.78 (m, 2H, 2 CH (Ar)), 7.90–7.96 (m, 4H, 4 CH(*Ar*-CN)), 8.33 (d, *J* = 7.7 Hz, 1H, CON*H*), 8.66 (t, *J* = 6.0 Hz, 1H, N*H*Bn), 8.78 (d, *J* = 8.4 Hz, 1H, CON*H*), 9.14 (s, 1H, Ar-O*H* (Tyr)), 10.25 (s, 1H, N*H*Ar); δ_C (100 MHz, DMSO-d_6) 21.6, 23.0, 24.3, 36.2, 40.6, 42.4, 52.2, 55.3, 110.0, 113.7, 114.9, 118.3, 118.8, 120.1, 126.7, 127.3, 128.1, 128.2, 128.2, 128.8, 129.2, 130.0, 131.8, 132.0, 132.4, 138.0, 138.6, 139.0, 139.9, 145.1, 145.1 155.7, 165.0, 168.1, 171.4, 171.4;LRMS (MS ES), calcd for $C_{44}H_{40}N_6O_5Na$ [M+Na] *m/z* = 755.31, found 755.15.

4.2.13. Methyl 2′-(benzylcarbamoyl)-5′-((*S*)-2-((*S*)-2-(4-cyanobenzamido)-3-(4-hydroxyphenyl)propanamido)-4-methylpentanamido)-[1,1′-biphenyl]-3-carboxylate (12ac)

Compound **11a** (142 mg, 0.2 mmol) was coupled to 4-methoxycarboxyphenylboronic-acid according to general procedure **G**. Crude material was purified by flash chromatography (2:1 CH_2Cl_2:(92:7:1 CH_2Cl_2/MeOH/NH_4OH)) and yielded final product **12ac** as a white solid (66 mg, 0.086 mmol, 43%): δ_H (400 MHz, DMSO-d_6) 0.91 (dd, *J* = 16.4 and 6.3 Hz, 6H, 2 CH_3 (Leu)), 1.52–1.75 (m, 3H, CH_2C*H* (Leu)), 2.82–2.88 (m, 1H, CH_2 (Tyr)), 3.01–3.08 (m, 1H, CH, CH_2 (Tyr)), 3.86 (s, 3H, COOCH_3), 4.25 (d, *J* = 6.0 Hz, 2H, CH_2Ph), 4.40–4.50 (m, 1H, C*H*(Leu)), 4.65–4.71 (m, 1H, C*H* (Tyr)), 6.61 (d, *J* = 8.4 Hz, 2H, *Ar*-OH (Tyr)), 7.05–7.06 (m, 2H, 2 CH (Ar)), 7.14 (d, *J* = 8.4 Hz, 2H, *Ar*-OH), 7.20–7.22 (m, 3H, 3 CH (Ar)), 7.42–7.44 (m, 2H, 2 CH (Ar)), 7.47–7.49 (m, 1H, CH (Ar)), 7.68–7.70 (m, 2H, 2 CH (Ar)), 7.88–7.94 (m, 6H, *Ar*-CN and Ar), 8.36 (d, *J* = 7.7 Hz, 1H, CON*H*), 8.64 (t, *J* = 6.0 Hz, 1H, N*H*Bn), 8.80 (d, *J* = 8.4 Hz, 1H, CON*H*), 9.19 (s, 1H, Ar-O*H* (Tyr)), 10.26 (s, 1H, N*H*Ar); δ_C (100 MHz, DMSO-d_6) 21.7, 23.0, 24.4, 36.3, 40.7, 42.5, 52.2, 52.3, 55.4, 113.7, 114.9, 118.1, 118.3, 120.2, 126.7, 127.3, 128.1, 128.2, 128.3, 128.4, 128.7, 128.8, 129.1, 130.0, 132.0, 132.4, 138.0, 139.0, 139.1, 139.9, 145.2, 155.8, 165.1, 166.2, 168.5, 171.5, 171.5; LRMS (MS ES), calcd for $C_{45}H_{43}N_5O_7Na$ [M+Na] *m/z* = 788.32, found 788.28.

4.2.14. *N*2-Benzyl-5-((*S*)-2-((*S*)-2-(4-cyanobenzamido)-3-(4-hydroxyphenyl)propanamido)-4-methylpentanamido)-[1,1′-biphenyl]-2,3′-dicarboxamide (12ad)

Compound **11a** (142 mg, 0.2 mmol) was coupled to 3-amidocarboxyphenylboronic-acid according to general procedure **G**. Crude material was purified by flash chromatography (92:7:1 CH_2Cl_2/MeOH/NH_4OH) and yielded final product **12ad** as a white solid (59 mg, 0.078 mmol, 39%): δ_H (400 MHz, DMSO-d_6) 0.91 (dd, *J* = 16.2 and 6.4 Hz, 6H, 2 CH_3 (Leu)), 1.52–1.73 (m, 3H, CH_2C*H* (Leu)), 2.83–2.89 (m, 1H, CH_2 (Tyr)), 3.02–3.06 (m, 1H, CH_2 (Tyr)), 4.27 (d, *J* = 6.0 Hz, 2H, CH_2Ph), 4.46–4.51 (m, 1H, C*H* (Leu)), 4.66–4.71 (m, 1H, C*H* (Tyr)), 6.61 (d, *J* = 6.8 Hz, 2H, *Ar*-OH (Tyr)), 7.07 (m, 2H, 2 CH (Ar)), 7.14 (d, *J* = 8.4 Hz, 1H, CH (Ar)), 7.20–7.27 (m, 3H, 3 CH (Ar)), 7.37–7.48 (m, 4H, 4 CH (Ar)), 7.66–7.67 (m, 2H, 2 CH (Ar)), 7.69–7.72 (m, 1H, CH (Ar)), 7.87 (d, *J* = 7.4 Hz, 1H, CH (Ar)), 7.91–7.95 (m, 5H, *Ar*-CN and Ar), 8.03 (s, 1H, CH (Ar)), 8.33 (d, *J* = 7.5 Hz, 1H, CON*H*), 8.59 (t, *J* = 6.0 Hz, 1H, N*H*Bn), 8.80 (d, *J* = 8.4 Hz, 1H, CON*H*), 9.14 (s, 1H, Ar-O*H* (Tyr)), 10.22 (s, 1H, N*H*Ar)); δ_C (100 MHz, DMSO-d_6) 21.6, 23.0, 24.3, 36.2, 40.7, 42.4, 52.2, 55.3, 113.7, 114.9, 117.6, 118.3, 120.5, 126.2, 126.5, 127.0, 127.7, 127.9, 128.2, 128.2, 128.7, 128.7, 128.8, 130.0, 131.1, 131.4, 131.5, 131.9, 132.4, 134.3, 138.0, 139.7, 139.8, 140.5, 155.7, 165.0, 167.7, 168.6, 171.4. LRMS (MS ES), calcd for $C_{44}H_{42}N_6O_6Na$ [M+Na] *m/z* = 773.32, found 773.23.

4.2.15 *N*-Benzyl-3′-cyano-5-((*S*)-2-((*S*)-2-(4-cyanobenzamido)-3-(4-hydroxyphenyl)propanamido)-4-methylpentanamido)-[1,1′-biphenyl]-2-carboxamide (12ae)

Compound **11a** (142 mg, 0.2 mmol) was coupled to 3-cyanophenylboronic-acid according to general procedure **G**. Crude material was purified by flash chromatography (2:1 CH_2Cl_2:(92:7:1 CH_2Cl_2/MeOH/NH_4OH)) and yielded final product **12ae** as a white solid (51 mg, 0.070 mmol, 35%): δ_H (400 MHz, DMSO-d_6) 0.91 (dd, *J* = 16.4 and 6.3 Hz, 6H, 2 CH_3 (Leu)), 1.51–1.71 (m, 3H, CH_2C*H* (Leu)), 2.82–2.89 (m, 1H, CH_2 (Tyr)), 3.00–3.05 (m, 1H, CH_2 (Tyr)), 3.86 (s, 3H, COOCH_3), 4.27 (d, *J* = 5.9 Hz, 2H, CH_2Ph), 4.44–4.50 (m, 1H, C*H* (Leu)), 4.65–4.70 (m, 1H, C*H* (Tyr)), 6.61 (d, *J* = 8.4 Hz, 2H, *Ar*-OH (Tyr)), 7.06–7.07 (m, 2H, 2 CH (Ar)), 7.13 (d, *J* = 8.4 Hz, 2H, *Ar*-OH (Tyr)), 7.20–7.29 (m, 3H, 3 CH (Ar)), 7.50–7.60 (m, 3H, 3 CH (Ar)), 7.66–7.67 (m, 1H, CH (Ar)), 7.70–7.72 (m, 2H, 2 CH (Ar)), 7.82 (d, *J* = 8.2 Hz, 1H, 1 CH (Ar)), 7.90–7.95 (m, 4H, 4 CH (Ar-CN)), 8.36 (d, *J* = 7.7 Hz, 1H, CON*H*), 8.69 (t, *J* = 6.1 Hz, 1H, N*H*Bn), 8.80 (d, *J* = 8.2 Hz, 1H, CON*H*), 9.16 (s, 1H, Ar-O*H* (Tyr)), 10.26 (s, 1H, N*H*Ar); δ_C (100 MHz, DMSO-d_6) 21.6, 23.0, 24.3, 36.2, 40.6, 42.4, 52.3, 55.3, 111.3, 113.7, 114.9, 118.2, 118.3, 118.7, 120.4, 126.7, 127.1, 128.2, 128.2, 128.2, 128.8, 129.4, 130.0, 131.0, 131.6, 131.7, 132.4, 133.2, 138.0, 138.2, 139.1, 139.9, 141.6, 155.7, 165.0, 168.2, 171.4, 171.4; LRMS (MS ES), calcd for $C_{44}H_{40}N_6O_5Na$ [M+Na] *m/z* = 755.31, found 755.15.

4.2.16. Methyl 2′-(benzylcarbamoyl)-5′-((*S*)-2-((*S*)-2-(4-cyanobenzamido)-3-(4-hydroxyphenyl)propan-amido)-4-methylpentanamido)-[1,1′-biphenyl]-3-carboxylate (12af)

Compound **11a** (142 mg, 0.2 mmol) was coupled to 3-methoxycarboxyphenylboronic-acid according to general procedure **G**. Crude material was purified by flash chromatography (2:1 CH_2Cl_2:(92:7:1 CH_2Cl_2/MeOH/NH_4OH)) and yielded final product **12af** as a white solid (81 mg, 0.11 mmol, 53%): δ_H (400 MHz, DMSO-d_6) 0.91 (dd, *J* = 16.4 and 6.3 Hz, 6H, 2 CH_3 (Leu)), 1.53–1.72 (m, 3H, CH_2C*H* (Leu)), 2.83–2.89 (m, 1H, CH_2 (Tyr)), 3.02–3.06 (m, 1H, CH_2 (Tyr)), 3.86 (s, 3H, COOCH_3), 4.25 (d, *J* = 6.0 Hz, 2H, CH_2Ph), 4.45–4.50 (m, 1H, C*H* (Leu)), 4.65–4.71 (m, 1H, C*H* (Tyr)), 6.61 (d, *J* = 8.4 Hz, 2H, *Ar*-OH (Tyr)), 7.03–7.05 (m, 2H, 2 CH (Ar)), 7.14 (d, *J* = 8.4 Hz, 2H, *Ar*-OH), 7.17–7.24 (m, 3H, 3 CH (Ar)), 7.47–7.51 (m, 2H, 2 CH (Ar)), 7.55–7.58 (m, 1H, CH (Ar)), 7.68–7.72 (m, 2H, 2 CH (Ar)), 7.92–7.95 (m, 6H, *Ar*-CN and Ar), 8.36 (d, *J* = 7.7 Hz, 1H, CON*H*), 8.62 (t, *J* = 6.0 Hz, 1H, N*H*Bn), 8.81 (d, *J* = 8.4 Hz, 1H, CON*H*), 9.15 (s, 1H, Ar-O*H* (Tyr)), 10.25 (s, 1H, N*H*Ar): δ_C (100 MHz, DMSO-d_6) 21.6, 23.0, 24.3, 36.2, 40.7, 42.4, 52.2, 52.3, 55.3, 113.7, 114.9, 117.8, 118.3, 120.2, 126.6, 127.1, 128.0, 128.1, 128.2, 128.7, 128.8, 128.9, 129.6, 130.0, 131.8, 132.3, 133.1, 138.0, 139.0, 139.1, 139.9, 140.8, 155.7, 165.0,

166.1, 168.5, 171.4, 171.4; LRMS (MS ES), calcd for $C_{45}H_{43}N_5O_7Na$ [M+Na] *m/z* = 788.32, found 788.28.

