JOC The Journal of Organic Chemistry

Note

pubs.acs.org/joc

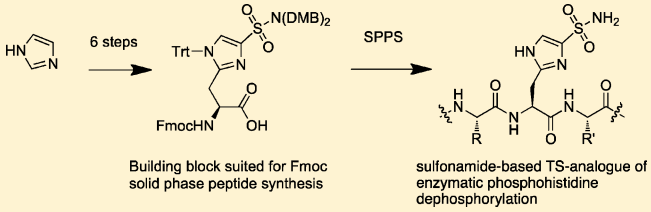
Design and Synthesis of an Fmoc-SPPS-Compatible Amino Acid Building Block Mimicking the Transition State of Phosphohistidine Phosphatase

Martijn F. Eerland and Christian Hedberg*

Department of Chemical Biology, Max Planck Institute for Molecular Physiology, Otto-Hahn Strasse 11, Dortmund D-44227, Germany

Supporting Information

ABSTRACT: The synthesis of a sulfonamide-based transition-state (TS) analogue of enzymatic phosphohistidine dephosphorylation as an amino acid building block is presented, together with the proof-of-concept of its incorporation into peptides. Key features include final global acidolytic protective group removal as well as full compatibility with standard Fmoc solid-phase peptide synthesis (SPPS). The peptides are designed as inhibitors of phosphohistidine phosphatase and as a pull-down probe for identification of phosphohistidine phosphatases, respectively.



N-Phosphorylation of histidine (Figure 1A) in vertebrates is a phenomenon generally overlooked, despite the importance of histidine *N*-phosphorylation in prokaryotes and protein phosphorylation in general.[1] Analysis and handling difficulties related to the acid-labile *N*-*P* (phosphoamidate) species, as well as the promiscuous nature of the enzymes responsible for *N*-phosphorylation, have hampered investigations in this field. Few reports have appeared on kinases and phosphatases responsible for phosphorylation/dephosphorylation of histidine, arginine, and lysine residues on eukaryotic proteins. Nucleoside diphosphate kinases (NDPKs) belong to a promiscuous family of kinases, which has been shown to phosphorylate histidine and aspartate residues.[2] However, NDPK's are primarily known for their role in nucleotide metabolism. Several serine/threonine protein phosphatases have been shown to lack discrimination between *O*- and *N*-phosphorylation and possesses significant phosphohistidine phosphatase activity.[3] Altogether, only one phosphohistidine-specific enzyme has been described so far. Discovered independently by the groups of Zetterqvist[4] and Klumpp[5] in 2002, phospho-histidine phosphatase, or PHP (a.k.a. PHP1 or PHP14), represents a unique enzymatic activity in mammalian cells. Studies based on overexpression of PHP in neuronal cells revealed a reduction in cell viability, most likely due to a decrease in ATP-citrate lyase (ACL) activity, a known target for PHP.[6] A recent study based on the shRNA knock-down of PHP expression in highly metastatic lung cancer cell lines (Cl1-0 and Cl1-5) showed reduced migration in solid agar medium.[7] Here, an effect on cytoskeletal reorganization was suggested as the mechanism of action. In two separate studies, the effect of overexpression or reduction (by shRNA) of the level of cellular PHP causes different effects.[6,7] This clearly shows the need for better tools to conditionally control PHP activity in living cells. In line with our ongoing investigations on eukaryotic *N*-phosphorylation, we hypothesized that PHP could be inhibited by small substrate-like peptides equipped with a nonhydrolyzable mimic of the phosphohistidine dephosphorylation transition state (TS). Corresponding peptides could be used as pull-down probes to further identify and characterize phosphohistidine phosphatase activity in cells.

A significant amount of indirect evidence has been published on the mechanism of action of PHP. The phosphohistidine substructure of the substrate protein is recognized through a strong hydrogen bond network around the phosphate, and protonation of the histidine imidazole moiety further destabilizes the *N*–*P* bond (Figure 1B).[8] His53 of PHP is most likely responsible for proton supply, since point mutation has shown this residue to be essential for enzyme activity.[9] Finally, hydrolysis of the weak *N*–*P* bond by nucleophilic attack of a water molecule removes the phosphate from the substrate.[10] Nonhydrolyzable phosphohistidine mimics based on furan or triazole phosphonic acids have been previously reported.[11−13] However, these heterocycles are suboptimal, being very different with regard to structure, as well as pK_a, compared to the native imidazole core of histidine. Here, we maintain the imidazole and exchange the phosphate for a sulfonamide, a known TS-mimic for inhibition of phosphatases.[14] In order to prevent hydrolysis, the histidine imidazole nitrogen is shifted one position, resulting in a feasible synthetic target (as shown in the box of Figure 1B). To allow incorporation of this transition state mimic into peptides, a building block suited for Fmoc solid-phase peptide synthesis (SPPS) was designed, relying on global acidolytic protective group removal.

