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JOURNAL OF THE AMERICAN CHEMICAL SOCIETY
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Site-Specifically Phosphorylated Lysine Peptides

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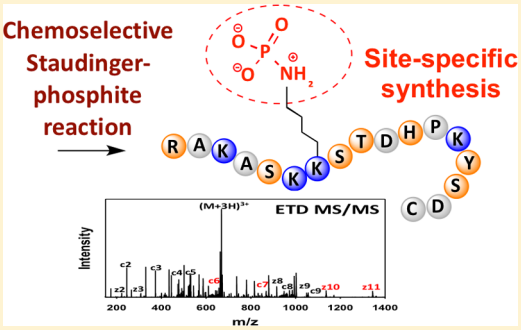
Supporting Information

ABSTRACT: Protein phosphorylation controls major processes in cells. Although phosphorylation of serine, threonine, and tyrosine and also recently histidine and arginine are well-established, the extent and biological significance of lysine phosphorylation has remained elusive. Research in this area has been particularly limited by the inaccessibility of peptides and proteins that are phosphorylated at specific lysine residues, which are incompatible with solid-phase peptide synthesis (SPPS) due to the intrinsic acid lability of the P(=O)−N phosphoramidate bond. To address this issue, we have developed a new synthetic route for the synthesis of site-specifically phospholysine (pLys)-containing peptides by employing the chemoselectivity of the Staudinger-phosphite reaction. Our synthetic approach relies on the SPPS of unprotected ε-azido lysine-containing peptides and their subsequent reaction to phosphoramidates with phosphite esters before they are converted into the natural modification via UV irradiation or basic deprotection. With these peptides in hand, we demonstrate that electron-transfer dissociation tandem mass spectrometry can be used for unambiguous assignment of phosphorylated-lysine residues within histone peptides and that these peptides can be detected in cell lysates using a bottom-up proteomic approach. This new tagging method is expected to be an essential tool for evaluating the biological relevance of lysine phosphorylation.



INTRODUCTION

Reversible protein phosphorylation of specific amino acid side chains is a key regulatory mark in most cellular processes.[1] The roles of this post-translational modification (PTM) have been most widely studied in the context of serine, threonine, and tyrosine, for which many synthetic, biochemical, and proteomic tools are available to probe the biological significance.[2−5] In addition to those, phosphorylation of N-containing amino acids, e.g., Arg, His, and Lys, is also known to occur in nature, but the acid lability of the P(=O)−N bond has prevented this modification from being studied extensively. Nevertheless, recent studies have indicated the potential importance of phosphohistidine[6−8] and phosphoarginine[9,10] in different biological signaling processes, pointing toward the necessity to focus on the least studied modification from this group, i.e., phospholysine (pLys).[11]

To date, the biological role of pLys still remains unclear. More than three decades ago, Smith et al. reported preliminary evidence of Lys-phosphorylation by looking at nuclear extracts from Walker-256 cells and regenerating rat liver.[12] Those contained acid-labile phosphates when they were supplemented with Histone H1 and γ-^{32}P-ATP. The putative kinase was isolated from the same nuclear extracts and assayed *in vitro* against Histone H1 showing phosphorylation on lysine residues after analyzing the protein digests by ^{32}P labeling or paper chromatography.[13−15] The detection of a potential lysine kinase raises the question of the existence of the complementary protein lysine phosphatase. Accordingly, Hiraishi et al. reported the isolation of a phosphatase capable of hydrolyzing P(=O)−N bonds with high specificity toward pLys residues.[16] Although these biochemical studies point toward the existence and importance of Lys-phosphorylation, the analytical techniques employed in those studies could not provide more detailed information such as the detection of the particular phosphorylation site or the analysis of specific binding partners. In pursuing this aim, it is therefore essential to develop enhanced phosphoproteomic tools that are well-suited for detection and identification of labile Lys-phosphorylation sites in proteins. The chemical properties of pLys, having a high-energy alkyl-phosphoramidate bond, might suggest a role in phosphoryl transfer reactions to other more stable entities.[11] Whether this is being exploited in nature in signaling networks, as it is in the case of pHis,[6,17] remains unknown.

Synthetic peptides are important probes for the study of PTMs.[18] Consequently, the accessibility of naturally modified peptides is a key requirement. However, the lability of certain post-translational modifications imposes a synthetic challenge and is often incompatible with standard solid-phase peptide synthesis (SPPS). Since P(=O)−N bonds in pArg, pHis, and