4.2.17. N^3-Benzyl-5-((*S*)-2-((*S*)-2-(4-cyanobenzamido)-3-(4-hydroxyphenyl)propanamido)-4-methylpentanamido)-[1,1′-biphenyl]-3,4′-dicarboxamide (12ba)

Compound **11b** (142 mg, 0.2 mmol) was coupled to 4-aminocarbonylphenyl boronic acid according to general procedure **G**. Crude material was purified by flash chromatography (92:7:1 CH_2Cl_2/MeOH/NH_4OH) and yielded final product **12ba** as a white solid (63 mg, 0.084 mmol, 42%): δ_H (400 MHz, DMSO-d_6) 0.90 (dd, *J* = 16.0 and 6.4 Hz, 6H, 2 CH_3 (Leu)), 1.56–1.75 (br m, 3H, CH_2C*H* (Leu)), 2.83–2.89 (m, 1H, CH_2 (Tyr)), 3.02–3.06 (m, 1H, CH_2 (Tyr)), 4.48–4.53 (m, 3H, C*H* and NHCH_2), 4.67–4.72 (m, 1H, C*H* (Tyr)), 6.51 (d, *J* = 8.4 Hz, 2H, *Ar*-OH (Tyr)), 7.14 (d, *J* = 8.4 Hz, 2H, *Ar*-OH (Tyr)), 7.23–7.28 (m, 1H, CH (Ar)), 7.31–7.36 (m, 4H, 4 CH (Ar)), 7.43 (s, 1H, CH (Ar)), 7.77 (d, *J* = 8.4 Hz, 2H, *Ar*-COONH$_2$), 7.93–7.95 (m, 5H, *Ar*-CN and *Ar*), 8.00 (d, *J* = 8.0 Hz, 2H, *Ar*-COONH$_2$), 8.03 (s, 1H, CH, (Ar)), 8.16 (d, *J* = 5.6 Hz, 2H, COONH$_2$), 8.36 (d, *J* = 7.4 Hz, 1H, CONH), 8.81 (d, *J* = 8.2 Hz, 1H, CONH) 9.14 (s, 1H, Ar-OH), 9.19 (t, *J* = 6.2 Hz, 1H, NHBn), 10.30 (s, 1H, NHAr); δ_C (100 MHz, DMSO-d_6) 22.0, 23.6, 26.0, 37.9, 41.7, 44.7, 54.3, 57.2, 116.2, 116.3, 119.1, 119.9, 122.7, 122.8, 128.3, 128.3, 128.7, 129.0, 129.4, 129.5, 129.6, 131.4, 133.5, 134.4, 137.3, 139.4, 140.1, 140.6, 142.4, 144.7, 157.4, 168.6, 169.7, 171.9, 173.4, 174.0; LRMS (MS ES), calcd for $C_{44}H_{42}N_6O_6Na$ [M+Na] *m/z* = 773.32, found 773.23.

4.2.18. *N*-Benzyl-4′-cyano-5-((*S*)-2-((*S*)-2-(4-cyanobenzamido)-3-(4-hydroxyphenyl)propanamido)-4-methylpentanamido)-[1,1′-biphenyl]-3-carboxamide (12bb)

Compound **11b** (142 mg, 0.2 mmol) was coupled to 4-cyanophenylboronic-acid according to general procedure **G**. Crude material was purified by flash chromatography (3:1 CH_2Cl_2:(92:7:1 CH_2Cl_2/MeOH/NH_4OH)) and yielded final product **12bb** as a white solid (59 mg, 0.080 mmol, 40%): δ_H (400 MHz, DMSO-d_6) 0.92 (dd, *J* = 15.8 and 6.5 Hz, 6H, 2 CH_3 (Leu)), 1.55–1.72 (br m, 3H, CH_2C*H* (Leu)), 2.82–2.89 (m, 1H, CHCH_2 (Tyr)), 3.02–3.07 (m, 1H, CHCH_2 (Tyr)), 4.46–4.53 (m, 3H, C*H* and NHCH_2), 4.65–4.72 (m, 1H, C*H* (Tyr)), 6.61 (d, *J* = 8.4 Hz, 2H, *Ar*-OH (Tyr)), 7.14 (d, *J* = 8.4 Hz, 2H, *Ar*-OH), 7.22–7.27 (m, 1H, CH (Ar)), 7.31–7.34 (m, 4H, 4 CH (Ar)), 7.89–8.00 (m, 9H, *Ar*-CN and *Ar*-CN and (Ar)), 8.17 (s, 1H, CH (Ar)), 8.20 (s, 1H, CH (Ar)), 8.37 (d, *J* = 7.4 Hz, 1H, CONH), 8.81 (d, *J* = 8.2 Hz, 1H, CONH), 9.15 (s, 1H, Ar-OH (Tyr)), 9.20 (t, *J* = 5.7 Hz, 1H, NHBn), 10.34 (s, 1H, NHAr); δ_C (100 MHz, DMSO-d_6) 21.6, 23.0, 24.3, 36.2, 40.7, 42.7, 52.3, 55.3, 110.5, 113.7, 114.9, 118.3, 118.8, 119.0, 120.2, 120.4, 126.8, 127.3, 127.7, 128.2, 128.2, 128.3, 130.1, 132.4, 133.0, 135.9, 138.0, 138.9, 139.5, 139.9, 144.0, 155.7, 165.1, 165.7, 171.4, 171.5; LRMS (MS ES), calcd for $C_{44}H_{40}N_6O_5Na$ [M+Na] *m/z* = 755.31, found 755.28.

4.2.19. Methyl 3′-(benzylcarbamoyl)-5′-((*S*)-2-((*S*)-2-(4-cyanobenzamido)-3-(4-hydroxyphenyl)propanamido)-4-methylpentanamido)-[1,1′-biphenyl]-4-carboxylate (12bc)

Compound **11b** (142 mg, 0.2 mmol) was coupled to 4-methoxycarbonylphenylboronic-acid according to general procedure **G**. Crude material was purified by flash chromatography (98:2 CH_2Cl_2/MeOH) and yielded final product **12bc** as a white solid (86 mg, 0.11 mmol, 56%): δ_H (400 MHz, DMSO-d_6) 0.91 (m, 6H, 2 CH_3 (Leu)), 1.60–1.72 (br m, 3H, CH_2C*H* (Leu)), 2.85–2.91 (m, 1H, CH_2 (Tyr)), 3.04–3.09 (m, 1H,CH_2 (Tyr)), 3.88 (s, 3H, COOCH_3), 4.49–4.54 (m, 3H, CH(Leu) and CH_2 (NHCH_2Ph)), 4.70–4.75 (m, 1H, CH (Tyr)), 6.63 (d, *J* = 8.4 Hz, 2H, *Ar*-OH (Tyr)), 7.16 (d, *J* = 8.4 Hz, 2H, *Ar*-OH (Tyr)), 7.23–7.26 (m, 1H, CH (Ar)), 7.31–7.35 (m, 4H, 4 CH (Ar)), 7.86 (d, *J* = 8.4 Hz, 2H, *Ar*-COOMe), 7.92–7.98 (m, 5H, 5 CH (*Ar*-CN) and (Ar)), 8.08 (d, *J* = 8.4 Hz, 2H, Ar-COOMe), 8.18 (s, 1H, CH, (Ar)), 8.22 (s, 1H, CH, (Ar)), 8.33 (d, *J* = 8.0 Hz, 1H, CONH), 8.78 (d, *J* = 8.4 Hz, 1H, CONH) 9.11 (s, 1H, Ar-OH (Tyr)), 9.19 (t, *J* = 6.5 Hz, 1H, NHBn), 10.30 (s, 1H, NHAr); δ_C (100 MHz, DMSO-d_6) 21.7, 23.0, 24.4, 36.3, 40.7, 42.8, 52.2, 52.3, 55.4, 113.7, 114.9, 118.3, 118.7, 120.2, 120.4, 126.8, 127.1, 127.3, 128.3 (2 C), 128.3, 128.9, 130.1, 132.4, 135.9, 138.1, 139.5, 139.5, 139.8, 144.1, 155.8, 165.1, 165.9, 166.0, 171.4, 171.5; LRMS (MS ES), calcd for $C_{45}H_{43}N_5O_7Na$ [M+Na] *m/z* = 788.32, found 788.21.

4.2.20. N^3-Benzyl-5-((*S*)-2-((*S*)-2-(4-cyanobenzamido)-3-(4-hydroxyphenyl)propanamido)-4-methylpentanamido)-[1,1′-biphenyl]-3,3′-dicarboxamide (12bd)

Compound **11b** (142 mg, 0.2 mmol) was coupled to 3-aminocarbonylphenyl boronic acid according to general procedure **G**. Crude material was purified by flash chromatography (92:7:1 CH_2Cl_2/MeOH/NH_4OH) and yielded final product **12bc** as a white solid (94 mg, 0.13 mmol, 63%): δ_H (400 MHz, DMSO-d_6) 0.91 (m, 6H, 2 CH_3 (Leu)), 1.55–1.67 (br m, 3H, CH_2C*H* (Leu)), 2.81–2.87 (m, 1H, CHCH_2 (Leu)), 3.01–3.05 (m, 1H, CHCH_2), 4.48–4.50 (m, 3H, C*H*(Leu) and CH_2 (NHCH_2Ph)), 4.66–4.70 (m, 1H, CH (Tyr)), 6.60 (d, *J* = 8.4 Hz, 2H, *Ar*-OH (Tyr)), 7.14 (d, *J* = 8.4 Hz, 2H, *Ar*-OH), 7.21–7.25 (m, 1H, CH (Ar)), 7.29–7.35 (m, 4H, 4 CH (Ar)), 7.49 (s, 1H, CH (Ar)), 7.58 (t, *J* = 7.7 Hz, 1H, CH (Ar)), 7.85 (d, *J* = 8.4 Hz, 1H, CH (Ar)), 7.93 (m, 7H, 5 CH (*Ar*-CN) and (Ar) and CONH$_2$), 8.13–8.17 (m, 3H, 3 CH (*Ar*)), 8.21 (s, 1H, CH (Ar)), 8.36 (d, *J* = 8.0 Hz, 1H, CONH), 8.81 (d, *J* = 8.4 Hz, 1H, CONH), 9.15 (s, 1H, Ar-OH), 9.21 (t, *J* = 6.5 Hz, 1H, NHBn), 10.31 (s, 1H, NHAr); δ_C (100 MHz, DMSO-d_6) 21.7, 23.0, 24.4, 36.3, 40.7, 42.8, 52.2, 55.4, 113.7, 114.9, 118.3, 118.7, 120.2, 120.4, 126.8, 127.1, 127.3, 128.3 (2C), 128.3, 128.9, 130.1, 132.4, 135.9, 138.1, 139.5, 139.5, 139.8, 144.1, 155.8, 165.1, 165.9, 166.0, 171.4, 171.5; LRMS (MS ES), calcd for $C_{45}H_{42}N_5O_7Na$ [M+Na] *m/z = 773.32, found 773.21.*

4.2.21. *N*-Benzyl-4′-cyano-5-((*S*)-2-((*S*)-2-(4-cyanobenzamido)-3-(4-hydroxyphenyl)propanamido)-4-methylpentanamido)-[1,1′-biphenyl]-3-carboxamide (12be)

Compound **11b** (142 mg, 0.2 mmol) was coupled to 3-cyanophenylboronic-acid according to general procedure **G**. Crude material was purified by flash chromatography (3:2 CH_2Cl_2:(92:7:1 CH_2Cl_2/MeOH/NH_4OH)) and yielded final product **12bc** as a white solid (75 mg, 0.10 mmol, 51%): δ_H (400 MHz, DMSO-d_6) 0.92 (dd, *J* = 15.8and 6.5 Hz, 6H, 2 CH_3 (Leu)), 1.55–1.73 (br m, 3H, CH_2C*H* (Leu)), 2.83–2.89 (m, 1H, CHCH_2 (Tyr)), 3.02–3.07 (m, 1H, CHCH_2 (Tyr)), 4.47–4.58 (m, 3H, C*H*(Leu) and NHCH_2), 4.66–4.72 (m, 1H, C*H* (Tyr)), 6.61 (d, *J* = 8.4 Hz, 2H, Ar-OH), 7.15 (d, *J* = 8.4 Hz, 2H, *Ar*-OH (Tyr)), 7.23–7.28 (m, 1H, CH (Ar)), 7.31–7.36 (m, 4H, 4 CH (Ar)), 7.72 (t, *J* = 7.8 Hz, 1H, CH (Ar)), 7.87–7.90 (m, 1H, CH (Ar)), 7.93–7.95 (m, 5H, Ar-CN and Ar), 8.01–8.03 (m, 1H, CH (Ar)), 8.17–8.18 (m, 3H, 3CH (Ar)), 8.38 (d, *J* = 7.4 Hz, 1H, CONH), 8.82 (d, *J* = 8.2 Hz, 1H, CONH) 9.15–9.19 (m, 2H, Ar-OH and NHBn), 10.33 (s, 1H, NHAr); δ_C (100 MHz, DMSO-d_6) 21.6, 23.0, 24.3, 36.2, 40.7, 42.7, 52.3, 55.3, 110.5, 113.7, 114.9, 118.3, 118.8, 119.0, 120.2, 120.4, 126.8, 127.3, 127.7, 128.2, 128.2, 128.3, 130.1, 132.4, 133.0, 135.9, 138.0, 138.9, 139.5, 139.9, 144.0, 155.7, 165.1, 165.7, 171.4, 171.5; LRMS (MS ES), calcd for $C_{44}H_{40}N_6O_5Ns$ [M+Na] *m/z* = 755.31, found 755.28.