Received: December 14, 2011
Published: January 26, 2012

ACS Publications © 2012 American Chemical Society

dx.doi.org/10.1021/jo2025702 | J. Org. Chem. 2012, 77, 2047−2052

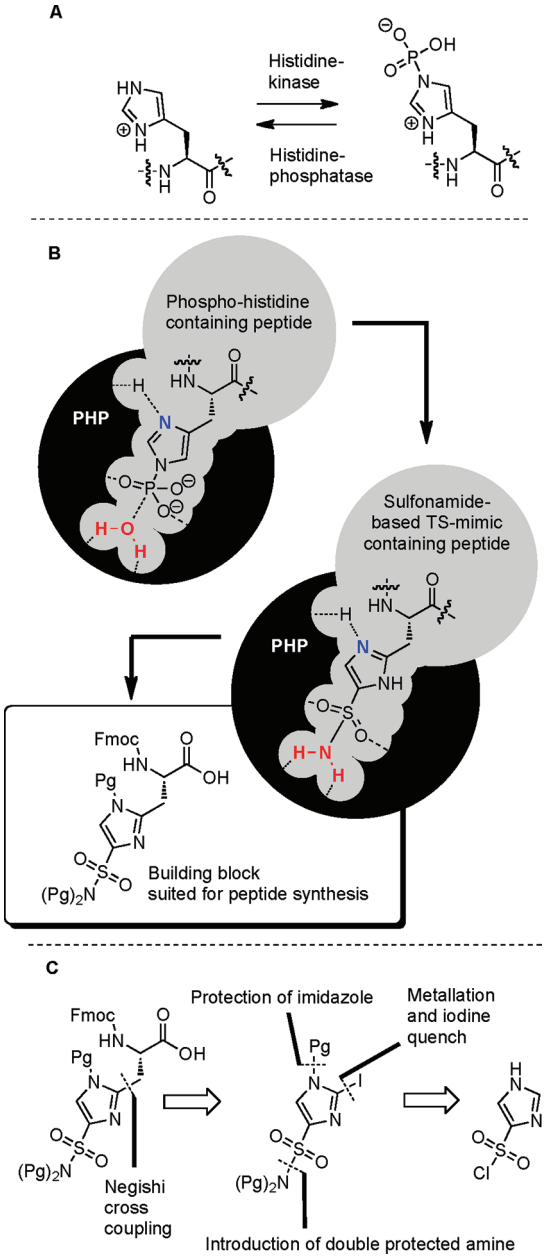
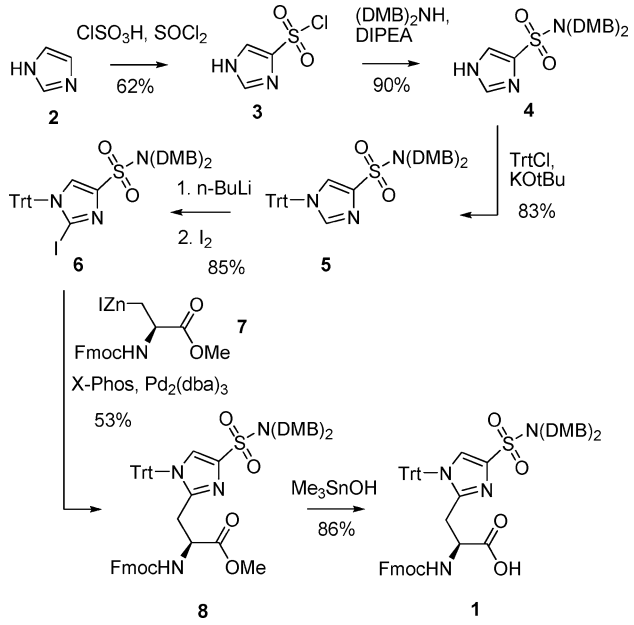


Figure 1. (A) Enzymatic phosphorylation/dephosphorylation of histidine. (B) Representation of the recognition of a phosphohistidine substrate by PHP, a sulfonamide-based TS-mimic, and the corresponding building block suited for Fmoc SPPS. (C) Retrosynthetic analysis of target building block.

A retrosynthetic analysis for the synthesis of this fully protected building block was carried out (Figure 1C). We focused on a convergent coupling approach where a zinc–iodine serine derivative was coupled to a fully protected 2-iodoimidazole via a Negishi cross-coupling reaction. Many different unnatural phenylalanine derivatives have been prepared in this fashion.[15−17] We choose to base our synthesis strategy on the orthogonal cleavage of the amino acid methyl ester by trimethyltin-hydroxide (Me_3SnOH) treatment, thus leaving the acid-labile protective groups of the imidazole sulfonamide moiety as well as the base-labile Fmoc protection of the N^{α}-position unaffected.[18] Bis(2,4-dimethoxybenzyl)-amine was used to acquire a protected sulfonamide by direct coupling with the imidazole sulfonyl chloride at an early stage of the synthesis. The 2,4-dimethoxybenzyl protective group has been proven to be an excellent choice for protection of sulfonamides, as well as for carboxamides, and can be removed easily under acidolytic cleavage conditions, similar to Boc removal.[19]

The synthesis (Scheme 1) commenced by heating imidazole (**2**) in chlorosulfonic acid. Subsequent addition of thionyl

