JOC The Journal of Organic Chemistry

Note

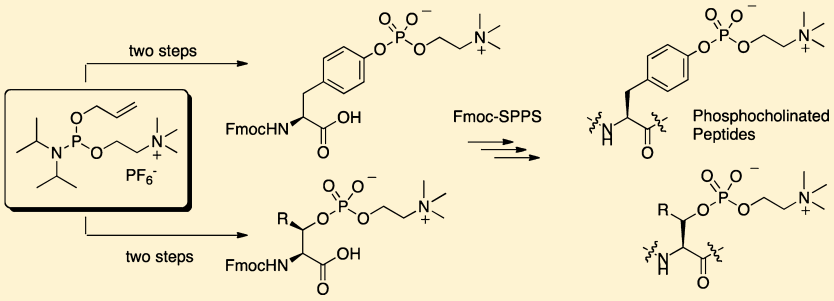
pubs.acs.org/joc

Amino Acid Building Blocks for Fmoc Solid-Phase Synthesis of Peptides Phosphocholinated at Serine, Threonine, and Tyrosine

Michael F. Albers and Christian Hedberg*

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

Supporting Information



ABSTRACT: Phosphocholination of eukaryotic host cell proteins has recently been identified as a novel post-translational modification important for bacterial pathogenesis. Here, we describe the first straightforward synthetic strategy for peptides containing phosphocholinated serine, threonine, or tyrosine residues using preformed functional amino acid building blocks, fully compatible with standard Fmoc solid-phase peptide synthesis.

During infection, the human pathogen *Legionella pneumophila* injects several hundred effector proteins into its host cell. Several of the released effector proteins target the small GTPases of the host cell.[1] Small GTPases act as molecular switches when hydrolyzing GTP to GDP, thus regulating signaling events at a cellular level.[2] By modulating the activity of host cell Rab GTPases, the pathogen redirects transport of vesicular compartments to its replication vacuole.[1,3,4] Recently, it was revealed that *Legionella* protein AnkX is covalently transferring a phosphocholine moiety from CDP-choline onto specific amino acid residues of the Switch II region of human GTPases of the Rab family (Ser76 in Rab1, Thr76 in Rab35).[5] As a consequence of the modifications, the Rab proteins are trapped in the inactive, GDP-bound state on the membrane, unable to interact with their endogenous cellular effectors, while *Legionella* effectors like LidA still show interaction.[6] Interestingly, *Legionella* protein Lem3 (lpg0696) was identified as a dephosphocholinase capable of hydrolyzing the covalent modification on the protein Rab1, thus making the modification reversible (Figure 1).[6,7]

However, specificity of phosphocholination and dephosphocholination as well as the distribution of this post-translational modification in a eukaryotic context is still unclear. We hypothesized that the generation of phosphcholinated peptides would provide excellent tools for the investigation of the intriguing biochemical activity of the protein pair AnkX and Lem3 as well as raising epitope-specific antibodies for phosphocholinated proteins. Phosphocholination shows similarities to adenylylation, which consists of post-translational addition of adenosine monophosphate (AMP) onto amino acid side chains of host cell proteins (small GTPases), mediated by adenylyltransferases from the invasive pathogen.[8,9] We have demonstrated that peptides adenylylated at tyrosine, threonine,

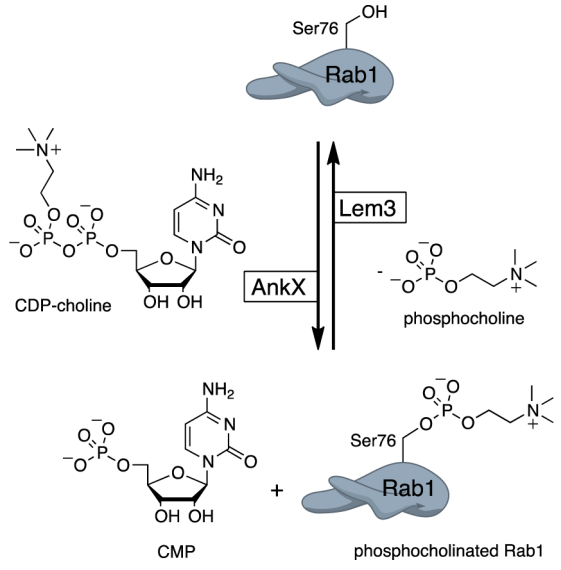


Figure 1. Covalent phosphocholination of Rab1 mediated by the phosphocholine transferase AnkX. The protein Lem3 reverts the modification by hydrolyzing the phosphodiester.