4.2.22. Methyl 3′-(benzylcarbamoyl)-5′-((*S*)-2-((*S*)-2-(4-cyanobenzamido)-3-(4-hydroxyphenyl)propanamido)-4-methylpentanamido)-[1,1′-biphenyl]-3-carboxylate (12bf)

Compound **11b** (142 mg, 0.2 mmol) was coupled to 4-methoxycarbonylphenylboronic-acid according to general procedure **G**. Crude material was purified by flash chromatography (98:2 CH_2Cl_2/MeOH) and yielded final product **12bf** as a white solid (93 mg, 0.12 mmol, 61%): δ_H (400 MHz, DMSO-d_6) δ_H (400 MHz, DMSO-d_6) 0.92 (dd, *J* = 15.5 and 6.4 Hz, 6H, 2 CH_3 (Leu)),

1.59.1.69 (br m, 3H, CH_2CH (Leu)), 2.84–2.90 (m, 1H, CH_2 (Tyr)), 3.05–3.08 (m, 1H, CHCH_2), 3.89 (s, 3H, COOCH_3), 4.48–4.53 (m, 3H, CH(Leu) and NHCH_2), 4.68–4.73 (m, 1H, CH (Tyr)), 6.62 (d, J = 8.4 Hz, 2H, *Ar*-OH (Tyr)), 7.16 (d, J = 8.4 Hz, 2H, *Ar*-OH (Tyr)), 7.22–7.27 (m, 1H, C_6H_5), 7.31–7.35 (m, 4H, 4 CH (Ar)), 7.67 (t, J = 7.8 Hz, 1H, *Ar*-COOMe), 7.94–7.95 (m, 5H, Ar-CN and Ar-COOMe), 7.99–8.02 (m, 2H, 2 CH (Ar-COOMe)), 8.17–8.25 (m, 3H, 3 CH (Ar)), 8.37 (d, J = 7.6 Hz, 1H, CON*H*), 8.81 (d, J = 8.3 Hz, 1H, CON*H*) 9.15 (s, 1H, Ar-O*H* (Tyr)), 9.24 (t, J = 6.0 Hz, 1H, N*H*Bn), 10.33 (s, 1H, N*H*Ar); δ_C (100 MHz, DMSO-d_6) 21.7, 23.0, 24.4, 36.2, 40.7, 42.7, 52.3, 52.3, 55.3, 113.7, 114.9, 118.3, 119.9, 120.1, 126.8, 127.1, 127.2, 128.3, 128.6, 128.7, 129.7, 130.1, 130.4, 131.6, 131.7, 132.4, 135.9, 138.1, 139.6, 139.6, 139.8, 140.0, 155.73, 165.0, 165.9, 166.1, 171.4, 171.4; LRMS (MS ES), calcd for $C_{45}H_{43}N_5O_7$Na [M+Na] m/z = 788.32, found 788.15.

4.2.23. 4-((*S*)-3-(((*S*)-1-((6-(Benzylcarbamoyl)-4′-carbamoyl-[1,1′-biphenyl]-3-yl)amino)-4-methyl-1-oxopentan-2-yl)-amino)-2-(4-cyanobenzamido)-3-oxopropyl)phenylbis-(dimethylamino) phosphordiamidate (13aa)

Phenol **12aa** (50 mg, 0.067 mmol) was treated according to general procedure **H**, and purified by flash column chromatography (1:1 CH_2Cl_2:(92:7:1 CH_2Cl_2/MeOH/NH_4OH)) to yield final product **13aa** as a white powder (25 mg, 0.028 mmol, 42%): δ_H (400 MHz, DMSO-d_6) 0.91 (dd, J = 16.2 and 6.4 Hz, 6H, 2 CH_3 (Leu)), 1.52–1.70 (m, 3H, CH_2CH(Leu)), 2.53–2.55 (dd, J = 10.1 and 1.5 Hz, 12H, N(Me$_3$)$_2$), 2.88–2.97 (m, 1H, CH_2 (Tyr)), 3.11–3.15 (m, 1H, CH_2 (Tyr)), 4.27 (d, J = 5.9 Hz, 2H, NHCH_2Ph), 4.46–4.51 (m, 1H, CH (Leu)), 4.73–4.79 (m, 1H, CH (Tyr)), 7.00–7.03 (m, 4H, 4 CH (Ar)), 7.19–7.25 (m, 3H, CH (*Ar*)), 7.31–7.33 (m, 2H, CH (Ar)), 7.37–7.41 (m, 3H, CH (Ar)), 7.45–7.47 (m, 1H, CH (Ar)), 7.68–7.70 (m, 2H, 2 CH (Ar)), 7.86–7.95 (m, 6H, 6 CH (Ar)), 8.03 (s, 1H, CH (Ar)), 8.41 (d, J = 7.5 Hz, 1H, CON*H*), 8.62 (t, J = 6.0 Hz, 1H, N*H*Bn), 8.85 (d, J = 8.4 Hz, 1H, CON*H*), 10.26 (s, 1H, N*H*Ar); δ_C (100 MHz, DMSO-d_6) 21.7, 23.0, 24.4, 36.2, 36.2, 36.2, 40.7, 42.4, 52.3, 54.9, 113.7, 117.8, 118.3, 119.7, 119.7, 120.2, 126.6, 127.1, 127.4, 128.1, 128.1, 128.2, 128.6, 130.2, 132.0, 132.4, 133.0, 133.9, 138.0, 139.2, 139.4, 139.8, 143.2, 149.5, 149.6, 155.7, 165.0, 167.5, 168.6, 171.1, 171.4; LRMS (MS ES), calcd for $C_{48}H_{53}N_8O_7$PNa [M+Na] m/z = 907.37, found 907.48.

4.2.24. 4-((*S*)-3-(((*S*)-1-((6-(Benzylcarbamoyl)-4′-cyano-[1,1′-biphenyl]-3-yl)amino)-4-methyl-1-oxopentan-2-yl)amino)-2-(4-cyanobenzamido)-3-oxopropyl)phenyl bis(dimethylamino) phosphordiamidate (13ab)

Phenol **12ab** (50 mg, 0.068 mmol) was treated according to general procedure **H**, and purified by flash column chromatography (2:1 CH_2Cl_2:(92:7:1 CH_2Cl_2/MeOH/NH_4OH)) to yield final product **13ab** as a white powder (37 mg, 0.042 mmol, 62%): δ_H (400 MHz, DMSO-d_6) 0.91 (dd, J = 16.6 and 6.4 Hz, 6H, 2 CH_3 (Leu)), 1.53–1.70 (m, 3H, CH_2CH (Leu)), 2.52–2.55 (dd, J = 10.1 and 1.5 Hz, 12H, N(CH_3)$_2$), 2.90–2.96 (m, 1H, CH_2 (Tyr)), 3.11–3.15 (m, 1H, CH_2 (Tyr)), 4.28 (d, J = 6.0 Hz, 2H, CH_2Ph), 4.45–4.51 (m, 1H, CH(Leu)), 4.73–4.79 (m, 1H, CH (Tyr)), 7.00 (d, J = 8.3 Hz, 2H, 2 CH (*Ar*)), 7.06–7.08 (m, 2H, 2 CH (Ar)), 7.23–7.32 (m, 5H, 5 CH (Ar)), 7.45 (m, 2H, 2 CH (Ar)), 7.49–7.51 (m, 1H, CH (Ar)), 7.69–7.71 (m, 2H, 3 CH (Ar)), 7.76–7.78 (m, 2H, CH (Ar)), 7.90–7.95 (m, 4H, 4 CH (Ar)), 8.42 (d, J = 7.7 Hz, 1H, CON*H*), 8.67 (t, J = 6.0 Hz, 1H, N*H*Bn), 8.85 (d, J = 8.4 Hz, 1H, CON*H*), 10.30 (s, 1H, N*H*Ar); δ_C (100 MHz, DMSO-d_6) 21.6, 22.9, 24.3, 36.2, 36.2, 36.2, 40.6, 42.4, 52.3, 54.9, 110.0, 113.7, 118.3, 118.8, 119.7, 119.7, 120.1, 126.7, 127.3, 128.1, 128.2, 128.8, 129.2, 130.2, 131.8, 132.0, 132.3, 133.9, 138.0, 138.6, 139.0, 139.4, 145.2, 149.5, 149.6, 165.0, 168.1, 171.1, 171.4; LRMS (MS ES), calcd for $C_{48}H_{51}N_8O_6$PNa [M+Na] m/z = 889.37, found 889.28.

4.2.25. Methyl 2′-(benzylcarbamoyl)-5′-((*S*)-2-((*S*)-3-(4-((bis-(dimethylamino)phosphoryl)oxy)phenyl)-2-(4-cyanobenzamido)propanamido)-4-methylpentanamido)-[1,1′-biphenyl]-4-carboxylate (13ac)

Phenol **12ac** (45 mg, 0.059 mmol) was treated according to general procedure **H**, and purified by flash column chromatography (2:1 CH_2Cl_2:(92:7:1 CH_2Cl_2/MeOH/NH_4OH)) to yield final product **13ac** as a white powder (41 mg, 0.046 mmol, 78%): δ_H (400 MHz, DMSO-d_6) 0.91 (dd, J = 16.5and 6.4 Hz, 6H, 2 CH_3 (Leu)), 1.55–1.70 (m, 3H, CH_2CH(Leu)), 2.52–2.55 (dd, J = 10.1 and 1.6 Hz, 12H, N(CH_3)$_2$), 2.90–2.96 (m, 1H, CH_2 (Tyr)), 3.11–3.15 (m, 1H, CH_2 (Tyr)), 3.89 (s, 3H, COOCH_3), 4.26 (d, J = 6.0 Hz, 2H, NHCH_2Ph), 4.45–4.51 (m, 1H, CH (Leu)), 4.73–4.79 (m, 1H, CH (Tyr)), 7.00 (d, J = 8.4 Hz, 2H, 2 CH (Ar)), 7.04–7.06 (m, 2H, 2 CH (Ar)), 7.21–7.24 (m, 3H, 3 CH (Ar)), 7.31 (d, J = 8.4 Hz, 2H, CH (Ar)), 7.41–7.43 (m, 2H, 2 CH (Ar)), 7.47–7.49 (m, 1H, CH (Ar)), 7.68–7.70 (m, 2H, 2 CH (Ar)), 7.88–7.94 (m, 6H, 6 CH (Ar)), 8.39 (d, J = 7.7 Hz, 1H, CON*H*), 8.64 (t, J = 6.0 Hz, 1H, N*H*Bn), 8.83 (d, J = 8.4 Hz, 1H, CON*H*), 10.28 (s, 1H, N*H*Ar)); δ_C (100 MHz, DMSO-d_6) 21.7, 23.0, 24.4, 36.2, 36.3, 40.7, 42.5, 52.2, 52.3, 54.9, 113.7, 118.1, 118.3, 119.7, 119.7, 120.1, 126.7, 127.3, 128.1, 128.2, 128.4, 128.7, 128.8, 129.1, 130.3, 132.0, 133.9, 138.0, 139.1, 139.1, 139.9, 145.1, 149.5, 149.6, 165.1, 166.1, 168.4, 171.2, 171.4; LRMS (MS ES), calcd for $C_{49}H_{54}N_7O_8$PNa [M+Na] m/z = 922.38, found 922.41.

4.2.26. 4-((*S*)-3-(((*S*)-1-((6-(Benzylcarbamoyl)-3′-carbamoyl-[1, 1′-biphenyl]-3-yl)amino)-4-methyl-1-oxopentan-2-yl)-amino)-2-(4-cyanobenzamido)-3-oxopropyl)phenyl bis(dimethylamino) phosphordiamidate (13ad)

Phenol **12ad** (45 mg, 0.061 mmol) was treated according to general procedure **H**, and purified by flash column chromatography (3:2CH_2Cl_2:(92:7:1 CH_2Cl_2/MeOH/NH_4OH)) to yield final product **13ad** as a white powder (24 mg, 0.028 mmol, 46%): δ_H (400 MHz, DMSO-d_6) δ_H (400 MHz, DMSO-d_6) 0.92 (dd, J = 16.5 and 6.4 Hz, 6H, 2 CH_3 (Leu)), 1.57–1.69 (m, 3H, CH_2CH (Leu)), 2.52–2.55 (dd, J = 10.1 and 1.5 Hz, 12H, N(CH_3)$_2$), 2.91–2.97 (m, 1H, CH_2 (Tyr)), 3.11–3.16 (m, 1H, CH_2 (Tyr)), 4.28 (d, J = 6.0 Hz, 2H, CH_2Ph) 4.45–4.51 (m, 1H, CH(Leu)), 4.73–4.79 (m, 1H, CH(Tyr)), 7.00 (d, J = 6.8 Hz, 2H, 2 CH (*Ar*)), 7.05 (d, J = 7.0 Hz, 2H, 2 CH (Ar)), 7.18–7.27 (m, 3H, 3 CH (Ar)), 7.31–7.33 (m, 2H, 2 CH (Ar)), 7.36–7.48 (m, 4H, 4 CH (Ar)), 7.67–7.72 (m, 2H, 2 CH (Ar)), 7.86–7.94 (m, 6H, 6CH (Ar)), 8.04 (s, 1H, CH (Ar)), 8.42 (d, J = 7.8 Hz, 1H, CON*H*), 8.61 (t, J = 5.8 Hz, 1H, N*H*Bn), 8.79 (d, J = 8.3 Hz, 1H, CON*H*), 10.26 (s, 1H, N*H*Ar); δ_C (100 MHz, DMSO-d_6) 21.7, 23.0, 24.3, 36.2, 36.2, 36.2, 40.7, 42.4, 52.3, 54.9, 113.7, 117.6, 118.3, 119.7, 119.7, 120.4, 126.2, 126.6, 127.1, 127.7, 127.9, 128.2, 128.2, 128.7, 130.2, 131.1, 131.9, 132.4, 133.9, 134.3, 138.0, 139.2, 139.7, 139.8, 140.5, 149.5, 149.6, 165.0, 167.6, 168.6, 171.1, 171.3; LRMS (MS ES), calcd for $C_{48}H_{53}N_8O_7$PNa [M+Na] m/z = 907.38, found 907.23.