Scheme 1. Synthesis of 1



chloride yielded 4(5)-imidazolesulfonyl chloride (**3**) in 62% after aqueous workup.[20] Reaction of **3** with *N,N*-bis(2,4-dimethoxybenzyl)amine (DMB_2NH) in the presence of DIPEA in DCM at 0 °C resulted in sulfonamide **4** in high yield (90%). We found it necessary to protect the imidazole (**4**) at *N*(1) before introducing the iodine-substituent in 2-position through deprotonation/I_2 quench. Several protective groups were investigated, tosyl, phenylsulfonyl, and *N,N*-dimethylsulfonamine, all of which resulted in poor regioselectivity in the deprotonation step (5- vs 2-position), as well as low reproducibility in terms of isolated yield. A *N*(1)-Boc protective group was investigated with good results but was found to be noncompatible with peptide synthesis. Scrambling of the Boc group between the imidazole and the N^{α}-position upon Fmoc deprotection during peptide synthesis terminated the peptide synthesis prematurely with significantly reduced yield as a result. Eventually, incorporation of a trityl (Trt) group at *N*(1) according to standard conditions resulted in compound **5** (83%), which underwent complete regioselective deprotonation on the 2-position with *n*-butyllithium at −30 °C, followed by quenching with I_2 (3 equiv). After optimization, the procedure resulted in a good yield (85%) of **6** on multigram scale. Next, with building block **6** in hand, we investigated the Negishi cross coupling with (*R*)-N^{α}-Fmoc-3-zinc iodoalanine methyl ester (**7**). Such coupling reactions are useful in the preparation of unnatural phenylalanine analogues, although *ortho*-substituted aryl halides have been reported to be difficult substrates.[15] Our trityl-protected iodoimidazole proved to be a very difficult substrate as well, and significant catalyst screening and optimization were required in order to reach isolatable amounts of product. First, for the generation of reactive

dx.doi.org/10.1021/jo2025702 | J. Org. Chem. 2012, 77, 2047−2052

Table 1. Negishi Cross-Coupling Results

entry	Pd precursor	equiv	ligand	equiv	T (°C) (for the coupling reaction)	% conv into coupling product[a]
1	$Pd(PPh_3)_4$	0.1			25	0
2	$Pd(PPh_3)_4$	0.1			40	8
3	$Pd(PPh_3)_4$	0.1			80	0
4	$PdCl_2(PPh_3)_2$	0.1			40	13
5	$PdCl_2(PPh_3)_2$	0.2			40	21
6	$PdCl_2(PPh_3)_2$	0.1			80	5
7	$Pd_2(dba)_3$	0.1	$P(o\text{-tol})_3$	0.4	40	0
8	$Pd(OAc)_2$	0.05	$P(o\text{-tol})_3$	0.2	40	7
9	$Pd(OAc)_2$	0.1	$P(o\text{-tol})_3$	0.2	40	23
10	$Pd(OAc)_2$	0.1	$P(o\text{-tol})_3$	0.4	40	20
11	$Pd(OAc)_2$	0.1	JohnPhos	0.2	40	0
12	$Pd(OAc)_2$	0.1	SPhos	0.2	40	19
13	$Pd(OAc)_2$	0.1	XPhos	0.2	40	43
14	$Pd_2(dba)_3$	0.1	JohnPhos	0.1	40	0
15	$Pd_2(dba)_3$	0.1	SPhos	0.1	40	22
16	$Pd_2(dba)_3$	0.1	XPhos	0.1	40	75 (53)[b]
17	$Pd_2(dba)_3$	0.05	XPhos	0.05	40	44

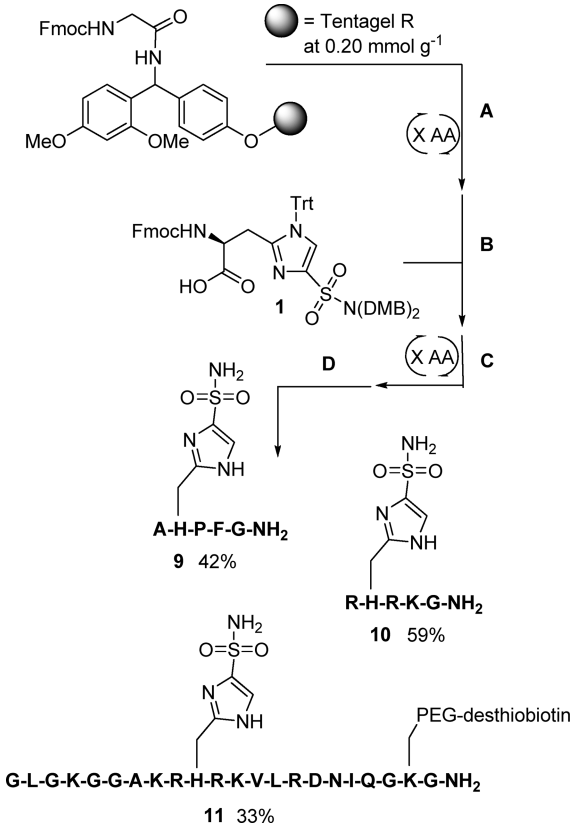
[a]HPLC-based as detected (absolute intensity) by a corona CAD detector. Percent conversion into coupling product = area coupling product/(area coupling product + area reduction product) × 100. Starting material fully converted in all cases. [b]Isolated yield after silica gel purification.