Received: November 27, 2012
Published: February 1, 2013

and serine could effectively and conveniently be synthesized relying on preformed adenylylated amino acid building blocks for Fmoc solid-phase peptide synthesis (SPPS).[10,11] Phosphocholination has only been reported on serine and threonine residues; however, we included tyrosine as well in our investigation, since phosphocholinated tyrosine is equally conceivable and might well be of biological relevance. The outlined building blocks (Figure 2) consist of the N-terminal

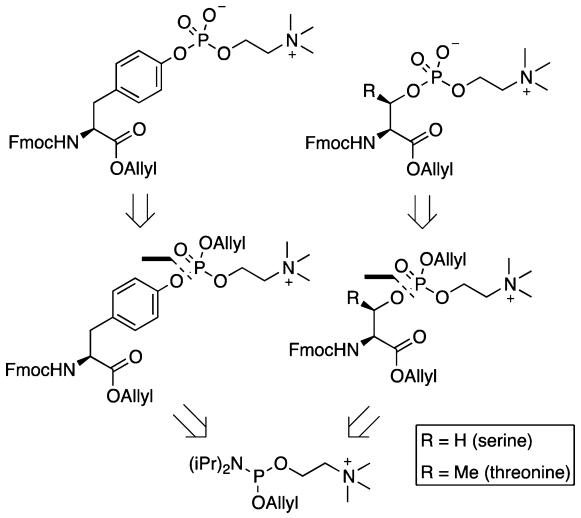


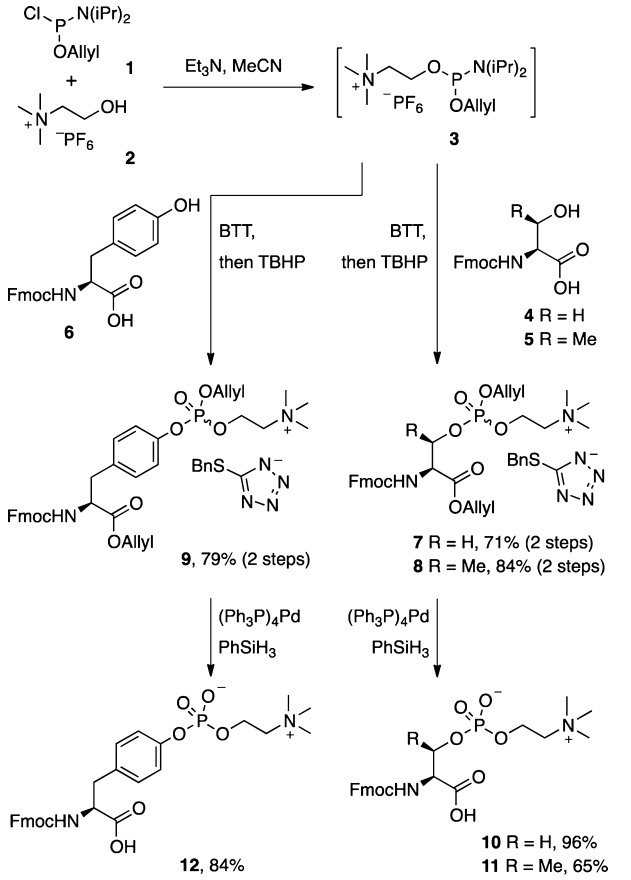
Figure 2. Retrosynthetic analysis of the target phosphocholine building blocks for Fmoc SPPS.

Fmoc protected amino acids onto which the choline moiety is attached via phosphodiesters in monoanionic form. In the case of serine and threonine, masking the phosphodiester with an additional protective group would abet β-elimination when exposed to the basic conditions in routine Fmoc SPPS. Thus, the use of the unprotected phosphodiester has been shown to suppress the formation of the β-elimination product, since the monoanionic form provides a much poorer leaving group.[11]

The phosphoimidate chemistry requires non-nucleophilic solvents, posing a challenge for the introduction of the cationic choline moiety, since the physicochemical properties of most choline salts allow only very limited solubility in suitable organic solvents. Schulz and co-workers reported the use of choline tosylate for the synthesis of phosphatidylcholine derivates according to the phosphoimidate protocol;[12] however, the tosylate interferes with the reactive phosphorus(III) species upon activation, thereby significantly lowering the yields. Upon investigating solubility of different choline salts with non-nucleophilic counterions in phosphoimidate-compatible solvents, we found that choline hexafluorophosphate shows excellent solubility in acetonitrile, which was chosen for further development.

As outlined in Scheme 1, the synthetic strategy commences with the reaction of 1-(allyloxy)-1-chloro-*N*,*N*-diisopropylphosphinamine[13] (**1**) with choline hexafluorophosphate (**2**) in acetonitrile. We found triethylamine to be best suited for this reaction, since subsequent removal of any excess of base in vacuo is important for the efficiency of the subsequent tetrazole coupling. N^{α}-Fmoc serine (**4**), threonine (**5**), and tyrosine (**6**), C-terminally allyl ester protected,[14] were immediately coupled to the highly unstable compound **3**, using 2 equiv of 5-(benzylthio)-1*H*-tetrazole (BTT) as activator.[15] In the case of the threonine, the coupling time had to be extended from 1 to 5

Scheme 1. Synthetic Approach to Phosphocholinated Amino Acid Building Blocks. BTT: 5-(benzylthio)-1*H*-tetrazole. TBHP: *tert*-butyl hydroperoxide.