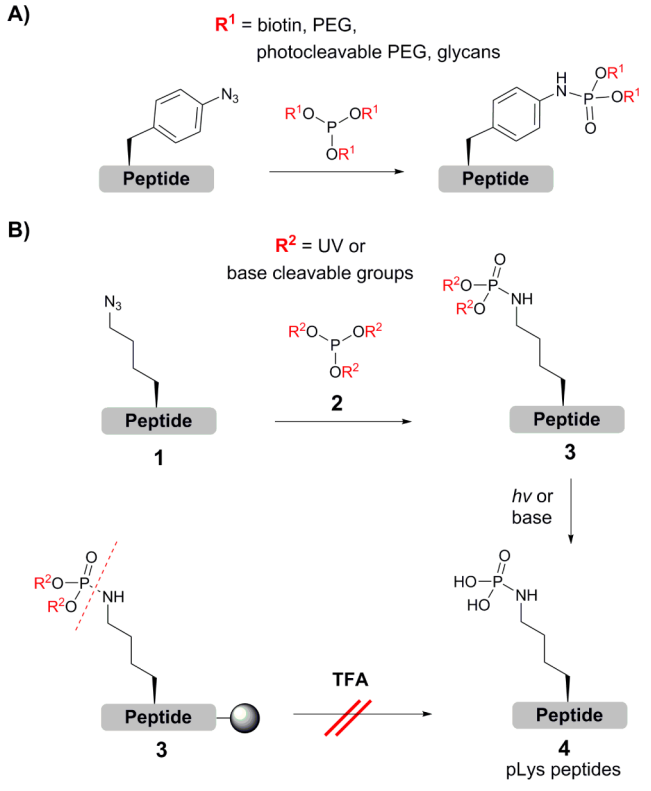
Received: January 20, 2014
Published: September 7, 2014

   dx.doi.org/10.1021/ja507886s | J. Am. Chem. Soc. 2014, 136, 13622−13628

pLys are unstable under acidic conditions, adjustments to the standard SPPS protocol are sought-after. Recently, pArg peptides were prepared by SPPS with a trichloroethyl-protected pArg building block, which increased the stability of the P(=O)−N bond under the acidic deprotection conditions required for SPPS.[19] In another example labile pyrophosphopeptides were prepared by reaction of a pSer peptides with a P(V) phosphorimidazolide reagent in solution.[20] To date the only known synthesis of phosphorylated-lysine polypeptides relies on the use of potassium phosphoramidate as a phosphorylation reagent.[21] This approach, however, does not only lead to the phosphorylation of all (accessible) lysine residues but also to the modification of other N-side chains like histidine, arginine, or the N-terminus. In addition, the already mentioned acid lability of the P(=O)−N bond has hampered its synthesis by standard SPPS using phospholysine as a building block due to the required final acidic deprotection.

Considering the lack of a synthetic route to access site-specifically phosphorylated Lys peptides, we now present an approach for their synthesis based on the Staudinger-phosphite reaction and their subsequent characterization by tandem mass spectrometry (MS/MS). Most importantly, our synthesis takes advantage of the chemoselectivity of the Staudinger reaction in solution, which is performed after the acidic deprotection of amino acid side chains and the release of peptides from the resin. Thereby, unprotected ε-azido lysine-containing peptides **1**, which are easily accessed by standard SPPS using *N*-Fmoc-6-azidonorleucine as a building block, can be reacted with a UV- or base-cleavable phosphite triester **2**, which delivers after uncaging or alkaline deprotection of the phosphoramidate **3** the corresponding pLys peptide **4** (Scheme 1B). In the recent past, our group has employed the Staudinger-phosphite reaction for the site-specific peptide and protein PEGylation, glycosylation, and biotinylation or the incorporation of a photocleavable aryl-phosphoramidate to render pTyr analogues, which already demonstrated the chemoselectivity of the reaction itself (Scheme 1A).[22a−e] Here, the reaction is applied in a similar fashion but using an aliphatic azidonorleucine instead of the previously applied aryl-azide moiety. Inspired by the outstanding scope of the earlier reported reaction, we decided to inspect the performance of the less reactive aliphatic azides in the Staudinger-phosphite reaction to form the corresponding phosphoramidates peptides **3**.

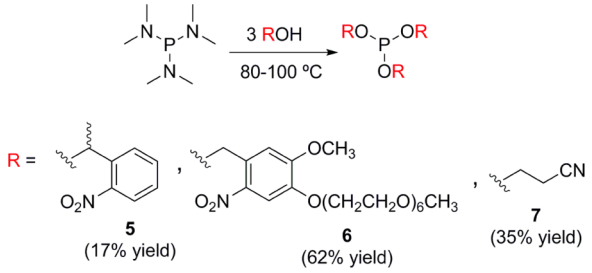
Scheme 1. Staudinger-Phosphite Reaction (A) of Aryl Azido-Containing Peptides and (B) on ε-azido Lysine-Containing Peptides



RESULTS AND DISCUSSION

Phosphite Synthesis. An important requirement for the efficient synthesis of the phosphorylated peptide **4** is an appropriate choice of deprotection conditions for the phosphoramidate ester **3** under which the P(=O)−N bond is not cleaved (Scheme 1B). Consequently, the phosphites employed for the Staudinger-phosphite reaction should already include cleavable groups that meet these requirements. Previously, our group and others have used 2-nitrobenzyl esters-type caged groups to access free phosphoramidates or phosphomonoesters by mild light-induced photolysis.[22a,d,23a,b] The presence of this masking group provides a photoactivatable approach, allowing spatial and temporal control over the release of the active modification. In this aspect, a 2-nitrophenyl ethyl (NPE) caging group is more suitable than 2-nitrobenzyl benzyl (NB), because the photolysis byproduct nitrosoacetophenone is less reactive than the corresponding nitrosobenzaldehyde.[23a,b] With this consideration in mind we synthesized two different photocleavable phosphites, **5** and **6** (Scheme 2).