4.2.27. 4-((*S*)-3-(((*S*)-1-((6-(Benzylcarbamoyl)-3′-cyano-[1,1′-biphenyl]-3-yl)amino)-4-methyl-1-oxopentan-2-yl)amino)-2-(4-cyanobenzamido)-3-oxopropyl)phenyl bis(dimethylamino) phosphordiamidate (13ae)

Phenol **12ae** (35 mg, 0.048 mmol) was treated according to general procedure **H**, and purified by flash column chromatography (2:1 CH_2Cl_2:(92:7:1 CH_2Cl_2/MeOH/NH_4OH)) to yield final product **13ae** as a white powder (33 mg, 0.038 mmol, 80%): δ_H (400 MHz, DMSO-d_6): δ_H (400 MHz, DMSO-d_6) 0.91 (dd, J = 16.6 and 6.4 Hz, 6H, 2 CH_3 (Leu)), 1.55–1.70 (m, 3H, CH_2CH (Leu)), 2.52–2.55 (dd, J = 10.1 and 1.5 Hz, 12H, N(CH_3)$_2$), 2.90–2.96 (m, 1H, CH_2 (Tyr)), 3.11–3.15 (m, 1H, CH$_2$ (Tyr)), 4.28 (d, J = 5.9 Hz, 2H, NHCH_2Ph), 4.45–4.51 (m, 1H, CH(Tyr)), 4.73–4.79 (m, 1H, CH (Tyr)), 7.00 (d, J = 8.4 Hz, 2H, 2 CH (*Ar*)), 7.06–7.07 (m, 2H, 2 CH (Ar)), 7.20–7.33 (m, 5H, 5 CH (Ar)), 7.50–7.57 (m, 2H, 2CH (Ar)), 7.60–7.62 (m,

1H, CH (Ar)), 7.68–7.72 (m, 3H, 3 CH (Ar)), 7.81–7.83 (m, 1H, 1 CH (Ar)), 7.90–7.95 (m, 4H, 4 CH (Ar)), 8.42 (d, *J* = 7.7 Hz, 1H, CON*H*), 8.70 (t, *J* = 6.0 Hz, 1H, N*H*Bn), 8.85 (d, *J* = 8.2 Hz, 1H, CON*H*), 10.29 (s, 1H, N*H*Ar); δ_C (100 MHz, DMSO-d_6) 21.6, 22.9, 24.3, 36.2, 36.2, 36.2, 40.6, 42.4, 52.3, 54.9, 111.2, 113.7, 118.2, 118.3, 118.7, 119.7, 119.7, 120.3, 126.7, 127.0, 128.2, 128.8, 129.4, 130.2, 131.0, 131.6, 131.7, 132.3, 133.2, 133.9, 137.9, 138.2, 139.1, 139.9, 141.6, 149.5, 149.6, 165.0, 168.1, 171.1, 171.3; LRMS (MS ES), calcd for $C_{48}H_{51}N_8O_6PNa$ [M+Na] *m/z* = 889.37, found 889.22.

4.2.28. Methyl 2′-(benzylcarbamoyl)-5′-((*S*)-2-((*S*)-3-(4-((bis(dimethylamino)phosphoryl)oxy)phenyl)-2-(4-cyanobenzamido) propanamido)-4-methylpentanamido)-[1,1′-biphenyl]-3-carboxylate (13af)

Phenol **12af** (60 mg, 0.078 mmol) was treated according to general procedure **H**, and purified by flash column chromatography (2:1 CH_2Cl_2:[92:7:1 CH_2Cl_2/MeOH/NH_4OH]) to yield final product **13af** as a white powder (45 mg, 0.050 mmol, 64%): δ_H (400 MHz, DMSO-d_6): δ_H (400 MHz, DMSO-d_6) 0.91 (dd, *J* = 16.5 and 6.4 Hz, 6H, 2 CH_3 (Leu)), 1.55–1.70 (m, 3H, CH_2C*H*(Leu)), 2.52–2.55 (dd, *J* = 10.1 and 1.5 Hz, 12H, N(CH_3)$_2$), 2.89–2.97 (m, 1H, CH_2 (Tyr)), 3.11–3.16 (m, 1H, CH_2 (Tyr)), 3.85 (s, 3H, COOCH_3), 4.26 (d, *J* = 6.0 Hz, 2H, CH_2Ph), 4.45–4.51 (m, 1H, C*H*(Leu)), 4.73–4.79 (m, 1H, C*H* (Tyr)), 6.99–7.04 (m, 4H, 4 CH (Ar)), 7.20–7.22 (m, 3H, 3 CH (Ar)), 7.32 (d, *J* = 8.4 Hz, 1H, CH (Ar)), 7.47–7.51 (m, 2H, 2 CH (Ar)), 7.56–7.78 (m, 1H, CH (Ar)), 7.79–7.72 (m, 2H, 2 CH (Ar)), 7.89–7.95 (m, 6H, 6 CH (Ar)), 8.43 (d, *J* = 7.5 Hz, 1H, CON*H*), 8.65 (t, *J* = 6.0 Hz, 1H, N*H*Bn), 8.86 (d, *J* = 8.3 Hz, 1H, CON*H*), 10.29 (s, 1H, N*H*Ar)); δ_C (100 MHz, DMSO-d_6) 21.7, 23.0, 24.4, 36.2, 36.3, 36.3, 40.7, 42.5, 52.3, 52.4, 54.9, 113.7, 117.9, 118.3, 119.7, 119.8, 120.3, 126.6, 127.1, 128.0, 128.1, 128.2, 128.7, 128.8, 129.0, 129.6, 130.3, 131.9, 132.4, 133.2, 133.9, 138.0, 139.1, 139.1, 139.9, 140.8, 149.5, 149.6, 165.1, 166.1, 168.5, 171.2, 171.4; LRMS (MS ES), calcd for $C_{49}H_{54}N_7O_8PNa$ [M+Na] *m/z* = 922.38, found 922.09.

4.2.29. 4-((*S*)-3-(((*S*)-1-((5-(Benzylcarbamoyl)-4′-carbamoyl-[1,1′-biphenyl]-3-yl)amino)-4-methyl-1-oxopentan-2-yl)-amino)-2-(4-cyanobenzamido)-3-oxopropyl)phenyl bis(dimethylamino) phosphordiamidate (13ba)

Phenol **12ba** (45 mg, 0.061 mmol) was treated according to general procedure **H**, and purified by flash column chromatography (1:1 CH_2Cl_2:(92:7:1 CH_2Cl_2/MeOH/NH_4OH)) to yield final product **13ba** as a white solid (34 mg, 0.039 mmol, 63%): δ_H (400 MHz, DMSO-d_6) 0.90–0.96 (m, 6H, 2 CH_3 (Leu)), 1.58–1.73 (br m, 3H, CH_2C*H*(Leu)), 2.52–2.55 (m, 12H, N(CH_3)$_2$), 2.92–2.98 (m, 1H, CH_2 (Tyr)), 3.13–3.17 (m, 1H, CH_2 (Tyr)), 4.48–4.53 (m, 3H, Leu-C*H* and NHCH_2), 4.75–4.81 (m, 1H, C*H* (Tyr)), 7.01 (d, *J* = 8.4 Hz, 2H, 2 CH (Ar)), 7.23–7.27 (m, 1H, CH (Ar)), 7.32–7.35 (m, 6H, 6 CH (Ar)), 7.43 (s, 1H, CH (Ar)), 7.77 (d, *J* = 8.4 Hz, 2H, 2 CH (Ar)), 7.93–7.95 (m, 5H, 5 CH (Ar)), 8.00 (d, *J* = 8.0 Hz, 2H, Ar-COONH_2), 8.05 (s, 1H, CH (Ar)), 8.17 (m, 2H, CH (Ar)), 8.41 (d, *J* = 7.4 Hz, 1H, CON*H*), 8.85 (d, *J* = 8.2 Hz, 1H, CON*H*), 9.20 (t, *J* = 6.2 Hz, 1H, N*H*Bn), 10.32 (s, 1H, N*H*Ar); δ_C (100 MHz, MeOD-d_4) 22.1, 23.6, 26.0, 36.8, 36.9, 37.9, 41.8, 44.7, 54.3, 56.7, 116.2, 119.0, 119.89, 121.3, 121.4, 122.7, 128.2, 128.3, 128.7, 129.4, 129.5, 129.9, 131.7, 132.4, 133.5, 133.7, 134.4, 135.0, 137.3, 139.3, 140.2, 140.7, 142.3, 144.6, 151.2, 151.3, 168.5, 169.4, 169.6, 171.8, 173.4, 173.7; LRMS (MS ES), calcd for $C_{48}H_{53}N_8O_7PNa$ [M+Na] *m/z* = 907.38, found 907.10.

4.2.30. 4-((*S*)-3-(((*S*)-1-((5-(Benzylcarbamoyl)-4′-cyano-[1,1′-biphenyl]-3-yl)amino)-4-methyl-1-oxopentan-2-yl)amino)-2-(4-cyanobenzamido)-3-oxopropyl)phenyl bis(dimethylamino) phosphordiamidate (13bb)

Phenol **12bb** (45 mg, 0.061 mmol) was treated according to general procedure **H**, and purified by flash column chromatography (1:1 CH_2Cl_2:(92:7:1 CH_2Cl_2/MeOH/NH_4OH)) to yield final product **13bb** as a white solid (42 mg, 0.047 mmol, 77%): δ_H (400 MHz, DMSO-d_6) 0.92 (dd, *J* = 16 and 6.4 Hz, 6H, 2 CH_3 (Leu)), 1.55–1.74 (br m, 3H, CH_2C*H* (Leu)), 2.52–2.55 (m, 12H, (N(CH_3)$_2$), 2.91–2.97 (m, 1H, CH_2 (Tyr)), 3.12–3.17 (m, 1H, CH_2 (Tyr)), 4.47–4.53 (m, 3H, Leu-C*H* and NHCH_2), 4.74–4.80 (m, 1H, C*H* (Tyr)), 7.00 (d, *J* = 8.0 Hz, 2H, 2 CH (Ar)), 7.23–7.27 (m, 1H, CH (Ar)), 7.31–7.34 (m, 6H, 6 CH (Ar)), 7.89–8.00 (m, 9H, 9 CH (Ar)), 8.18 (s, 1H, CH (Ar)), 8.21 (s, 1H, CH (Ar)), 8.41 (d, *J* = 7.4 Hz, 1H, CON*H*), 8.86 (d, *J* = 8.2 Hz, 1H, CON*H*), 9.21 (t, *J* = 5.7 Hz, 1H, N*H*Bn), 10.35 (s, 1H, N*H*Ar); δ_C (100 MHz, DMSO-d_6) 21.6, 22.9, 24.3, 36.2, 36.2, 36.2, 40.6, 42.4, 52.3, 54.9, 110.0, 113.7, 118.3, 118.8, 119.7, 119.7, 120.1, 126.7, 127.3, 128.1, 128.2, 128.8, 129.2, 130.2, 131.8, 132.0, 132.3, 133.9, 138.0, 138.6, 139.0, 139.9, 145.2, 149.5, 149.6, 165.0, 168.1, 171.1, 171.4; LRMS (MS ES), calcd for $C_{48}H_{51}N_8O_6PNa$ [M+Na] *m/z* = 889.37, found 889.22.

4.2.31. Methyl 3′-(benzylcarbamoyl)-5′-((*S*)-2-((*S*)-3-(4-((bis(dimethylamino)phosphoryl)oxy)phenyl)-2-(4-cyanobenzamido) propanamido)-4-methylpentanamido)-[1,1′-biphenyl]-4-carboxylate (13bc)

Phenol **12bc** (60 mg, 0.078 mmol) was treated according to general procedure **H**, and purified by flash column chromatography (2:1 CH_2Cl_2:(92:7:1 CH_2Cl_2/MeOH/NH_4OH)) to yield final product **13bc** as a white solid (48 mg, 0.053 mmol, 68%): δ_H (400 MHz, DMSO-d_6) 0.92 (dd, *J* = 16.0 and 6.0 Hz, 6H, 2 CH_3 (Leu)), 1.58–1.73 (br m, 3H, CH_2C*H* (Leu)), 2.52–2.55 (m, 12H, N(CH_3)$_2$), 2.89–2.97 (m, 1H, CH_2 (Tyr)), 3.11–3.17 (m, 1H, CH_2 (Tyr)), 3.88 (s, 3H, COOCH_3), 4.48–4.53 (m, 3H, Leu-C*H* and NHCH_2C$_6$H$_5$), 4.73–4.81 (m, 1H, C*H* (Tyr)), 7.01 (d, *J* = 8.4 Hz, 2H, 2 CH (Ar)), 7.22–7.28 (m, 1H, CH (Ar)), 7.32–7.34 (m, 6H, 6 CH (Ar)), 7.86 (d, *J* = 8.4 Hz, 2H, 2 CH (Ar-COOMe)), 7.93 (s, 4H, 4 CH (Ar-CN)), 7.97 (s, 1H, CH, (Ar)), 8.09 (d, *J* = 8.4 Hz, 2H, 2 CH (Ar-COOMe)), 8.17 (s, 1H, CH (Ar)), 8.22 (s, 1H, CH (Ar)), 8.42 (d, *J* = 8.0 Hz, 1H, CON*H*), 8.86 (d, *J* = 8.4 Hz, 1H, CON*H*), 9.22 (t, *J* = 6.5 Hz, 1H, N*H*Bn), 10.35 (s, 1H, N*H*Ar); δ_C (100 MHz, CCl_3D) 22.3, 22.7, 24.8, 36.5, 36.5, 36.8, 40.9, 44.0, 52.1, 53.1, 55.6, 114.9, 117.9, 118.4, 120.1, 120.1, 121.3, 121.5, 127.0, 127.4, 127.6, 128.0, 128.6, 129.3, 130.0, 130.3, 132.0, 132.5, 136.0, 137.3, 138.3, 139.1, 140.9, 144.1, 150.0, 150.1, 166.1, 166.7, 167.6, 170.8, 171.8; LRMS (MS ES), calcd for $C_{49}H_{54}N_7O_8PNa$ [M+Na] *m/z* = 922.38, found 922.15.