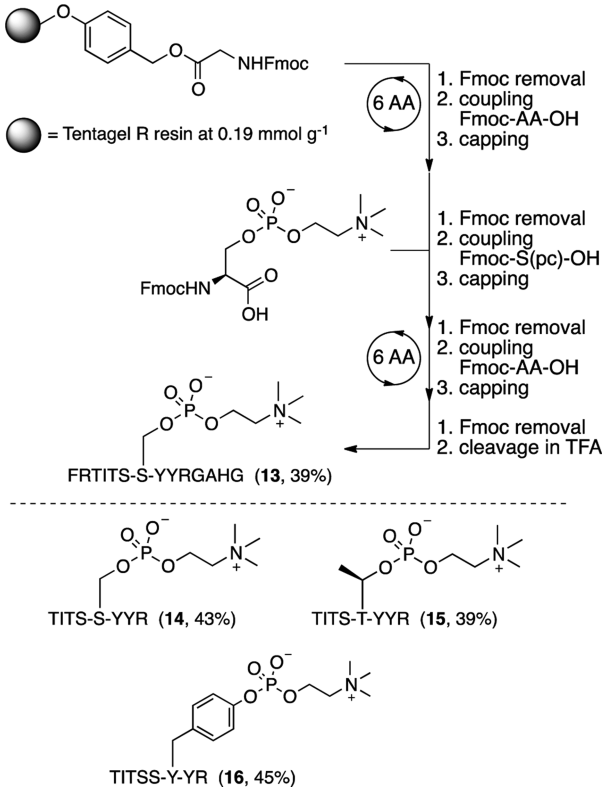


h, possibly due to steric hindrance. In situ oxidation with *tert*-butyl hydroperoxide (TBHP) yielded the corresponding phosphotriesters **7**–**9** in 1:1 diastereomeric mixtures at phosphorus. We applied reversed-phase (C18) cartridges for purification, eluting the products (water-to-acetonitrile gradient) in form of the mono-5-(benzylthio)-1*H*-tetrazolide salts. Next, the compounds were deallylated simultaneously at the carboxylic allyl ester and the allyl phosphotriester with 5 mol % tetrakis(triphenylphosphine)palladium as catalyst, phenylsilane as nucleophile, and 2,6-lutidine as mild base.[16] Purification of the final products **10**–**12** was carried out by reversed-phase (C18) Sep-Pak cartridge purification, which yielded material in sufficiently pure form for direct use in Fmoc SPPS. For **10**, main contaminations were triphenylphosphine oxide (6%) and 5-(benzylthio)-1*H*-tetrazole (25%), which do not interfere with the Fmoc SPPS. All three amino acids showed similar results; variations in yield were in an acceptable range (96–65%).

The synthesized building blocks were applied to Fmoc SPPS (Scheme 2). An automated peptide synthesizer was used to couple Fmoc amino acids (10 equiv), applying Tentagel PHB resin and standard HBTU/HOBt activation.[17−19] Phosphocholinated amino acid building blocks (**10**–**12**, 2 equiv) were coupled manually, using HATU/HOAt activation, followed by elongation of the sequence according to the automated protocol.[20] We choose to prepare the sequence FRTITS-S(pc)-YYRGAHG (**13**) and the corresponding tryptic fragment TITS-S(pc)-YYR (**14**) from the switch II region of the GTPases Rab1 and TITS-T(pc)-YYR (**15**) from Rab35 to

dx.doi.org/10.1021/jo302587g | J. Org. Chem. 2013, 78, 2715−2719

Scheme 2. Fmoc Solid-Phase Peptide Synthesis of Phosphocholinated Peptides with Building Blocks 10−12[a]



[a]Yields calculated on the basis of resin loading.

evaluate the synthetic strategy, since to date these are the only known sites of phosphocholination. So far, phosphocholination on a tyrosine residue has not been experimentally verified; however, for evaluation of our strategy, we modified the Rab1 sequence, yielding TITSS-Y(pc)-YR (**16**) as an representative example. Cleavage of the peptides from the resin and global deprotection was performed in trifluoroacetic acid/triisopropylsilane/water (90:5:5). Subsequently, concentration in vacuo, precipitation with diethyl ether, and purification by preparative reversed-phase (C18) HPLC yielded the phosphocholine−peptides in pure form. We did not observe β-elimination products of the peptides, nor any truncations due to insufficient coupling of building blocks **10-12**. The yields (39−45%) were comparable to the corresponding nonphosphocholine−peptides.