organozinc species from alkyl iodides like iodoalanines, several methods have been described.[15] In our hands, Zn-activation through ultrasonic treatment or through Knochel's procedure using 1,2-dibromoethane and chlorotrimethylsilane gave variable/irreproducible results. We found direct in situ activation of Zn by addition of a small amount of iodine to be the best method.[21] In line with previous reports, we also observed a general increase in yield when the reaction was performed in DMF instead of THF.[15] For the Negishi cross-coupling reaction leading to arylalanines, a number of different palladium sources and ligand combinations have been reported in literature. With our demanding substrate (**6**), we observed a strong preference for reduction of the 2-iodoimidazole leading to rapid formation of the 2*H*-imidazole as a competing reaction. This is most likely caused by preferential β-hydride elimination/reduction competing with product-forming reductive elimination as previously described in literature.[22] A correlation was found between the amount of equivalents of zinc reagent added and the yield for the palladium-catalyzed coupling reaction. Raising the amount of zinc reagent usually led to higher yields, indicating that the β-hydride elimination/reduction is quick compared to effective coupling. Eventually, we chose 2 equiv as a reasonable compromise between higher yield and excessive use of the iodoalanine precursor. For the coupling reaction, different palladium catalysts and ligands were investigated (Table 1). $Pd(PPh_3)_4$ generally showed only traces of product formation at room temperature; at 40 °C, 8% product was formed. At this temperature, complete consumption of the iodoimidazole (**6**) was usually achieved after 2 h. Use of $PdCl_2(PPh_3)_2$ also did not result in useful amounts of the coupling product. It appeared that only a single turnover took place per equivalent of catalyst (entry 4 and 5). Increasing the temperature to 80 °C (entry 3 and 6) resulted in rapid decomposition of organozinc reagent **7**, and no significant product formation was observed.[23] A well-documented palladium catalyst−ligand combination described for Negishi coupling reactions is $Pd(OAc)_2$ or $Pd_2(dba)_3$ with $P(o\text{-tol})_3$. Again, we only managed to reach reasonable yield when using higher loading of palladium catalysts. Next, we investigated electron-rich biaryl phosphine ligands, which have been reported to perform well and with high substrate tolerance even for sterically hindered (*ortho*-substituted) aryliodides.[24] We maintained a ratio between palladium and ligand of 1:1.[21] JohnPhos, SPhos, and XPhos showed large differences when used in combination with $Pd(OAc)_2$ or $Pd_2(dba)_3$. In both cases, XPhos showed the highest conversion (entries 13 and 16). This is not entirely in agreement with the results from earlier studies where SPhos was shown to be superior over XPhos concerning product formation over β-hydride elimination/reduction.[22] Eventually, entry 16 gave us the best result with acceptable catalyst loading, while lowering the amount of palladium resulted in reduced product formation (entry 17). Next, we scaled up the reaction to multigram scale, resulting in consistent yields. Cleavage of the methyl ester functionality of **8** with trimethyltin hydroxide proved viable, thus providing building block **1** in 86% yield. Overall, the synthesis sequence resulted in 18% of building block **1** over six steps.

Next, we evaluated building block **1** in standard SPPS according to the Fmoc protocol (Scheme 2). We decided to prepare a peptide with the sequence AH*PFG originating from Zetterqvists original studies on PHP (peptide **9**). This peptide is likely to be recognized by PHP and might impair its function. The peptide was synthesized on Tentagel-resin functionalized with a RAM-anchored Fmoc-glycine (Scheme 2, **A**−**D**). Coupling of the Fmoc-amino acids (10 equiv) (**A** and **C**) was carried out using standard HBTU/HOBt activation, except for the protected sulfonamide building block **1** (2 equiv of **B**), which was coupled employing HATU/HOAt as activating reagents. We found it important not to cap after coupling of building block **1**, as treatment with acetic anhydride/HOBt resulted in *N*-acetylation and Trt removal at the imidazole moiety, leading to acyl migration from the imidazole core to the N^{α}-position after subsequent Fmoc removal. This shift stops further amino acid coupling and significantly reduces yield. Cleavage from the resin (**D**), as well as global deprotection, was carried out by applying a mixture of trifluoroacetic acid (TFA)/triisopropylsilane (TIPS)/H_2O (90:5:5). After filtration from the resin, an additional 10% H_2O was added to the cleavage mixture, which was aged for 30 min to ensure complete hydrolysis of the DMB-protective groups from the sulfonamide

dx.doi.org/10.1021/jo2025702 | J. Org. Chem. 2012, 77, 2047−2052

Scheme 2. SPPS of Peptides 9−11



moiety. Concentration under reduced pressure at ambient temperature and trituration with diethyl ether yielded the crude peptide. After preparative reverse phase HPLC purification (C18) and subsequent lyophilization, peptide **9** was isolated in 42% yield (from resin loading). To verify the generality of the methodology, we synthesized one additional peptide sequence (derived from Histone H4) carrying the sulfonamide histidine (RH*RKG, peptide **10**, 59%) as well as a longer peptide derived from the histone H4 sequence carrying the sulfonamide histidine in the middle of the sequence and equipped with a PEG-linked desthiobiotin (GLGKGGAKRH*RKVLRD-NIQGK*G, peptide **11**, 33%). This peptide will be used as a pull-down probe in order to identify novel proteins possessing phosphohistidine phosphatase activity.