4.2.32. (4-((*S*)-3-((*S*)-1-((5-(Benzylcarbamoyl)-3′-carbamoyl-[1,1′-biphenyl]-3-yl)amino)-4-methyl-1-oxopentan-2-yl)amino)-2- (4-cyanobenzamido)-3-oxopropyl)phenylbis(dimethylamino) phosphordiamidate (13bd)

Phenol **12bd** (70 mg, 0.096 mmol) was treated according to general procedure **H**, and purified by flash column chromatography (1:1 CH_2Cl_2:(92:7:1 CH_2Cl_2/MeOH/NH_4OH)) to yield final product **13bd** as a white solid (41 mg, 0.047 mmol, 49%): δ_H (400 MHz, DMSO-d_6) 0.90–0.96 (m, 6H, 2 CH_3 (Leu)), 1.56–1.71 (br m, 3H, CH_2C*H* (Leu)), 2.52–2.55 (m, 12H, N(CH_3)$_2$), 2.92–2.98 (m, 1H, tyr-CHCH_2), 3.14–3.16 (m, 1H, tyr-CHCH_2), 4.46–4.53 (m, 3H, Leu-C*H* and NHCH_2), 4.76–4.80 (m, 1H, tyr-C*H*), 7.01 (d, *J* = 8.4 Hz, 2H, 2 CH (Ar)), 7.24–7.26 (m, 1H, CH (Ar)), 7.32–7.34 (m, 6H, 6 CH (Ar)), 7.47 (s, 1H, CH (Ar)), 7.58 (t, *J* = 7.5 Hz, 1H, CH (Ar)), 7.84 (d, *J* = 8.4 Hz, 2H, *Ar*-COONH_2), 7.90–7.94 (m, 5H, 5 CH (Ar)), 8.11–8.20 (m, 4H, CH (Ar)), 8.39 (d, *J* = 7.6 Hz, 1H, CON*H*), 8.84 (d, *J* = 8.2 Hz, 1H, CON*H*), 9.20 (t, *J* = 6.2 Hz, 1H, N*H*Bn), 10.31 (s, 1H, N*H*Ar); δ_C (100 MHz, DMSO-d_6) 21.7, 23.0, 24.3, 30.7, 36.2, 36.2, 36.2, 40.7, 42.7, 55.3, 113.7, 113.9, 118.1, 118.3, 119.7, 120.2, 126.0, 126.8, 127.2, 128.2, 128.2, 128.3, 128.7, 129.0, 129.5, 130.2, 132.3, 133.9, 135.1, 135.8, 138.1 139.6, 139.7, 140.2, 151.2, 151.3, 165.0, 166.0, 167.7, 171.1; LRMS (MS ES), calcd for $C_{48}H_{53}N_8O_7PNa$ [M+Na] *m/z* = 907.38, found 907.36.

4.2.33. 4-((*S*)-3-(((*S*)-1-((5-(Benzylcarbamoyl)-3′-cyano-[1,1′-biphenyl]-3-yl)amino)-4-methyl-1-oxopentan-2-yl)amino)-2-(4-cyanobenzamido)-3-oxopropyl)phenyl bis(dimethylamino) phosphordiamidate (13be)

Phenol **12be** (50 mg, 0.068 mmol) was treated according to general procedure **H**, and purified by flash column chromatography (97.5:2.5 CH_2Cl_2/MeOH) to yield final product **13be** as a white solid (45 mg, 0.052 mmol, 76%): δ_H (400 MHz, MeOD-d_4) 0.95 (dd, J = 15.7 and 6.4 Hz, 6H, 2 CH_3 (Leu)), 1.67–1.77 (br m, 3H, CH_2CH (Leu)), 2.60–2.62 (dd, J = 15.1 and 1.0 Hz, 12H, N(CH_3)$_2$), 3.02–3.08 (m, 1H, CH_2 (Tyr)), 3.27–3.28 (m, 1H, CH_2 (Tyr)), 4.59–4.63 (m, 3H, CH and CH_2 (Leu)), 4.89–4.92 (m, 1H, CH (Tyr)), 6.99 (d, J = 8.4 Hz, 2H, CH (Ar)), 7.22–7.38 (m, 7H, 7 CH (Ar)), 7.61–7.65 (m, 1H, CH (Ar)), 7.72–7.77 (m, 3H, 3 CH (Ar)), 7.82.7.87 (m, 3H, 3 CH (Ar)), 7.94–7.96 (m, 1 H, CH (Ar-COOMe)), 8.01–8.02 (m, 1H, CH (Ar)), 8.05–8.06 (m, 1H, CH (Ar)), 8.11–8.12 (s, 1H, CH (Ar)); δ_C (100 MHz, MeOD-d_4) 22.0, 23.5, 26.0, 36.8, 36.9, 37.9, 41.8, 44.8, 54.3, 56.7, 114.3, 126.2, 119.0, 119.6, 120.2, 121.4, 121.4, 122.5, 128.3, 128.7, 129.4, 129.6, 131.3, 131.7, 131.8, 133.5, 135.0, 137.5, 139.4, 140.1, 140.9, 141.2, 142.7, 151.2, 151.3, 168.5, 169.5, 173.4, 173.7; LRMS (MS ES), calcd for $C_{48}H_{51}N_8O_6PNa$ [M+Na] m/z = 889.37, found 889.16.

4.2.34. Methyl 3′-(benzylcarbamoyl)-5′-((S)-2-((S)-3-(4-((bis-(dimethylamino)phosphoryl)oxy)phenyl)-2-(4-cyanobenzamido)propanamido)-4-methylpentanamido)-[1,1′-biphenyl]-3-carboxylate (13bf)

Phenol **12bf** (70 mg, 0.091 mmol) was treated according to general procedure **H**, and purified by flash column chromatography (92:7:1 CH_2Cl_2/MeOH/NH_4OH) to yield final product **13bf** as a white solid (47 mg, 0.052 mmol, 57%): δ_H (400 MHz, DMSO-d_6) 0.92 (dd, J = 16.0 and 6.5 Hz, 6H, 2 CH_3 (Leu)), 1.59–1.71 (br m, 3H, CH_2CH(Leu)), 2.52–2.55 (m, 12H, N(CH_3)$_2$), 2.91–2.98 (m, 1H, CH_2 (Tyr)), 3.13–3.17 (m, 1H, CH_2 (Tyr)), 3.90 (s, 3H, COOCH_3), 4.47–4.53 (m, 3H, Leu-CH and NHCH_2 (Leu)), 4.74–4.80 (m, 1H, CH(Tyr)), 7.00 (d, J = 8.4 Hz, 2H, 2 CH (Ar)), 7.23–7.27 (m, 1H, CH (Ar)), 7.31–7.34 (m, 6H, 6 CH (Ar)), 7.66–7.70 (m, 1H, CH (Ar-CN)), 7.93–7.94 (m, 5H, 5 CH (Ar)), 7.99–8.02 (m, 2H, CH (Ar)), 8.16–8.17 (m, 1H, CH (Ar)), 8.19–8.20 (m, 1H, CH (Ar)), 8.24–8.25 (m, 1H, CH (Ar)), 8.43 (d, J = 7.5 Hz, 1H, CONH), 8.89 (d, J = 8.4 Hz, 1H, CONH), 9.17 (t, J = 6.0 Hz, 1H, NHBn), 10.35 (s, 1H, NHAr); δ_C (100 MHz, CCl_3D) 22.2, 22.7, 24.7, 36.5, 36.5, 36.8, 40.8, 44.0, 52.2, 53.1, 55.6, 115.0, 117.9, 117.9, 120.2, 120.2, 121.1, 121.4, 127.4, 127.7, 128.1, 128.6, 128.9, 128.9, 130.3, 130.7, 131.6, 132.1, 132.4, 135.9, 137.4, 138.2, 139.1, 140.1, 141.1, 150.1, 150.1, 166.1, 166.8, 167.5, 170.7, 171.7; LRMS (MS ES), calcd for $C_{49}H_{54}N_7O_8PNa$ [M+Na] m/z = 922.38, found 922.15.

4.2.35. 4-((*S*)-3-((*S*)-1-(6-(Benzylcarbamoyl)-4′-carbamoylbiphenyl-3-ylamino)-4-methyl-1-oxopentan-2-ylamino)-2-(4-cyanobenzamido)-3-oxopropyl)phenyl dihydrogen phosphate (14aa)

Phosphoramidate **13aa** (20 mg, 0.022 mmol) was treated according to general procedure **H**, to yield final product **14aa** as a white lyophilized powder (18 mg, 0.021 mmol, 95%): mp 254–259 °C; δ_H (400 MHz, DMSO-d_6) 0.91 (dd, J = 16.1 and 6.2 Hz, 6H, 2 CH_3 (Leu)), 1.53–1.71 (m, 3H, CH_2CH (Leu)), 2.88–2.95 (m, 1H, CH_2 (Tyr)), 3.07–3.11 (m, 1H, CH_2 (Tyr)), 4.27 (d, J = 5.9 Hz, 2H, NHCH_2Ph), 4.44–4.50 (m, 1H, CH (Leu)), 4.68–4.73 (m, 1H, CH (Tyr)), 7.00–7.03 (m, 4H, Ar and Ar-OH (Tyr)), 7.19–7.24 (m, 5H, 5 CH (Ar)), 7.37–7.39 (m, 3H, CH (Ar)), 7.44–7.46 (m, 1H, CH (Ar)), 7.68–7.69 (m, 2H, 2 CH (Ar)), 7.86–7.92 (m, 6H, 6 CH (Ar)), 8.05 (s, 1H, CH (Ar)), 8.43 (d, J = 7.6 Hz, 1H, CONH), 8.66 (t, J = 5.9 Hz, 1H, NHBn), 8.87 (d, J = 8.1 Hz, 1H, CONH), 10.27 (s, 1H, NHAr); δ_C (100 MHz, DMSO-d_6) 21.6, 23.0, 24.4, 34.3, 40.6, 42.4, 52.4, 55.2, 113.7, 117.8, 118.4, 118.9, 119.5, 119.5, 120.2, 126.6, 127.1, 127.4, 128.1, 128.2, 128.2, 128.7, 129.6, 132.0, 132.4, 133.0, 138.0, 139.2, 139.4, 139.8, 143.2, 165.1, 167.6, 168.6, 171.3, 171.4. HRMS (MS- ES), calcd for $C_{44}H_{44}N_6O_9P$ [M+H] m/z = 831.2901, found 831.2866; *rp*HPLC t_R: condition (I) 17.361 (II) 30.160 min, purity 97.2%and 97.6%.

4.2.36. 4-((*S*)-3-(((*S*)-1-((6-(Benzylcarbamoyl)-4′-cyano-[1,1′-biphenyl]-3-yl)amino)-4-methyl-1-oxopentan-2-yl)amino)-2-(4-cyanobenzamido)-3-oxopropyl)phenyl dihydrogen phosphate (14ab)

Phosphoramidate **13ab** (25 mg, 0.029 mmol) was treated according to general procedure **H**, to yield final product **14ab** as a white lyophilized powder (22 mg, 0.027 mmol, 94%): mp 232–234 °C; δ_H (400 MHz, DMSO-d_6) 0.90 (dd, J = 15.7 and 6.3 Hz, 6H, 2 CH_3 (Leu)), 1.54–1.70 (m, 3H, CH_2CH (Leu)), 2.89–2.95 (m, 1H, CH_2 (Tyr)), 3.07–3.11 (m, 1H, CH_2 (Tyr)), 4.26 (d, J = 5.8 Hz, 2H, NHCH_2Ph), 4.44–4.49 (m, 1H, CH (Leu)), 4.68–4.76 (m, 1H, CH(Tyr)), 7.00–7.07 (m, 4H, 4 CH (Ar)), 7.23–7.28 (m, 5H, 5 CH (Ar)), 7.44–7.51 (m, 3H, 3 CH (Ar)), 7.69–7.77 (m, 4H, 4 CH (Ar)), 7.91 (m, 4H, 4 CH (Ar-CN)), 8.42 (d, J = 7.5 Hz, 1H, CONH), 8.72 (t, J = 5.8 Hz, 1H, NHBn), 8.87 (d, J = 8.3 Hz, 1H, CONH), 10.31 (s, 1H, NHAr); δ_C (100 MHz, DMSO-d_6) 21.6, 22.9, 24.3, 34.3, 40.6, 42.4, 52.4, 55.2, 110.0, 113.7, 118.3, 118.8, 119.6, 119.6, 120.1, 126.7, 127.3, 128.1, 128.2, 128.8, 129.3, 129.7, 131.8, 132.0, 132.4, 138.0, 138.6, 139.1, 140.0, 145.2, 165.2, 168.2, 171.3, 171.5; HRMS (MS ES), calcd for $C_{44}H_{42}N_6O_8P$ [M+H] m/z = 813.2796, found 813.2790; *rp*HPLC t_R: condition (I) 19.446 (II) 35.840 min, purity 94.5% and 96.3%.