In conclusion, we demonstrated the first synthetic strategy for peptides bearing a phosphocholine modification at a serine, threonine, or tyrosine side chain. The building block approach grants the advantages of simplicity, efficiency, and flexibility, allowing incorporation of phosphocholine moieties in any desired position of a given peptide sequence.

EXPERIMENTAL SECTION

Choline Hexafluorophosphate (2). Choline chloride (10.17 g, 72.8 mmol) was dissolved in water (5 mL). A saturated solution of potassium hexafluorophosphate in water (14.75 g in 100 mL) was added dropwise to the well-stirred solution of choline chloride. Stirring was continued for 16 h. The mixture was cooled to 0 °C for 1 h and filtered, and the solid material was washed with ice-cold water followed by extensive drying (48 h) under oil pump vacuum. A white crystalline powder was obtained (9.37 g, 37.6 mmol, 52%). ^{1}H NMR (400 MHz, CD_3CN) δ: 3.93 (m, 2H), 3.38−3.32 (m, 3H), 3.09 (s, 9H). ^{13}C NMR (101 MHz, CD_3CN) δ: 68.5 (t, J = 3.0 Hz), 56.7, 54.8 (t, J = 4.0 Hz). ^{19}F NMR (377 MHz, CD_3CN) δ: −73.02 (d, J = 706.7 Hz). ^{31}P NMR (162 MHz, CD_3CN) δ: −143.46 (hept, J = 706.9 Hz). Mp: 252−254 °C.

***O*-Allyl-*N*$^{\alpha}$-Fmoc-L-serine-*O*-allylphosphorylcholine (7).** Choline hexafluorophosphate (1.00 g, 4.0 mmol) and freshly distilled triethylamine (2.14 mL, 16.1 mmol, 4 equiv) were dissolved in anhydrous acetonitrile (10 mL) under an argon atmosphere in a dry Schlenk tube. The solution was cooled to 0 °C, and (allyloxy)-(diisopropylamine)chlorophosphine (0.99 g, 4.4 mmol, 1.1 equiv) was added slowly over 10 min. During addition, a white precipitate formed. The reaction mixture was allowed to warm to rt. After 30 min at rt, the reaction mixture was filtered under argon atmosphere through a sintered glass frit, and the resulting filtrate was evaporated to dryness in a predried Schlenk tube. Subsequently, the mixture was coevaporated twice with dry toluene, yielding a sticky, white substance ((allyloxy)(choline)(diisopropylamine)phosphine (**3**), quantitative conversion based on ^{31}P NMR, residual impurity with triethylammonium chloride), which was immediately used for subsequent reaction steps. Note: This material is highly sensitive and must be handled under inert atmosphere. ^{1}H NMR (400 MHz, CD_3CN) δ: 6.05−5.90 (m, 1H), 5.38−5.11 (m, 2H), 4.29−4.10 (m, 2H), 4.07−3.95 (m, 2H), 3.70−3.57 (m, 2H), 3.57−3.48 (m, 2H), 3.15 (s, 9H), 1.23−1.14 (m, 12H). ^{31}P NMR (162 MHz, CD_3CN) δ: 149.61 (s), −143.42 (hept, J = 707.2 Hz, PF_6^-).

General Procedure for Phosphoimidate Coupling. Crude (allyloxy)(choline)(diisopropylamine)phosphine (**3**) (1.55 g, 3.55 mmol) was dissolved in anhydrous MeCN (15 mL) under an argon atmosphere. *N*-Fmoc-L-serine allyl ester (**4**) (1.18 g, 3.20 mmol, 0.9 equiv) was added, followed by addition of 5-(benzylthio)-1*H*-tetrazole (1.37 g, 7.10 mmol, 2 equiv). After 1 h, TLC analysis (ethyl acetate/cyclohexane 1:1) showed complete conversion of the amino acid ester, and the reaction mixture was cooled to 0 °C. *tert*-Butyl hydroperoxide (TBHP, 5.5 M solution in decane, 1.29 mL, 7.10 mmol, 2 equiv) was added dropwise over 10 min. The reaction mixture was allowed to warm to rt and subsequently stirred for 1 h. Afterward, the reaction mixture was evaporated to dryness and purified by applying a reversed-phase (C18) cartridge (10 g column material per gram crude reaction mixture). A gradient from 100% water to 50% MeCN in water was used. Elution fractions containing product were combined and lyophilized, yielding the product in the form of the 5-(benzylthio)-1*H*-tetrazolide salt (1.93 g, 2.53 mmol, 71%) as a 1:1 diastereomeric mixture at phosphorus. For characterization, an analytical amount was purified by preparative reversed-phase HPLC (Supporting Information, program 1), yielding the pure product in form of the trifluoroacetic acid salt. $[\alpha]_D^{20}$ = −5.4 (c = 1.0 CH_3CN). ^{1}H NMR (400 MHz, CD_3CN) δ: 7.84 (d, J = 7.5 Hz, 2H), 7.72−7.66 (m, 2H), 7.43 (t, J = 7.4 Hz, 2H), 7.38−7.31 (m, 2H), 6.91−6.84 (m, 1H), 6.02−5.87 (m, 2H), 5.42−5.20 (m, 4H), 4.65 (d, J = 5.0 Hz, 2H), 4.61−4.50 (m, 3H), 4.48−4.28 (m, 6H), 4.25 (t, J = 6.9 Hz, 1H), 3.67−3.60 (m, 2H), 3.12 (s, 9H). ^{13}C NMR (151 MHz, CD_3CN) δ: 169.8, 169.7, 157.1, 145.0, 144.9, 142.0, 133.4, 132.9, 128.7, 128.1, 126.19, 126.15, 120.9, 119.0, 118.7, 69.6, 69.6, 68.0, 67.99, 67.96, 67.93, 67.6, 66.9, 66.3, 62.1, 55.4, 54.69, 54.66, 54.64, 47.8. ^{31}P NMR (162 MHz, CD_3CN) δ: −1.24, −1.29. HRMS (ESI-Orbitrap) m/z: $[M]^+$ calcd for $C_{29}H_{38}N_2O_8P$ 573.23603, found 573.23558.