Scheme 2. Phosphite Synthesis



Those phosphites were accessible by the condensation of hexamethylphosphorous triamide and the corresponding alcohol.[24] The stability of the water-soluble PEG-photocleavable phosphite **6** in aqueous media allowed us to perform the reaction in aqueous buffers at physiological pH.[22a] Additionally, we synthesized the base cleavable tris(2-cyanoethyl) phosphite **7** (Scheme 2) to check whether basic conditions release the free phosphoramidate but also whether the P(=O)−N bond remains stable in the process.

Synthesis of Phosporamidate Lysine-Containing Peptides. At the outset of our studies we checked whether solid-supported phosphoramidate esters **3** survive acidic deprotection conditions necessary for standard SPPS. As a model peptide we chose a short peptide sequence (**8**) from Histone H1.4 (K23ARKSAGA30, Figure 1A), a linker Histone previously reported to harbor phosphorylated lysines.[11] The model peptide was synthesized via standard Fmoc-based SPPS on a Rink Amide resin and contained 4-chloro-7-nitrobenzofurazan

dx.doi.org/10.1021/ja507886s | J. Am. Chem. Soc. 2014, 136, 13622−13628

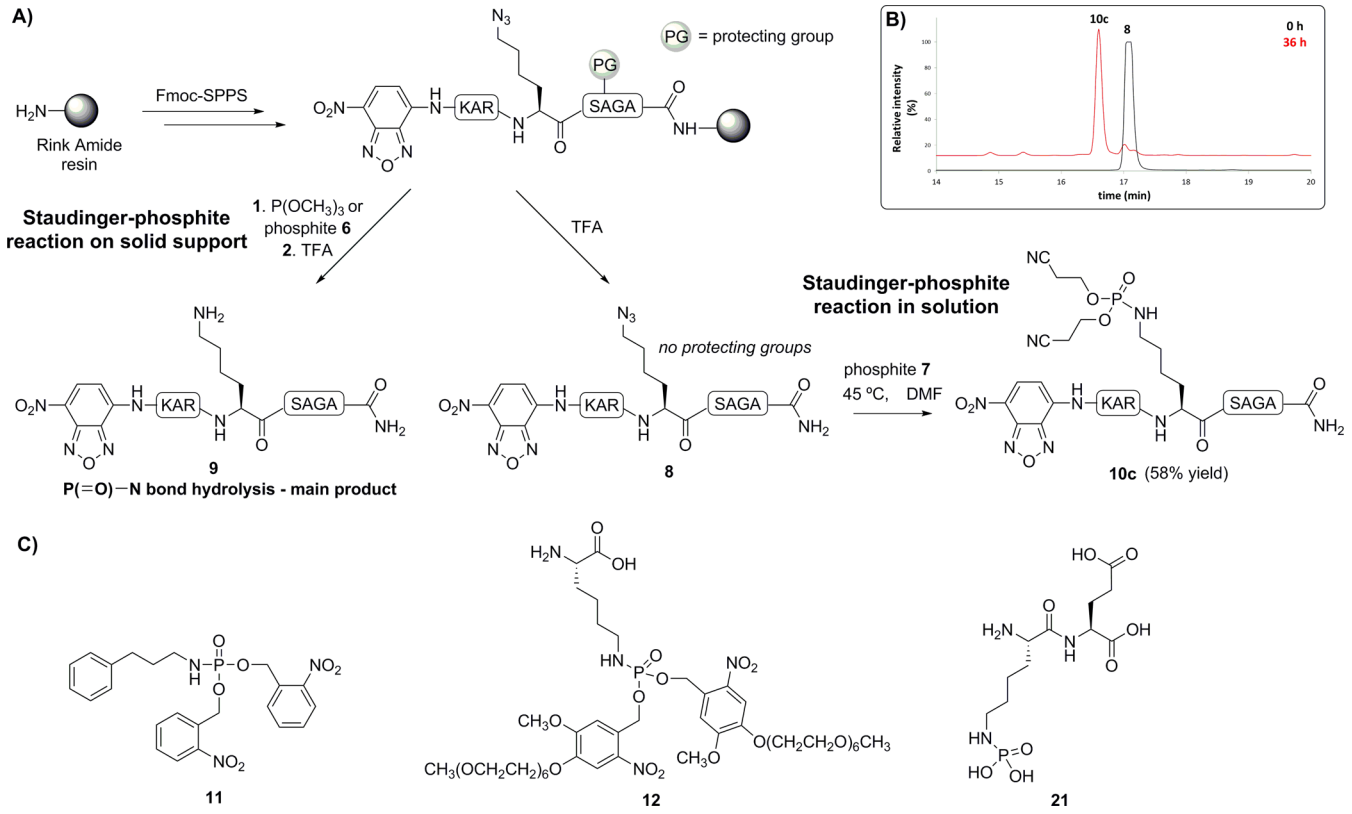


Figure 1. Staudinger-phosphite reactions with ε-azido lysine peptides. (A) Staudinger-phosphite reactions on the solid support and in solution. (B) HPLC-FLD of the reaction mixture of peptide **8** and phosphite **7** at t = 0 h (black) and after 36 h (red). (C) Phosphoramidate esters (**11** and **12**) and phosphorylated dipeptide **21**.