4.2.37. Methyl 2′-(benzylcarbamoyl)-5′-((*S*)-2-((*S*)-2-(4-cyanobenzamido)-3-(4-(phosphonooxy)phenyl)propanamido)-4-methylpentanamido)-[1,1′-biphenyl]-4-carboxylate (14ac)

Phosphoramidate **13ac** (30 mg, 0.033 mmol) was treated according to general procedure **H**, to yield final product **14ac** as a white lyophilized powder (26 mg, 0.031 mmol, 94%): mp 253–259 °C; δ_H (400 MHz, DMSO-d_6) 0.90 (dd, J = 16.5and 6.4 Hz, 6H, 2 CH_3 (Leu)), 1.55–1.69 (m, 3H, CH_2CH (Leu)), 2.88–2.94 (m, 1H, CH_2 (Tyr)), 3.07–3.10 (m, 1H, CH_2 (Tyr)), 3.88 (s, 3H, COOCH_3), 4.25 (d, J = 5.8 Hz, 2H, NHCH_2Ph), 4.43–4.49 (m, 1H, CH (Leu)), 4.68–4.73 (m, 1H, CH (Tyr)), 7.00–7.06 (m, 4H, 4 CH (Ar)), 7.20–7.24 (m, 5H, 5 CH (Ar)), 7.41–7.43 (m, 2H, 2CH (Ar)), 7.46–7.49 (m, 1H, CH (Ar)), 7.68–7.72 (m, 2H, 2CH (Ar)), 7.87–7.91 (m, 6H, 6 CH (Ar)), 8.43 (d, J = 7.5 Hz, 1H, CONH), 8.69 (t, J = 5.8 Hz, 1H, NHBn), 8.89 (d, J = 8.4 Hz, 1H, CONH), 10.30 (s, 1H, NHAr); δ_C (100 MHz, DMSO-d_6) 21.6, 23.0, 24.4, 34.3, 40.6, 42.4, 52.2, 52.4, 55.3, 113.7, 118.1, 118.3, 119.6, 120.1, 126.6, 127.3, 128.1, 128.2, 128.4, 128.7, 129.0, 129.7, 131.9, 132.4, 138.0, 139.0, 139.1, 139.9, 145.2, 165.2, 166.1, 168.4, 171.3, 171.5; HRMS (MS ES), calcd for $C_{45}H_{45}N_5O_{10}P$ [M+H] m/z = 846.2898, found 846.2906; *rp*HPLC t_R: condition (I) 19.673 (II) 36.585 min, purity 95.6% and 97.5%.

4.2.38. 4-((*S*)-3-(((*S*)-1-((6-(Benzylcarbamoyl)-3′-carbamoyl-[1,1′-biphenyl]-3-yl)amino)-4-methyl-1-oxopentan-2-yl)-amino)-2-(4-cyanobenzamido)-3-oxopropyl)phenyl dihydrogen phosphate (14ad)

Phosphoramidate **13ad** (18 mg, 0.020 mmol) was treated according to general procedure **H**, to yield final product **14ad** as a white lyophilized powder (16 mg, 0.019 mmol, 95%): mp 196–201 °C; δ_H (400 MHz, DMSO-d_6) 0.91 (dd, J = 16.4 and 6.2 Hz, 6H, 2 CH_3 (Leu)), 1.57–1.70 (m, 3H, CH_2CH(Leu)), 2.90–2.96 (m, 1H, CH_2 (Tyr)), 3.09–3.12 (m, 1H, CH_2 (Tyr)), 4.27 (d, J = 5.8 Hz, 2H, NHCH_2Ph), 4.47–4.48 (m, 1H, CH(Leu)), 4.69–4.75 (m, 1H, CH (Tyr)), 7.01–7.06 (m, 4H, 4 CH (Ar)), 7.19–7.29 (m, 5H, 5 CH (Ar)), 7.36–7.48 (m, 4H, 4CH (Ar)), 7.68–7.72 (m, 2H, 2CH (Ar)), 7.86–7.94 (m, 6H, 6CH (Ar)), 8.07 (s, 1H, CH (Ar)), 8.44 (d, J = 7.4 Hz, 1H, CONH), 8.65 (t, J = 5.8 Hz, 1H, NHBn), 8.90 (d, J = 7.9 Hz, 1H,

CONH), 10.28 (s, 1H, NHAr); δ_C (100 MHz, DMSO-d_6) 21.6, 23.0, 24.4, 34.3, 40.7, 42.4, 52.4, 55.3, 113.7, 117.7, 118.4, 119.6, 119.6, 120.5, 126.2, 126.6, 127.1, 127.7, 128.0, 128.3, 128.7, 129.8, 131.2, 131.9, 132.4, 132.6, 134.3, 138.0, 139.3, 139.7, 140.5, 151.1, 151.1, 158.2, 158.5, 165.2, 167.8, 168.7, 171.3, 171.4; HRMS (MS ES), calcd for $C_{44}H_{44}N_6O_9P$ [M+H] m/z = 831.2901, found 831.2870; *rp*HPLC t_R: condition (I) 17.854 (II) 31.489 min, purity 95.9% and 97.4%.

4.2.39. 4-((*S*)-3-(((*S*)-1-((6-(Benzylcarbamoyl)-3′-cyano-[1,1′-biphenyl]-3-yl)amino)-4-methyl-1-oxopentan-2-yl)amino)-2-(4-cyanobenzamido)-3-oxopropyl)phenyl dihydrogen phosphate (14ae)

Phosphoramidate **13ae** (25 mg, 0.029 mmol) was treated according to general procedure **H**, to yield final product **14ae** as a white lyophilized powder (22 mg, 0.027 mmol, 94%): mp 179–183 °C; δ_H (400 MHz, DMSO-d_6) 0.90 (dd, J = 15.7 and 6.3 Hz, 6H, 2 CH_3 (Leu)), 1.56–1.67 (m, 3H, CH_2CH(Leu)), 2.89–2.95 (m, 1H, CH_2 (Tyr)), 3.07–3.10 (m, 1H, CH_2 (Tyr)), 4.27 (d, J = 5.9 Hz, 2H, NHCH_2Ph), 4.44–4.50 (m, 1H, CH(Leu)), 4.68–4.74 (m, 1H, CH(Tyr)), 7.00–7.07 (m, 4H, 4 CH (Ar)), 7.19–7.28 (m, 5H, 5 CH (Ar)), 7.49–7.56 (m, 2H, 2 CH (Ar)), 7.60–7.62 (m, 1H, CH (Ar)), 7.67–7.72 (m, 3H, 3 CH (Ar)), 7.80–7.82 (m, 1H, CH (Ar)), 7.91 (s, 4H, 4 CH (Ar)), 8.42 (d, J = 7.5 Hz, 1H, CONH), 8.73 (t, J = 5.9 Hz, 1H, NHBn), 8.88 (d, J = 8.0 Hz, 1H, CONH), 10.30 (s, 1H, NHAr); δ_C (100 MHz, DMSO-d_6) 21.6, 22.9, 24.3, 34.3, 40.6, 42.4, 52.4, 55.2, 111.2, 113.7, 118.2, 118.3, 118.7, 119.6, 119.6, 120.3, 126.7, 127.0, 128.2, 128.8, 129.4, 129.6, 131.0, 131.6, 131.7, 132.3, 133.2, 138.0, 138.2, 139.2, 140.0, 141.6, 165.2, 168.2, 171.3, 171.4; HRMS (MS ES), calcd for $C_{44}H_{42}N_5O_8P$ [M+H] m/z = 813.2796, found 813.2814;*rp*HPLC t_R: condition (I) 19.314 (II) 35.499 min, purity 96.4% and 97.5%.

4.2.40. Methyl 2′-(benzylcarbamoyl)-5′-((*S*)-2-((*S*)-2-(4-cyanobenzamido)-3-(4-(phosphonooxy)phenyl)propanamido)-4-methylpentanamido)-[1,1′-biphenyl]-3-carboxylate (14af)

Phosphoramidate **13af** (35 mg, 0.039 mmol) was treated according to general procedure **H**, to yield final product **14af** as a white lyophilized powder (31 mg, 0.037 mmol, 94%): mp 188–195 °C; δ_H (400 MHz, DMSO-d_6) 0.90 (dd, J = 14.2and 6.0 Hz, 6H, 2 CH_3 (Leu)), 1.56–1.68 (m, 3H, CH_2CH(Leu)), 2.89–2.95 (m, 1H, CH_2 (Tyr)), 3.07–3.11 (m, 1H, CH_2 (Tyr)), 3.85 (s, 3H, COOCH_3), 4.25 (d, J = 5.5 Hz, 2H, NHCH_2Ph), 4.46–4.48 (m, 1H, CH(Leu)), 4.69–4.71 (m, 1H, CH (Tyr)), 7.02–7.04 (m, 4H, 4 CH (Ar)), 7.19–7.25 (m, 5H, 5CH (Ar)), 7.46–4.49 (m, 2H, 2CH (Ar)), 7.55–7.57 (m, 1H, CH (Ar)), 7.68–7.72 (m, 2H, 2CH (Ar)), 7.90–7.94 (m, 6H, 6 CH (Ar)), 8.42 (d, J = 6.6 Hz, 1H, CONH), 8.66 (t, J = 5.5 Hz, 1H, NHBn), 8.89 (d, J = 8.3 Hz, 1H, CONH), 10.29 (s, 1H, NHAr); δ_C (100 MHz, DMSO-d_6) 21.6, 23.0, 24.4, 34.3, 40.6, 42.4, 52.2, 52.4, 55.3, 113.7, 117.9, 118.3, 119.6, 119.6, 120.3, 126.6, 127.1, 128.0, 128.1, 128.2, 128.7, 128.8, 129.0, 129.6, 129.7, 131.9, 132.4, 133.2, 138.0, 139.1, 139.1, 139.1, 139.9, 140.8, 158.0, 158.3, 165.2, 166.1, 168.5, 171.4, 171.5; HRMS (MS ES), calcd for $C_{45}H_{45}N_5O_8P$ [M+H] m/z = 846.2898, found 846.2904; *rp*HPLC t_R: condition (I) 19.806 (II) 36.905 min, purity 95.9% and 97.0%.

4.2.41. 4-((*S*)-3-(((*S*)-1-((5-(Benzylcarbamoyl)-4′-carbamoyl-[1,1′-biphenyl]-3-yl)amino)-4-methyl-1-oxopentan-2-yl)amino)-2-(4-cyanobenzamido)-3-oxopropyl)phenyl dihydrogen phosphate (14ba)

Phosphoramidate **13ba** (25 mg, 0.028 mmol) was treated according to general procedure **H**, to yield final product **14ba** as a white lyophilized powder (23 mg, 0.027 mmol, 97%): mp 167–171 °C; δ_H (400 MHz, DMSO-d_6) 0.92 (dd, J = 15.4 and 6.2 Hz, 6H, 2 CH_3 (Leu)), 1.65–1.73 (br m, 3H, CH_2CH (Leu)), 2.92–2.95 (m, 1H, CH_2 (Tyr)), 3.07–3.11 (m, 1H, CH_2 (Tyr)), 4.46–4.51 (m, 3H, 3 CH (Leu)), 4.69–4.73 (m, 1H, CH (Tyr)), 7.01 (d, J = 8.4 Hz, 2H, 2 CH (Ar)), 7.21–7.26 (m, 3H, CH (Ar)), 7.31–7.35 (m, 5H, 5 CH (Ar)), 7.40 (s, 1H, CH, (Ar)), 7.77 (d, J = 8.4 Hz, 2H, 2 CH (Ar)), 7.90–7.94 (m, 5H, 5 CH (Ar)), 8.01 (d, J = 8.4 Hz, 2H, 2 CH (Ar)), 8.10 (s, 1H, CH (Ar)), 8.16–8.18 (m, 2H, 2 CH (Ar)), 8.41 (d, J = 7.4 Hz, 1H, CONH), 8.88 (d, J = 8.0 Hz, 1H, CONH), 9.20 (m, 1H, NHBn), 10.33 (s, 1H, NHAr); δ_C (100 MHz, DMSO-d_6) 21.6, 23.0, 24.3, 34.3, 40.7, 42.7, 52.4, 55.2, 113.6, 118.3, 119.5, 119.9, 120.2, 126.5, 126.8, 127.2, 128.2, 128.3, 128.6, 129.5, 130.4, 131.7, 132.3, 133.5, 138.0, 139.6, 139.8, 142.0, 157.6, 157.9, 165.1, 167.0, 167.4, 171.3, 171.4; HRMS (MS ES), calcd for $C_{44}H_{44}N_6O_9P$ [M+H] m/z = 831.2901, found 831.2897; *rp*HPLC t_R: condition (I) 19.490 (II) 36.040 min, purity 93.1% and 97.8%.