***O*-Allyl-*N*$^{\alpha}$-Fmoc-L-threonine-*O*-allylphosphorylcholine (8).** Prepared as above, except for 5-h reaction time. Yield as the 5-(benzylthio)-1*H*-tetrazolide salt 84% (1.04 g, 1.34 mmol), as 1:1 diastereomeric mixture at phosphorus. For characterization an analytical amount was purified by preparative reversed-phase HPLC (Supporting Information, program 1), yielding the pure product in form of the trifluoroacetic acid salt. $[\alpha]_D^{20}$ = −1.2 (c = 1.0 CH_3CN). ^{1}H NMR (400 MHz, CD_3CN) δ: 7.84 (d, J = 7.5 Hz, 2H), 7.74−7.66 (m, 2H), 7.43 (t, J = 7.4 Hz, 2H), 7.34 (t, J = 7.4 Hz, 2H), 6.61−6.41 (m, 1H), 6.04−5.88 (m, 2H), 5.43−5.21 (m, 4H), 5.06−4.95 (m, 1H), 4.72−4.58 (m, 2H), 4.57−4.44 (m, 3H), 4.43−4.34 (m, 4H), 4.26 (t, J = 7.0 Hz, 1H), 3.65 (s, 2H), 3.14 (s, 10H), 1.38 (t, J = 6.5 Hz, 2H). ^{13}C NMR (101 MHz, CD_3CN) δ: 170.5, 170.2, 157.6, 145.0, 144.9, 142.1, 133.63, 133.56, 133.03, 132.98, 128.7, 128.1, 126.2, 121.0,

dx.doi.org/10.1021/jo302587g | J. Org. Chem. 2013, 78, 2715−2719

118.9, 118.8, 69.58, 69.55, 69.52, 69.49, 67.6, 67.0, 66.4, 62.0, 59.4, 54.8, 54.8, 54.7, 48.0, 18.7. ^{31}P NMR (162 MHz, CD_3CN) δ: −1.74, −1.88. HRMS (ESI-Orbitrap) m/z: $[M]^+$ calcd for $C_{30}H_{40}N_2O_8P$ 587.25168, found 587.25112.

***O*-Allyl-*N*$^{\alpha}$-Fmoc-L-tyrosine-*O*-allyl-phosphorylcholine (9).** Prepared as above, except for 2 h reaction time. Yield as the 5-(benzylthio)-1*H*-tetrazolide salt 79% (1.99 g, 2.36 mmol), as a 1:1 diastereomeric mixture at phosphorus. For characterization an analytical amount was purified by preparative reversed-phase HPLC (Supporting Information, program 1), yielding the pure product in the form of the trifluoroacetic acid salt. $[\alpha]_D^{20}$ = −12.7 (c = 1.0 CH_3CN). ^{1}H NMR (400 MHz, CD_3CN) δ: 7.83 (d, J = 7.5 Hz, 2H), 7.62 (d, J = 7.5 Hz, 2H), 7.42 (t, J = 7.5 Hz, 2H), 7.36−7.30 (m, 2H), 7.27 (m, 2H), 7.15 (m, 2H), 6.29−6.22 (m, 1H), 6.03−5.84 (m, 2H), 5.42−5.20 (m, 4H), 4.71−4.56 (m, 4H), 4.54−4.48 (m, 2H), 4.48−4.39 (m, 1H), 4.27 (d, J = 7.0 Hz, 2H), 4.18 (t, J = 6.9 Hz, 1H), 3.65−3.59 (m, 2H), 3.20−3.13 (m, 1H), 3.07 (s, 9H), 3.01−2.94 (m, 1H). ^{13}C NMR (101 MHz, CD_3CN) δ: 172.2, 156.9, 150.2, 145.0, 142.1, 135.7, 133.31, 133.25, 133.1, 131.8, 128.7, 128.1, 126.2, 121.0, 120.9, 119.3, 118.6, 70.24, 70.18, 67.3, 66.4, 62.6, 62.5, 56.5, 54.80, 54.76, 54.7, 47.9, 37.3. ^{31}P NMR (162 MHz, CD_3CN) δ: −5.84, −5.86. HRMS (ESI-Orbitrap) m/z: $[M]^+$ calcd for $C_{35}H_{42}N_2O_8P$ 649.26733, found 649.26691.