(NBD) at the N-terminus to monitor the Staudinger-phosphite reaction products by fluorescence detection (FLD). *N*-Fmoc-6-azidonorleucine was incorporated as a building block and placed four residues from the N-terminus. After reaction with commercially available trimethyl phosphite and phosphite **6** (Scheme S8), the peptides were cleaved from the resin with 95% trifluoroacetic acid (TFA), 2.5% triisopropylsilane (TIS), and 2.5% thioanisole (TA). The cleaved peptides were then analyzed by HPLC-UV-MS and HPLC-FLD (Figures S10 and S11). Amine formation (**9**) was observed as the main product (Table S2) in both cases, which indicated hydrolysis of the P(═O)−N bond and confirmed that pLys peptides are not accessible by reaction with protected solid-supported peptides.

Thus, we decide to study the stability of the phosphoramidate ester bond under different conditions with two different substrates (**11** and **12**) (Figure 1C, Schemes S3 and S4). As observed previously, the P(═O)−N bond does not survive harsh acidic conditions (Figure S1, Table S1); however the phosphoramidate bond is highly stable at 0.1% TFA concentration (Figure S1), a widely used condition to analyze and purify peptides by HPLC.

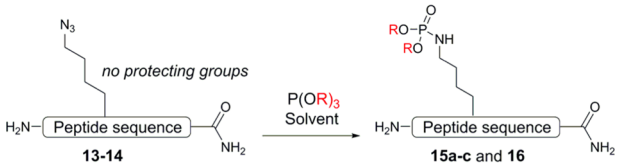
As mentioned before, the Staudinger-phosphite reaction is a chemoselective reaction and therefore compatible with unprotected peptide side chains.[22d] Therefore, we aimed for an approach in solution to generate phosphoramidate-containing peptides, for which the same ε-azido lysine-containing H1.4 peptide was cleaved from the resin and purified by semipreparative HPLC (Figure 1A, Scheme S5). First, we tested the reactivity of peptide **8** with phosphite **6** using Tris-HCl buffer (pH 8.2) as a solvent (Scheme S9). As mentioned before, we expected lower reactivity of the aliphatic azides relative to the previous reported aryl azides in the Staudinger-phosphite reaction.[22d] At micromolar concentrations, using a similar phosphite we observed that after 24 h and at room temperature, only 19% conversion to phosphoramidate **10b** was formed (Figure S12). This is in contrast to aryl azides, which proceed in almost quantitative conversion to aryl phosphoramidate in less than 8 h.[22d] Therefore, we probed the reactivity of peptide **8** at milimolar concentrations and at higher temperature (45 °C) (Figure S15). After 24 h peptide **8** was nearly quantitatively transformed into phosphoramidate **10b** using 50 equiv of phosphite **6** (Figure S15). Note that using dimethylformamide (DMF) as a solvent the same trend was observed (data not shown). In accordance with these data, the unprotected peptide **8** was reacted in DMF with phosphite **7** (Figure 1A, Scheme S10) and the formation of the phosphoramidate Lys peptide **10c** was monitored by HPLC-UV-MS and HPLC-FLD (Figure 1B). After 36 h full conversion was achieved, and peptide **10c** was isolated by semiprepartive HPLC in good yield (Figure 1A, Table S3). As shown in the HPLC-FLD trace (Figure 1B) the desired phosphoramidate was the major product, and no amine (**9**) or other peptidic compounds were observed. This result supports our strategy to generate site-specifically phosphoramidate-Lys-residues on peptides by the Staudinger-phosphite reaction utilizing simple and easily accessible starting materials.

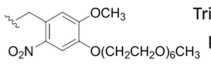
Encouraged by these results, we aimed to synthesize a larger peptide sequence containing more diverse functionalities and specifically other basic amino acids. Consequently, we prepared a peptide (**13**) belonging to the N-terminal domain of Histone H1 (H1.0 isoform) of the sequence K17ASKK(N_3)-STDHPKYS29, in which the *N*-Fmoc-6-azidonorleucine

dx.doi.org/10.1021/ja507886s | J. Am. Chem. Soc. 2014, 136, 13622−13628

building block was placed instead of Lys21. To test the compatibility of the reaction with cysteine-containing peptides, we also prepared the same peptide sequence (**14**) containing a cysteine residue among others amino acids (R15AKASKK-(N_3)STDHPKYSDC31). Analogous to peptide **8**, peptides **13** and **14** were cleaved from the resin and successfully isolated by semipreparative HPLC (Schemes S6 and S7, Figures S7 and S9). Peptide **13** was then reacted with phosphites **5** and **7** in DMF and with phosphite **6** in Tris-HCl buffer, while peptide **14** was reacted with phosphite **5** in DMF (Table 1). The

Table 1. Synthesis of Phosphoramidate Peptides 15a−c and 16.