4.2.42. 4-((*S*)-3-(((*S*)-1-((5-(Benzylcarbamoyl)-4′-cyano-[1,1′-biphenyl]-3-yl)amino)-4-methyl-1-oxopentan-2-yl)amino)-2-(4-cyanobenzamido)-3-oxopropyl)phenyl dihydrogen phosphate (14bb)

Phosphoramidate **13bb** (30 mg, 0.035 mmol) was treated according to general procedure **H**, to yield final product **14bb** as a white lyophilized powder (27 mg, 0.034 mmol, 98%): mp 150–157 °C; δ_H (400 MHz, DMSO-d_6) 0.91 (dd, J = 14.7 and 6.2 Hz), 6H, 2 CH_3 (Leu)), 1.55–1.72 (br m, 3H, CH_2CH(Leu)), 2.89–2.95 (m, 1H, CH_2 (Tyr)), 3.07–3.11 (m, 1H, CH_2 (Tyr)), 4.46–4.52 (m, 3H, CH (Leu) and CH_2 (NHCH_2Ph)), 4.68–4.74 (m, 1H, CH(Tyr)), 7.01 (d, J = 8.1 Hz, 2H, 2 CH (Ar)), 7.22–7.24 (m, 3H, 3 CH (Ar)), 7.31–7.34 (m, 4H, 4CH (Ar)), 7.88–7.98 (m, 9H, 9CH (Ar)), 8.20–8.21 (m, 2H, CH (Ar)), 8.45 (d, J = 7.8 Hz, 1H, CONH), 8.91 (d, J = 8.2 Hz, 1H, CONH), 9.25 (t, J = 5.8 Hz, 1H, NHBn), 10.43 (s, 1H, NHAr); δ_C (100 MHz, DMSO-d_6) 21.6, 23.0, 24.3, 34.2, 40.7, 42.7, 52.4, 55.4, 110.5, 113.6, 118.3, 118.8, 119.0, 119.5, 120.2, 120.4, 126.8, 127.3, 127.7, 128.2, 128.3, 129.5, 131.4, 132.3, 133.0, 135.9, 138.0, 138.8, 139.5, 139.9, 144.0, 157.9, 158.2, 165.1, 165.7, 171.4, 171.5; HRMS (MS ES), calcd for $C_{44}H_{42}N_6O_8P$ [M+H] m/z = 813.2796, found 813.2774; *rp*HPLCt_R: condition (I) 20.975 (II) 40.195 min, purity 73.8% and 90.3%.

4.2.43. Methyl 3′-(benzylcarbamoyl)-5′-((S)-2-((S)-2-(4-cyanobenzamido)-3-(4-(phosphonooxy)phenyl)propanamido)-4-methylpentanamido)-[1,1′-biphenyl]-4-carboxylate (14bc)

Phosphoramidate **13bc** (35 mg, 0.039 mmol) was treated according to general procedure **H**, to yield final product **14bc** as a white lyophilized powder (31 mg, 0.037 mmol, 95%): mp 138–145 °C; δ_H (400 MHz, DMSO-d_6) 0.92 (dd, J = 16.2 and 6.2 Hz, 6H, 2 CH_3 (Leu)), 1.57–1.73 (m, 3H, CH_2CH (Leu)), 2.89–2.95 (m, 1H, CH_2 (Tyr)), 3.07–3.12 (m, 1H, CH_2 (Tyr)), 3.89 (s, 3H, COOCH_3), 4.49–4.52 (m, 3H, CH (Leu) and CH_2 (NHCH_2Ph)), 4.69–4.75 (m, 1H, CH (Tyr)), 7.01–7.03 (m, 2H, 2CH (Ar)), 7.22–7.24 (m, 3H, 3CH (Ar)), 7.33–7.34 (m, 4H, 4CH (Ar)), 7.65–7.69 (m, 1H, CH (Ar)), 7.89–7.94 (m, 5H, 5 CH (Ar)), 7.99–8.00 (m, 2H, 2 CH (Ar)), 8.16 (s, 1H,CH (Ar)), 8.20 (s, 1H, CH (Ar)), 8.24 (s, 1H, CH (Ar)), 8.42 (d, J = 7.3 Hz, 1H, CONH), 8.88 (d, J = 8.7 Hz, 1H, CONH), 9.25 (t, J = 6.0 Hz, 1H, NHBn), 10.38 (s, 1H, NHAr); δ_C (100 MHz, DMSO-d_6) 21.6, 23.0, 24.4, 34.4, 40.7, 42.7, 52.3, 52.3, 55.3, 113.6, 118.3, 119.5, 119.5, 119.9, 120.1, 126.8, 127.1, 127.2, 128.2, 128.3, 129.5, 129.6, 130.4, 131.6, 132.3, 135.9, 138.0, 139.6, 139.8, 140.0 157.7, 158.0, 165.1, 165.9, 166.1, 171.4, 171.4; HRMS (MS ES), calcd for $C_{45}H_{45}N_5O_{10}P$ [M+H] m/z = 846.2898, found 846.290;*rp*HPLCt_R: condition (I) 21.573 (II) 41.866 min, purity 96.5% and 96.6%.

4.2.44. 4-((*S*)-3-(((*S*)-1-((5-(Benzylcarbamoyl)-3′-carbamoyl-[1,1′-biphenyl]-3-yl)amino)-4-methyl-1-oxopentan-2-yl) amino)-2-(4-cyanobenzamido)-3-oxopropyl)phenyl dihydrogen phosphate (14bd)

Phosphoramidate **13bd** (30 mg, 0.034 mmol) was treated according to general procedure **H**, to yield final product **14bd** as

a white lyophilized powder (27 mg, 0.034 mmol, 96%): mp 152–157 °C; δ_H (400 MHz, DMSO-d_6) 0.90–0.95 (m, 6H, 2 CH_3 (Leu)), 1.57–1.73 (br m, 3H, CH_2CH (Leu)), 2.88–2.94 (m, 1H, CHCH_2 (Tyr)), 3.07–3.11 (m, 1H, CHCH_2 (Tyr)), 4.47–4.52 (m, 3H, CH (Leu) and CH_2 (NHCH_2Ph)), 4.69–4.74 (m, 1H, CH (Tyr)), 7.01 (d, J = 8.4 Hz, 2H, 2 CH (Ar)), 7.20–7.26 (m, 3H, 3 CH (Ar)), 7.31–7.35 (m, 4H, 4CH (Ar)), 7.47 (s, 1H, CH, (Ar)), 7.56–7.60 (m, 1H, CH (Ar)), 7.83–7.85 (m, 1H, CH (Ar)), 7.89–7.95 (m, 6H, 6CH (Ar)), 8.14–8.20 (m, 4H, 4 CH (Ar)), 8.40 (d, J = 7.4 Hz, 1H, CONH), 8.86 (d, J = 8.2 Hz, 1H, CONH), 9.22–9.25 (m, 1H, NHBn), 10.35 (s, 1H, NHAr); δ_C (100 MHz, DMSO-d_6) 21.6, 23.0, 24.4, 34.2, 40.7, 42.7, 52.4, 55.3, 113.7, 118.1, 118.8, 119.5, 119.5, 120.1, 126.0, 126.8, 126.9, 127.3, 128.2, 128.3, 129.0, 129.5, 131.3, 132.3, 132.6, 135.1, 135.8, 138.0, 139.6, 139.7, 140.2, 158.0, 158.3, 165.1, 166.0, 167.8, 171.3, 171.4; HRMS (MS ES), calcd for $C_{44}H_{44}N_6O_9P$ [M+H] m/z = 831.2901, found 831.2921; *rp*HPLC t_R: condition (I) 19.665 (II) 36.574 min, purity 87.6% and 88.1%.

4.2.45. 4-((*S*)-3-(((*S*)-1-((5-(Benzylcarbamoyl)-3′-cyano-[1,1′-biphenyl]-3-yl)amino)-4-methyl-1-oxopentan-2-yl)amino)-2-(4-cyanobenzamido)-3-oxopropyl)phenyl dihydrogen phosphate (14be)

Phosphoramidate **13be** (30 mg, 0.035 mmol) was treated according to general procedure **H**, to yield final product **14be** as a white lyophilized powder (27 mg, 0.034 mmol, 97%): mp 125–133 °C; δ_H (400 MHz, DMSO-d_6) 0.92 (dd, J = 14.5 and 6.5 Hz, 6H, 2 CH_3 (Leu)), 1.57–1.72 (br m, 3H, CH_2CH (Leu)), 2.89–2.95 (m, 1H, CHCH_2 (Tyr)), 3.08–3.11 (m, 1H, CHCH_2 (Tyr)), 4.46–4.53 (m, 3H, CH(Leu) and CH_2 (NHCH_2Ph)), 4.68–4.74 (m, 1H, CH (Ar)), 7.01 (d, J = 7.7 Hz, 2H, 2 CH (Ar)), 7.22–7.24 (m, 3H, 3 CH (Ar)), 7.31–7.36 (m, 4H, 4 CH (Ar)), 7.68–7.73 (m, 1H, CH (Ar)), 7.86–7.94 (m, 6H, 6 CH (Ar)), 8.00–8.03 (m, 1H, CH (Ar)), 8.16–8.17 (m, 2H, 2 CH (Ar)), 8.21 (s, 1H, CH (Ar)), 8.46 (d, J = 7.5 Hz, 1H, CONH), 8.82 (d, J = 8.4 Hz, 1H, CONH), 9.22 (t, J = 6.0 Hz, 1H, NHBn), 10.42 (s, 1H, NHAr); δ_C (100 MHz, DMSO-d_6) 21.6, 23.0, 24.3, 34.2, 40.7, 42.7, 52.4, 55.4, 112.2, 113.6, 118.3, 118.7, 119.5, 119.5, 120.1, 120.3, 126.8, 127.2, 128.2, 128.3, 129.5, 130.3, 131.3, 131.5, 131.6, 132.3, 135.8, 138.0, 138.6, 139.5, 139.9, 140.7, 157.9, 158.2, 165.1, 165.7, 171.4, 171.5; HRMS (MS ES), calcd for $C_{44}H_{42}N_6O_8P$ [M+H] m/z = 813.2796, found 813.2777; *rp*HPLC t_R: condition (I) 20.965 (II) 40.147 min, purity 93.3% and 95.9%.

4.2.46. Methyl 3′-(benzylcarbamoyl)-5′-((*S*)-2-((*S*)-2-(4-cyano benzamido)-3-(4-(phosphonooxy)phenyl)propanamido)-4-methylpentanamido)-[1,1′-biphenyl]-3-carboxylate (14bf)

Phosphoramidate **13bf** (30 mg, 0.033 mmol) was treated according to general procedure **H**, to yield final product **14bf** as a white lyophilized powder (28 mg, 0.033 mmol, 98%): mp 136–144 °C; δ_H (400 MHz, DMSO-d_6) 0.92 (dd, J = 15.7and 6.4 Hz, 6H, 2 CH_3 (Leu)), 1.59–1.71 (br m, 3H, CH_2CH(Leu)), 2.92–2.98 (m, 1H, CHCH_2 (Tyr)), 3.11–3.16 (m, 1H, CH_2 (Tyr)), 3.88 (s, 3H, COOCH_3), 4.47–4.522 (m, 3H, CH (Leu) and CH_2 (NHCH_2Ph)), 4.72–4.78 (m, 1H, CH(Tyr)), 7.03–7.05 (m, 4H, 4 CH (Ar)), 7.17 (m, 1H, CH (Ar)), 7.30–7.34 (m, 5H, 5 CH (Ar)), 7.85 (d, J = 8.4 Hz, 2H, 2 CH (Ar)), 7.92–7.93 (m, 3H, 3 CH (Ar)), 7.97 (s, 1H, CH (Ar)), 8.09 (d, J = 8.4 Hz, 2H, 2CH (Ar)), 8.17 (s, 1H, CH (Ar)), 8.21 (s, 1H, CH (Ar)), 8.41 (d, J = 8.0 Hz, 1H, CONH), 8.89 (d, J = 8.4 Hz, 1H, CONH), 9.23 (t, J = 6.5 Hz, 1H, NHBn), 10.36 (s, 1H, NHAr); δ_C (100 MHz, DMSO-d_6) 21.6, 23.0, 24.4, 34.3, 40.7, 42.7, 52.2, 52.3, 55.0, 113.7, 118.3, 118.7, 119.6, 119.7, 120.1 120.3, 126.8, 127.1, 127.3, 128.2, 128.3, 128.9, 129.9, 130.0, 132.4, 133.6, 135.9, 138.0, 139.4, 139.5, 139.8, 144.0, 157.9, 158.2, 166.1, 166.8, 167.5, 170.7, 171.7; HRMS (MS ES), calcd for $C_{45}H_{45}N_5O_{10}P$ [M+H] m/z = 846.2898, found 846.2911;*rp*HPLC t_R: condition (I) 21.525 (II) 41.764 min, purity 98.3% and 98.3%.