***N*$^{\alpha}$-Fmoc-L-serine-*O*-phosphorylcholine (10): General Procedure for Deallylation Reaction.** *O*-Allyl-*N*$^{\alpha}$-Fmoc-L-serine-*O*-allylphosphorylcholine 5-(benzylthio)-1*H*-tetrazolide (**7**) (1.93 g, 2.52 mmol) was dissolved in THF (15 mL) under an argon atmosphere and cooled to 0 °C. Lutidine (0.59 mL, 5.05 mmol, 2 equiv) was added together with phenylsilane (0.93 mL, 7.57 mmol, 3 equiv). A solution of (tetrakis)(triphenylphosphine)palladium (146 mg, 126 μmol, 0.05 equiv) in THF (2 mL) was added to the reaction mixture. After addition, the reaction was allowed to warm to rt and stirred for 3 h in the dark. Afterward, the reaction mixture was evaporated to dryness, coevaporated with toluene twice, dissolved in water/MeCN, and lyophilized, yielding the crude product. Reversed-phased (C18) cartridge purification (100% water to 40% MeCN) and subsequent lyophilization yielded the product in sufficient purity (impurities: 25% 5-(benzylthio)-1*H*-tetrazole and 6% triphenylphosphine oxide, based on ^{1}H NMR) for Fmoc SPPS (1.8 g, 2.44 mmol, 96%). The compound can be stored at least several months at −20 °C prior use. For characterization, a small amount was purified by preparative reversed-phase HPLC (program 1), yielding the analytical pure product in form of the trifluoroacetic acid salt. $[\alpha]_D^{20}$ = −4.7 (c = 1.0 DMF). ^{1}H NMR (500 MHz, DMF-d_7) δ: 8.13 (d, J = 7.8 Hz, 1H), 7.92 (d, J = 7.5 Hz, 2H), 7.84−7.78 (m, 2H), 7.44 (t, J = 7.4 Hz, 2H), 7.40−7.34 (m, 2H), 4.51−4.42 (m, 3H), 4.40−4.24 (m, 5H), 3.87 (s, 2H), 3.38 (s, 9H). ^{13}C NMR (126 MHz, DMF-d_7) δ: 172.6, 157.5, 145.34, 145.28, 142.2, 128.8, 128.3, 126.6, 121.1, 67.6, 67.0, 66.6, 60.7, 56.5, 54.6, 48.1. ^{31}P NMR (121 MHz, DMF-d_7) δ: −0.77. HRMS (ESI-Orbitrap) m/z: $[M + H]^+$ calcd for $C_{23}H_{30}N_2O_8P$ 493.17343, found 493.17314.

***N*$^{\alpha}$-Fmoc-L-threonine-*O*-phosphorylcholine (11).** As above from **8** (0.97 g, 1.25 mmol), 65% (0.79 g, 0.80 mmol). Impurities: 29% 5-(benzylthio)-1*H*-tetrazole and 19% triphenylphosphine oxide, based on ^{1}H NMR. Storable at −20 °C. For characterization a small amount was purified by preparative reversed-phase HPLC (program 1), yielding the pure product in form of the trifluoroacetic acid salt. $[\alpha]_D^{20}$ = 15.2 (c = 1.0 DMF). ^{1}H NMR (400 MHz, DMF-d_7) δ: 7.92 (d, J = 7.5 Hz, 2H), 7.84 (t, J = 7.3 Hz, 2H), 7.72 (m, 1H), 7.44 (t, J = 7.4 Hz, 2H), 7.36 (t, J = 7.4 Hz, 2H), 4.87 (s, 1H), 4.44−4.34 (m, 3H), 4.33−4.25 (m, 3H), 3.92−3.84 (m, 2H), 3.40 (s, 9H), 1.40 (d, J = 6.0 Hz, 3H). ^{13}C NMR (101 MHz, DMF-d_7) δ: 173.0, 158.0, 145.4, 145.3, 142.2, 128.8, 128.3, 126.7, 121.1, 73.2, 67.7, 67.0, 60.6, 54.6, 48.2, 19.5. ^{31}P NMR (121 MHz, DMF-d_7) δ: −1.71. HRMS (ESI-Orbitrap) m/z: $[M + H]^+$ calcd for $C_{24}H_{32}N_2O_8P$ 507.18908, found 507.18845.