Compound	Azido-Peptide	R	Solvent	Isolated yield[a] (%)
15a[b]	13		DMF	33 (9)
15b[b]	13		Tris Buffer pH 7.6	40 (10)
15c[c]	13		DMF	48 (12)
16[b]	14		DMF	30 (8)

[a]Overall peptide synthesis yield (incl. Fmoc-SPPS) in brackets. [b]Subsequent addition of phosphite (2 × 5 equiv) over 24−48 h, 45 °C. [c]Subsequent addition of phosphite (3 × 5 equiv) over 9 h, 45 °C.

reactions were followed by HPLC-UV-MS (Figure S16), and the reaction mixtures were left until depletion of the ε-azido lysine-containing peptides **13** and **14**. Within the range of 9 and 48 h the corresponding phosphoramidates **15a−c** and **16** were obtained in excellent conversions, and the reaction mixtures were purified again by semipreparative HPLC. Peptides **15a−c** and **16** were isolated in good yields (Table 1, Figures S18−S21) and characterized by high-resolution (HR)-MS and ^{31}P NMR (Figures S57−S60). As mentioned before, the phosphoramidate ester bond was stable during analysis and purification by HPLC under standard conditions.

Uncaging of Photocleavable Phosphoramidates Lysine-Containing Peptides. After the isolation of the caged peptides **15a**, **15b**, and **16**, our goal was to cleave the phosphoramidate ester under mild conditions to release the natural pLys modification without harming the acid-labile phosphoramidate bond. Following our reported UV-deprotection of nitrobenzyl ester-type caged aryl phosphoramidates,[22a,d] we were able to uncage all peptides, **15a**, **15b**, and **16**, thus providing the pLys peptides **17** and **18** as main products (Schemes S13−S15, Figures S22−S24). Interestingly, irradiation of **15a** at 355 nm for 15 s rendered full deprotection as observed by HPLC-UV (Figure 2A,B). Phosphoramidate **15b** was fully deprotected after irradiation under the same conditions for 30 s (Figure S23). The faster uncaging rate observed for **15a** is in agreement with the already published photolysis of caged pSer.[25]

No other peptidic products were observed after photolysis. Note that for the cysteine-containing peptide **16**, dithiothreitol (DTT) was added to prevent side reactions of the released nitrosoacetophenone with the peptide or phosphate during UV irradiation (Figure S24).[25] To our knowledge, these results show, for the first time, the site-specific phosphorylation of Lys residues on a peptide level.

Basic Deprotection of bis(2-Cyanoethyl) Phosphoramidate Lysine-Containing Peptides. The base-labile 2-cyanoethyl group has been widely used as a protecting group (PG) in phosphoramidites for solid-phase synthesis of oligonucleotides.[26] This base-labile PG can be removed quickly in concentrated solutions of hydroxide anions via β-elimination. Nevertheless, we were concerned about the reactive electrophilic acrylonitrile that is formed after the deprotection, which could react with the nucleophilic side chains of the unprotected histone peptide.

Incubation of peptide **15c** in 250 mM sodium hydroxide solution revealed full deprotection after 20 min (Figure 2A,C, Scheme S16). The main observed product was the desired pLys peptide **17**, whereas no other peptidic side-products were observed. Remarkably, only a very minimal amount of P(=O)−N hydrolysis was detected (<7% by LC-UV) (Figure S25), which was in accordance with the higher stability of *N*-alkyl phosphoramidates under basic as opposed to acidic conditions.[11]

Stability of the Phosphoramidate Bond in Unprotected Phosphorylated Lys-Peptides. A first attempt to isolate pLys peptide **17** by semipreparative HPLC using an acetonitrile/water gradient in 0.1% TFA led to the dephosphorylation of peptide **17** (data not shown). We therefore decided to check the stability of the P(=O)−N bond under different conditions. To accomplish this we synthesized a phosphorylated-lysine dipeptide **21** (Figure 1C) obtained by basic deprotection of the bis(2-cyanoethyl) phosphoramidate **20** (Scheme S17). The crude dipeptide **21** was incubated under neutral, slightly acidic and acidic conditions (Tables S4 and S5). The decay of the P(=O)−N bond was monitored by ^{1}H, ^{31}P-HMBC NMR. In <2 h, half of the phosphoramidate bond in dipeptide **21** was hydrolyzed both in 0.05% formic acid and in 0.1% trifluoroacetic acid (Table S5, Figure S31). In contrast, after 18 h, the phosphoramidate dipeptide **21** was still the major product at pH 4.5 (59%) and pH 7.5 (69%) (Table S4, Figure S30). Although these data confirm the instability of the P(=O)−N bond at very acidic pH, it also suggests that Lys-phosphorylated peptides could be analyzed by short-gradient HPLC runs employing acidic solvents.