In conclusion, we have developed a facile and efficient synthesis of peptides containing a sulfonamide-based TS-mimic of the enzymatic phosphohistine dephosphorylation reaction, which was introduced by standard Fmoc-based SPPS. The general availability of this building block gives access to any desired sequence containing the TS-mimic targeting enzymatic phosphohistidine phosphatase activity. The synthesized peptides are currently under investigation in our laboratory and will be reported in due course.

EXPERIMENTAL SECTION

1*H*-Imidazole-4-sulfonyl Chloride (3). 20.0 g (294 mmol) imidazole was melted (theoretical mp = 90 °C/heated to 115 °C) in an argon-flushed 250 mL flask. A 60.0 mL (900 mmol) portion of $ClSO_3H$ was added dropwise (Caution! the first 15 mL reacted violently, with excessive HCl(g) formation). The reaction mixture was heated at 140 °C for 16 h. The resulting dark reaction mixture (RM) was cooled to 40 °C before addition of 22.0 mL (302 mmol) of thionyl chloride dropwise over 5 min (immediate gas evolution visible). The RM was heated until gas evolution ceased, during which time the temperature was slowly raised to 125 °C. The RM was allowed to cool to rt before pouring it into a slurry of 300 g of crushed ice with 20.0 g NaCl. A white precipitate was formed, and the suspension was filtered after the ice had completely melted. The white solid was washed with a small amount of cold water and dried overnight under vacuum over KOH. The filtrate was refiltered once and the collected solid was washed with a small amount of water and dried overnight under vacuum over KOH. Combining the two solids yielded 30.2 g (62%) **3** as a fine white powder: ^{1}H NMR(400 MHz, DMSO-d_6) δ 14.22 (broad s, 1H), 8.99 (d, J = 1.2 Hz, 1H), 7.64 (d, J = 1.2 Hz, 1H) Analytical data in agreement with literature.[25]

***N,N*-Bis(2,4-dimethoxybenzyl)-1*H*-imidazole-4-sulfonamide (4).** A 4.00 g (24.0 mmol) portion of **3** was added in small portions to a solution of 6.34 g of *N,N*-bis(2,4-dimethoxybenzyl)amine (20.0 mmol) in 100 mL of DCM with 6.60 mL (5.17 g/40 mmol) of DIPEA at 0 °C. The RM was stirred at 0 °C for 30 min, followed by 2 h at rt. When TLC indicated complete conversion, the RM was poured into 20.0 mL of 5% citric acid and was shaken once before addition of 200 mL of satd $NaHCO_3$(aq) and careful shaking (gas evolution). The layers were separated, and the DCM was washed with brine, dried over $MgSO_4$(s), concentrated, and dried under vacuum. The product was purified by SiO_2 column chromatography and eluted with EtOAc to obtain 8.02 g of **4** as a pale yellow foam (90% yield from bis(2,4-dimethoxybenzyl)amine): R_f = 0.46 (DCM/MeOH 9:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.92 (d, J = 1.2 Hz, 1H), 7.23 (d, J = 1.2 Hz, 1H), 7.05 (d, J = 8.4 Hz, 2H), 6.28 (dd, J = 8.4 Hz J = 2.4 Hz, 2H), 9.19 (d, J = 2.4 Hz, 2H), 5.35 (broad s, 2H), 4.34 (s, 4H), 3.66 (s, 6H), 3.54 (s, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 160.3, 158.1, 137.3, 136.4, 130.4, 120.7, 116.6, 104.0, 97.7, 55.2, 54.9, 46.6; ESI HRMS calcd for $C_{21}H_{26}O_6N_3S$ $[M + H]^+$ = 448.15368; Mass found $[M + H]^+$ = 448.15358.

(*N,N*-Bis(2,4-dimethoxybenzyl)-1-trityl-1*H*-imidazole-4-sulfonamide (5). In a 100 mL round-bottom flask, 2.00 g (4.47 mmol) of **4** was dissolved in 30.0 mL of dry DMF. After addition of 1.00 g (8.91 mmol) of KOtBu (RM became yellow) and stirring (20 min), 1.87 g (6.71 mmol) of trityl-Cl was added at 0 °C and the RM stirred for 18 h at rt. The DMF was evaporated, and the residue was suspended in satd $NaHCO_3$(aq) and extracted with EtOAc twice. The combined EtOAc layers were washed with brine, dried over $MgSO_4$(s), concentrated, and dried under vacuum. The product was purified by SiO_2 column chromatography and eluted with toluene/EtOAc (85:15) to obtain 2.53 g of **4** as a white/yellow foam (83% yield from **4**): R_f = 0.69 (toluene/EtOAc 1:1); ^{1}H NMR(400 MHz, $CDCl_3$) δ 7.46 (d, J = 1.2 Hz, 1H), 7.34 (m, 10H), 7.20 (d, J = 8.4 Hz, 2H), 7.05 (m, 6H), 6.33 (dd, J = 8.4 Hz, J = 2.4 Hz, 2H), 6.29 (d, J = 2,4 Hz, 1H), 4.48 (s, 4H), 3.74 (s, 6H), 3.62 (s, 6H); ^{13}C NMR(101 MHz, $CDCl_3$) δ 160.2, 158.3, 141.8, 140.2, 140.1, 130.3, 129.9, 128.7, 128.5, 124.8, 117.9, 104.1, 98.1, 76.5, 55.5, 55.2, 46.3; ESI HRMS calcd for $C_{40}H_{40}O_6N_3S$ $[M + H]^+$ = 690.26323; Mass found $[M + H]^+$ = 690.26366.