***N*$^{\alpha}$-Fmoc-L-tyrosine-*O*-phosphorylcholine (12).** As above from **9** (1.84 g, 2.23 mmol), 84% (1.49 g, 1.85 mmol). Impurities: 16% 5-(benzylthio)-1*H*-tetrazole and 15% triphenylphosphine oxide, based on ^{1}H NMR. Storable at −20 °C. For characterization a small amount was purified by preparative reversed-phase HPLC (program 1), yielding the pure product in form of the trifluoroacetic acid salt. $[\alpha]_D^{20}$ = −2.1 (c = 1.0 DMF). ^{1}H NMR (500 MHz, DMF-d_7) δ: 7.96−7.88 (m, 2H), 7.79−7.73 (m, 2H), 7.72−7.65 (m, 1H), 7.48−7.40 (m, 2H), 7.40−7.21 (m, 6H), 4.48−4.36 (m, 3H), 4.31−4.21 (m, 3H), 3.82−3.73 (m, 2H), 3.26 (s, 9H), 3.18 (m, 1H), 3.08−2.98 (m, 1H). ^{13}C NMR (126 MHz, DMF-d_7) δ: 174.4, 157.4, 153.3, 145.3, 145.2, 142.1, 133.5, 131.1, 128.8, 128.2, 126.6, 121.1, 67.3, 66.8, 60.7, 57.3, 54.3, 48.1, 37.6. ^{31}P NMR (121 MHz, DMF-d_7) δ: −5.98. HRMS (ESI-Orbitrap) m/z: $[M + H]^+$ calcd for $C_{29}H_{34}N_2O_8P$ 569.20473, found 569.20460.

Fmoc Solid-Phase Peptide Synthesis (Fmoc SPPS). An automated peptide synthesizer was used for synthesis of the peptides. Tentagel PHB resin was used as solid phase. Each coupling step was employed with 10 equiv of amino acid and 10 equiv of HBTU. The phosphocholine building blocks were coupled manually in the minimum amount of solvent using following procedure: The preswollen resin (DCM) was deprotected with 20% piperidine in DMF (3 × 10 min). After the resin was washed with DMF 3 times, 2 equiv of phosphocholine-building block with 2 equiv of HBTU, 2 equiv of HOAt, and 6 equiv of DIPEA in DMF were used for coupling. The phosphocholine-building blocks were coevaporated with DMF prior use to remove traces of water after lyophilization. The mixture was shaken for 3 h at rt and subsequently washed 5 times with DMF. After the peptide sequence was finished, the resin was washed with DMF, methanol, diethyl ether, and DCM (5 times each). For cleavage and global deprotection a cleavage mixture, consisting of 5% water and 5% TIPS-H in TFA, was added. Cleavage was carried out twice for 1 h at rt. After being filtered from the resin, the cleavage solution was evaporated to dryness and coevaporated with toluene twice. The remaining solid was triturated with diethyl ether and subsequently dissolved in water/MeCN. After lyophilization, the crude peptide was dissolved again in water/MeCN and purified by preparative HPLC. Peptides: FRTITS-S(pc)-YYRGAHG (**13**) Synthesis by Fmoc-based SPPS on Tentagel R-PHB-Gly resin at 0.08 mmol scale. The pure peptide was obtained by preparative HPLC using program 2 (56 mg, 31 μmol, 39% based on resin load). HRMS (ESI-Orbitrap) m/z: $[M + H]^{2+}$ calcd for $C_{77}H_{120}N_{23}O_{24}P$ 890.93016, found 890.93039. ^{31}P NMR (D_2O/CD_3CN 7:3, 162 MHz) δ: 0.34. TITS-S(pc)-YYR (**14**) Synthesis by Fmoc-based SPPS on Tentagel R-PHB-Arg(Pbf) resin at 0.05 mmol scale. The pure peptide was obtained by preparative HPLC using program 2 (25 mg, 21.6 μmol, 43% based on resin load). HRMS (ESI-Orbitrap) m/z: $[M + H]^{2+}$ calcd for $C_{49}H_{81}N_{12}O_{18}P$ 578.27592, found 578.27640. ^{31}P NMR (D_2O/CD_3CN 7:3, 162 MHz) δ: 0.33. TITS-T(pc)-YYR (**15**) Synthesis by Fmoc-based SPPS on Tentagel R-PHB-Arg(Pbf) resin at 0.05 mmol scale. The pure peptide was obtained by preparative HPLC using program 2 (23 mg, 19.5 μmol, 39% based on resin load). HRMS (ESI-Orbitrap) m/z: $[M + H]^{2+}$ calcd for $C_{50}H_{83}N_{12}O_{18}P$ 585.28375, found 585.28461. ^{31}P NMR (D_2O/CD_3CN 7:3, 162 MHz) δ: −0.66. TITSS-Y(pc)-YR (**16**). Synthesis by Fmoc-based SPPS on Tentagel R-PHB-Arg(Pbf) resin at 0.05 mmol scale. The pure peptide was obtained by preparative HPLC using program 2 (26 mg, 22.5 μmol, 45% based on resin load). HRMS (ESI-Orbitrap) m/z: $[M + H]^{2+}$ Calcd for $C_{49}H_{81}N_{12}O_{18}P$ 578.27592, found 578.27663. ^{31}P NMR (D_2O/CD_3CN 7:3, 162 MHz) δ: −4.20.