We then performed the purification with an acetonitrile/water gradient in alkaline aqueous buffer (pH 9.2) as a mobile phase (Figure S33). Under these conditions, no hydrolysis of the P(=O)−N bond was observed, and peptides **17** and **18** were successfully isolated in 42% and 38% yield, respectively (Figures S33 and S34). Similar conditions have been used previously for the purification of phosphorylated-arginine peptides without indication of hydrolysis.[19] With peptide **17** in hand, we also studied the thermal stability of the phosphorylated-lysine peptide (Scheme S18, Figure S32). As expected, higher temperatures (60−85 °C) caused rapid hydrolysis of the P(=O)−N bond, whereas lower temperatures (2 °C) seemed to be the best conditions to store the

dx.doi.org/10.1021/ja507886s | J. Am. Chem. Soc. 2014, 136, 13622−13628

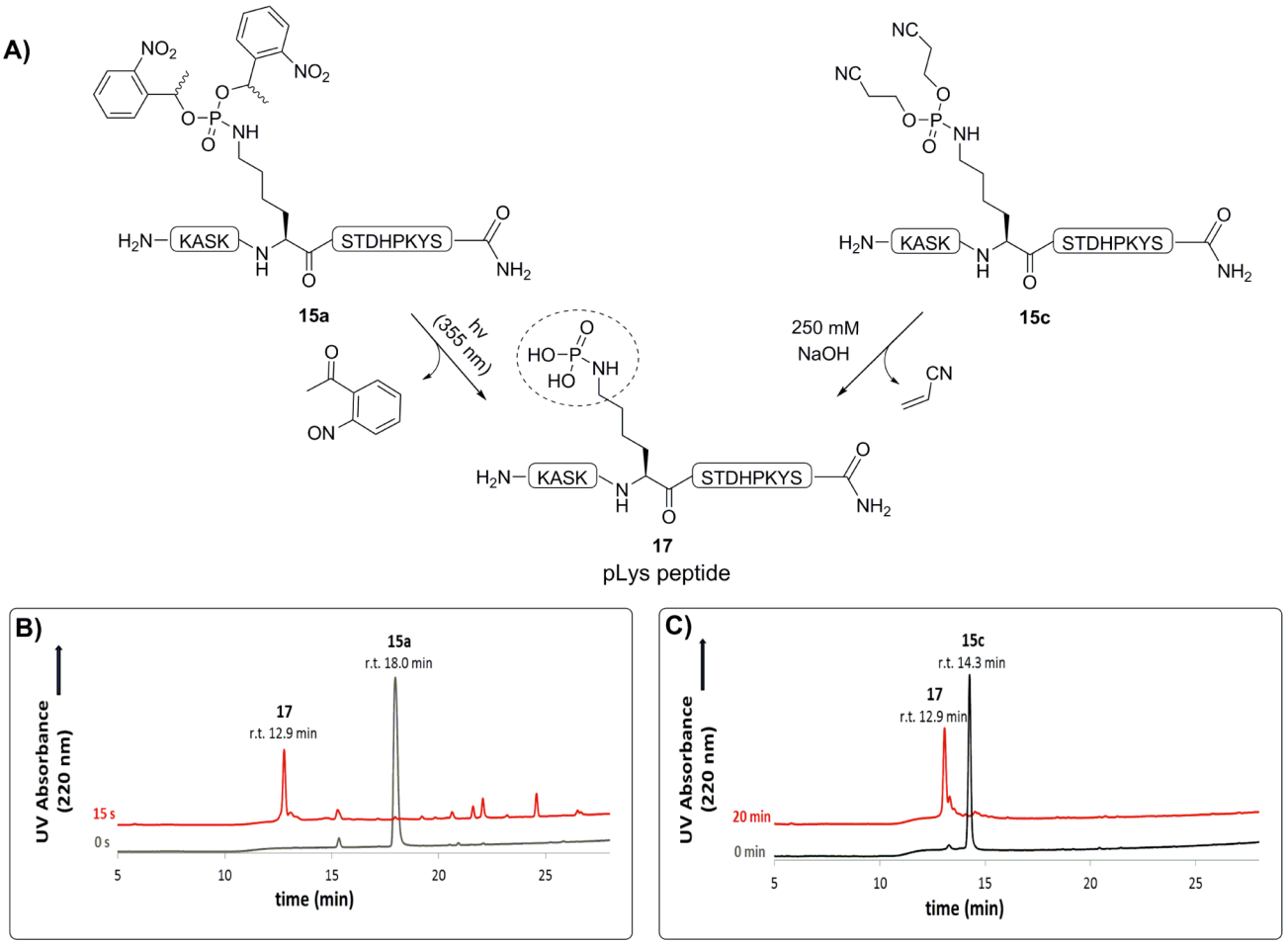


Figure 2. (A) Uncaging of peptide **15a** (left) and basic deprotection of peptide **15c** (right). (B and C) HPLC-UV traces before and after deprotection.

phosphorylated-lysine peptides. At room temperature and pH 7.4, phosphoramidate hydrolysis in **17** occurred (31% by LC-UV), similarly to the dipeptide **21** under the same conditions (Figures S30 and S32).