***N,N*-Bis(2,4-dimethoxybenzyl)-2-iodo-1-trityl-1*H*-imidazole-4-sulfonamide (6).** A 1.00 g (1.45 mmol) portion of **5** was dissolved in 20.0 mL of dry THF in a flame-dried Schlenk flask. The reaction mixture was cooled to −30 °C under argon atmosphere. A 1.20 mL (1.74 mmol) portion of a 1.41 M *n*-butyl lithium solution in hexane was added dropwise at −30 °C, and the RM became dark red. After 20 min, 1.00 g of iodine (3 equiv) dissolved in 5.00 mL of THF was added dropwise. The first equivalent of iodine was consumed as seen by disappearance of color. An additional 1 mL of iodine solution was added. The RM was stirred at −30 °C for 10 min before the cooling bath was removed and the RM allowed to warm for 10 min. The still relatively cold (−5 °C) RM was poured into satd $Na_2S_2O_3$ solution and was extracted twice with EtOAc. The combined EtOAc layers were washed with brine, dried over $MgSO_4$(s), concentrated, and dried under vacuum. The product was purified by SiO_2 column chromatography and eluted with toluene/EtOAc (95:5) to obtain 1.01 g of **4** as a white foam (85% yield from **5**): R_f = 0.73 (toluene/EtOAc 1:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34 (m, 10H), 7.20 (d, J = 8.4 Hz, 2H), 7.05 (m, 6H), 6.33 (dd, J = 8.4 Hz, J = 2.4 Hz, 2H), 6.29 (d, J = 2,4 Hz, 1H), 4.48 (s, 4H), 3.74 (s, 6H), 3.62 (s, 6H); ^{13}C

dx.doi.org/10.1021/jo2025702 | J. Org. Chem. 2012, 77, 2047−2052

NMR (101 MHz, $CDCl_3$) δ 160.3, 158.5, 142.1, 141.0, 130.8, 130.6, 128.6, 128.3, 128.2, 117.7, 104.1, 98.1, 92.1, 78.1, 55.5, 55.3, 46.2; ESI HRMS calcd for $C_{40}H_{39}O_6N_3IS$ $[M + H]^+$ = 816.15988; Mass found $[M + H]^+$ = 816.16011.

(*S*)-Methyl-2-(((9*H*-fluoren-9-yl)methoxy)carbonylamino)-3-(4-(*N*,*N*-bis(2,4-dimethoxybenzyl)sulfamoyl)-1-trityl-1*H*-imidazol-2-yl)propanoate (8). A 65 mg (1 mmol) portion of Zn(0) (dust) was weighted into a pear-shaped flask and flame-dried. Twenty milligrams of I_2 dissolved in 3 mL of dry DMF was added (the yellow color disappeared after approximately 1 min). A 160 mg (0.36 mmol) portion of (*R*)-methyl 2-(((9*H*-fluoren-9-yl)methoxy)carbonylamino)-3-iodopropanoate **7** together with 20 mg of iodine was added, and the RM was stirred until TLC indicated full consumption of **7**.[26] The stirring was stopped, and the unreacted Zn was allowed to precipitate. Two milliliters of the supernatant was taken out in a syringe and directly added to a prestirred solution of 6 mg (0.006 mmol) of $Pd_2(dba)_3$, 6 mg (0.012 mmol) of Xphos, and 100 mg (0.12 mmol) of **6** suspended in 1 mL of dry DMF. The RM was aged for 2 h at 40 °C, poured into satd $NaHCO_3$(aq), and extracted twice with EtOAc. The combined EtOAc layers were washed with satd NaCl(aq), dried over $MgSO_4$(s), concentrated, and dried under vacuum. The product was purified by SiO_2 column chromatography and eluted with toluene/EtOAc (9:1) to obtain 66 mg of **8** as a pale yellow foam (53% yield from **6**): R_f = 0.63 (toluene/EtOAc 1:1); $[\alpha]^{20}_D$ +22.2 (c = 0.4, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.73 (d, J = 8.0 Hz, 2H), 7.58 (t, J = 8.0 Hz, 2H), 7.35 (m, 11H), 7.22 (m, 5H), 7.06 (m, 6H), 6.45 (d, J = 8.8 Hz, 1H), 6.39 (dd, J = 8.4 Hz, J = 2.4 Hz, 2H), 6.28 (d, J = 2.4 Hz, 1H), 4.60 (d, 16.0 Hz, 1H), 4.45 (d, 16.0 Hz, 1H), 4.39 (m, 2H), 4.20 (m, 2H), 3.74 (s, 6H), 3.71 (s, 3H), 3.58 (s, 6H), 2.57 (dd, J = 17.2 Hz, J = 4.0 Hz, 1H)), 2.07 (dd, J = 17.2 Hz, J = 4.0 Hz, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.7, 160.2, 158.4, 156.5, 149.2, 144.2, 144.0, 141.4, 141.4, 141.1, 137.7, 130.5, 130.1, 128.7, 128.6, 127.9, 127.8, 127.4, 127.4, 125.6, 125.5, 125.0, 120.1, 117.9, 104.3, 98.1, 76.3, 67.6, 55.5, 55.2, 52.7, 51.7, 47.3, 46.5, 32.8; ESI HRMS calcd for $C_{59}H_{57}O_{10}N_4S$ $[M + H]^+$ = 1013.37899. Mass found $[M + H]^+$ = 1013.37950.