4.3. Biophysical and biological assays

4.3.1. Cells and reagents

Normal mouse fibroblasts (NIH3T3) and counterparts transformed by v-Src (NIH3T3/v-Src) or overexpressing the human epidermal growth factor (EGF) receptor (NIH3T3/hEGFR), the murine thymus epithelial stromal cells, and the human breast cancer (MDA-MB-231) and pancreatic cancer (Panc-1) cells have all been previously reported.[28,33] Antibodies against STAT3, pY705STAT3, Erk1/2, and pErk1/2 are from Cell Signaling Technology (Danvers, MA).

4.3.2. Cloning and protein expression

Coding regions for the murine STAT3 protein and STAT3 SH2 domain were amplified by PCR and cloned into vectors pET-44 Ek/LIC (Novagen) and pET SUMO (Invitrogen), respectively. The primers used for amplification were: STAT3 Forward: GACGACGACAAGAT GGCTCAGTGGAACCAGCTGC; STAT3 Reverse: GAGGAGAAGCCC GGTTATCACATGGGGGAGGTAGCACACT; STAT3-SH2 Forward: ATGGGTTTCATCAGCAAGGA; STAT3-SH2 Reverse: TCACCTACAG TACTTTCCAAATGC. Clones were sequenced to verify the correct sequences and orientation. His-tagged recombinant proteins were expressed in BL21(DE3) cells, and purified on Ni-ion sepharose column.

4.3.3. STAT1, STAT3, and STAT5 fluorescence polarization binding assays

A fixed concentration of the fluorescently-labeled peptide probe (10 nM) was incubated with increasing concentration of the STAT1, STAT3, and STAT5 protein for 30 min at room temperature in the buffer, 50 mM NaCl, 10 mM HEPES, 1 mM EDTA, 0.1% Nonidet P-40, and the fluorescent polarization measurements were determined using an M1000 TECAN Infinite (TECAN, NC), with the set gain adjustment at 35 mP. The Z′ value was derived per the equation $Z' = 1-(3SDbound + 3SDfree)/(mPbound - mPfree)$, where SD is the standard deviation and mP is the average of fluorescence polarization. The bound state for STAT1 was achieved using 10 nM 5-carboxyfluorescein-GpYDKPHVL-NH_2incubated with 80nM STAT1 protein; the unbound state used the same mixture except 10 μM of unlabeled probe (Ac-GpYDKPHVL-NH_2) was added. In the bound state, 10 nM 5-carboxyfluorescein-GpYLPQTV-NH_2 was incubated with 150 nM purified STAT3 protein, while the free (unbound) state was the same mixture, but incubated with an additional 10 μM unlabeled Ac-GpYLPQTV-NH_2. The use of 10nM 5-carboxyfluorescein-GpYLVLDKW-NH_2 incubated with 105 nM STAT5 protein achieved the STAT5 bound state; the same mixture plus 10 μM of STAT5 unlabeled probes (Ac-GpYLVLDKW-NH_2) produced the free state following incubation. For evaluating agents, STAT1 (80nM), STAT3 (150 nM), and STAT5 (105 nM) proteins were individually incubated with serial concentrations of inhibitors at 30 °C for 60 min in the indicated assay buffer conditions. Prior to the addition of their respective fluorescent probes, the protein:inhibitor mixtures were allowed to equilibrate at room temperature for 15 min. Probes were added at a final concentration of 10 nM and incubated for 30 min at room temperature following which the FP measurements were taken using the TECAN M1000 polarimeter, with the set gain adjustment at 35 mP.

4.3.4. Nuclear extract preparation and gel shift assays

Nuclear extract preparation from v-Src-transformed fibroblasts (NIH3T3/v-Src) or mouse fibroblasts overexpressing the human epidermal growth factor receptor (NIH3T3/hEGFR) and electrophoretic mobility shift assay (EMSA) were carried out as previously described.[8] Nuclear extracts of equal total protein content

were pre-incubated with different concentrations of compounds for 30 min at room temperature prior to incubation with the radiolabeled probe. The ^{32}P-labeled oligonucleotide probe used is hSIE (high affinity sis-inducible element from the *c-fos* gene, m67 variant, 5′-AGCTTCATTTCCCGTAAATCCCTA) that binds STAT1 and STAT3.[37]

4.3.5. Surface Plasmon Resonance (SPR) binding experiments

SensiQ and its analysis software Qdat (ICX Technologies, Oklahoma City, OK) were used to analyze the interaction between agents and the STAT3 protein and to determine the binding affinity. Purified STAT3 was immobilized on a HisCap Sensor Chip by injecting 50 μg/ml of STAT3 onto the chip. Various concentrations of inhibitors in running buffer (1X PBS, 0.5% DMSO) were passed over the sensor chip to produce response signals. The association and dissociation rate constants were calculated using the Qdat software. The ratio of the association and dissociation rate constants was determined as the affinity (K_D).

4.3.6. Permeability and efflux analysis in Caco-2 models

Human, epithelial Caco-2 cells were seeded at a density of 75,000 cells/cm^2, on high-density PET membrane inserts, (1.0 μm pore size, 0.31 cm^2 surface area) and utilized on day 25 (post-seeding). At this stage of growth, cell monolayers were fully polarized and differentiated. All compounds were tested at a final concentration of 10 μM, under non-gradient pH conditions (pH 7.4/7.4) for 90 min as previously described.[1] Narrow-window mass extraction LC/MS analysis was performed for all samples from this study using a Waters Xevo quadrupole time-of-flight (QTof) mass spectrometer and an ACQUITY UPLC system, to determine relative peak areas of parent compound. The percent of transported drug was calculated based on these peak areas, relative to the initial, dosing concentration.

Acknowledgements

The authors gratefully acknowledge the OICR medicinal chemistry group for generously performing pharmacological experiments. For financial support of this work, the authors would like to thank the University of Toronto (P.T.G.), NSERC (P.T.G.) NIH Grants CA106439 and CA128865 (J.T. and P.G.) and the LLSC (P.T.G. and J.T.). Dr. A.D.S. is a Leukemia and Lymphoma Society Scholar in Clinical Research.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2010.12.010.

References and notes

1. Zhong, Z.; Wen, Z.; Darnell, J. E., Jr. *Science* **1994**, *264*, 95.
2. Darnell, J. E., Jr.; Kerr, I. M.; Stark, G. R. *Science* **1994**, *264*, 1415.
3. Garcia, R.; Yu, C.; Hudnall, A.; Catlett, R.; Nelson, K. L.; Smithgall, T.; Fujita, D. J.; Ethier, S. P.; Jove, R. *Cell Growth Differ.* **1997**, *8*, 1267.
4. Turkson, J.; Bowman, T.; Garcia, R.; Caldenhoven, E.; De Groot, R. P.; Jove, R. *Mol. Cell. Biol.* **1998**, *18*, 2545.
5. Bowman, T.; Garcia, R.; Turkson, J.; Jove, R. *Oncogene* **2000**, *19*, 2474.
6. Bromberg, J. F.; Wrzeszczynska, M. H.; Devgan, G.; Zhao, Y.; Pestell, R. G.; Albanese, C.; Darnell, J. E., Jr. *Cell* **1999**, *98*, 295.
7. Siddiquee, K. A. Z.; Turkson, J. *Cell Res.* **2008**, *18*, 254.
8. Turkson, J.; Ryan, D.; Kim, J. S.; Zhang, Y.; Chen, Z.; Haura, E.; Laudano, A.; Sebti, S.; Hamilton, A. D.; Jove, R. *J. Biol. Chem.* **2001**, *276*, 45443.
9. Gunning, P. T.; Katt, W. P.; Glenn, M.; Siddique, K.; Kim, J. S.; Jove, R.; Sebti, S. M.; Turkson, J.; Hamilton, A. D. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 1875.
10. (a) Fletcher, S.; Turkson, J.; Gunning, P. T. *ChemMedChem* **2008**, *3*, 1159; (b) Fletcher, S.; Drewry, J. A.; Shahani, V. M.; Gunning, P. T. *Biochem. Cell Biol.* **2009**, *87*, 825–833; (c) Avadisian, M.; Haftchenary, S.; Gunning, P. T. *Anti-Cancer Drugs*, doi:10.1097/CAD.0b013e328341185b.; (d) Page, B. D. G.; Ball, D.; Gunning, P. T. *Expert Opin. Ther. Pat.* **2011**, *21*, 1–19.
11. Coleman, D. R., IV; Kaluarachchi, K.; Ren, Z.; Chen, X.; McMurray, J. S. *Int. J. Pept. Res. Ther.* **2008**, *14*, 1.
12. Chen, J.; Bai, L.; Bernard, D.; Nikolovska-Coleska, Z.; Gomez, C.; Zgang, J.; Yi, H.; Wang, S. *ACS Med. Chem. Lett.* **2010**, *1*, 85.
13. Schust, J.; Sperl, B.; Hollis, A.; Mayer, T. U.; Berg, T. *Chem. Biol.* **2006**, *13*, 1235.
14. Fletcher, S.; Singh, J.; Zhang, X.; Yue, P. B.; Page, B. D. G.; Sharmeen, S.; Shahani, V. M.; Zhao, W.; Schimmer, A. D.; Turkson, J.; Gunning, P. T. *ChemBioChem* **2009**, *10*, 1959.
15. Siddiquee, K. A. Z.; Gunning, P. T.; Glenn, M.; Katt, W. P.; Zhang, S.; Schroeck, C.; Sebti, S. M.; Jove, R.; Hamilton, A. D.; Turkson, J. *ACS Chem. Biol.* **2007**, *2*, 787.
16. Gunning, P. T.; Glenn, M. P.; Siddiquee, K. A. Z.; Katt, W. P.; Masson, E.; Sebti, S. M.; Turkson, J.; Hamilton, A. D. *ChemBioChem* **2008**, *9*, 2800.
17. Mandal, P. K.; Ren, Z.; Chen, X.; Xiong, C.; McMurray, J. S. *J. Med. Chem.* **2009**, *52*, 6126.
18. Turkson, J.; Kim, J. S.; Zhang, S. M.; Yuan, J.; Huang, M.; Glenn, M.; Haura, E.; Sebti, S.; Hamilton, A. D.; Jove, R. *Mol. Cancer Ther.* **2004**, *3*, 261.
19. Coleman, D. R., IV; Ren, Z.; Mandal, P. K.; Cameron, A. G.; Dyer, G. A.; Muranjan, S.; Campbell, M.; Chen, X.; McMurray, J. S. *J. Med. Chem.* **2005**, *48*, 6661.
20. Jones, G.; Willett, P.; Glen, R. C.; Leach, A. C.; Taylor, R. *J. Mol. Biol.* **1997**, *267*, 727.
21. Becker, S.; Groner, B.; Muller, C. W. *Nature* **1998**, *394*, 145.
22. Schust, J.; Berg, T. *Anal. Biochem.* **2004**, *330*, 114.
23. Pengguang, W.; Brasseur, M.; Schindler, U. *Anal. Biochem.* **1997**, *249*, 29.
24. Muller, J.; Schust, J.; Berg, T. *Anal. Biochem.* **2008**, *375*, 249.
25. Turkson, J. *Expert Opin. Ther. Targets* **2004**, *8*, 409.
26. Dourlat, J.; Liu, W. Q.; Sancier, F.; Edmonds, T.; Pamonsinlapatham, P.; Cruzalegui, F.; Garbay, C. *Biochimie* **2009**, *91*, 996.
27. Mandal, P. K.; Liao, W. S. L.; McMurray, J. S. *Org. Lett.* **2009**, *11*, 3394.
28. Zhang, X.; Yue, P.; Fletcher, S.; Zhao, W.; Gunning, P. T.; Turkson, J. *Biochem. Pharm.* **2010**, *79*, 1398.
29. (a) Hidalgo, I. J.; Raub, T. J.; Borchardt, R. T. *Gastroenterology* **1989**, *96*, 736; (b) Artursson, P. *J. Pharm. Sci.* **1990**, *79*, 476.
30. Alimirah, F.; Chen, J.; Basrawala, Z.; Xin, H.; Choubey, D. *FEBS Lett.* **2006**, *580*, 2294.
31. Lieber, M.; Mazzetta, J.; Nelson-Rees, W.; Kaplan, M.; Todaro, G. *Int. J. Cancer* **1975**, *15*, 741.
32. (a) Filmus, J.; Trent, J. M.; Pollak, M. N.; Buick, R. N. *Mol. Cell. Biol.* **1987**, *7*, 251; (b) Garcia, R.; Bowman, T. L.; Nui, G.; Yu, H.; Minton, S.; Muro-Cacho, A. A.; Cox, C. E.; Falcone, R.; Fairclough, R.; Parsons, S.; Laudano, A.; Gazit, A.; Levitzki, A.; Kraker, A.; Jove, R. *Oncogene* **2001**, *20*, 2499; (c) Mora, L. B.; Buettner, R.; Seigne, J.; Diaz, J.; Ahmad, N.; Garcia, R.; Bowman, T.; Falcone, R.; Fairclough, R.; Cantor, A.; Muro-Cacho, C.; Livingston, S.; Karras, J.; Pow-Sang, J.; Jove, R. *Cancer Res.* **2002**, *22*, 6659.
33. Wagner, M.; Kleeff, J.; Friess, H.; Buchler, M. W.; Korc, M. *Pancreas* **1999**, *19*, 370.