■ ASSOCIATED CONTENT

Supporting Information

^{1}H, ^{31}P, ^{13}C NMR, and LC−MS spectra for all new compounds (**2**,**3**, **7**−**12**) and ^{31}P NMR, HPLC traces, and LC−MS spectra for the peptides **13**−**16**. 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 acknowledge Prof. Dr. Herbert Waldmann (Max-Planck Inst. Dortmund) and Prof. Dr. Roger S. Goody (Max-Planck Inst. Dortmund) for their unconditional support. We thank the DFG for support through Grant No. SPP1623. M.F.A. was supported through the Max-Planck International Research School (IMPRS) by a graduate fellowship. We thank Mr. Sascha Gentz (Max-Planck Inst. Dortmund) for operating the automated peptide synthesizer.

■ REFERENCES

(1) Isberg, R. R.; O'Connor, T. J.; Heidtman, M. *Nature Rev. Microbiol.* **2009**, *7*, 13−24.
(2) Itzen, A.; Goody, R. S. *Semin. Cell Dev. Biol.* **2011**, *22*, 48−56.
(3) Ingmundson, A.; Delprato, A.; Lambright, D. G.; Roy, C. R. *Nature* **2007**, *450*, 365−369.
(4) Machner, M. P.; Isberg, R. R. *Dev. Cell* **2006**, *11*, 47−56.
(5) Mukherjee, S.; Liu, X.; Arasaki, K.; McDonough, J.; Galan, J. E.; Roy, C. R. *Nature* **2011**, *477*, 103−106.
(6) Goody, P. R.; Heller, K.; Oesterlin, L. K.; Muller, M. P.; Itzen, A.; Goody, R. S. *EMBO J.* **2012**, *31*, 1774−1784.
(7) Tan, Y.; Arnold, R. J.; Luo, Z. Q. *Proc. Natl. Acad. Sci. U.S.A.* **2011**, *108*, 21212−21217.
(8) Yarbrough, M. L.; Li, Y.; Kinch, L. N.; Grishin, N. V.; Ball, H. L.; Orth, K. *Science* **2009**, *323*, 269−272.
(9) Muller, M. P.; Peters, H.; Blumer, J.; Blankenfeldt, W.; Goody, R. S.; Itzen, A. *Science* **2010**, *329*, 946−949.
(10) Smit, C.; Blumer, J.; Eerland, M. F.; Albers, M. F.; Muller, M. P.; Goody, R. S.; Itzen, A.; Hedberg, C. *Angew. Chem., Int. Ed.* **2011**, *50*, 9200−9204.
(11) Albers, M. F.; van Vliet, B.; Hedberg, C. *Org. Lett.* **2011**, *13*, 6014−6017.
(12) Wichmann, O.; Schultz, C. *Chem. Commun.* **2001**, *23*, 2500−2501.
(13) Wippo, H.; Reck, F.; Kudick, R.; Ramaseshan, M.; Ceulemans, G.; Bolli, M.; Krishnamurthy, R.; Eschenmoser, A. *Bioorg. Med. Chem.* **2001**, *9*, 2411−2428.
(14) Ficht, S.; Payne, R. J.; Guy, R. T.; Wong, C. H. *Chem.—Eur. J.* **2008**, *14*, 3620−3629.
(15) Pitsch, S.; Weiss, P. A.; Jenny, L.; Stutz, A.; Wu, X. L. *Helv. Chim. Acta* **2001**, *84*, 3773−3795.
(16) Dessolin, M.; Guillerez, M. G.; Thieriet, N.; Guibe, F.; Loffet, A. *Tetrahedron Lett.* **1995**, *36*, 5741−5744.
(17) Dourtoglou, V.; Gross, B.; Lambropoulou, V.; Zioudrou, C. *Synthesis* **1984**, 572−574.
(18) Tentagel PHB resin was aquired from Rapp Polymere GmbH, www.rapp-polymere.com.
(19) Automated peptide synthesis was carried out on an Applied Biosystems peptide synthesizer.
(20) Carpino, L. A. *J. Am. Chem. Soc.* **1993**, *115*, 4397−4398.