(*S*)-2-(((9*H*-Fluoren-9-yl)methoxy)carbonylamino)-3-(4-(*N*,*N*-bis(2,4-dimethoxybenzyl)sulfamoyl)-1-trityl-1*H*-imidazol-2-yl)-propanoic Acid (1). A 500 mg (0.49 mmol) portion of **8** was dissolved in 10 mL of DCE. An 894 mg (5.0 mmol) portion of $Sn(Me)_3OH$ was added. The RM was heated at 65 °C for 18 h, when TLC indicated complete disappearance of SM. The RM was poured into citric acid and extracted with DCM (2 × 20 mL). The DCM was dried over $MgSO_4$(s), concentrated, and dried under vacuum. The product was purified by SiO_2 column chromatography and eluted with toluene/EtOAc (1:1) to obtain 425 mg of **1** as a pale yellow foam (86% yield from **7**): R_f = 0.20 (toluene/EtOAc 1:1); $[\alpha]^{20}_D$ +19.8 (c = 0.3, $CHCl_3$) ; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 (d, J = 8.0 Hz, 2H), 7.56 (d, J = 8.0 Hz, 1H), 7.51 (d, J = 8.0 Hz, 1H), 7.35 (m, 11H), 7.22 (m, 5H), 7.06 (m, 6H), 6.45 (d, J = 8.8 Hz, 1H), 6.39 (dd, J = 8.4 Hz, J = 2.4 Hz, 2H), 6.28 (d, J = 2.4 Hz, 1H), 4.60 (d, 16.0 Hz, 1H), 4.45 (d, 16.0 Hz, 1H), 4.39 (m, 2H), 4.20 (m, 2H), 3.74 (s, 6H), 3.71 (s, 3H), 3.58 (s, 6H), 2.57 (dd, J = 17.2 Hz, J = 4.0 Hz, 1H)), 2.07 (dd, J = 17.2 Hz, J = 4.0 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.2, 160.4, 158.5, 155.6, 148.9, 144.1, 144.0, 141.5, 141.4, 140.6, 137.0, 130.8, 130.0, 128.8, 128.1, 128.0, 127.5, 127.3, 125.4, 125.3, 124.9, 120.2, 117.5, 104.3, 98.3, 67.4, 55.5, 55.4, 53,7, 51.7, 47.2, 46.6, 34.2, 29.9; ESI HRMS calcd for $C_{58}H_{55}O_{10}N_4S$ $[M + H]^+$ = 999.36334; Mass found $[M + H]^+$ = 999.36386.

Peptides 9−11. Peptide **11** was synthesized using Tentagel Rink-amide resin as solid phase employing 10 equiv of amino acid and 10 equiv of HBTU for all amino acids except **1** (2 equiv) which was coupled using 2 equiv of HOAt, 2 equiv of HATU, and 5 equiv of DIPEA in DMF. The coupling reaction was allowed to proceed overnight at room temperature. The resin was pre-swollen by treatment with DCM (15 min). Removal of the Fmoc protecting group was performed by treatment with 20% piperidine in DMF (3 times 10 min). The resin was washed with DMF three times. Peptides **9** and **10** were synthesized manually on a Tentagel Rink-amide resin preloaded with glycine using the same procedures as described for peptide **11**. Before cleavage of the peptides from the resin, the resin was washed thoroughly with diethyl ether (5 times), methanol (5 times), and DCM (5 times). The peptides were cleaved with 5% TIPS and 5% water in TFA (3 times 1 h). The cleavage mixture was evaporated to dryness in vacuo. The resulting solid was triturated with diethyl ether, dissolved in water/acetonitrile and subsequently lyophilized overnight. Pure peptides were obtained after purification by preparative HPLC. For peptide **9**; ESI HRMS calcd for $C_{25}H_{36}O_7N_9S$ $[M + H]^+$ = 606.24529; Mass found $[M + H]^+$ = 606.24433. For peptide **10**; ESI HRMS calcd for $C_{26}H_{51}O_7N_{16}S$ $[M + H]^+$ = 731.38419; Mass found $[M + H]^+$ = 731.38311. For peptide **11**; ESI HRMS calcd for $C_{118}H_{217}O_{34}N_{44}S$ $[M + 5H]^{5+}$ = 565.32594; Mass found $[M + 5H]^{5+}$ = 565.32579.