Localization of Phosphorylation by Use of Electron-Transfer Dissociation Mass Spectrometry (ETD-MS). Having succeeded in establishing a synthetic route to access site-specifically phosphorylated-lysine peptides, we focused on the characterization of the corresponding phosphorylated-lysine peptides. First, we used NMR to characterize the ^{31}P NMR signal for peptides **17** and **18**. A unique signal peak was observed in the ^{31}P NMR spectra for both peptides (Figures S61 and S62). To demonstrate that this ^{31}P NMR signal was attached to a lysine residue, ^{1}H−^{31}P HMBC was performed. The ε-methylene hydrogen atoms from the lysine side-chain showed coupling to the phosphorus signal peak, thus confirming that the phosphate was attached to a lysine side chain (Figures S61 and S62). However, localization of the phosphorylated-lysine residue using NMR would require a full sequential assignment of all peptide resonances. We therefore decided to use mass spectrometry (MS), with the intention of unambiguous localization of the site of phosphorylation. Tandem mass spectrometry (MS/MS) has become the method of choice for analysis of PTMs of peptides or proteins because of the high sensitivity and high-throughput capabilities when coupled to nanoliquid chromatography (nLC).[27] Collision induced-dissociation (CID) is the most common fragmentation technique for modified peptides, specifically for those containing O-phosphorylated residues.[28] Although CID has been applied successfully in many studies, the analysis is often hampered by a neutral loss of the phosphate group, thus preventing the unambiguous assignment of the modification site.[29] This complication is even more severe in the case of the more labile phosphoramidate bond for N-phosphorylated peptides.[30]

For the analysis of labile PTMs such as arginine and lysine-phosphorylation radical-driven fragmentation techniques such as electron capture dissociation (ECD)[31] and electron-transfer dissociation (ETD)[32] were found to be more useful than CID due to the reduced elimination of phosphoric acid.[9,30] In fact, ECD was applied to determine the phosphorylation site of different lysine-phosphorylated peptides, which were synthesized by using a nonsite-specific approach.[21] Compared to CID, ECD showed fragment ion spectra, allowing the localization of N-phosphorylation in the corresponding pLys peptides. However, the lack of a synthetic route to access site-specifically phosphorylated-lysine peptides together with the high instabilty of the P(=O)−N bond in the gas phase suggested that the phospho-site assignment by ECD should be done with caution.[21]

With these considerations in mind, we proceeded with the analytical characterization of peptides **17** and **18** (Figures 2A and 3A). As expected, CID showed complete neutral loss of phosphate (data not shown). No phosphorylated fragment ions

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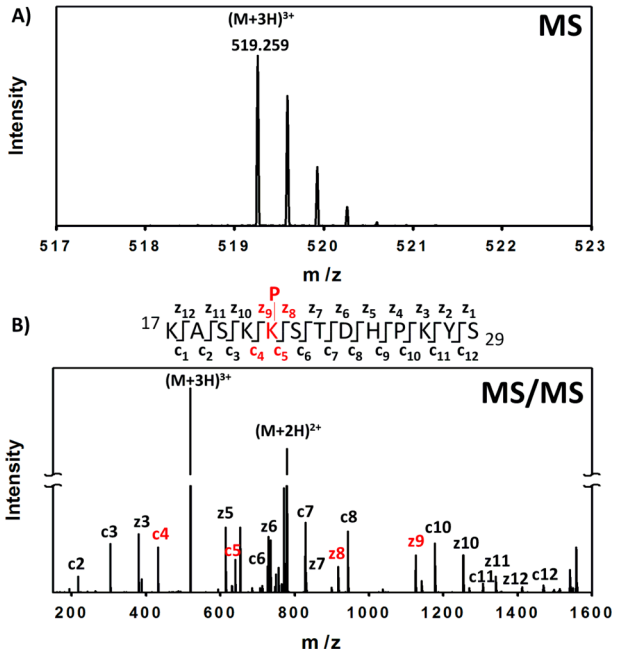

Figure 3. (A) ESI-MS spectra showing the triply charged precursor ion of **17**. (B) ETD fragment ion spectra of peptide **17**. Individual fragments are labeled according to the c- or z-ion nomenclature. The characteristic mass difference of the phosphorylated Lys21 is shown in the Figures S38 and S39.