■ ASSOCIATED CONTENT

Supporting Information

^{1}H and ^{13}C NMR, LC−MS, and HR-MS spectra for all new compounds and HPLC, LC−MS, and HR-MS spectra for the peptides. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: christian.hedberg@mpi-dortmund.mpg.de.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We are grateful to Prof. Dr. Herbert Waldmann, Department of Chemical Biology, Max-Planck Institute of Molecular Physiology, for his generous and unconditional support. C.H. thanks AstraZeneca AB, Sweden, for financial support. We thank Mr. Sascha Gentz for operating the automated peptide synthesizer.

■ REFERENCES

(1) Klumpp, S.; Krieglstein, J. *Sci. Signaling* **2009**, *2*, No. pe13.
(2) Besant, P. G.; Attwood, P. V. *Biochim. Biophys. Acta, Proteins Proteomics* **2005**, *1754*, 281.
(3) Klumpp, S.; Bechmann, G.; Maeurer, A.; Krieglstein, J. *Top. Curr. Genet.* **2004**, *5*, 131.
(4) Ek, P.; Pettersson, G.; Ek, B.; Gong, F.; Li, J.-P.; Zetterqvist, O. *Eur. J. Biochem.* **2002**, *269*, 5016.
(5) Klumpp, S.; Hermesmeier, J.; Selke, D.; Baumeister, R.; Kellner, R.; Krieglstein, J. *J. Cereb. Blood Flow Metab.* **2002**, *22*, 1420.
(6) Krieglstein, J.; Lehmann, M.; Maeurer, A.; Gudermann, T.; Pinkenburg, O.; Wieland, T.; Litterscheid, S.; Klumpp, S. *Neurochem. Int.* **2008**, *53*, 132.
(7) Xu, A.; Hao, J.; Zhang, Z.; Tian, T.; Jiang, S.; Hao, J.; Liu, C.; Huang, L.; Xiao, X.; He, D. *Lung cancer* **2010**, *67*, 48.
(8) Gong, W.; Li, Y.; Cui, G.; Hu, J.; Fang, H.; Jin, C.; Xia, B. *Biochem. J.* **2009**, *418*, 337.
(9) Ma, R.; Kanders, E.; Sundh, U. B.; Geng, M.; Ek, P.; Zetterqvist, O.; Li, J.-P. *Biochem. Biophys. Res. Commun.* **2005**, *337*, 887.
(10) Solution structure and catalytic mechanism of human protein histidine phosphatase **1**, see: Gong, W.; Li, Y.; Cui, G.; Hu, J.; Fang, H.; Jin, C.; Xia, B. *Biochem. J.* **2009**, *418*, 337−344.
(11) Schenkels, C.; Erni, B.; Reymond, J.-L. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 1443.
(12) Kee, J.-M.; Villani, B.; Carpenter, L. R.; Muir, T. W. *J. Am. Chem. Soc.* **2010**, *132*, 14327.
(13) McAllister, T. E.; Nix, M. G.; Webb, M. E. *Chem. Commun. (Cambridge, U. K.)* **2011**, *47*, 1297.
(14) Chen Yen, T.; Xie, J.; Seto Christopher, T. *J. Org. Chem.* **2003**, *68*, 4123.
(15) Jackson, R. F. W.; Moore, R. J.; Dexter, C. S.; Elliott, J.; Mowbray, C. E. *J. Org. Chem.* **1998**, *63*, 7875.

(16) Oswald, C. L.; Carrillo-Marquez, T.; Caggiano, L.; Jackson, R. F. W. *Tetrahedron* **2007**, *64*, 681.
(17) Jackson, R. F. W.; Wishart, N.; Wood, A.; James, K.; Wythes, M. J. *J. Org. Chem.* **1992**, *57*, 3397.
(18) Nicolaou, K. C.; Estrada, A. A.; Zak, M.; Lee, S. H.; Safina, B. S. *Angew. Chem., Int. Ed.* **2005**, *44*, 1378.
(19) Hill, B.; Liu, Y.; Taylor, S. D. *Org. Lett.* **2004**, *6*, 4285.
(20) Baker, G. P.; Bourne, I. A.; Ford, M. J.; Foster, R. W. G.; Jackson, T. H.; Pannell, R. W.; Whitmore, M. W. *Org. Process Res. Dev.* **1999**, *3*, 104.
(21) Ross, A. J.; Lang, H. L.; Jackson, R. F. W. *J. Org. Chem.* **2010**, *75*, 245.
(22) Han, C.; Buchwald, S. L. *J. Am. Chem. Soc.* **2009**, *131*, 7532.
(23) Jackson, R. F. W. *ACS Symp. Ser.* **2009**, *1009*, 2.
(24) Milne, J. E.; Buchwald, S. L. *J. Am. Chem. Soc.* **2004**, *126*, 13028.
(25) Salituro, F. G.; Saunders, J. O. (Agios Pharmaceuticals, Inc.). Application: WO, 2010/118063 A2.
(26) A significant difference observed compared to the procedure described in literature (ref 21) was the absence of an exotherm for the insertion and generally longer reaction time needed to complete the insertion reaction.