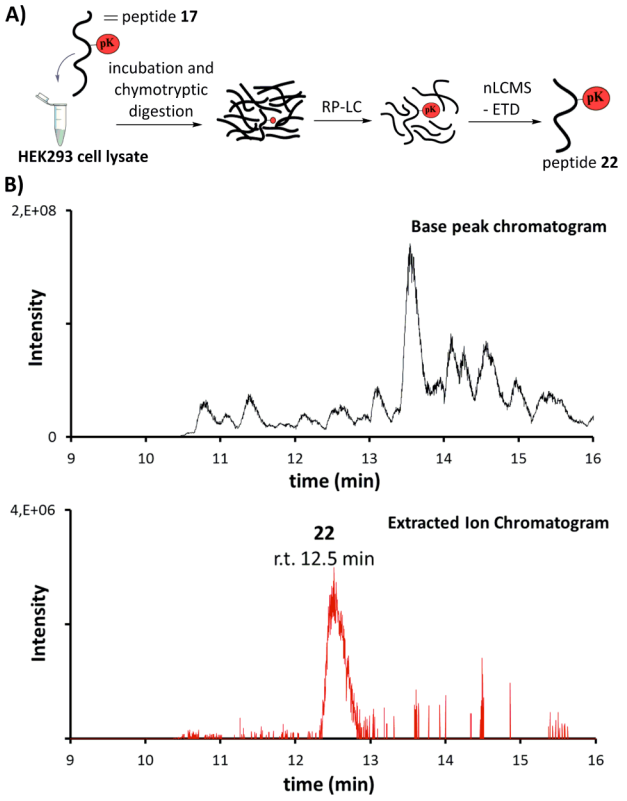

Figure 4. (A) Detection workflow of chymotryptic peptide **22** in a cell lysate context. (B) Base peak intensity chromatogram of chymotryptic peptide mixture. Extracted ion chromatogram of the doubly/triply/quadriply charged precursor ion of phosphorylated-lysine peptide **22**.

for the identification of phosphorylated lysine could be found in any tandem MS fragment preventing the localization of phosphorylation with this method. In contrast, ETD fragmentation verified phosphorylation at the lysine side chain in position 21 (Figures 3B, S38, S39, S43, and S44). Fragment ions indicating phosphorylation of other amino acid side-chains such as Arg15, Lys17, Lys20, Lys27, Ser19, Ser22, Thr23, His25, Tyr28, Ser29, Asp30, or Cys31 could not be observed. This result confirms the chemoselectivity of the Staudinger-phosphite reaction and indicates that migration of the phosphate in the gas phase does not play a role during ETD analysis of peptides **17** and **18**.

These results confirm that with the applied UV light and basic deprotection conditions the phosphorylation remains on the desired modification site to a larger extent and no phosphate migration to other phosphate acceptors occurs.

Detection of a Synthetic Phosphorylated-Lysine Peptide in a Proteome Context. Having established both synthetic and analytical tools to study Lys-phosphorylation, we aimed to detect phosphorylated-lysine peptides in a more complex sample. Our goal was not only to detect phosphorylated-lysine peptides in a cell lysate but also to ensure that phosphorylated-lysine peptides survive the conditions of a bottom-up proteomic analysis. We therefore incubated synthetic peptide **17** at various concentrations in a whole-cell lysate of HEK293 cells (Figure 4A, Scheme S19, and Table S6). After incubation for 1 h, proteins were digested with chymotrypsin. Because of the basic character of the histone-derived Lys-phosphorylated peptide, chymotrypsin instead of trypsin was used for enzymatic digestion. The resulting peptide mixture was analyzed using a two-dimensional reversed-phase (2-D) LC-MS/MS approach.[33] The expected chymotryptic peptide **22** was detected at a concentration as low as 0.4 μM (Figure 4B, S45, and Table S6). Characterization by ETD confirms the phosphorylation site. However, despite the addition of a phosphatase inhibitor (Na_3VO_4), a significant loss of phosphate was observed which cannot be explained based on the intrinsic instability of phosphorylated-lysine peptides at neutral pH (Figures S30 and S32). Possibly other enzymes might have an influence on the stability of phosphorylated-lysine peptides in a cell lysate context.

CONCLUSION

In summary, we have developed the first synthesis of site-specifically phosphorylated-lysine peptides. Our synthetic route takes advantage of the chemoselectivity of the Staudinger-phosphite reaction of straightforwardly accessible unprotected ε-azido lysine-containing peptides with phosphites. The method circumvents the final acidic treatment of the acid-sensitive alkyl-phosphoramidate moiety. Furthermore, we employed phosphoramidate esters as precursors for the natural modification, which can be liberated by photolysis or applying basic conditions. Finally, we were able to demonstrate the site-specific localization of phosphorylated lysine by ETD tandem MS. Referring to previous ECD results,[21] our data indicate that the stability of the phosphoramidate bond during the ETD process is high, keeping the pLys side-chain completely intact during fragmentation. This makes ETD mass spectrometry particularly suitable for analysis of phospholysine peptides. Consequently, ETD can also be used for proteomic studies of Lys-phosphorylated proteins. With this chemical approach in hand, we expect to contribute in the near future toward understanding the role of lysine phosphorylation and its

dx.doi.org/10.1021/ja507886s | J. Am. Chem. Soc. 2014, 136, 13622−13628

biological impact. Further studies in that direction are currently underway in our laboratories.

■ ASSOCIATED CONTENT

Supporting Information

Experimental procedures including characterization data for all new compounds, NMR spectra, HPLC-UV or FLD traces as well as MS and tandem MS spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The authors acknowledge support from the DFG (SFB 765 and SPP 1623), the Fonds der Chemischen Industrie, the Einstein Foundation, and the Boehringer-Ingelheim Foundation (Plus 3 award). We thank Dr. Gerrits, Dr. Majkut, and Iris Claußnitzer the use of the laser (Spectron Laser Systems). We would like to dedicate the article to Prof. Hans Reißig on the occasion of his 65th birthday.

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